Summary Progress
Report

MASTER

100-111111 LIMITED

100

#C00-3521-128

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

SUMMARY PROGRESS REPORT TO
THE U.S. ATOMIC ENERGY COMMISSION
CONTRACT NO. AT930-1) 910

BIOLOGICAL EFFECTS OF RADIATION, AND RELATED
BIOCHEMICAL AND PHYSICAL STUDIES

PERIOD OF REPORT: JULY 1, 1950 - SEPTEMBER 30, 1971

LEO WADE, M.D.
ACTING DIRECTOR

DATE OF REPORT: SEPTEMBER 30, 1971

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH
410 EAST 68th STREET, NEW YORK, NEW YORK 10021

NOTICE
This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

MASTER

TABLE OF CONTENTS
AEC CONTRACT AT(30-1) 910

<u>DIVISION OF BIOPHYSICS</u> (Proposals #1, 2, 3, and 4)	<u>Page</u>
I. Radiation Mechanisms in Mammalian Systems	2
II. Theoretical Studies of Radiation Absorption	13
III. Free Radical Production in Biologically Significant Compounds	22
IV. Radioactive Isotope Instrumentation, Data Analysis and Organ Scanning Studies	33
V. Metabolic and Organ Scanning Studies (Non-Osseous)	83
VI. Metabolic and Organ Scanning Studies (Osseous)	118
VII. Cyclotron Operation and Radionuclide Production	169
VIII. Graduate Students Trained	186
IX. Bibliography (Includes Division of Medical Research - Section 5604)	187
 <u>DIVISION OF BIOLOGICAL CHEMISTRY</u> (Proposals #5 and 6)	
I. Divisional Summary	211
II. Chemical and Biological Studies on Nucleic Acids and Derivatives	217
III. Physical - Chemical Studies on Nucleic Acids and Derivatives	224
IV. Graduate Students Trained, Degrees Granted and Postdoctoral Tenures Completed	227
V. Bibliography	227
VI. Opinion as to the Present State of Knowledge in this Area of Research, Significance and Needed Future Investigations:	
Proposal #5	268
Proposal #6	270

TABLE OF CONTENTS (Continued)

	<u>Page</u>
VII. Present Division of Federal Support	271

<u>Proposal #</u>	<u>Department</u>	<u>Investigator</u>	<u>Co-Investigator</u>
1	Division of Biophysics	John S. Laughlin, Ph.D. Member	Ira Pullman, Ph.D. Associate Nikitas Kassaris, Ph.D. Associate
2	Division of Biophysics	John S. Laughlin, Ph.D. Member	Jerrold Fried, Ph.D. Associate Bozidar Djordjevic, Ph.D. Associate Alan Gelbard, Ph.D. Research Associate
3	Division of Biophysics and Division of Medical Research	John S. Laughlin, Ph.D. Member	George Brown, Ph.D. Member Helen Woodard, Ph.D. Consultant Richard Benua, M.D. Associate Member Louis Zeitz, Ph.D. Associate Member Klaus Mayer, M.D. Associate W. Gordon Monahan, Ph.D. Associate Edmund Rothschild, M.D. Associate Charles LaMonte, M.D. Assistant Clinician Ernest Greenberg, M.D. Visiting Investigator J. Wes Beattie, M.E.E. Research Associate Alexandra Dimich, M.D. Research Associate Marvin Friedman, Ph.D. Research Associate

<u>Personal</u>	<u>Department</u>	<u>Investigator</u>	<u>Co-Investigator</u>
4	Division of Biophysics	John S. Laughlin, Ph.D. Member	Roy S. Tilbury, Ph.D. Associate Thomas Kuo, Ph.D. Research Associate
5	Division of Biological Chemistry	George B. Brown, Ph.D. Member	Aaron Bendich, Ph.D. Member
6	Division of Biological Chemistry	George B. Brown, Ph.D. Member	Mary L. Petermann, Ph.D. Member Liebe F. Cavalieri, Ph.D. Member

Summary of Activities 1950 - 1971 related to

AEC Contract AT(30-1)910

- PROPOSAL # 1. Radiation Studies: Theoretical Investigation of Electron LET Spectra and Dose Relationship for Ionizing Radiations.
- PROPOSAL # 2. Radiation Studies: Radiation Mechanisms in Mammalian Systems.
- PROPOSAL # 3. Metabolic Studies in Cancer with Radioactive Isotopes.
- PROPOSAL # 4. Cyclotron Facility and Radionuclide Production

John S. Laughlin, Ph.D.

Division of Biophysics

DIVISION OF BIOPHYSICS

Over the past twenty-one years, the Division of Biophysics has carried out studies in a number of projects concerned with the biological, chemical, physical as well as clinical effects of radiations from accelerators and from radioactive isotopes. The present report places most emphasis on the research accomplishments of the current program and does not attempt to cover all of the past achievements which were described in the 18-year report submitted in September of 1968.

In particular, the achievements in radiation chemistry and radiation dosimetry studies are not reported here. Also omitted is much of the technical development of the high energy gamma-ray scanning system and the original clinical studies with Ca-47 initiated in 1958. Also, the kinetic studies of iodine metabolism carried out to establish quantitative correlations between clinical abnormalities and physiological parameters, and the dosimetry method developed for prescribing the largest relative safe dose of I-131 in the therapy of functioning metastatic thyroid cancer which are described in the 1968 report, are not included here.

I. RADIATION MECHANISMS IN MAMMALIAN SYSTEMS

John S. Laughlin, Ph.D., Member
Bozidar Djordjevic, Ph.D., Associate

A. Radiosensitivity Studies with Synchronized Populations of Mammalian Cells in Culture

During the preceding period, the following objectives have been fulfilled using mainly synchronized cultures of HeLa cells.

1) A new method of synchronization using vinblastine-sulfate was perfected (1). This method allows for larger quantities of synchronized cells to be utilized, which is particularly important for biochemical work.

2) The role of early and late synthesized RNA and protein in initiation of DNA synthesis was investigated with double labeling with C-14 and H-3 thymidine and double layer autoradiography, and appropriate metabolic inhibitors (2).

It was found that inhibition of RNA synthesis by actinomycin retarded DNA synthesis in cells which had not entered the DNA synthetic period during actinomycin treatment, but that once DNA synthesis started, it was little affected by actinomycin D. Similarly, it was confirmed that protein synthesis is a prerequisite for initiation of DNA synthesis. These findings are relevant to the problem of the role of DNA synthesis in radiosensitivity.

3) Changes in RNA and protein metabolism were determined following X-irradiation (3).

Doses of 200 rads produce a prompt depression of protein synthesis, whereas RNA synthesis is not affected for the first few hours after exposure of cells to X-irradiation. The inhibition of protein synthesis is a consequence of a delayed release of newly synthesized polypeptides from the polyribosome. The earliest alteration that can be detected in RNA metabolism is a slow rate of transformation of rapidly labeled 45S RNA into smaller molecular species of ribosomal RNA.

4) The periodic synthesis of deoxycytidine monophosphate (dCMP) deaminase during the cell cycle have been observed. The level of dCMP deaminase is found to be very low immediately after cell division. It rises during the S period, and reaches a peak at 18 hours after mitosis. Upon entering into the next division, cells rapidly lose dCMP deaminase activity, and repeat the rise in activity during the subsequent S period (4).

5) The effect of X-irradiation on the periodic synthesis of thymidine kinase was studied (5). Exposure of the synchronous cells in early G₁ phase to doses of up to 2,000 rads had no effect on the

rise of thymidine kinase during the cell cycle, suggesting that ionizing radiation does not interfere with the synthesis of this enzyme. Irradiation did interfere with the periodic fluctuation of thymidine kinase level by preventing the decay of enzyme activity. A similar pattern of prolonged elevated activity was also obtained when synchronous cells were treated with inhibitors of DNA synthesis (hydroxyurea or excess thymidine) or with colcemid, a mitotic spindle inhibitor. The one common characteristic of the cell population exposed to these inhibitors and to 2,000 rads is that the cells are prevented from reaching a stage of the division cycle (G_1) which is characterized by low enzyme activity. Thus the phenomenon observed apparently results from the inhibition of cell division rather than from a direct effect on the synthesis or activity of the enzyme.

6) Fluctuations in toxicity by several metabolic inhibitors have been studied in synchronized HeLa cells during the division cycle, and interesting differences in the toxicity pattern have been observed. 5-Bromodeoxyuridine (BUdR), an analog of thymidine, varied in its toxicity proportionally to the intensity of DNA synthesis during the cycle (6). Hydroxyurea was also found most toxic in the S period, when given to synchronous cells at $10^{-2}M$ during a 4-hour incubation period (7). On the other hand, phleomycin, a DNA binding agent, was found most toxic during the G-2 phase of the division cycle (8). Mitomycin C is most toxic during the G-1 phase, while actinomycin D is again most toxic during the S phase (9). Daunomycin is most toxic during the S period (10). Information from such studies is expected to contribute to a better understanding of the periodic fluctuation of radiosensitivity during the division cycle.

7) Studies on "unbalanced growth" caused by metabolic inhibitors have been conducted. The effect of 1-B-D-arabinofuranosylcytosine (11), its fluorinated derivative (12), deoxyadenosine, and excess thymidine (13), on nucleic acid metabolism and viability of HeLa cells, have been reported by this laboratory. Also, a report on the mechanism of the "unbalanced growth" syndrome has recently been completed (14). Again, such studies are expected to provide a better understanding of radiobiological phenomena.

B. Effects of Exposure of Mammalian Cells to Selected Metabolic Inhibitors on their Radiosensitivity and Recovery

1) Effect of inhibitors on RNA synthesis (15). Puromycin-aminonucleoside (PAN), an inhibitor of ribosomal RNA synthesis, increases the effect of X-irradiation in synchronized cells irradiated in the S phase but not in the G-1 phase. Optimal conditions (increased radiosensitivity without toxicity to unirradiated cells) for the maximum effect of PAN were found.

2) Effect of inhibitors of DNA and protein synthesis on radiosensitivity.

It was found that inhibition of DNA synthesis per se will

increase survival of irradiated cells, particularly those in the S phase. High concentration of thymidine (which inhibits DNA synthesis), hydroxyurea and arabinofuranosylcytosine have that effect. Also, acetoxycycloheximide, an inhibitor of both DNA and protein synthesis, restores viability in irradiated cells. Puromycin, an inhibitor of protein synthesis, on the contrary, decreases survival, particularly in the S phase (16). In this category belongs also work on the effect of inhibitors of protein synthesis on the recovery of sublethally irradiated HeLa cells (17).

3) Molecular changes caused by cytotoxic and radiation response-modifying drugs.

In an effort to correlate the differential cytotoxic effect of mitomycin during the division cycle with its assumed primary mode of action, an CS_2SO_4 density gradient analysis of DNA extracted from cells treated with mitomycin during the division cycle was performed. Mitomycin has been shown to cross-link DNA and this is thought to be the primary cause of toxic action in bacteria (18). Cross-linking of DNA strands can be followed in density gradients, as it facilitates renaturation of heat denatured DNA, rendering it lighter and thus discernible in a gradient. When DNA was extracted from cells treated for one hour with 40 $\mu\text{g}/\text{ml}$ mitomycin in the later part of the cycle, more cross-linking (per gram of DNA) was observed than in DNA from cells treated earlier in the cycle (G_1). Since toxicity is highest in the early part of the cycle, there appears to be no direct correlation between cross-linking and toxicity, at least in our system.

C. Relative Biological Effectiveness of Electrons

The biological response to electrons has been studied as a function of depth in tissue equivalent material irradiated with high energy electrons. Three criteria were used with irradiated HeLa cells in vitro: clonal size distribution, survival curve analysis (Do and extrapolation number), and degree of recovery as indicated by split dose experiments. On the basis of all of these criteria, the biological effectiveness of high energy electrons did not change with depth, despite the major change in energy (from 24 million electron volts to essentially zero energy (19).

D. Unscheduled DNA Synthesis and Radiation Response

There is a certain amount of DNA synthesis occurring outside the main period of DNA synthesis (S phase) in HeLa cells; this is known as unscheduled DNA synthesis (U.D.S.) and is considerably increased after U.V. and X-irradiation (20,21).

In view of the current interest in "metabolic" DNA (22) and the possibility of involvement of U.D.S. as an error correcting mechanism during all parts of the division cycle, we have studied the metabolic stability of unscheduled synthesized DNA and the sensitivity of this synthesis to an agent (acriflavine) which is non-toxic to unirradiated cells but decreases the viability of irradiated ones. We have made

the following observations:

1) We have found that significant amounts of 3-H thymidine are incorporated into DNA of HeLa cells (involving about 1% of the total DNA) without an extracellular stimulus, at a time when normal semiconservative DNA synthesis does not occur (i.e., in the early G₁ phase). This newly synthesized DNA is localized on the chromosomes and is not due to mycoplasma contamination. This DNA synthesis can be suppressed by non-toxic concentrations of acriflavine, a DNA complexing agent (23).

2) Ultraviolet irradiation induced an excess of unscheduled DNA synthesis (UDS) in the G₁ phase, using a dose causing only slight killing. Acriflavine at non-toxic concentrations depresses U.D.S. at low doses of U.V. light, but not at higher doses. On the other hand, acriflavine did not depress survival at low U.V. doses, but only at higher doses (24).

3) Both "spontaneous" and U.V. induced U.D.S. are metabolically stable over one generation time. The two differ, however, in their response to increased temperature. While U.V. induced U.D.S. is stimulated by increased incubation temperature (42°C versus 37°C) spontaneous U.D.S. is depressed (25).

It is apparent from the foregoing that the role of U.D.S. has yet to be determined.

E. Radiobiological Studies of Mammalian Cells Using High Intensity Pulsed Electrons

In our system of HeLa cells attached to transposable mylar discs, cell migration from radiation protected areas was prevented, a possible source of artefacts in systems of other workers. No break was seen in the survival curve of HeLa cells irradiated in air with up to 2,000 rads of 3 nanosecond electron pulses. With lesser oxygen concentration in nitrogen (less than 1%), survival curves in their final parts were parallel to those obtained under pure nitrogen, indicating that oxygen was depleted with pulsed irradiation under these conditions.

F. Neutron Radiobiology

We have developed an hypoxic cell culture system in which S3 HeLa cells retain excellent viability for 24 hrs. or longer. With this system, an OER of about 1.5 was obtained using cyclotron-produced neutron irradiation at 2.4 cm depth in tissue equivalent material; using Co-60 gamma irradiation, the OER was 3.25.

Investigation of the recovery of cells from neutron-induced damage by means of split dose irradiation revealed that there was little or no recovery in the period immediately following the first neutron exposure (Fig. 1). This tended to confirm the information obtained from neutron survival curves which showed an almost complete lack of shoulder following correction for cell multiplicity. However, differences did exist in the shape of the recovery curves obtained from irradiating cells at different depth, viz., the survival was consistently higher for cells irradiated at 8 cm. compared to 2.4 cm. This might be due to the higher RBE

of the beam at 2.4 cm. and is unlikely to be due to the slightly larger gamma contribution to the total dose at 8 cm (15%, versus 11% at 2.4 cm.).

We have recently found that for S3 HeLa cells in tissue culture the RBE of a neutron beam (modal energy 6 MeV and average LET 30/keV/u) appears to decrease with increasing depth in tissue equivalent material (26) (Table I). Moreover, the survival following neutron irradiation, and hence the RBE values, were found to be independent of both dose rate and field size, at least for dose rates in the range 4 to 15 rads/min and field sizes from infinity to 10 x 10 cm. These RBE data were supported by investigations on LET changes using a Rossi-type counter which showed a monotonic shift in the LET distribution to lower values with increasing depth in the phantom.

G. Studies with Cf-252 Sources

Preliminary work on californium sources is essentially completed. Arrangements for irradiation (including geometry of sources, shielding, holders for tubes containing cell suspensions) have been worked out. Our newly developed system of chronically hypoxic HeLa cells should prove uniquely suited for irradiation with the low dose rate emission of mixed gamma rays and neutrons from californium sources.

H. Finally, the work on L-asparaginase performed by this laboratory, should be mentioned. The inhibitory effect of asparaginase from Escherichia Coli on HeLa cells has been determined (27). A predictive test on the efficacy of treatment of leukemia cells by L-asparaginase, has been elaborated (28).

Future Investigations: We plan to continue work on O.E.R. of fast neutron irradiation from the Institute's cyclotron and mixed gamma and neutron irradiation from californium sources, using our newly developed system of chronically hypoxic HeLa cells. We will also study the movement of synchronized HeLa cells through the division cycle when maintained in an hypoxic state by co-incubation with heavily irradiated oxygen-consuming cells. Also we will irradiate these synchronized cells in various parts of the division cycle with X-rays.

Finally, we are planning to continue work on density controlled 3T3 cells, using this system as a model for dormant tumor cells. We will seek to synchronize these cells and irradiate them in various parts of the division cycle. A major goal of these studies will be to investigate differences in the radiation response (including repair and recovery) of cells in the various growth phases (including stationary phase).

Present State of Knowledge: Sensitization of biological material to ionizing radiation by molecular oxygen is not well understood. While the so-called oxygen enhancement ratio (O.E.R.) is evident in both procaryotic and eucaryotic organisms, in simpler systems such as bacteriophage or transforming DNA which are protected against indirect radiation effects, an oxygen effect is evident only after addition of radioprotective substances such as cysteamine (20). Consequently,

the oxygen effect in whole cells has been attributed to free SH (radioprotective) groups within the cell which "repair" target sites inactivated under anoxic condition, but are prevented from doing so in the presence of oxygen (30). In investigating the mechanisms of damage and repair, it may be wise not to confine consideration to effects on DNA alone. Indeed, such radiation induced lesions as single DNA strand breaks are generally considered to be of little biological importance (31). While this does not preclude involvement of DNA in radiation response, it may be imagined that a radiation-changed cellular component may interact with DNA in such a way as to change its structure and function. The nuclear membrane may be a candidate for such a role, as it is composed of easily oxidizable lipoproteins and its role in DNA replication has been implicated (32).

Apart from the above considerations it is obvious that oxygen is the most ubiquitous and important of radiosensitizers. Since depression of the oxygen content is held responsible for the high radioresistance of certain tumor cells (33), O.E.R. studies, including the effects of high LET irradiation (to which cells are less sensitized by molecular oxygen (34), are thus highly relevant to radiotherapy.

References

1. KIM, J.H. and STAMBUK, B.D., Synchronization of HeLa cells by vinblastine sulfate, *Experimental Cell Research* 44: 631-634, 1966.
2. KIM, J.H., GELBARD, A.S. and PEREZ, A.G., Inhibition of DNA synthesis by actinomycin D and cycloheximide in synchronized HeLa cells, *Experimental Cell Research* 53: 478-487, 1968.
3. KIM, S.H., KIM, J.H., and DJORDJEVIC, B., Effects of X-irradiation on RNA and protein synthesis in HeLa cells, *Radiation Research* 42: 577-589, June, 1970.
4. GELBARD, A.S., KIM, J.H., and PEREZ, A.G., Fluctuations in deoxycytidine monophosphate deaminase activity during the cell cycle in synchronous populations of HeLa cells, *Bioch. Biophys. Acta* 182: 564-566, 1969.
5. GELBARD, A.S., PEREZ, A.G., KIM, J.H. and DJORDJEVIC, B., The Effect of X-irradiation on Thymidine Kinase Activity in Synchronous Populations of HeLa Cells, *Radiation Research* 46: 334-342, 1971.
6. KIM, J.H., GELBARD, A.S., PEREZ, A.G., and EIDINOFF, M.L., Effect of 5-bromodeoxyuridine on nucleic acid and protein synthesis and viability in HeLa cells, *Biochem. Biophys. Acta* 134: 338-394, 1967.
7. KIM, J.H., GELBARD, A.S. and PEREZ, A.G., Action of hydroxyurea on the nucleic acid metabolism and viability of HeLa cells, *Cancer Research* 27: 1301-1305, 1967.

8. DJORDJEVIC, B. and KIM, J.H., Lethal effect of phleomycin in different stages of the division cycle of HeLa cells, *Cancer Research* 27: 2255-2260, 1967.
9. DJORDJEVIC, B. and KIM, J.H., Differential effects of mitomycin C and actinomycin D during the division cycle of HeLa cells, *J. Cell Biology* 38: 477-484, 1968.
10. KIM, J.H., GELBARD, A.S., DJORDJEVIC, B., KIM, S.H. and PEREZ, A.G., Action of daunomycin on the nucleic acid metabolism and viability of HeLa cells, *Cancer Research* 28: 2437-2442, 1968.
11. KIM, J.H., and EIDINOFF, M.L., Action of 1- β -D-arabinofuranosylcytosine on the nucleic acid metabolism and viability of HeLa cells, *Cancer Research* 25: 698-702, 1965.
12. KIM, J.H., EIDINOFF, M.L. and FOX, J.J., Action of 1- β -D-arabinofuranosyl-5-fluorocytosine on the nucleic acid metabolism and viability of HeLa cells, *Cancer Research* 26: 1661-1664, 1966.
13. KIM, J.H., KIM, S.H. and EIDINOFF, M.L., Cell viability and nucleic acid metabolism after exposure of HeLa cells to excess thymidine and deoxyadenosine, *Biochem. Pharmacol.* 14: 1821-1829, 1965.
14. KIM, J.H., PEREZ, A.G. and DJORDJEVIC, B., Studies on unbalanced growth in synchronized HeLa cells, *Cancer Research* 28: 2443-2447, 1968.
15. DJORDJEVIC, B., KIM, J.H. and KIM, S.H., Modification of radiation response in synchronized HeLa cells by Metabolic inhibitors. I. Effect of an inhibitor of RNA synthesis, puromycin aminonucleoside, *Int. J. Radiat. Biol.* 14: 1-7, 1968.
16. DJORDJEVIC, B. and KIM, J.H., Modification of radiation response in synchronized HeLa cells by metabolic inhibitors. II. Effects of inhibitors of DNA and protein synthesis. *Radiation Research* 37: 435-450, 1969.
17. KIM, J.H., EIDINOFF, M.L. and LAUGHLIN, J.S., Recovery from sublethal X-ray damage of synchronized HeLa cells during inhibition of protein synthesis, *Int. J. Rad. Biol.* 11: No. 5, 509-511, 1966.
18. SZYBALSKI, W., and IYER, V.N., The Mitomycins and porfiromycins in antibiotics, Vol. I, 211-245, GOTTLIEB, D., and SHAW, P.D. EDS. Springer Verlag, New York, 1967.
19. KIM, J.H., PINKERTON, A. and LAUGHLIN, J.S., Studies on the biological effectiveness of high-energy electron beams at different depths in bone-absorbing material, *Radiation Research* 33: 419-425, 1968.
20. RASMUSSEN, R.E., and PAINTER, R.B., Evidence for repair of ultra-

violet damaged deoxyribonucleic acid in cultured mammalian cells, Nature 203: 1360-1362, 1964.

21. PAINTER, R.B., and CLEAVER, J.E., Repair replication in HeLa cells after large doses of X-irradiation, Nature 216: 369-370, 1967.
22. PELC, S.R., Turnover of DNA and function, Nature 219: 162-163, 1968.
23. DJORDJEVIC, B., EVANS, R.G., PEREZ, A.G. and WEILL, M.K., Spontaneous unscheduled DNA synthesis in GI HeLa cells, Nature, 224: 803-804, 1969.
24. EVANS, R. and DJORDJEVIC, B., Effect of non-toxic concentrations, of acriflavin on unscheduled DNA synthesis and survival of synchronized HeLa cells exposed to ultraviolet light, Int. J. Radiat. Biol. 15: 239-244, 1969.
25. EVANS, R.G., DJORDJEVIC, B., and PEREZ, A.G., Temperature Dependence of Thymidine Uptake and Stability of Thymidine Incorporated during Unscheduled DNA synthesis in Irradiated and Unirradiated HeLa cells, International Journal of Radiation Biology 18: 277-280, 1970.
26. EVANS, R.G., PINKERTON, A., DJORDJEVIC, B., MAMACOS, J., and LAUGHLIN, J.S., Changes in Biological Effectiveness of a Fast Neutron Beam with Depth in Tissue-Equivalent Material, Radiation Research 45: 235-243, 1971.
27. KIM, J.H., BOYSE, E.A., OLD, L.J. and CAMPBELL, H.A., Inhibition of HeLa cell cultures by preparations of E. coli, L-asparaginase, Biochem. Biophys. Acta 158: 476-479, 1968.
28. OETTGEN, H.F., OLD, L.J., BOYSE, E.A., CAMPBELL, H.A., PHILIPS, F.S., CLARKSON, B.D., TALLAL, L., LEEPER, R.D., SCHWARTZ, M.K. and KIM, J.H., Inhibition of leukemias in man by L-asparaginase, Cancer Research 27: 2619-2631, December, 1967.
29. HOWARD-FLANDERS, P., Effect of oxygen on the radiosensitivity of bacteriophage in the presence of sulphhydryle compounds, Nature 186: 485-487, 1960.
30. BACQ, Z.M., Chemical protection against ionizing radiation, Charles C. Thomas, Publ., Springfield, Ill. 1965, p.224.
31. ORMEROD, M.G., and STEVENS, U., The rejoining of X-ray-induced strand breaks in the DNA of a murine lymphoma cell, Biochem. Biophys. Acta 232: 72-82, 1971.
32. FRIEDMAN, D.L., and MUELLER, G.C., Studies on the nature of replicating DNA of HeLa cells, Biochem. Biophys. Acta 174: 253-263, 1969.
33. POWERS, W.E., and TOLMACH, L.J., Demonstration of an anoxic

component in a mouse tumor-cell population by in vivo assay of survival following irradiation, Radiology 83: 328-336, 1964.

34. BARENDSEN, G.W., Modification of radiation damage by fractionation of the dose, anoxia and chemical protectors in relation to LET, Ann. N.Y. Ac. Sci. 114: 96-114, 1964.

FIGURE 1. Relative survival of S3 Hela cells following split doses of radiation at different depths in tissue-equivalent medium.

TABLE I

RBE OF NEUTRON BEAM COMPARED

TO ⁶⁰Co AT DIFFERENT DEPTHS

Depth in cm	RBE values at different surviving fractions			
	0.80	0.50	0.37	0.20
2.4	3.8	3.4	3.2	3.1
8.0	3.2	2.9	2.7	2.6
16.0	2.4	2.3	2.2	2.2

II. THEORETICAL STUDIES OF RADIATION ABSORPTION

John S. Laughlin, Ph.D., Member
Nikitas D. Kessaris, Ph.D., Research Associate

A. Energy and Linear Energy Transfer (LET) spectra from X and gamma-ray sources

The object of this investigation was the theoretical calculation of the energy spectrum and LET spectrum of electrons in tissue-like matter subjected to external X-ray and gamma-ray sources. The large volume was uniformly irradiated by a known initial spectrum of photons. The photons, in the process of penetrating and being scattered in the medium, produce electrons with a variety of energies, and these electrons, while slowing down by collision, produce secondary electrons, which themselves possess various energies. Knowledge of the spectrum of the electrons as a function of energy as well as a function of LET is important in understanding the biophysical and biological effects of radiation of tissue-like matter.

The calculations were performed for seven photon spectra: (1) 130 Kevp, 5 mm. Al h.v.l.; (2) 200 Kevp, 0.5mm. Cu. h.v.l.; (3) 250 Kevp, 1 mm. Cu. h.v.l.; (4) 250 Kevp, 2 mm. Cu. h.v.l.; (5) 1 Mevp, 3.4mm. Pb h.v.l.; (6) 25 Mevp (betatron) X-rays, uncompensated; (7) Co-60 gamma-rays. Photon spectra 1-5 were derived with use of a simplified linear Kramer's plot for the continuous thick target emission spectrum, with the attenuation of interposed filtering material taken into consideration. The K-line radiation from the target, and Compton scattered radiation in the irradiated material were not included. The results of Cormack and Johns for spectra 6 and 7 were used. Spectrum 6 includes the absorption by the betatron donut monitor ionization chamber and 4 cm. of lucite, and ignores the neutron production of the betatron. Spectrum 7 does not include gammas scattered by the collimator on the cobalt itself.

The calculation proceeded in the following steps: From the initial photon spectrum, $I(R)$, normalized per roentgen of incident radiation, the primary electron source distribution $N(T)$, is derived. This is integrated to obtain the integrated primary electron source distribution $Q(T)$. The ratio of total to primary electron flux is subsequently employed to obtain the total electron source distribution, $Q(T)$. The primary and total electron energy spectra, $n(T)$ and $n(T)$, are obtained by dividing $Q(T)$ and $Q(T)$ by the total stopping power. The LET distribution functions $n(S)$ and $n(SA)$, corresponding to the identification of LET with the collision stopping power $S(T)$ and the local excitation stopping power, $SA(T)$ respectively, were finally derived from the total electron energy spectrum. The application of an upper limit Δ to the energy loss associated with any collision event, and the resulting LET spectra provide a picture of the instantaneous spatial distribution of collisions which corresponds more closely to the physical reality. The appropriate choice of a Δ depends critically on the dimensional features of the particular reaction (target size, migration of radicals, etc.).

These energy spectra can be utilized to obtain two very important biophysical quantities: D/J , the ratio of rads to density of ionization, and D/r , the conversion factor from roentgens to rads. These ratios were obtained for each of the seven photon spectra considered.

A paper describing the results of this work has been published in Radiology (1).

B. The Penetration, Scattering, and Absorption of High Electron Beams

The penetration, scattering, and absorption of electron beams of energy between 10 and 20 Mev in water have been mathematically investigated by the moment method. The beam geometry investigated first was a plane infinite monodirectional and monoenergetic beam incident on a water medium.

The moment method of solving the Lewis equation furnishes the mathematical moments of any given desirable quantity, such as the flux or the depth dose. The numerical values of the moments in this case were obtained by numerical evaluation of a set of multiple integrals resulting from the original equation. This can be best accomplished with the aid of computers. Initially, inaccessibility to a computer necessitated our using a desk calculator, but with a variety of computers subsequently available, (such as Bendix G-15, the IBM 650, and the IBM 9090), the project was greatly accelerated, and the construction of a large variety of biophysically interesting quantities became possible. It was also possible to expand the scope of the project in two directions: a) in calculating not only the scattering and absorption of the incident (primary) electrons, but also the scattering and absorption of the secondary electrons - those set in motion by the primaries, and b) in investigating a more complicated beam geometry, specifically the point monodirectional beam, also incident on an infinite water medium.

Thus, the following quantities have been obtained as a function of penetration depth for the plane parallel beam case:

- 1) The flux of electrons
- 2) The transmission function
- 3) The rate of charge accumulation
- 4) The rate of energy dissipation
- 5) The rate of bremsstrahlung production
- 6) The rate of bremsstrahlung absorption
- 7) The rate of energy absorption (depth-dose)
- 8) The angular distribution

We also found the relation between average depth and residual track length.

Finally, the important energy and LET spectra at various depths were obtained by solving a two-dimensional moment problem.

In addition, the following quantities were obtained for the point-monodirectional case:

- 1) the root-mean-square average radial spread of the flux as a function of the residual track length.
- 2) the root-mean-square average radial spread of the flux as a function of depth.
- 3) the root-mean-square average radial spread of the energy dissipation as a function of depth.
- 4) the average depth of penetration at a given radial distance (distance from the axis of the beam).

The solution of the Lewis equation also affords numerical values of the moments of a variety of other physically meaningful quantities.

This work was carried out for three distinct beam energies: 39, 30, 20 m_0c^2 , corresponding to 20, 15.3, and 10.2 Mev respectively.

Papers describing various parts of this work were published in Radiation Research (2,3) and Physical Review(4).

C. High Intensity Pulsed Radiations

The object of this investigation is the mathematical analysis of certain physical and biophysical phenomena that occur under conditions of very high intensity pulsed irradiation of tissue-like matter. In particular the aim was to attain:

- 1) The instantaneously effective linear energy transfer (LET) and the density of ions produced in the medium, and
- 2) The rediffusion of oxygen into the medium immediately after the latter was irradiated by a very high intensity pulse of electrons.

The biological effect of any type of radiation is dependent upon the linear energy transfer (LET), that is, upon the ion density produced by the incoming ionizing particle along its track. Thus, the same dose from two types of different LET produces biological effects that are usually different. The higher the LET, the more pronounced the biological effects are expected to be.

Very high LET can be attained in essentially two ways: 1) by employing radiations with inherently high LET (i.e., protons, α -particles), and 2) by employing very high intensity low LET radiation, delivered instantaneously (in a fraction of a microsecond, for example). Thus, in any given microscopic volume, the high intensity instantly produces an overlap of ion tracks or clusters, which instantaneously results in a high LET in the volume.

A mathematical analysis has been carried out to obtain the probability distribution of dose and effective LET in a microscopic volume when a given macroscopic dose is delivered. The results are in the form of a family of modified Poisson distributions. For low doses (a few rads or less for electrons) a considerable probability exists for no dose at all in the volume (no particle traverses the microscopic volume). This probability is negligible at the higher doses that are of primary interest in this project.

Subsequently, the study proceeded to analyze and obtain the oxygen rediffusion, as a function of time, into a thin aqueous cellular layer after the layer was irradiated with an electron pulse delivering a dose in the range of kilorads to a megarad in a few nanoseconds. Such extremely high dose rates are obtainable from field emission units that have been installed at our laboratory. Experimental investigations at our laboratory (5) and elsewhere (6-8) show a serious modification of the survival curves for bacterial systems under such dose rates: The curves exhibit a well defined change in slope at a certain dose (proportional to the oxygen concentration in the medium prior to irradiation) below which the slope is that of oxygenated and above which it is that of anoxic conditions. This is attributed to the total depletion of oxygen at and above the break-point dose, due to the large number of radiochemical reactions initiated by the irradiation. The medium remains anoxic until oxygen from the oxygen-containing gaseous volume above the cell-layer begins to rediffuse. By means of a double pulse experiment an experimental curve of this oxygen diffusion with time was obtained by Epp, et al. at our Institute. The curve exhibits a semiplateau of intermediate times and covers about five decades in time for saturation (10^{-4} to 10 sec). Such behaviour is unobtainable, with ordinary diffusion.

To understand this type of oxygen diffusion a model was set up that assumed the presence of a mechanism that resulted in partial removal of the rediffusing oxygen. The agents of this removal can be a set of long lived radicals produced by the radiation and interacting with the oxygen. In the process of interaction the simple assumption was made that both the oxygen and radical molecules are removed from further consideration in the diffusion process. This model was translated into mathematical terms by deriving the rate of oxygen removal (absorption) which was inserted into the diffusion equation and the latter was solved by the iterative method. Preliminary calculations are being made at this time, corresponding to different doses and equilibrium oxygen concentrations in order to compare the theory with additional experiments currently under way.

A paper on the methods and results of this investigation will be published in Radiation Research.

D. Relation Between Absorbed Dose and Cavity Ionization

The object of this project was to obtain the ratio of absorbed dose to cavity chamber ionization at various depths of penetration of high energy electron beams incident on water and carbon.

The widespread use and convenience of the method of cavity chamber ionization for measuring absorbed dose on one hand, and the increasing application of high energy electron beams for biological and therapeutic purposes on the other, requires an accurate and easily accessible method of converting cavity ionization into dose at any desired depth of penetration into a tissue-like medium at energies above a few Mev. The ratio of absorbed dose to cavity ionization is sensitive to the electron spectrum at the point of interest, and varies strongly with depth. The variation is due to the presence of the polarization effect in liquid and solid media but not in gases (such as those employed in cavity chambers). The calculation also takes into account the production and subsequent partial or total absorption of bremsstrahlung which is produced by the fast moving electrons. The calculation was carried out for three initial beam energies: 10, 15, 20 Mev, and the calculations were extrapolated (with a small associated uncertainty) to energies of 30, 40 and 50 Mev. The basic spectra employed were those of this laboratory (3), and those of Harder (9). The media considered were water and carbon.

The conversion of cavity ionization, J , into absorbed dose, D , at a given depth, Z , in the medium utilized the following basic expression:

$$\frac{D(Z)}{J(Z)} = 0.869 \frac{\int_{\Delta}^{T_0} I_0(Z,T) S_{med}(T,\Delta) dT}{\int_{\Delta}^{T_0} I_0(Z,T) S_{air}(T,\Delta) dT}$$

where $I_0(Z,T)$ is the total spectrum of electrons per energy interval at the desired depth Z , $S_{med}(T,\Delta)$, $S_{air}(T,\Delta)$ are the local stopping powers for the medium and cavity gas respectively, both restricted to include energy losses less than Δ , Δ being determined by cavity size, and 0.869 is the conversion factor of rads to esu/cm³.

The advantage of this formulation over other (9, 12, 13) consists of weighing the stopping powers by the energy spectrum and integrating over all energies, as is shown in the expression above, rather than evaluating the stopping powers at some estimated average or peak of the primaries.

The results of this work are exhibited in Tables I, II, for a water and carbon medium respectively.

An estimate of the minimum and maximum cavity sizes for which the theory is valid is obtained. At normal gas pressures, the minimum size is of the order of three tenths of a millimeter; the maximum size depends mainly on the degree of atomic number mismatch between medium and cavity gas. For closely matched medium and gas, the maximum size is so large (tens of centimeters or more) as to impose no practical experimental restrictions. For a poor match, the maximum size drops down to a few centimeters.

A paper describing the results of this work was published (10).

E. LET and RBE Studies in Water for Cyclotron Produced Neutron Beams

The current availability of relatively high neutron fluxes from installations such as the cyclotron at our laboratory, and elsewhere, has made possible the use of high energy neutron beams for biological experimentation and clinical applications. Such beams possess the deep penetration ability of X-rays and have a radio-biological effectiveness (RBE) well above that of X-rays; in addition the oxygen enhancement ratio is about 1.5 compared to about twice this for X-rays. Thus, neutron beams can be particularly effective in cases of deep-seated anoxic (radioresistant) tumors. The elevated values of RBE are due to the high values of the linear energy transfer (LET) of the recoil protons (tens of Kev per micron) and the still higher LET of the heavier ion recoils (hundreds of Kev per micron).

In this study the LET spectra in water for very small depths (first collisions only) and large depths (multiple collisions) were obtained for a variety of monoenergetic neutron beams covering a range of energies of most practical interest. These results and additional information in the literature was subsequently utilized to obtain the dependence of radio-biological effectiveness (RBE) on certain basic physical parameters of the radiation spectrum at the point of interest: The depth in the medium, the relative dose fraction of proton, heavy recoils and γ -ray components of the spectrum and the average LET of each of these components. In the analysis the RBE was assumed to increase up to about 100 Kev/ μ and decrease for LET greater than 100 Kev/ μ . This behavior is well confirmed experimentally. Simple increasing and decreasing mathematical functions were used to describe this behavior.

To obtain the possible range of RBE change with depth, dose fractions and average LET's with values covering most cases of practical interest were used at small as well as at large depths. In all cases considered so far, that did not include the γ -ray component, the RBE tends to increase or remain practically constant with increasing depth. The increase was small: about ten percent or less.

RATIO OF ABSORBED DOSE TO CAVITY IONIZATION (rads in water/cm²-cu⁻³ in air)
FOR HIGH ENERGY ELECTRON BEAMS IN WATER

Depth (g/cm ²)	10 MeV	15 MeV	20 MeV	25 MeV	30 MeV	35 MeV	40 MeV	45 MeV	50 MeV
1	0.890	0.856	0.836	0.820	0.807	0.796	0.787	0.780	0.774
2	0.907	0.871	0.848	0.831	0.818	0.807	0.799	0.790	0.783
3	0.922	0.884	0.861	0.843	0.828	0.816	0.806	0.798	0.790
4	0.941	0.899	0.871	0.853	0.837	0.824	0.813	0.8045	0.797
5	0.958	0.913	0.884	0.860	0.844	0.831	0.821	0.811	0.803
6	-----	0.943	0.898	0.8695	0.852	0.838	0.827	0.818	0.810
7		0.980	0.916	0.880	0.859	0.845	0.833	0.824	0.815
8		-----	0.955	0.895	0.867	0.852	0.840	0.828	0.820
9			0.974	0.912	0.878	0.860	0.845	0.832	0.824
10			0.953	0.931	0.891	0.869	0.853	0.840	0.831
11			-----	0.960	0.904	0.879	0.861	0.847	0.834
12				0.953	0.920	0.892	0.870	0.854	0.842
13				-----	0.940	0.906	0.860	0.861	0.848
14					0.974	0.921	0.890	0.870	0.855
15					0.947	0.938	0.904	0.879	0.862
16					-----	0.969	0.917	0.889	0.876
17						0.943	0.921	0.900	0.8775
18						-----	0.954	0.914	0.887
19							0.950	0.929	0.896
20							-----	0.949	0.909
21								0.957	0.927
22								0.935	0.918
23								-----	0.959
24									0.932
25									-----
26									
27									

TABLE I

RATIO OF ABSORBED DOSE TO CAVITY IONIZATION (rads to carbon/cm² to sic)

FOR HIGH ENERGY ELECTRON BEAMS IN CARBON

Depth (g/cm ²)	10 MeV	15 MeV	20 MeV	25 MeV	30 MeV	35 MeV	40 MeV	45 MeV	50 MeV
1	0.777	0.731	0.733	0.719	0.707	0.698	0.689	0.681	0.674
2	0.792	0.741	0.743	0.731	0.719	0.710	0.701	0.693	0.686
3	0.807	0.771	0.752	0.740	0.728	0.718	0.709	0.700	0.693
4	0.823	0.782	0.762	0.748	0.735	0.724	0.715	0.707	0.700
5	0.838	0.795	0.770	0.754	0.741	0.730	0.720	0.712	0.706
6	-----	0.810	0.780	0.762	0.747	0.735	0.725	0.717	0.710
7		0.826	0.790	0.769	0.752	0.740	0.730	0.721	0.715
8		0.841	0.800	0.776	0.759	0.746	0.735	0.726	0.719
9		-----	0.816	0.784	0.764	0.751	0.739	0.730	0.722
10			0.835	0.792	0.771	0.756	0.744	0.735	0.727
11			0.843	0.801	0.777	0.762	0.749	0.739	0.731
12			-----	0.812	0.784	0.768	0.755	0.744	0.735
13				0.826	0.792	0.773	0.760	0.749	0.739
14				0.840	0.801	0.779	0.765	0.755	0.743
15				-----	0.813	0.786	0.770	0.757	0.747
16					0.833	0.793	0.775	0.762	0.751
17					-----	0.802	0.780	0.766	0.755
18						0.814	0.786	0.771	0.759
19						0.840	0.794	0.776	0.763
20						-----	0.801	0.781	0.768
21							0.819	0.788	0.772
22							0.830	0.794	0.777
23							-----	0.807	0.783
24								0.829	0.788
25								-----	0.796
26									0.809
27									0.810

TABLE II

Inclusion of the γ -ray component and consideration of incident of neutron spectra in the low Mev region tend to produce a small decrease in RBE with increasing depth in calculation under way. So far, however, no large RBE changes have been obtained in either direction.

References

- 1) DANZKER, M., KESSARIS, N.D., LAUGHLIN, J.S., Absorbed dose and linear energy transfer in radiation experiments, *Radiology* 72: 51-56, 1959.
- 2) KESSARIS, N.D., Calculated absorbed dose for electrons, *Rad. Res.* 23: 63-64, 1964.
- 3) KESSARIS, N.D., Spectra of high energy electron beams in water *Rad. Res.* 43: 281-291, 1970.
- 4) KESSARIS, N.D., Penetration of high energy electron beams in water, *Physical review*, 145: 164-175, 1966.
- 5) EPP, E.R., Weiss H., SANTOMASSO, A., The oxygen effect in bacterial cells irradiated with high intensity pulsed electron *Rad. Res.* 23: 320-325, 1968.
- 6) HOWARD FLANDERS, P, MOORE, D. The time interval after pulsed irradiation injury to bacteria can be modified by dissolved oxygen *Rad. Res.* 9, 422-437, 1958.
- 7) DEWEY, D.L., BOAG, T.W., Modifications of the oxygen effect when Bacteria are given long pulses of radiation, *Nature*, 183: 1450-1451 (1959)
- 8) TODD, P.W., WINCHELL, H.S., FEOLA, F.M., and JONES, G.Z. Inactivation by pulsed high intensity Rays, of human cells cultured in vitro, 15th Annual Meeting of Radiation Research Society, San Juan, Puerto Rico, May 1967.
- 9) HARDER, D., FEIST, H., Energy distribution of high energy electron beams, *Chem. Phys.* April 1963.
- 10) KESSARIS, N.D., Absorbed dose and cavity ionization for high-energy electron beams, *Rad. Res.*, 43: 288-301, 1970
- 11) BERGER, M.J., Calculation of energy dissipations by electrons in water, NBS Report 8678, April, 1965.
- 12) ALMOND, P.R., The physical measurements of electron beams from 6 to 18 Mev: Absorbed dose and energy calibrations. *Phys. Med. Biol.* 12: 13-24, 1967.
- 13) SVENSSSEN, H., and PETERSON, G., Absorbed dose calibrations of thimble chambers with high energy electrons at different phantom depths. *Ark. Fys. (Sweden)* 34: 377-383, 1967.

III. FREE RADICAL PRODUCTION IN BIOLOGICALLY SIGNIFICANT COMPOUNDS

John S. Laughlin, Ph.D., Member
Ira Pullman, Ph.D., Associate

Introduction: Free radicals have been implicated for a long time as one of the most reactive products formed when ionizing radiation interacts with aqueous and biological systems. Free radicals would be expected to play a significant role in producing radiation damage, and perhaps be involved in the initial stages of cancerigenesis. Since their existence is usually of exceedingly short duration in solution, their presence has been inferred, in the past, from measurements of end products and postulated reaction sequences. The invention of electron spin resonance spectroscopy has presented radiation chemistry and radiobiology with an exceedingly sensitive and unique tool for the direct detection of free radicals, and determination of their structure and kinetics. The Division of Biophysics, has, therefore, built a sensitive and flexible instrument in order to apply this form of spectroscopy to selected problems in radiobiology and cancerigenesis.

Spectrometer Design: The electron spin resonance spectrometer was designed to have high sensitivity at low power and high resolution for the purpose of studying the yield, structure, and kinetics of free radicals and other paramagnetic species which may be formed in cancerigenic and related processes, such as the interaction of ionizing and other types of radiation with biological systems.

The spectrometer, which operates at X-band microwave frequencies, uses 30 MHz superheterodyne phase detection for low power operation. High sensitivity in the microwatt power region is achieved so that the saturation effects which are common in organic free radical signals at higher power levels can be avoided or studied. The signal klystron, local oscillator klystron, and sample cavity are phase-locked together, using two 30 MHz crystal controlled phase discriminators. This provides stable, low-noise operation for long periods of time (of the order of hours) even when the cavity is subjected to the disturbing effects of temperature changes, liquid flow, and irradiation procedures. It also permits higher power operation than can be achieved in ordinary superheterodyne detection. Audio frequency modulation of the magnetic field with narrow band amplification, phase-locked detection, and adjustable RC filter network output is applied in the usual way to further improve the signal to noise ratio. The audio modulating coils were designed to fit over the pole faces of the magnet so that a uniform modulating field is applied over the entire maximum sample volume. Electronic integrating circuits have been constructed to obtain the absorption spectrum and the total intensity spectrum from the first derivative spectrum signal. All spectra can be simultaneously recorded.

Equipment has been built for measuring the magnetic field strength and the microwave frequency to one part in 10^5 or better, thus providing accurate determination of spectral parameters.

Sample cavities have been designed and built for special purposes such as the handling of aqueous solution; a commercial Varian cavity is also in use. Quartz dewars for studying samples at various temperature from 77° up have been constructed.

A high intensity, 60 Kev X-ray source which can produce dose rates up to 400 kilorads per minute is available in the laboratory. A jig to permit irradiation within the cavity has been constructed. UV sources for in situ irradiation are also available.

Computer methods have been applied to increase the sensitivity of the spectrometer and to aid in the analysis of the data. The spectrometer has been put on line to the SKI IBM-1800 computer which has been programmed to signal average the data. The spectra can be integrated with base line shift corrections for more accurate intensity determinations. Multiple differentiation by the Fourier transform method can be carried out to increase the resolution. Spectra can be subtracted or added to eliminate background and to analyze mixtures.

In addition, a Varian CAT is located in the laboratory for signal averaging when the IBM-1800 computer is not available for the more sophisticated data treatment.

Applications: The spectrometer is being applied to the following problems:

A. Studies on the mechanism of radiation protection of nucleic acid systems by sulfhydryl compounds.

In the first phase of our work we decided to study the interaction between nucleic acid systems and the well known radioprotector cysteamine, in order to complement the work published in the literature which demonstrated spin transfer between proteins and cysteamine. We demonstrated a spin transfer effect from DNA to cysteamine when a dried, evacuated mixture of the two compounds (containing one mole of cysteamine per four nucleotides) is irradiated with X-rays at 105°K and then warmed up to room temperature. A large build-up of sulfur radicals was seen, much greater than could be accounted for by the amount of cysteamine present. This is similar to the spin transfer effect between irradiated proteins or nucleoproteins and cysteamine reported by other workers.

When we repeated the experiment using the components of the nucleic acids, we found appreciable spin transfer only from nucleotides containing 2,4 diketo groups in the bases (thymidylic acid, deoxyuridylic acid, and uridylic acid). Spin transfer was not observed

from thymine or thymidine, but full transfer was seen between deoxyribose phosphate and cysteamine in an equimolecular mixture.

The appearance of the sulfur radical in the cysteamine coupled with the disappearance of the radical signals from the nucleic acid, suggests a possible repair process. Radiation damage to the nucleic acid caused by removal or addition of a hydrogen atom may be repaired by addition of the sulfhydryl hydrogen atom or by removal of the extra hydrogen atom as hydrogen gas.

The phosphate group in the nucleotide is believed to serve as an electrostatic binding site for the cysteamine at its amino group. The spectrum of an irradiated pure nucleotide resembles that of its irradiated base, but the G value for radical production in the nucleotide corresponds to that for the irradiated sugar component. We have observed a suppression of the sulphur radical at temperatures below 250°K. Thus, at low temperatures the radical formation may occur primarily in the sugar component, moving over to the base and then back to the sulfhydryl group as the temperature is raised.

Experiments have been carried out on the diastereoisomeric pairs of salts prepared from optically pure diammonium thymidylate with L-cysteine and D-cysteine hydrochloride. The relative rates of spin transfer combined with knowledge of the configurations of the diastereoisomeric salts should give some indication of the structure of the radicals initially formed from the nucleotide molecule by the radiation.

The ESR spectrum of each x-irradiated diastereoisomer appears to be a superposition of the spectra of the pure components, although the nucleotide contribution is greater than the cysteine contribution. However, the yield of radicals per rad per mole was considerably higher in the mixture than for the pure nucleotide and was comparable to that for pure cysteine. This anomaly, which is not yet understood, was also observed in similar experiments carried out with the radioprotector thiourea in place of cysteine.

Diastereoisomer samples using L-cysteine hydrochloride were prepared by freeze drying and by solution evaporation. Their X-ray powder diffraction patterns were taken through the courtesy of Dr. E. Handler of the Research Laboratories of the Hospital for Special Surgery, and proved to be identical. Thus, freeze drying does not produce a more amorphous solid than solution evaporation, so that either procedure is suitable for sample preparation. Observations of birefringence under the polarizing microscope indicate a larger crystal size for the solution evaporated material but this should not effect the spin transfer phenomenon.

A comparison of the X-ray powder diffraction pattern of the diastereoisomer containing L-cysteine hydrochloride, with the patterns of the individual components, showed that a compound was formed with a yield approaching 100%, as was also the case with cysteine replaced by cysteamine. A detailed enumeration of the possible chemical

reactions which could take place indicated that they would be electrostatic in nature with the final compounds ionic or salt-like. Reactions involving the phosphate group of the nucleotide would produce ammonium chloride while reactions at the enolate group of the base would produce hydrochloric acid. The powder pattern showed that in the case of cysteamine no ammonium chloride was formed but appreciable amounts were found when L-cysteine was used. Thus the two radioprotectors bind differently to ammonium thymidylate and this may be related to the difference in their spin transfer.

B. ESR Studies on irradiated bone

There is very little published work on free radicals in irradiated bone, and there is uncertainty as to whether the stable radicals are formed in the mineral or organic components of bone. We have carried out experiments in which rat femur and foetal calf bone and their components and synthetic mixtures were irradiated as lyophilized powders at 105°K, and then annealed to higher temperatures, a treatment similar to that of the nucleic acid systems described in the previous section (A). This work was done in collaboration with the Laboratory for Ultrastructural Biochemistry at the Hospital for Special Surgery and Cornell University Graduate School of Medical Sciences, where the samples were prepared. Our results may be summarized as follows:

1) A method was developed for the determination in bone of the percentage of amorphous calcium phosphate relative to the crystalline hydroxyapatite content. The method is based on the observation that hydrogen atoms are produced on irradiating the mineral component with X-rays at 105°K, and that the intensity of this signal disappears completely when amorphous calcium phosphate is annealed to 273°K, whereas only a small, reproducible decrease is seen in crystalline apatite. The quantitative analyses are in excellent agreement with analyses obtained by X-ray diffraction and infrared spectroscopy. The ESR method has the advantage that corrections do not have to be made for the amount of organic component present.

2) Superhyperfine lines have been observed as a structure of two to four extra lines in the wings of the hydrogen atom lines in irradiated hydroxyapatites of large crystal size. These lines are attributed to simultaneous flipping of hydroxyl protons near the main hydrogen atom signal source. Irradiated hydroxyapatites of smaller crystal size are believed to be somewhat deficient in hydroxyl content, and do show a weaker superhyperfine structure. Irradiated fluorapatite has no superhyperfine structure, but does have strong hydrogen lines. The origin of the main hydrogen line is not known at present. At present we attribute it to water that is not removed on heating to 300°C and then evacuated, and is apparently bound stoichiometrically.

3) When dried bone is irradiated at 105°K, the main part of the spectrum distributed around $g = 2$ is similar to that of irradiated

hydroxyapatite. On annealing to 273°K, the spectrum changes to a doublet resembling that of collagen, which is attributed to the glycine component of the protein. Further quantitative measurements showed that the yield of unpaired spins in irradiated bone was about 2 to 3 times the number in a mechanical mixture of bone components even at 105°K. The spectral shape of the mechanical mixture very closely approached that of the weighted average of the individual components. When annealed to room temperature, the proteinlike signal in bone corresponded to 3 times the signal expected from the amount of protein present. Thus, a spin transfer has occurred from the mineral phase to the organic phase of the bone under the annealing conditions. This suggests a close bonding between the phases. There is essentially no other direct evidence for such bonding. In another experiment, a mechanical mixture imitating bone was made wet, rehyphenized, and irradiated. There was no spin transfer, similar to the results for the dry, mechanical mixture. Therefore, we assume that water bridges are not involved in the spin transfer.

In another series of experiments, we attempted to observe spin transfer to sulfur atoms in X-irradiated and annealed samples of normal and calcified elastin. These samples were received from Dr. E. Schiffman of the National Institute of Dental Research, NIH, who has reported bonding of apatite to elastin through sulfhydryl residues in the protein during calcification. We did not see the build-up of a sulfur atom signal, possibly because the concentration of sulfhydryl groups was too low or because the spin transfer may have taken place from the sulfhydryl group to the apatite structure. This latter effect, which is the opposite of what we observed in DNA-cysteamine mixtures, was seen when cysteamine-apatite mixtures were X-irradiated and annealed to room temperature. This may mean that there are available spin states which have a lower energy in apatite than in cysteamine. If this can be verified, one could speculate that radioprotection of an organism might be achieved by adding cysteamine bound to a better acceptor such as apatite so that less of the toxic cysteamine need be used. Apatite itself may not be suitable because tissue calcification might be an undesirable side effect.

In our most recent studies on the structure of bone, we have applied the principles of stable paramagnetic probes to study the binding sites in apatite-collagen (mineral-matrix) systems. Changes in the ESR spectra of the probe can indicate the type of configuration of the labeled molecules and the nature of the bonding at the probe site. Both organic nitroxides and transition element ions have been used as probes with emphasis at first on the latter for the following reasons. It is known that vanadium is a trace element in bone and that in demineralization studies the vanadium follows the mineral, i.e. the amount of vanadium present is a direct function of the amount of mineral present. Thus vanadium with its complex paramagnetic spectrum has the properties for a probe into the interaction between mineral and matrix.

It was found possible to obtain vanadyl ion doped mineral by precipitating calcium phosphate from Tris buffered solutions of

calcium chloride and disodium hydrogen phosphate (at $\text{Ca} \times \text{P}$ ion product = 132 (mM)^2) in the presence of VO_2SO_4 at a level such that $\text{Ca}/\text{VO} = 24$. The precipitates were washed 3 times with distilled water and lyophilized previous to examination in the ESR spectrometer. The spectra were typical of a "powder" spectrum due to random orientation of the vanadyl ions and existing axial symmetry in a clearly resolved hyperfine structure. The following values of magnetic resonance parameters were measured for the doped calcium phosphate:

$$\begin{aligned}g_{11} &= 1.9393 \pm .0005 \\g_1 &= 1.9989 \pm .0005 \\g_0 &= 1.9790 \pm .0005 \\A_{11} &= 194.9 \pm 0.8 \text{ gauss} \\A_1 &= 62.4 \pm 0.8 \text{ gauss} \\A_0 &= 106.6 \pm 0.8 \text{ gauss}\end{aligned}$$

Intensity measurements via electronic double integration indicated that about 0.2 μmoles of paramagnetic vanadium species per mg mineral had been incorporated into our samples.

In order to investigate the mineral interaction with the organic phase, soluble macromolecules were added either to the calcium or phosphate solutions and were allowed to coprecipitate with the mineral phase in the presence of vanadyl ions as before.

Macromolecules used;

1. Collagen - chick skin, neutral salt soluble protein obtained as a gift from Dr. J. Termine of the NIDR at Bethesda. This is the chief protein of bone.
2. Gelatin - Bacto gelatin from Difco Company - a denatured form of collagen.
3. Lysozyme - a basic protein of well characterized structure known to act as a nucleating agent in synthetic calcium phosphate systems.
4. Bovine Serum Albumen - an acidic protein of known amino acid composition.
5. Phosvitin - a phosphoprotein with phosphate groups attached at serine residues.
6. Chondroitin - SO_4 - a mucopolysaccharide typical of the ground substance of bone
7. Sodium polyacrylate - a non-nitrogen containing poly-carboxylic polymer

8. Polyamino acids - to provide various specific protein side chain residues in isolation from other kinds of residues:

acidic: polyglutamic acid, sodium salt

basic: polylysine, polyarginine

neutral: poly-alanine

peculiar to collagen: polyproline (Sigma type II)
polyhydroxyproline

All macromolecular additives were used at 10 mg in a 50 ml system of CaP product = 132, Ca/P = 1.1 of reacting calcium and phosphate ions. From recorded ESR spectra studies were done on the perturbation of magnetic parameters due to presence of the macromolecule.

For example, it was possible to measure the Fermi isotropic contact term (K) to within ± 0.007 error. This number is a measure of the amount of unpaired electron density at the vanadium nucleus. The results indicate a value of $K = 0.761$ for mineral with no additive. Addition of collagen leaves this value unchanged ($K = 0.760$) as might be expected, the vanadyl ion finding the mineral and collagen environments very similar. However, it is interesting to note that gelatin shows a value of $K = 0.738$, significantly lower than for mineral alone indicating increased delocalization of the electron from the vanadium nucleus.

Another somewhat surprising result shows up in comparing polyproline with polyhydroxyproline. For the former, the K value is 0.766, the same as for pure mineral in experimental error limits. The latter however shows $K = 0.736$ a significant increase in delocalization of unpaired electron from vanadium nucleus comparable to that seen in gelatin.

Studies of a similar nature were performed for manganese doped calcium carbonate, though much less bonding information was obtained here, since the only purpose in looking at this system was to develop a probe for carbonate to be used in later work on the influence of carbonate on calcium phosphate systems. The manganese hyperfine lines are much sharper and better resolved than the vanadium lines and should prove to be a sensitive probe in carbonate systems. However, manganese does not yield spectra as well resolved as vanadium in phosphate systems.

Further studies are underway using stable nitroxides to label different functional groups in the organic component of the mineral-matrix model systems described above. These systems are also being irradiated with both UV and X-rays to determine if the spin transfer effect can serve as a labeling technique to characterize the nature of the bonding between the system components.

C. Irradiated Purine N-oxides

(In collaboration with Dr. George B. Brown and co-workers of the Division of Biological Chemistry)

Certain purine N-oxides, such as 3-hydroxyxanthine (I) have been shown to be oncogenic in the rat by Dr. G. B. Brown and collaborators and both ionic and free radical reactions have been postulated in the metabolism of (I). On exposure of powdered (I) to UV radiation the compound turns purple and a stable free radical is formed. A radical yield of 12 to 15% can be achieved and chemical analysis of the product shows that two-thirds of (I) has been converted to zanthine. Our studies have been directed to understanding the nature of the free radical produce in (I) and its derivatives and the relationship of this radical to the metabolic activity of (I).

The yields of free radicals formed by UV irradiation in the solid state were found to be 25 to 100 times higher for (I) and its potentially metabolically active ester, 3-acetoxyxanthine, than for other derivatives such as 8-methyl, 3-hydroxyxanthine, and the analogue 1-hydroxyxanthine. The reason for this is not known and is somewhat surprising.

At sufficiently low UV dose to prevent distortion of the spectrum by exchange narrowing (I) exhibits a three line asymmetric spectrum with an overall spacing of 33 G and a g value of 2.005. These results suggest nitrogen hyperfine coupling produced by loss of the 3-hydroxyl group, leaving an odd electron at the 3-nitrogen position. A similar spectrum is obtained from UV irradiated 3-acetoxyxanthine. X-rays produce the same three line spectrum in (I). Then through allylic shifts the odd electron could move to the 5 or 8 position (Fig. 1). Thus we would expect to see hyperfine interaction with the nitrogen at the 3 position and with substituents at the 8 position. A high spin density at the 5 position where there is no hyperfine interaction could be responsible for the relatively narrow overall width.

It would have been desirable to measure the spectra in irradiated single crystals or in solutions of the radicals for the purpose of determining their structure but this has not yet proven technically feasible. In aqueous solution the radical was destroyed too rapidly to be detected. In dibutyl sulfoxide the radical was also not detectable but the purple color was not discharged. In solutions of dibutyl sulfoxide containing sodium dodecyl sulfate a transient complex spectrum was observed which has not yet been analyzed. Here the technique of letting the solid free radical slowly dissolve in solution from micelles of an amphiphilic compound offers promise of stabilizing the radical long enough for detection.

In another approach toward the analysis of the free radical structure, a number of N-methyl derivatives of (I) were irradiated as powdered solids and their spectra compared to see if changes in

the hyperfine spectra correlated with the nature of the substituent and the postulated odd electron distribution. Also it was demonstrated that the lines in (I) did belong to the same (nitrogen) hyperfine multiplet rather than arising from separate radicals. This was accomplished by measuring the spectrum at two different frequencies in the X-band region using cavities constructed in the Institute's machine shop. The frequencies differed by 9% while the separation of the lines remained constant within experimental error ($<2\%$).

The results from the analysis of the N-methyl derivatives support the postulated structure of the radical (Fig. 1) although there are some discrepancies which still must be resolved. The 1-methyl and 7-methyl derivatives showed spectra essentially identical to that of (I). This indicates that there is no interaction of the radical with the hydrogen at N-1 and that methyl substitution at N-7 prevents the resonance exchange $\text{III} \leftrightarrow \text{IV}$ (Fig. 1). The 8-methyl derivative gave a spectrum more complex than (I) although lines at position corresponding to those in the spectrum (I) were present. The overall width was about twice that of (I) and this coupled with the presence of additional lines indicates that the radical is interacting with protons in the C-8 methyl group. Here the electron donating capacity of the 8-methyl group must contribute to stabilizing the radical at C-8 and would explain the greater contribution of the resonance from IV (Fig. 1).

D. Carcinogenic Polycyclic Hydrocarbons

A study of the free radical structure of the negative ions of some polycyclic hydrocarbons is being carried out to determine if the carcinogenic properties of the compounds can be related to their magnetic resonance spectra. The negative ions were prepared by reacting the hydrocarbons with alkali metal in tetrahydrofuran solvent. The spectral analysis involves measuring the hyperfine coupling constants, and comparing these with theoretical values calculated by means of the Huckel molecular orbital (M.O.) method and by means of McLachlan's perturbation refinement of the method which allows for negative spin densities due to exchange polarization. (1)

At the time this work was initiated, theoretical studies by the Japanese workers who developed the frontier electron theory, predicted that the electron density distribution of the highest occupied molecular orbital could be used to calculate carcinogenic activity indices. The electron densities are proportional to the coupling constants.

All theoretical models of carcinogenesis at the molecular level so far reported have incorrectly predicted the carcinogenic activity of some compounds. However, Poole and Griffith (2) have recently shown that the nitration reactivities of the various positions in phenanthrene correlated better with the hyperfine coupling constants than with various theoretical indices, such as the Huckel molecular orbital spin densities, free valence index, etc. Thus it becomes important to obtain accurate measurements of the coupling constants of the more complex polycyclic hydrocarbons. Unfortunately, many of

the compounds of interest which we have studied produce ESR spectra too complex to be completely resolved for an unambiguous assignment of coupling constants by simply measuring line positions and intensities and comparing these with a stick spectrum. The experimental spectral lines are the result of the superposition of many stick lines, each of which has been broadened by natural line width, concentration effects, and magnetic field modulation. Attempts to increase the resolution by the use of inverse Fourier transform methods did not succeed because the method is too sensitive to noise in the recorded spectrum, even with the application of smoothing functions.

We have now developed a computer program which takes into account line width effects and field modulation effects separately to calculate a spectrum which in practice can become indistinguishable from the experimental spectrum. This gives more confidence to the estimated coupling constants. Further confirmation results when a run at a different modulation amplitude is available for comparison. The program also calculates a modified standard deviation and correlation coefficient for line positions and line intensities between experimental and calculated spectra in order to get quantitative estimates of the goodness of fit.

New analyses of the coupling constants for anthracene, anthanthrene and benzo(a)pyrene have been complete. The results for anthracene and anthanthrene have been published (3). The results for benzo(a)-pyrene are given in Table 1.

Table 1

Experimental and Calculated Hyperfine Coupling
Constants for Benzo(a)pyrene Negative Ion.

Coupling Constants, gauss				Successive ratios of theoretical spin densities by	
Original	Successive Ratios a_n/a_{n+1}	Reassigned Values	Successive Ratios a_n/a_{n+1}	Huckel M.O.	McLachlan M.O.
a_1 4.65	1.08	4.65	1.08	1.04	1.065
a_2 4.31	1.18	4.31	1.86	1.16	1.47
a_3 3.64	1.56	2.32	2.34	3.83	2.36
a_4 2.34	2.36	0.99	1.48	3.96	1.82
a_5 0.99	1.43	0.67	2.48	35.0	3.4
a_6 0.69		0.27			

The successive ratios of the reassigned coupling constants agree remarkably well with the ratios of spin densities calculated by McLachlan's M.O. method.

The results of analyses such as these can serve as a check on the various semi-empirical correlations between spin densities and coupling constants which have been reported for the simpler hydrocarbons. A correlation could then be attempted between the coupling constants and the more quantitative evaluation of carcinogenic activity based on the Blandings Index reported by Hoffman and Wynder (4).

References

- 1) WOOD, R. An Electron Spin Resonance Study of Aromatic Hydrocarbon Negative Ions. A Thesis Presented to the Faculty of the Graduate School of Medical Sciences, Cornell University, for the Degree of Doctor of Philosophy. January 1961.
- 2) POOLE, C.F., JR. and GRIFFITH, O.F., III., Relationship between chemical reactivity and the hyperfine structure of polycyclic hydrocarbons, J. Physical Chem. 71: 3672-3676, 1967.
- 3) WOOD, R., CHABROW, F. & PULLMAN, I. A Method for Determining Hyperfine Coupling Constants from Unresolved ESR Spectra of Hydrocarbon Ions. Journal of Magnetic Resonance 2, 88-98 (1970).
- 4) HOFFMAN, D. and WYNDER, E.L., Beitrag zur carcinogenen Wirkung von Dubenzopyrenen, Zeitschrift Fur Krebsforschung 68: 137-149, 1966.

IV. RADIOACTIVE ISOTOPE INSTRUMENTATION, DATA ANALYSIS, AND ORGAN SCANNING STUDIES

1. Quantitative Scanning

Objectives and Historical Background

The overall objective of this effort has been to develop quantitative and automatic computational methodology for the study of the metabolism of radioactive elements and labeled compounds by the body. Such studies are designed to define both normal and abnormal metabolism in the study of cancer.

In many areas, the evolution of an art to a science has depended on the recognition of pertinent parameters and the ability to measure their roles quantitatively. In other areas as well as in the uses of radionuclides, the Division of Biophysics has long been concerned with making studies possible by the identification of parameters and the development of quantitative methodology.

The initial application of automatic computational methods to radiation treatment planning¹ was made in 1954 and during the past decade, such methods have also been applied to radionuclide counting and scanning. In the case of external radiation treatment, the calculation of dose distributions throughout plane cross-sections of the patient, taking into consideration such inhomogeneous structures as the lung, would be prohibitively difficult and time consuming on a regular basis without the use of automatic computational methods. Similar methods have been developed for the computation of the dose distribution produced by the mechanical insertion of radionuclides inside the patient. The computer can provide the dose distribution for any plane at any angle for any array of radionuclide sources and has been programmed for dose distributions produced by iodine-125, cobalt-60, cesium-137, iridium-192, radium, and radon. These applications for both external and internal radiation have been described by our staff.²⁻⁵ Computer facilities at the Institute and much larger computers elsewhere are utilized on a time-sharing basis. The advantages of this type of computation and experience have been made available to other hospitals by means of teletype and telecopier used over telephone lines.

With respect to metabolic studies with radionuclides, quantitative methods were initially used in the dosimetry of radioiodine. Quantitative methods applied to tracer studies provided the basis for determination of appropriate therapeutic doses, and often allowed the safe use of much larger doses than would otherwise have been permissible.⁶ In order to study calcium metabolism and accommodate the use of calcium-47 following its production in 1955,⁷ a scanning system with specific features was designed. The High Energy Gamma-Ray System (HEG) which has been described in the literature⁸⁻¹¹ included provision for high energy gamma rays, total body scanning with line spacing adjustable from 5mm to 7.5 cm in 12 steps, quantitative display to scale, as well as computer analysis. This system has been in continuous use in patient studies over the past decade.

The features of the quantitative and computer-analyzed approach have been discussed previously^{12,13} and are briefly summarized here.

1. Quantitative methods permit a comparison of sequential studies. This is necessary for metabolic investigation. Such quantitative comparison of point counts of a specific organ or of scans can be carried out over a period of hours, days, weeks, months, or even years (Figure 1).
2. The use of multiple radionuclides in the same patient is facilitated by quantitative data handling.
3. The scan data can be displayed on a statistical basis so that the range of activity is represented by alphabetical or numerical symbols. The transitions between one symbol and the next are a preselectable number of standard deviations of the mean count rate in that range of activity (Figure 1).
4. Quantitative scanning may be of help in the differential diagnosis of abnormal findings. An example of this possibility is given below for liver malfunction.
5. Computer-analyzed scanning has facilitated a variety of data manipulation procedures including: the subtraction within the same scan of the counts due to one radionuclide from the distribution of another; correction for physical decay during the scan period; data smoothing by interpolation; summation over irregular areas; the subtraction of activity contributed by specific organs. Computational methods have been developed to improve the scan resolution or sharpness when the data are available in numerical form.
6. The dynamic range of the quantitative method allows processing of all data regardless of whether the count rate is

L.B. ♀ 300 μ Ci Se - 75 Methionine
Serial Neck Scans
Level Spacing : 2 σ

Fig. 1. Serial scans of the neck and superior mediastinum following intravenous ⁷⁵Se selenomethionine (300 μ Ci). Patient had been recently explored with failure to find adenoma. Arrow head identifies the sternal notch and vertical line indicates midline, both determined and metered on tape during scan. Increment size was 0.2 inch and a 37 hole tungsten focusing collimator was used. Count rate levels are separated by 2 γ intervals.

high or low. These features have also eliminated the present film calibration necessary with analog scanning.

Since the original Institute HEG system was developed,² rectilinear systems with the same computer-analysis features, have been placed in use at Memorial Hospital. A large double headed gamma camera system has been built at the Institute and a small commercial gamma camera system has been purchased by the Hospital. All of these systems have been equipped with the same data processing capabilities as developed for the HEG system.

A feature of the HEG system is its ability to provide a measure of total body activity in a patient so that over any given period of time the retention of a labeled compound or radionuclide can be followed. In this way, the retention of patients for such radionuclides as calcium-47, strontium-85, and selenium-75 labeled methionine has been followed. Total body retention as measured by integration of radioactivity has been compared with the retention calculated from collection of urine and feces, and has been demonstrated to be reliable without the hazards of lost or incomplete collections. The measurement takes approximately 15 minutes and may be done on outpatients.

Research Accomplishments

During the period commencing with the design of the original HEG system in 1957, a system has been developed for use with radioactive elements and labeled compounds administered to patients which achieve all of the objectives mentioned above.

The present system has been modernized electronically and mechanically (1970) in order to accommodate and record data at a higher rate. Conversion to a Canberra digital interface to serialize data electronically and a computer compatible magnetic tape unit for storage has enabled us to record approximately 24 data blocks per second. Each data block includes X and Y positional information, elapsed time, patient identification, anatomical landmarks and accumulated data from eight scalars. Using a PEG magnetic tape unit, many scans can be recorded on one reel, i.e., 800 bytes/inch vs. 10 bytes/inch, thereby permitting more efficient permanent storage and retrieval of data. In order to obtain improved mechanical capability in terms of speed range, smoothness of motion, increased rigidity and safety features, an oversized C frame with upper and lower lift mechanisms, as well as X and Y drive motors, was purchased from Ohio Nuclear, Inc. The scanning area is 30" x 77" and speed is continuously variable up to 200 inches/minute. Line spacings from 0.1" to 1.1" in 0.1"

steps are available. The original crystals, photomultiplier tubes, and shielding were retained, and an oversized C frame was specified to accommodate the detector separation required for total body retention scans. Mounting was also designed to preserve the rotation and angulation of the upper head. The control chassis displays X and Y locations of the detectors (3 digits each) and elapsed scan time (6 digits). Scan and stepping speeds as well as step direction are accessible. Thumbwheel switches to control scan width digitally, line spacing, data accumulation increment and scaler accumulation time for analyzer peaking are also readily available.

With the additional data storage capabilities (four channels on each head) multiple nuclide scanning is facilitated. A three-window technique can be used to formulate a variable subtraction factor. Studies on depth localization by calculation of the ratio of low and high energy gamma rays from a given nuclide are planned. The X and Y positional information, which is now recorded for each increment, facilitates scan analysis by irregular area summation. The system is also capable of recording accumulated data on magnetic tape at 0.1/sec time intervals. This enables it to be used as a multi-probe uptake monitor unit with up to 8 detectors.

An IBM 1800 computer is employed to analyze the quantitative scan data and programs have been continuously developed and improved to facilitate a variety of data manipulations and to provide different types of display.

Multiple nuclide separation is one of several procedures greatly facilitated by this quantitative method, and a few examples are given here.

For the simultaneous comparison of bone-seeking nuclides, Ca-47 (4.53 day half life) and F-18 (109.7 min half life) were used. Their respective gamma ray energies are 1.3 MeV and 511 keV. The localization of each was studied simultaneously by examining the separated scan distributions. It was well demonstrated, using this method, that the skeletal resolution using the Ca-47 at 1 day after injection approaches that achieved by the F-18 at 4 hours after injection (Figure 2). For this nuclide combination, a 100 keV window was employed for the F-18 photopeak, and the normalizing factor for the Ca-47 scatter correction was of the order of 0.5.

The daughter product of Ca-47, Sc-47 (3.43 day half life) was also investigated to determine the possibility of using this nuclide as a bone scanning agent. Sc-47 has the advantage of a low gamma ray energy at 160 keV and presents less problems in collimation than the 1.3 MeV gamma of the parent nuclide. While injected scandium was found to be

DS. 9484 μ c F-18 and 40 μ c Co-47
F-18 Anterior Total Body Search Scan
4 Hour Post Injection
Level Spacing: 3 σ

Fig. 2a. F-18 total body search scan at 4 hours post injection. Count rate levels are separated by 3 σ intervals.

DS 484 μ c F-18 and 40 μ c Co-47
Co-47 Anterior Total Body Search Scan
4 Hour Post Injection
Level Spacing: 3 σ

Fig. 2b. Ca-47 total body search scan obtained simultaneously
with Figure 2a.

Fig. 2c. Ca-47 total body search scan at one day post injection
using the same scanning parameters as in the 4 hour scans.

taken up mainly by the liver, or excreted, Sc-47 produced by Ca-47 in the bone seemed to remain in the bone.

In the combined study of extramedullary hematopoiesis and reticuloendothelial function, Fe-59 and Cr-51 tagged red blood cells were used. When no separation procedure was applied, very poor organ resolution was achieved, i.e., in many instances, the high iron content in the liver contributed considerable scatter into the spleen region. To test the nuclide separation procedure, the Fe-59 was scanned alone with energy discriminator settings normally used for Cr-51 to measure the iron scatter content in that region of the spectrum. The normalization factor was then averaged over the chest area and was of the order of 0.55. When the subtraction procedure was applied, almost all of the iron scatter activity was removed in the liver, except at its outer edges where the ratio of scatter/injected activity was greatest. Good spleen resolution was observed using the subtraction procedure.

With the availability of Fe-52 ferrous citrate from the Institute's cyclotron, the nuclide combination of Fe-52 (511 keV, 169 keV) and Cr-51 (320 keV) was employed. However, considerable interest was expressed in determining the contribution from the Fe-52 alone, since its daughter product Mn-52m, also decays by positron emission. The question arose as to whether the Mn-52m remained within the red cell. On scanning both the 169 keV photopeak and the 511 keV photopeak no significant difference in the corrected distributions of the parent and daughter nuclides was observed.

When the Cr-51 was introduced for the simultaneous study, the three photopeaks were used. In examining the in-vivo spectra for this combination, considerable scatter fell into the 169 keV photopeak owing to the energy proximity of the Cr-51 gamma rays. Furthermore, when the in-vivo spectra was observed close to the edge of the liver, the scatter content had increased. However, when considerable care was taken to average the normalization factors in that particular region, good scan resolution was achieved in each case.

In pancreas scanning, two nuclides were used. Se-75 methionine was employed for pancreas visualization and Tc-99m for definition of liver and spleen. For the separation procedure, scans were obtained in sequence on the Nuclear Chicago camera. The selenium distribution was obtained on the 265 keV photopeak. Following this, the scan matrix of the scatter contribution within the energy discriminator settings for the Tc-99m was stored. Upon injecting Tc-99m, the true scan matrix of this isotope was obtained by subtracting the scatter matrix. When a calculated percentage of the net technetium counts was subtracted from the selenium, the pancreas image was clearly seen (Figure 3) in a number of clinical cases.

MAG TAPE NO. 4 = 2

fraction of ^{99m}Tc at 141 Kev Photopeak
Level Spacing 3σ

Fig. 3. Computer analyzed scan of the pancreas using ^{75}Se labeled selenomethionine. Liver and spleen have been subtracted out by administering $^{99\text{m}}\text{Tc}$ sulfur colloid.

Since the data are in digital form, the application of a resolution correction procedure as well as data smoothing procedures is facilitated. Okumura⁴ has demonstrated the improvement possible in image presentation by the application of resolution improvement techniques based on modulation transfer function (MTF) analysis, in this system.

Figure 4 shows the Total Organ Kinetic Imaging Monitor (TOKIM) images of the Picker liver phantom in the "normal" state, and with lesions. The images, for both cases, are in the form of raw data, averaged image, and the corrected image resulting from MTF analysis.

It is obvious that it would be difficult to draw meaningful contours in the original data. By comparison with the actual phantom (or phantom with lesions), the corrected image is a more realistic depiction than the averaged one in that it diminishes the artificial spread of activity to outlying areas, and it does not smear out the important structures.

This work is being extended to include resolution correction for all of the several different collimator systems and counting geometries employed.

In addition to the development of this quantitative system and the associated methodology, the system has been employed in many quantitative metabolic studies. These have included a kinetic study of different bone-seeking radionuclides used simultaneously in patients which defined quantitatively the important features of fluorine-18, calcium-47, strontium-85, strontium-87m for scanning¹⁵. Erythropoiesis and phagocytosis have been contrasted with multiple nuclides employed simultaneously in the same patient. The localization of parathyroid adenomas have been substantially assisted by this technique. A variety of calcium kinetic studies have been carried out with this system. Since these are discussed elsewhere in this report, they are not reviewed here.

Significance and Conclusion

A quantitative and computer analyzed system has been developed to make metabolic studies of cancer in man feasible with radioactive nuclides and labeled compounds. The original HEG system was designed for this purpose in 1957 and incorporated the earlier computer assisted treatment planning concept initiated in this laboratory. This system has progressively evolved while being applied to a variety of cancer metabolic studies. Methods of data correction, more interpretable displays, and incorporation of solid state detection apparatus are being further developed.

Fig. 4. TOKIM images of a normal Picker liver phantom and one with lesions. The original, averaged, and corrected displays follow vertically, for each instance.

PICKER LIVER PHANTOM

ORIGINAL

AVERAGED

CORRECTED

PICKER LIVER PHANTOM WITH LESIONS

ORIGINAL

AVERAGED

CORRECTED

References

1. Laughlin, J.S.: Annual Report of the Division of Physics and Biophysics, April 1, 1954 to March 31, 1955, Sloan-Kettering Institute.
2. Tsien, K.C.: The application of automatic computing machines to radiation treatment planning. Brit. J. Radiol. 28:432, 1955.
3. Nelson, R.F., and Meurk, M.L.: The use of automatic computing machines for implant dosimetry. Radiology 70: 90, 1958.
4. Holt, G., Hilaris, B., Balter, S., Ragazzoni, G.D., Philips, R.F., and Laughlin, J.S.: Experience with computerized implant dosimetry. Amer. J. Roentgen. 102:688-693, 1968.
5. Laughlin, J.S., Realistic Treatment Planning. Cancer, 22: 716-729, 1968.
6. Berman, M., Rall, J.R., Heslin, J.: Some physical considerations governing the choice of internally administered radioisotopes for therapy. Phys. Med. Biol. 1:243, 1957.
7. Lyon, W.S., and Kahn, B.: Decay of scandium-47. Phys. Rev. 99:728, 1955.
8. Laughlin, J.S., Beattie, J.W., Corey, K.R., Isaacson, A., and Kenny, P.J.: Total body scanner for high energy gamma rays. Radiology 74:108, 1960.
9. Beattie, J.S., and Bradt, G.: Digital printout system for whole body scanner. IRE Transactions on Bio-Medical Electronics BME-8 No. 1 (1961) 24.
10. Laughlin, J.S., Ritter, F.W., Dwyer, A.J., Mayer, K., Greenberg, E.J., Dimich, A.B., Hasan, S., Rothschild, E., and Myers, W.P.L. Development and Applications of Quantitative and Computer-Analyzed Counting and Scanning. Cancer, 25: 2, 395-405, February, 1970.
11. Laughlin, J.S., Weber, D.A., Kenny, P.J., Corey, K.R., and Greenberg, E.: Total body scanning. Brit. J. Radiol. 37: 287-296, 1964.
12. Weber, D.A., Kenny, P.J., Fochaczewsky, R., Corey, K.R., and Laughlin, J.S.: Liver scans with digital readout. J. Nucl. Med. 6:528, 1965.

13. Benua, R.S., Weber, D.A., Kenny, P.J., and Laughlin, J.S.: Digital scanning compared with photo-scanning in liver examination. J. Nucl. Med. 9:135, 1968.
14. Okumura, Y.: The Application of the modulation Transfer Function to Reduce Unsharpness in Scintigrams. Int. J. Applied Rad. and Isotopes. Vol. 22, 49-52, 1971.
15. Weber, D.A., Greenberg, E. J., Dimich, A., Kenny, P.J., Rothschild, E.O., Myers, W.P.L., and Laughlin, J.S.: Kinetics of radionuclides used for bone studies. J. Nucl. Med. 10:8, 1969.

2. Total Organ Kinetic Imaging Monitor (TOKIM)

Objectives

The original objective of this project was to construct instrumentation of the Anger-camera type for quantitative, stationary imaging of radionuclide distributions in-vivo for both single gamma emitters and positron emitters, and to provide on-line operation to an IBM-1800 computer. A second objective was to study the spectral and spatial resolutions of such a system at different energies and its count rate handling capacity. Since its completion, the main objective for this project has been the use of the TOKIM system (Figure 5) in a variety of metabolic isotope studies using radionuclides produced by the Institute's cyclotron. Three modes of operation have been explored with this system: (1) positron, (2) 90° correlation, and (3) multi-probe detector for dynamic studies. The only changes required for these three modes of operation are in collimators on the detectors and soft-ware for the computer.

Research Accomplishments

The TOKIM system has proved to be a versatile tool for research in nuclear medicine. The change-over from one mode of operation to another requires at most ten minutes. For discrete or sharply defined source distributions, the positron mode gives unambiguous information about the exact location of the source and its absolute activity. However, since this mode depends on measuring coincidences between annihilation photons, without collimators on the detectors, the input data rate is limited by electronic pile-up. Figure 6 shows the measurements made to determine singles pile-up dead time for the entire TOKIM system. We have measured the maximum coincidence counting rate to be about 3000 counts per second for ideal geometry, which corresponds to 15 microcuries of activity. In practice, however, with extended geometry about 4 to 5 times this amount of activity can be used in the field of view of the detectors. This means that the positron mode has at least 10 times more sensitivity than a single-headed gamma camera equipped with a multi-hole collimator.

In the 90°-correlation mode, because collimators are used, the singles counting rates do not cause pile-up problems. However, the coincidence efficiency is very low, so the desired real to random coincidence ratio limits the counting rate. For a 50 microcurie point source and a real to random ratio of 5 to 1, the resultant coincidence rate was found to be only 5 counts per second. Preliminary work with this mode indicated good three dimensional resolution for point source distributions. This early work was performed using coincidence electronics with a 70 nanosecond resolving time. It will be necessary to achieve at least an order of magnitude improvement in the electronics resolving time before experiments with extended source distri-

Fig. 5. Block diagram of TOKIM system.

Fig. 6. Experiment to determine singles pile-up dead time for entire TOKIM system. Count rate versus time for a sample of $^{15}\text{O}_2$.

butions will be feasible.

The multi-probe detector mode has been used in dynamic studies to test hepatic and pulmonary function in dogs. The singles counting rate for dynamic studies is limited, by an overall system dead time of 22 microseconds, to about 10,000 counts per second. The curve used to determine this dead time is illustrated in Figure 7.

In all of these modes, data input is accomplished by sending the coordinates of each scintillation event packed into 12 bits of a 16 bit word to a digital input of the 1800. The input program, operating in 4K words of core, stores data in two 1050-word buffers. The buffers are chained, so that as one becomes full it is dumped to magnetic tape while data input continues uninterrupted to the other one. The IBM multi-programming system permits background processing of jobs during data input.

Data handling for this system can be separated into two categories: pictorial display and dynamic function studies. The data records on magnetic tape are replayed and accumulated in a 64 x 64 matrix. This matrix can be displayed in a variety of ways: (1) an alphanumeric print-out using intervals of constant standard deviation, (Figure 8) (2) density displays similar to scintiphotos, and (3) an isometric plot (Figure 9). The latter two are presented on an 8" x 10" storage oscilloscope and photographed using 4" x 5" Polaroid film. For dynamic function studies the matrices are first printed out in two-standard-deviation increments, so that areas of interest can be selected. The data is then replayed and the counts over these areas are accumulated for a fixed time increment and a least-squares fit is performed to a series of exponential functions.

Future Investigations

A new improved version of the TOKIM system is planned for the multiprobe detector mode that will permit: (1) a factor of five times higher data rates and (2) both detectors to be used in dynamic studies with several single channel analyzers looking at various parts of a gamma ray spectrum. With the addition of new coincidence electronics, we will be able to investigate extended source distributions in the 90°-correlation mode. The performance of the positron mode has been disappointing for distributed sources. However, we hope to be able to analyze this system further and to make changes which will correct the problems of image degradation due to pulse pile-up.

Fig. 7. Curve used to determine "effective dead time" of 22 μ sec for the TOKIM system.

Fig. 8. Printed output for positron mode showing six planes, separated by 1.0 inch, of the upper thoracic region of a dog with ^{18}F .

Fig. 9. Isometric display of ^{133}Xe ventilation study (posterior-anterior view).

3. Methodology of Absolute Quantitative Organ Scanning

Objective

To establish a methodology whereby a specific organ activity and organ size can be determined absolutely on the basis of the organ's digital scan image irrespective of the particular in-vivo geometry involved.

Research Accomplishments

A methodology has been developed which makes it possible to determine the absolute amount of radioactivity in an organ or region in-vivo, based on external count rate measurements. The method employs computer analyzed external scan matrices obtained with a dual headed rectilinear scanner and required the development of a dual collimator design whose response was depth independent throughout a patient thickness of 20 to 25 cm. Dual foci collimators, 28 hole F (14 cm, 18 cm), were designed, with the size and geometry of the apertures optimized to achieve depth independence, by means of a computer program for simulating collimator response. The computer program was unusual in that it could simulate the response of collimators with more than one focus as has been previously reported (See Section A3 of 1971 progress report for Proposal #3). Collimator sets have been fabricated suitable for the gamma-ray energy of Cr-51 (320 keV). The measured response of this system was depth independent to within $\pm 11.5\%$ and $\pm 15\%$ as measured by the full width of half maximum (F.W.H.M.) of line spread functions over the full separation of the dual detectors at 22.5 and 32.5 cm, respectively. These measurements were all taken in a (water) scattering medium (see Figure 10). The 2π r Δ r x point sensitivity response, a measure of the "beam like" response of the system, varied within $\pm 7\%$ over the detector separation of 22.5 cm. For a more comprehensive evaluation of this system and to establish a method that can be clinically applied to splenic uptake, spleen phantom sources were scanned at different orientations and locations within water tanks representing patient thicknesses of 20 and 30 cm.

Conversion factors were computed which related the total summed counts within the image contours to the microcuries of Cr-51 present. A revised system of programs was generated to compute and tabulate these calibrations and a graphical method was then applied for choosing an appropriate conversion factor for any spleen size, irrespective of its location or patient thickness. A procedure was also derived to correct for active tissue background on the basis of further calibrations with tanks uniformly filled with active water. Computed conversion factors related counts per unit area to the column of activity beneath. This was applied clinically by choosing an area (well removed) from any active

Fig. 10. Summed detector scan image of phantom source #3 positioned in the 20 and 30 cm water tanks. The unsmoothed scan data is displayed at 2 standard deviations between the mean of each level.

organ in the abdomen and determining the counts per unit area. Hence, knowing the patient thickness, the correct conversion factor can be applied. From the comprehensive phantom analysis, it was estimated that an organ size and activity could be determined accurately to within $\pm 10\%$.

However, to justify clinical use of this method of organ scanning, scheduled splenectomy patients were scanned just before operation and the in-vivo and in-vitro prediction of splenic activity was compared. This ideal clinical evaluation has been investigated in detail, and previously reported (See Section B 3b of 1971 progress report for Proposal #3).

Significance and Present State of Knowledge

This is a relatively new field in radioactive-isotope organ activity scanning, employing a dual headed rectilinear scanner, where emphasis is placed on deriving an absolute rather than a relative measure of organ function. In essence, the basic requirement is to establish a means of determining actual microcurie content of an organ in-vivo, on the basis of the organ's digital computer scan image. This procedure should also allow a correction for the surrounding active tissue background. The objective of obtaining an absolute measure is particularly complicated since, in general, external scanning methods using conventional focusing collimators are inherently dependent on the particular in-vivo activity distribution and measuring geometry involved for each patient. Earlier attempts have been made, using these detectors (1, 2, 3) but restricting their size for employment with small crystals (2" x 3" diameter) and having long focal lengths (15 cm). Their restriction in size, was to achieve as far as possible, a summed "beam like" or depth independent response where the externally measured count rate would be directly proportional to the activity present. However, the major criterion for a response of this nature is to achieve uniformity in the low response regions off the detector axis and this condition is not met by single focus collimators. Emphasis should be placed on the volume response of the system, as opposed to its resolution, normally expressed as the F.W.H.M. of the line or point spread functions, or in terms of their modulation transfer functions (MTF). Some progress has been made with the above approach (4) but its clinical usefulness has been limited. Other researchers have used the geometric means of the response of dual detectors, but this method complicates the correction for the surrounding active medium and is largely dependent on organ size (5,6). The current need in this research is dual collimator-detector systems designed specifically for organ uptake measurement and a precise methodology developed for clinical studies.

Future Investigations

The proposed development of organ activity scanning

in the coming year will cover the following:

(a) Application of the MTF to improve the "quantitative" resolution of an organ.

The MTF may be used to reduce image degradation due to "poor" resolution (7) essentially through a determination of the response of a camera to a point source. This response function is "inverted" and mathematically applied to a camera image, resulting in a better representation of the original nuclide distribution. Computer programs have already been generated in our laboratory to derive and apply this correction to the digital image of the TOKIM camera and an extension to the HEG scanner is presently being pursued. This computation can ideally be applied to the summed response of the dual collimator system discussed, since the point response function (in a scattering medium) and hence the derived correction, remains unchanged along the axis of the detectors. It is clear that by correcting for the finite resolution diameter or "beam diameter," sharper quantitative resolution of an organ can be obtained. A significant improvement is expected, since the F (14cm, 18cm) collimators were designed for Cr-51 (9% γ -emission, 320 keV) and to retain sufficient sensitivity, relatively poor spatial resolution was unavoidable. It is important however to achieve a statistically significant integral count rate over an organ in order to obtain an accurate assessment of activity present. In complex clinical cases, this improved organ image can be extremely useful. Where the organ scan image is not isolated from its surrounding, i.e., in measuring splenic uptake, high liver uptake is experienced and its close proximity to the spleen does not allow an unambiguous quantitative resolution of that organ. Furthermore, since the image corrected to source size will be reduced in area, the extent of tissue background subtraction would be considerably reduced. This is particularly important, since in tagged red cell survival studies, the tissue background can change both longitudinally and laterally across the patient's abdomen.

(b) To develop organ activity scanning procedures for the nuclides Tc-99m and Fe-52.

Since further interest has been expressed in quantitating the absolute extent of Tc-99m (141 keV) sulfur colloid and Fe-52 (511 keV, 169 keV) ferrous citrate in sites of reticuloendothelial function, particularly in the bone marrow, it is planned to develop procedures to include these nuclides. Ideally, their simultaneous comparison would clinically be of definite advantage; the lower gamma emission of Fe-52 has a similar attenuation as Tc-99m. With the high photon yield available, fine hole, high resolution detectors are possible. The general approach taken would be to incorporate several foci in their design, with each ring of holes focusing a greater distance along the detector axis. The computer program for simulating collimator response will be used to optimize these collimator design parameters for depth independence in resolution.

A further comprehensive series of phantom calibrations, covering the in-vivo measured geometries of the liver, spleen, and bone marrow will be repeated for both nuclides to establish a methodology for clinical application.

REFERENCES

1. Hisada, K., Hiraki, T., Ohba, S. and Matsudaira, M. Simultaneous performance of isosensitive scanning and bilaminoscaning. Radiology 88: 129-134, 1967.
2. Hisda, K., Ohba, S., and Matsudaira, M. Isosensitive radioisotope scanning. Radiology 88: 124-128, 1967.
3. Glass, H., Williams, W., Arnold, R., and Degarreta, A. A dual detector scanner for quantitative uptake and organ volume studies. Medical Radioisotope Scintigraphy, IAEA, Vienna, Vol. I: 665-683, 1969.
4. Szur, L., Glass, H., Lewis, S., Grammaticos, P., and Degarreta, A. Quantitative estimation of red cell uptake in the spleen using Rb-81 and Cr-51 labeled red cells. Brit. J. Radiol. 41: 819-825, 1968.
5. Arimizu, N. and Morris, A.C. Quantitative measurement of radioactivity in internal organs by area scanning. J. Nucl. Med. 10: 265, 1969.
6. Arimizu, N., Kakehi, H., and Morris, Jr., A.C. Area scanning for quantitative measurement of radioactivity in internal organs. Medical Radioisotope Scintigraphy, IAEA, Vienna, Vol. I 653-663, 1969.
7. Beck, R.N. A theory of radioisotope scanning systems. Medical Radioisotope Scanning. IAEA, Vienna, Vol. I: 35, 1964.

4. Dosimetry

Objective

Absorbed dose calculations have been done for radionuclides in current use, for the purpose of comparison with those presently used, and with nuclides being considered for possible future use. As part of the evaluation of the potential toxic effects of medically useful radionuclides, an estimate must be made of the amount of energy deposited in the organ of interest and in the surrounding total body tissue. This is referred to as the absorbed dose. It is a measure of the potential risk to the patient which must be weighed against the probable gains from the procedure.

Research Accomplishments and Significance

All dose calculations for the past 3-4 years have been done by the method of the absorbed fraction, described by the Medical Internal Radiation Dose (MIRD) Committee in Pamphlet 1, Supplement Number 1 of the Journal of Nuclear Medicine, February, 1968. The absorbed doses due to the bone-seeking nuclides Ca-47, Sr-85, and F-18, are of primary interest. The Ca-47 calculation includes the dose due to the daughter Sc-47, and a contaminant, Ca-45. Sr-87m and Ca-68 were done for purposes of comparison. Of these five bone-seeking nuclides, the Ca-47 calculations were done for two separate cases: 1) no biological loss, and 2) biological loss as well as physical decay. The retained activity versus time curve, from which the effective half-life was obtained, was plotted from a typical total body retention study. Likewise, Sr-87m and F-18 were done for both cases cited above, but using an effective decay constant, $\lambda_{\text{effective}}$, computed by other investigators.

The absorbed dose due to iron-52, to be used in the localization of sites of erythropoiesis, involved two additional calculations: one for the daughter, manganese-52m, and the other, for an impurity, iron-55. Iron-59 was done for comparison.

By tagging damaged red cells with chromium-48, phagocytic function of the reticuloendothelial system can be studied. Thus, the absorbed dose due to chromium-48 and its daughter, vanadium-48, was required.

Calculations were done for cesium-127, cesium-129, rubidium-81, and rubidium-82m. Cesium-127 is a possible scanning agent in the diagnosis of myocardial disorders, and rubidium-81 will hopefully be used in spleen scanning. For the last four nuclides, relative values of absorbed dose were of primary interest, hence, the simplest and worst possible case was taken. That is, it was assumed that: 1) there was

no biological loss, 2) uptake was instantaneous, and 3) uptake in the organ of interest was 100%.

For each nuclide, except chromium-48, the absorbed dose for the total body was computed in addition to that of the organ or organs of interest.

Lung function studies with oxygen-15 labeled CO_2 , CO and O_2 and carbon-11 labeled CO and CO_2 necessitated absorbed dose calculations for these radionuclides.

All absorbed dose values in Table I have been converted to millirads per microcurie $\frac{\text{m rad}}{\mu \text{ Ci}}$ in order to facilitate comparison, and are $(\beta + \gamma)$ doses. Only the values for 100% uptake (except where otherwise stated), and no biological loss, are presented.

Future Investigations:

All the bone-seeking nuclide calculations were done before the MIRD Pamphlet 5 was published. This pamphlet contains estimates of absorbed fractions for monoenergetic photon sources uniformly distributed in various organs of a heterogeneous phantom. For an approximately elliptical source organ the value of ϕ can be estimated, as from pamphlet 3. However, for the skeleton, this approximation is not close, and the calculations are to be redone. Beside the increased accuracy in the skeletal approximation, the dose to the marrow will be computed using the estimates for ϕ given in MIRD Pamphlet 5.

Dose calculations will be done for nitrogen-13 labeled ammonia which localizes in the kidneys and liver.

There will be a need for dose calculations for several new radionuclides to be produced in the SKI cyclotron. Gallium-67, Se-73, and In-111 will be used for soft tumor localization. There will be an additional computation for the long-lived In-114m, a resulting impurity in In-111 production. Indium-111 and In-114m, already computed in an approximate manner in order to obtain relative dose values, will be repeated, using the latest absorbed fraction estimates. Finally P-30 in phosphate form is to be examined for possible localization sites in humans, and as phosphene gas, will be applied in an experimental evaluation for animal studies.

TABLE I

I	ORGAN(S) of Interest	Absorbed Dose ($\frac{\text{mrad}}{\mu\text{Ci}}$)	TOTAL BODY Absorbed Dose ($\frac{\text{mrad}}{\mu\text{Ci}}$)
Ca-47 +Sc-47 +Ca-45	SKELETON (Backscatter from surrounding body accounted for)	161	17.7
Sr-85	"	55.3	12.9
F-18	"	0.29	0.05
Sr-87m	"	0.16	0.02
Ga-68	"	4.2	0.6
Fe-52 +Mn-52m +Fe-55	LIVER (80% uptake in liver and 20% uptake in surrounding body)	25.3	7.2
Fe-59	"	416	24
Fe-52 +Mn-52m +Fe-55	SPLEEN (40% uptake in spleen and 60% uptake in surrounding body)	1142	
Fe-59	"	1522	
Fe-52 +Mn-52m +Fe-55	BLOOD (80% uptake in blood)	76.8	
Fe-59	"	152	
Fe-52 +Mn-52m +Fe-55	RED MARROW (20% uptake in red marrow)	67	
Fe-59	"	124	
Cr-48 +V-48	SPLEEN (Backscatter from surrounding body accounted for)	2537	
	BLOOD	157	
C-17	BLOOD	0.45	0.056
Cs-129	BLOOD	2.2	0.16
Rb-81	BLOOD	0.62	0.069
Rb-82m	BLOOD	3.7	0.25

5. Measurement of Bone Mineral by Photon Absorptiometry

Objectives

To design, build, evaluate and calibrate an instrument using single-energy photon absorptiometry to obtain a quantitative index which is a sensitive measure of bone mineralization in humans. The bone mineral index being employed is the integral value. For the present it is designated relative bone mineral index (RBMI). The integral value, or RBMI, is defined by the expression
$$\Delta \sum_{i=1}^n \ln (I^*/I)_i$$
, where Δ

is the distance traveled per integration interval, n is the number of integration intervals in a bone width ($n \cdot \Delta$ = bone width w), I^* is the intensity transmitted through "soft-tissue" plus tissue equivalent material and I is the intensity transmitted through "soft-tissue," tissue equivalent material and bone,

To begin accumulating quantitative indexes of bone mineral content on normal individuals and patients with bone demineralization disease.

To carry out serial studies of changes in the bone mineral index following therapeutic intervention in patients with a demineralizing bone condition.

To use this quantitative index in conjunction with calcium kinetics studied by means of ^{45}Ca uptake to express the kinetic state of calcium in the body relative to the state of mineralization of the skeleton rather than body weight. This should give a sounder interpretation of calcium kinetics and lead to a better understanding of their control in normal and disease states affecting the skeleton.

Research Accomplishments

An instrument for bone mineral determinations by the direct photon absorptiometry method of Cameron (1) was designed, constructed and is being evaluated. The instrument has been used primarily for linear scans of the forearm bones. It can be modified for scans of phalanges, humerus, fibula, tibia or os calcis. A modification to allow for scans of the phalanges has recently been made.

A scan is made across the entire arm. The data obtained on paper tape printout from a digital recorder is transferred to punched cards. The results of the processed data are displayed on a CALCOMP plotter, (Fig. 11), and the calculated bone mineral content in grams per cm^2 and grams per cm of bone length of both radius and ulna are displayed on the computer printout (Fig. 12). The computer program uses

Fig. 11. Scan of left forearm 13.4 cm from distal end of radius.

LEFT RADIUS
INTERPOLATED IZERO* = 787.50
BONE WIDTH = 1.4346 CM (28 TO 57)
INTEGRAL VALUE = 27.3561 MM (30 INCREMENTS)
MEAN BONE RADIO DENSITY = 1.6653
MEAN EFFECTIVE BONE THICKNESS = 0.4071 CM
EFFECTIVE BONE MINERAL /UNIT AREA = 0.7532 GM/CM**2
EFFECTIVE BONE MINERAL/UNIT LENGTH = 12.3737 GM/CM

LEFT ULNA
INTERPOLATED IZERO* = 843.00
BONE WIDTH = 1.1608 CM (89 TO 113)
INTEGRAL VALUE = 23.8580 MM (25 INCREMENTS)
MEAN BONE RADIO DENSITY = 1.7429
MEAN EFFECTIVE BONE THICKNESS = 0.4261 CM
EFFECTIVE BONE MINERAL /UNIT AREA = 0.7883 GM/CM**2
EFFECTIVE BONE MINERAL/UNIT LENGTH = 10.7915 GM/CM

Fig. 12. Computer print-out of results for the scan of the arm shown in Fig. 11.

modified Fortran IV for an IBM 1800. The algorithm is essentially the same as used by Cameron and Sorenson. The bone edges are found by three successively decreasing points, the first of which is less than or equal to $I_0^* - 3\sqrt{I_0^*}$. The

limits of the bone are marked on the plotted scan by a triangle symbol. The actual bone width, W, is determined from the relation $W = (\text{number of intervals} + 2) \times \text{interval length} - \text{diameter of collimated beam}$.

More details on the computer program and the other important parameters of the instrument can be found in the Proceedings of the Bone Mineral Conference held in Chicago in May, 1970 (2).

The long term precision in measuring the integral value which is directly proportional to the bone mineral content, for both radius and ulna at the midshaft of the forearm, was a percent standard deviation of about 2%.

With the aid of a bone standard obtained from the Radiology Department of the University of Wisconsin (3), we were able to show (see Table II) (a) that the integral values obtained in the two laboratories agree to within experimental error for two of the standards with a small difference for the third (<5%), (b) that in our hands, the variance in the measure of the integral value is smaller than that of bone widths.

By comparison of a fat-free phantom arm to several human arms as shown in Figure 13, we have found that increased transmitted intensity toward the arm edges due to the presence of fat can have a considerable effect on the accuracy of the bone mineral determination. A study using standards with various fat configurations, whose results are shown in Figure 14, has resulted in a better understanding of the magnitude of the error due to the presence of fat in the arms of obese individuals. This study has also shown that the error in bone mineral determination is least when, in the expression of the integral

value $\Delta \sum_{i=1}^n \ln(I_i^*/I_0)$, I_0^* is determined as an average of

integrated counts adjacent to both edges of the bone. For the obese individual 1, of Figure 13, the integral value obtained in this manner, and hence the bone mineral content determination, can differ by as much as 15% from that obtained with an instrument used in some laboratories which determines the I_0 only on one side of the bone edge as defined in method (d) of Table III.

A proportionality constant relating bone mineral content in grams of bone mineral per cm of bone length to the integral value was determined on the basis of the measurement of calcium mass in segments of excised human extremity bones

TABLE II

COMPARISON OF STANDARDS

(Standards provided by Medical Physics Section, Radiology Dept., U. of Wisc.)

Measurements at Sloan-Kettering Institute Scan speed - 0.267 mm/sec. Integration time (sampling time) 2 sec. Readout time - 0.052 sec. Distance traveled/interval (2.052 sec) - 0.547 mm Beam diameter $\sim 1/8"$					Measurements at Rad. Dept. U. of Wisc. Scan speed - 2.4 mm/sec. Integration time - 0.4 sec. Readout time - 0.2 sec. Distance traveled/interval (0.6 sec) - 1.44 mm Beam diameter -			
SIMULATED BONE	Width $\pm \sigma^{(a)}$ (mm)	# of inter-vals $\pm \sigma^{(a)}$	$\sum_{i=1}^n \ln\left(\frac{I_i^*}{I}\right)_L$ $\pm \sigma^{(a)}$	Integral Value ^(b) $\pm \sigma^{(a)}$	Width $\pm \sigma$ (mm)	# of inter-vals $\pm \sigma$	$\sum_{i=1}^n \ln\left(\frac{I_i^*}{I}\right)_L$ $\pm \sigma$	Integral Value $\pm \sigma$
Small	6.41 ± 0.23	15.5 ± 0.54	14.30 ± 0.05	7.82 ± 0.03	7.20 $\pm$	5.0 $\pm$	5.5 ± 0.2	7.92 ± 0.3
Inter-mediate	12.80 ± 0.28	27.2 ± 0.55	23.81 ± 0.21	13.02 ± 0.11	12.96 $\pm$	9.0 $\pm$	9.3 ± 0.2	13.4 ± 0.3
Large	15.99 ± 0.31	33.0 ± 0.63	50.84 ± 0.40	27.81 ± 0.22	16.4 ± 0.7	11.4 ± 0.5	20.3 ± 0.4	29.2 ± 0.50

(a) Standard deviations based on six replicate runs.

(b) Integral value = $\left[\frac{1}{n} \sum_{i=1}^n \ln\left(\frac{I_i^*}{I}\right)_L \right] \cdot n \Delta = \Delta \cdot \sum_{i=1}^n \ln\left(\frac{I_i^*}{I}\right)_L$, where n is the number of intervals in width, W, and Δ is the length of each interval.

Fig. 13. Scans of four women with varying degrees of adipose tissue. The weight and heights are (1) 233 lbs., 5ft.7in., (2) 177 lbs., 5ft.4in., (3) 134lbs., 5ft., (4) 108 lbs., 5ft.1in. The scan of the phantom arm consisting of dried human bone, tissue equivalent water and a thin plastic enclosure is shown as an example of a fat-free arm. This scan is purposely displaced downward and the ulna is artificially placed to the right of its actual scan position to avoid confusion of the various scans.

PER CENT ERROR IN DETERMINATION OF INTEGRAL VALUE			
CONFIGURATION OF FAT	10 ⁸ BASED ON THE MEAN OF SIX VALUES		
	BETWEEN BONES ^(a)	REGION BEYOND EXTERNAL EDGE ^(b)	REGIONS ON BOTH SIDES OF BONE ^(c)
(a) APPROXIMATELY UNIFORM THICKNESS OF 2.5 cm	+1	+1	+1
(b)	-9	+3	-3
(c)	-32	-32	-32
(d)
	1	-6	+2
	2	-9	+5
	4	-15	+13
(e)
	1		+1
	2		-7
	3		-6
	4		+3
	5		+8
	6		+7
	7		+10
	8		+1

Fig. 14.

TABLE III

Integral Values (in mm) for Radius of Individual 1 of Fig. 13 Using
Different Methods to Obtain I_0

Method employed to obtain I_0	Medium in which forearm is main- tained in tissue equivalent material	
	Super Stuff in plastic box	In water immersion chamber
a) Mean between radius and ulna	17.7 (-12%)	17.7 (-7%)
b) Mean of six successive counting intervals starting on the third interval from the edge of the radius away from the ulna	22.9 (+14%)	20.2 (+6%)
c) Means of six intervals, three on each side of radius starting on the third interval from the edges of the bone	20.1	19.0
d) Value taken at a point outside the bone- 3 mm from the edge of the radius away from the ulna	23.3 (+16%)	20.5 (+9%)
e) Value taken at a point outside the bone 3 mm from the edge of the radius toward the ulna	17.5 (-13%)	17.7 (-7%)

which had been defatted and ashed. Our linear regression equation, based on a limited sample of 18 segments from 4 males ranging in age from 14 to 21, was $Y = .0332X + 0.265$ where X is the integral value and Y is the bone mineral content in grams of bone per cm bone length. The regression equation reported from Cameron's Laboratory at University of Wisconsin based on the measure of the ashed mass of 85 radius and ulna bones, was $Y = .0377X + .0747$. In comparing the bone mineral content determinations obtained using the two different linear regression equations, our equation gives determinations of grams of bone mineral per cm of bone length which range from 19% higher for radius bones with small integral values (ca. 15 mm) to 5% for large values (ca. 30mm).

Significance and Present State of Knowledge

The relatively precise readily obtained in-vivo measurement of bone mineral content available with single-energy photon absorptiometry makes possible studies of bone mineralization which were not easily attainable in the past with X-radiography. The dose to the patient of about 6 mrad in a scan of the forearm bone makes it possible to carry out serial studies of mineral content on patients with hypo- and hyper- calcemia following therapeutic intervention. The same would be true for measurements of other bones such as phalanges, humerus, fibula, tibia and os calcis. One can use this technique with rates of accretion obtained by calcium kinetic studies based on ^{45}Ca uptake plus a metabolic balance determination to determine rates of accretion and resorption as a function of RBMI (see Research Accomplishments), where the RBMI for localized bone scans may prove to be an index proportional to skeletal weight. If this is shown to be the case, then the possibility exists for expressing calcium kinetics as a function of skeletal weight which is a more meaningful measurement for patients with generalized increase or decrease of skeletal mineralization.

It has been recognized that the presence of fat decreases the accuracy of the bone mineral content measurement obtained with single-energy photon absorptiometry (4). The magnitude of the error introduced by the presence of fat is not well defined for in-vivo scans of different human bones. Studies described under Research Accomplishments should contribute to a better understanding of the magnitude of this error (5). An instrumental development proposed in the section "Future Investigations" may result in a convenient technique which would greatly improve the accuracy of the bone mineral content measurement for obese individuals.

Future Investigations

The accumulation of data on normal individuals of both sexes in all age groups will continue and will serve as

standards of comparison. Studies of calcium metabolism will now routinely be combined with RBMI determinations and interpreted in the light of this new parameter. Studies of RBMI, calcium accretion rate and calcium balance on osteoporotic patients undergoing calcium infusion treatment will be carried out as they become available.

Theoretical considerations suggest that the error introduced by the presence of fat can be made relatively small by the use of two different "single-energy" photon X-ray beams with constant thickness specimens, where the constant thickness is maintained by the use of tissue equivalent material. It is proposed that a two-photon instrument be constructed by modifying the present SKI bone mineral analyzer. The two-photon technique will be evaluated with bone phantom standards and various configurations of fat as was done to evaluate the error due to presence of fat (see Research Accomplishments).

References

1. Cameron, J.R., et al, Measurement of Bone Mineral in-vivo: An Improved Method, Science 142:230-232, October, 1963.
2. Zeitz, L. and Freed, B., Design and calibration of Sloan-Kettering Institute osteodensitometer, USAEC Report CONF-700515.
3. Witt, R.M., Mazess, R.B. and Cameron, J.R., Standardization of Bone Mineral Measurements, USAEC Report CONF-700515, p. 303, 1970.
4. The bone phantom reference standard was kindly supplied by Dr. J.R. Cameron's Laboratory and is described in Witt, R.M., Mazess, R.B., and Cameron, J.R., Standardization of Bone Mineral Measurements, Proc. of Bone Measurement Conference, May 22-23, 1970, AEC CONF-700515, p. 303.
5. Zeitz, L., The Effect of Subcutaneous Fat on Bone Mineral Content Measurements with the "single-energy" photon absorption technique. To be submitted for publication in Investigative Radiology.

6. Feasibility of Chemical Bonding Studies with X-ray Spectra

Objectives

To determine the feasibility of using an accelerated ionized helium beam as an excitation source to be used with our vacuum curved crystal X-ray spectrometer (MAC Model 400) in studies of the chemical bonding of iron and zinc, and of some of the lighter elements such as sulfur, calcium, magnesium, sodium and phosphorus which are of interest in biological components.

The study is to ascertain if by using excitation by charged alpha-particles it will be possible to observe changes in X-ray spectral lines, such as, line intensity, line energy or line shape changes which correlate with changes in the chemical environment or chemical bonding of certain select elements found in relatively low percentage in isolated biological moieties.

The initial measure of sensitivity will be to compare the sensitivity for the detection of the titanium- $K_{\alpha 1,2}$ line, by means of an X-ray crystal spectrometer, produced by a beam of cyclotron accelerated alpha-particles (15 MeV) to that obtained with a beam of accelerated electrons (25 keV).

Research Accomplishments

The vacuum curved crystal spectrometer (MAC Model 400) used in our conventional X-ray emission instrument (1) gives sufficient resolution as is indicated in Figure 15 to detect line shape changes in the K_{β} -band spectra of sulfur. These spectra were obtained with X-ray excitation using the instrumental arrangement previously reported (1). The resolution shown in Figure 15 of about 0.1 percent full width at half maximum was obtained with entrance and exit slit widths of 0.004".

The chamber constructed to allow this spectrometer to be used with the α -particle beam available at the exit port of the Sloan-Kettering Institute cyclotron is shown in Figure 16. The chamber contains two 0.318 cm diameter tantalum collimators separated by 14.5 cm and 14.5 cm from the target. A bellows separates the chamber from the flange at the beam transport exit port. This permits angular adjustment of the chamber to align the axis of the collimators parallel to the α -particle beam which makes an angle of 90° with the target.

The count rate in the TiK_{α} line recorded with this X-ray spectrometer per unit target α -particle current was determined in the following manner. First, the spectrometer

Fig. 15. S-K_β band spectra of various sulfur compounds.

Fig. 16. Arrangement of the vacuum curved crystal X-ray spectrometer and chamber with attached bellows to permit use of the spectrometer at the exit port of the SKI cyclotron.

was aligned and focused with a 0.5 cm diameter 10mCi ^{55}Fe source positioned in place of the target. The distance from the source to the entrance slit was 7.0 cm with the spectrometer entrance slit width equal to .015" and no slit on the detector. After focusing in this manner, a target consisting of nearly pure titanium 0.953 cm in diameter and 0.15 cm thick embedded centrally in a copper disk was placed in the position of the ^{55}Fe source. The scan of the $\text{TiK}_{\alpha 1,2}$ lines taken

with an α -particle beam current of about 3×10^{-9} amps is shown in Figure 17. The target current was measured with a Brookhaven Inst. Corp. Model 1000 electrometer (integrator) between the target and ground. The same electrometer could be used in conjunction with a scaler-timer (M-P Model #5590A) as a current integrator to measure the total α -particle beam current impinging in a given interval. The target was at essentially ground potential with no bias applied to prevent escape of secondary electrons produced by the impinging α -particles.

The efficiency of detection of X-rays with this particular geometry of sample and spectrometer remains nearly invariant if the X-ray source is within a 1/4" diameter of the center of the target (2). The position of the impinging α -particle beam was determined by radioautography of the target where the film is primarily detecting the radiation from ^{51}Cr produced in the α, n reaction $^{48}\text{Ti}(\alpha, n)^{51}\text{Cr}$. The α -particle beam was found to be well within a 1/4" diameter circle centered on the disk.

A plot of the scan of the $\text{TiK}_{\alpha 1,2}$ lines for a measured target current of 3×10^{-9} amps is shown in Figure 17. A corrected $\text{TiK}_{\alpha 1,2}$ line intensity of 3.7×10^6 counts/sec/ μA

was obtained. The background level was diminished over that shown in Figure 17 by surrounding the X-ray spectrometer with 1/8" thick pieces of lead. With this added lead, the line to background ratio was 32/1 based on the background intensity obtained with a copper target. A $\text{TiK}_{\alpha 1,2}$ line intensity of

9×10^5 counts/sec/ μA with a line/background ratio of 950/1 with iron as a background target was reported in the performance sheet supplied by the spectrometer manufacturer for the same type of spectrometer using a PET crystal with 0.015" slit on the detector and a narrow pencil (ca. 1 micron diameter) of 25 keV electrons. The estimated line/background ratio if copper had been used instead of iron is 820/1 based on the continuum intensity varying directly with atomic number. This result indicates that 15 MeV α -particle excitation gives a $\text{TiK}_{\alpha 1,2}$ line intensity, per unit target current, emitted from

the sample which is about 4 times that for 25 KeV electrons.

Fig. 17. Scan of $\text{TiK}_{\alpha_{1,2}}$ lines produced by an α -particle target current of 3×10^{-9} amps.

As compared to 15 MeV α 's, 25 keV electron excitation gives about 150 times the intensity in the emitted TiK_{α} lines for equal energy dissipation in the target.

For the geometry of the MAC 400 electron microprobe specimen chamber, less than 10% of the TiK_{α} radiation produced by 25 keV electrons, whose effective range for the production of TiK_{α} radiation in titanium is about 2 μ , is absorbed in the target before being emitted in the direction of the crystal monochromator. A much larger fraction of the TiK_{α} radiation is self-absorbed with 15 MeV α -particles whose range in titanium is about 100 μ .

A rough estimate of this self-absorption was obtained with aid of differential energy loss for α -particles in titanium obtained from tables of Williamson et al (3) and the efficiency for the production of K-shell ionizations at varying alpha-particle energies taken from the curves of energy versus cross section for K-shell ionizations by heavy particles developed by Merzbacher and Lewis (4). This estimate shows that in titanium only about 20% of the TiK_{α} photons produced by 15 MeV α 's which leave the target in the direction of the monochromator reach it as compared to nearly 100% for the case of 25 keV electron excitation. Correcting for self-absorption, we can say that for equal energy dissipation in the target, 25 keV electron excitation gives about 30 times (see p. 77) the number of TiK_{α} photons produced in a titanium target as does 15 MeV α -particles. For equal energy dissipation in the target and at equal particle energies, the background in the spectrometer due to the continuum should be about 5×10^7 times lower with α -particles than with electrons because of the $(m/M)^2$ factor (5).

The magnitude of the background with 15 MeV α -particle excitation was much larger than that expected from the electron excitation data which had been corrected for the $(m/M)^2$ factor and the self absorption of continuum radiation in the region of the TiK_{α} line. Experimentally it was also shown that a large fraction of the measured background was not due to radiation produced in the target. Under circumstances where all of the α -particle beam was stopped by the tantalum collimators before striking the target, about 70% of the background intensity remained. This coupled with the finding that the background intensity is diminished by the addition of lead shielding around the spectrometer, indicates that much of the reported spectrometer background is not due to the continuum produced in the titanium target. It is the sum of contributions from radiation produced by (a) α 's stopped in the tantalum collimators, (b) scattered α -particles striking the walls of the beam transport tube and (c) general radiation existing in the target room. The contribution to the background from non-target sources must be

almost completely eliminated to obtain the higher sensitivity, as compared to electrons, inherently available with heavy charged particle excitation because of the greatly diminished intensity of the continuous spectrum.

Methods of decreasing this extraneous background which are being considered include focusing of the charged particle beam so that physical collimation is not required and coincidence shielding with large scintillation crystals in the vicinity of the proportional counter.

Significance and Present State of Knowledge

This study will employ an ionized-helium beam in the excitation of specific elements to determine their bonding in compounds of significance in biology. The feasibility of this approach, delineating its potential and limitation, will be obtained. It will allow for a comparison of sensitivities, i.e., the minimum amount of element required to obtain useful x-ray spectra, using ionized helium and electron excitation.

The possibility of determining changes in bonding of zinc and sulfur as a result of dithizon-zinc complexing would add a new tool for the localization of chelating action and, in particular, could be of considerable value in studies of the effects of chelating agents on certain select tissues such as the mammalian prostate. The same would be true for changes of the K-lines of the sulfur in dithizon.

It is known that the catalytic action of many enzymes requires the presence of one of a number of cations, e.g., activation of polyribonucleotide phosphorylase and deoxyribonuclease by Mg^{++} or the activation of myosin adenosine triphosphatase by Ca^{++} (6). In these metal-activated enzyme systems, it is not known if the activating cation is involved in the formation of the enzyme substrate complex. Studies of the K-line parameter changes of these enzyme activating cations upon enzyme-substrate complexing could add to our understanding of the role of the metal ion in enzyme activity.

The information obtained from studies of the x-ray spectral line changes of the metallic ions when complexed with purine-N-oxides can be of value in choosing between different proposed bonding structures for these metal complexes.

The ability to correlate type of bonding with parameters of the K or L-lines of elements of importance in biological problems presents a potentially unique approach. This uniqueness is inherent in the use of a charged particle microprobe, either a charged helium or an electron beam, which would make possible inferences concerning the type of bonding which exists for certain select elements within localized

regions in tissue sections. The spatial localization could be of the order of a micron with presently available electron microprobes, and a similar degree of spatial resolution may become available with accelerated ionized helium beams.

It has been known for some time that the energy, intensity, and shape of the K-emission lines of elements as high in atomic number as sulfur (7,8), and the L-emission lines of some heavier elements (9), are effected by the degree of covalency, coordination number (number of nearest neighbors), valence state, oxidation number and other chemical influences.

Attempts to develop a theory to explain the effects of chemical bonding on electron transitions between inner levels have had very limited success (10). However, the effect of chemical combination on X-ray emission spectra has been used in an empirical manner in studies in materials science and other fields. Recently Takahashi and Yabe (11) systematically separated the SK_{β} band structure into four

component peaks. This approach clarified the relationship between chemical bond and the SK_{β} band structure. They

showed (a) a high degree of correlation between half band-width of the SK_{β} band and the ionic bonding, with the band-width decreasing with increasing ionic character of the bond and (b) as the positive charge of the sulfur atom increased the intensity of one of the component peaks increased.

Future Investigations

Methods which will be investigated to diminish extraneous background include (a) focusing the α -particles in the collimator material, and (b) the use of coincidence shielding surrounding the detector.

To optimize the line to background ratio for the α -production of characteristic K-radiation of the lighter elements requires going to relatively thin samples, i.e., about 5 μ for a titanium target with 15 MeV α -particles. The optimum thickness will be experimentally determined for titanium.

References

1. Zeitz, L. and Lee, R., Element analysis in labeled DNA by X-ray fluorescence. *Anal. Biochem.* 23: 442, 1968.
2. Zeitz, L. and Lee, R., Zinc analysis in biological specimens by X-ray fluorescence. *Anal. Bioch.*, 31: 123, 1969.
3. Williamson, C.F., Boujot, J.P. and Picard, J., Tables of range and stopping power of chemical elements for charged particles of range 0.05 to 500 MeV. Rapport CEA-R3042, 1966.
4. Merzbacher, E. and Lewis, H.W., X-ray production by heavy charged particles, in *Handbuch der Physik*, Ed. S. Flügge, 34: 166, 1958.
5. Heitler, W., in The Quantum Theory of Radiation, Oxford Press, London, 1954, p.375.
6. Steiner, R.F. The Chemical Foundations of Molecular Biology, D. Van Nostrand Co., Inc. Princeton, p.214, 1965.
7. Lindh, A.E. and Lundquist, Uber die Struktur der K_α Linie der elemente Schwefel und phosphor, *Arbiv, Mat. Astron. Physik* 18: No. 34, 1924.
8. Baun, W.L., and Fischer, D. W., Influence of chemical combination on aluminum K diagram and non-diagram lines. *Nature* 204: 642, 1964.
9. Fischer, D.W. and Baun, W.L., Effect of chemical combination on the soft X-ray L-emission spectra of K, Cl and S using a stearate soap film crystal. Tech. Report No. AFML-TR-65-139, Wright-Patterson Air Force Base, Ohio, December, 1964.
10. Anhenius, G., Chemical bond effect in electron transitions between inner levels, in *Optique des Rayons et Micro-analyse*, Castaing, R., Deschamps, P., et Philbert, J., eds., Edition Scientifiques; Hermann, Paris 1967.
11. Takahashi, Y. and Yabe, K., An X-ray emission investigation of the chemical bond of sulfur. III. The profile of SK band in sulfur compounds. *Bull. of the Chem. Soc. of Japan*, 42: 3064, 1969.

V. METABOLIC AND ORGAN SCANNING STUDIES (NON-OSSEOUS)

1. $^{13}\text{NH}_3$ Metabolism

Objectives

The objective of this work is part of an overall research goal to produce new radiopharmaceuticals which will have useful applications in medicine. Ammonia metabolism is especially noted for its central role in organ physiology of the kidney, liver and brain and in the biochemical pathways related to amino acids, purines and urea. Function of these organs and the related metabolic pathways are involved to some extent in virtually all human diseases.

Research Accomplishments

This is a preliminary report based on a small number of dog studies. These studies indicate that N-13 labeled ammonia has several potential uses: (1) as a short-lived tumor scanning agent, (2) as a kidney function test and (3) for static imaging of myocardium and liver. Our work suggests that $^{13}\text{NH}_3$ will prove valuable in organ scanning and function studies of clinical patients. We have been able to produce millicurie quantities of N-13 labeled ammonia from the relatively simple reaction of deuterons on methane gas. The $^{13}\text{NH}_3$, dissolved in saline, is of high purity (>99%) and good specific activity (up to 10 millicuries/cc). The large photon flux has made possible dynamic studies in normal dogs and dogs with malignant neoplasms for periods of more than 30 minutes with the Total Organ Kinetic Imaging Monitor (TOKIM) and the High Energy Gamma (HEG) scanner systems. Our work has shown that N-13 labeled ammonia localizes in myocardium following intravenous injection as ammonium chloride or after inspiration as gaseous ammonia. Although the mechanism for the uptake in myocardium is not understood, it is thought to be by either exchange for K^+ or incorporation into myocardial cells via glutamine synthesis. If the second hypothesis is correct, a dynamic study of N-13 in myocardium could also yield information about high-energy phosphate utilization.

Approximately 10-20% of the injected ammonium chloride activity is removed quickly from the bloodstream by the kidneys and collected in the urinary bladder. The mean half-times for clearance from the kidneys of the three dogs studied are 10.6 minutes and 7.7 minutes for the right and left kidneys respectively. The right kidney is adjacent to the liver which also accumulates N-13 and therefore contributes to the count rate for the selected area over the kidney. This explains the longer half-time for clearance from the right kidney.

The final application of $^{13}\text{NH}_4^+$ which we have explored is its use in detecting neoplasms. This work has been limited to scanning three dogs which had suspected spontaneous tumors. Of the three only one had a tumor - a thyroid carcinoma - which was confirmed at necropsy. In the ventral and lateral neck scans performed in this dog, ten minutes after the injection of N-13 labeled ammonium chloride, the tumor mass was clearly visible, as seen in Figure 18.

Significance and Present State of Knowledge

The significance of local myocardial ischemia in man makes it desirable to evaluate the distribution of coronary blood flow. The techniques required to gather information on coronary blood flow are often complex and not without risk, especially in seriously ill patients in whom the information may be particularly useful. It is therefore desirable to devise methods which are less invasive and still permit conclusions on the state of the myocardium. It is possible that N-13 labeled ammonia will provide us with such a myocardial scanning agent. This nuclide provides an excellent information to radiation dose ratio. For kidney and liver function studies $^{13}\text{NH}_4^+$ is a new physiologic tracer. And finally, we might speculate that $^{13}\text{NH}_4^+$ will become a useful soft-tissue tumor scanning agent.

Future Investigations

Since a non-invasive technique for viewing myocardium would provide a much needed tool in medicine, we plan to concentrate on this aspect of N-13 labeled ammonia. We are going to study acute coronary occlusions in large mongrel dogs by scanning with the TOKIM system after inhalation of $^{13}\text{NH}_3$. We plan to compare our results with the invasive technique of injecting xenon-133 into the coronary artery or its branches. We hope to be able to correlate our quantitative images with the ischemic or necrotic volumes obtained at necropsy.

The other aspects of N-13 labeled ammonia that will be investigated are: 1) the study of the mechanism of myocardial uptake via synthesized N-13 labeled glutamine, and 2) the detection of spontaneous tumors in the dog.

2. Pulmonary Function Studies with Gaseous Tracers

Objectives

In general, this project was initiated to develop a simple but effective isotopic procedure for the visualization and dynamic quantitation of regional ventilation and perfusion

I. D. AMC - 1 Run No. 5 Date: 3/23/71
Amt. Inj. 5 mCi Time A. I. 10 min View: Ventral
Tot. Cts. 300 K Scan Time: 9 min Output: 2 σ

Fig. 18. Ventral scan of the neck of a dog with a thyroid carcinoma following injection of N-13 labeled ammonium chloride

for the following investigations: (1) alveolar-capillary gas transport, (2) acute irradiation reaction, (3) pre- and post-operative pulmonary status, (4) homologous dog lung transplantation, and (5) effectiveness of lower costal expansion in producing preferential ventilation of the lower lung field unilaterally.

Research Accomplishments

(1) Baseline measurements of normal clearance half-times of oxygen, carbon dioxide and carbon monoxide labeled with cyclotron produced O-15 have been made on five dogs using the digital dynamic capability of the Total Organ Kinetic Imaging Monitor (TOKIM). The weighted mean clearance half-time was greatest for O-15 (17.39 sec.), and it had the highest percent standard deviation (9.1%). There is slight difference between the weighted mean clearance half-time (6.46 sec.) and associated percent standard deviation (7.3%) for $Cl^{15}O_2$ and those for $Cl^{15}O$ (7.45 sec. and 7.6%). An illustration of a clearance curve and the digital output with the lung field outlined is given in Figure 19.

(2) Preliminary results indicate that quantitative measurements of clearance half-times for $^{15}O_2$, $Cl^{15}O_2$ and $Cl^{15}O$ is an index of pulmonary irradiation reaction. However, 3 of the 5 dogs studied so far have died from extra-pulmonary difficulties before the O-15 labeled gas dynamic studies indicated lung malfunction. One dog is still alive 24 weeks after 2000 rads of Co-60 to the right lung. Differences between the clearance rates of irradiated and control lungs did not become significant until 100 days post therapy. A dog receiving 3000 rads to the right lung showed dynamic changes beginning around 25 days post irradiation and death occurred at 44 days. Further studies are necessary before this large time-dose effect can be documented.

(3) In April, 1969, a multi probe data acquisition and processing system was set up, consisting of seven 2-inch NaI crystals coupled with a nine channel magnetic tape recorder and a model 2115A Hewlett Packard computer. Techniques were adopted or developed and put into operation for dispensing Xe-133 either as a solution or as a gas; for delivering the gas in a single breath or by a rebreathing technique for ventilation studies; and for safely disposing of the used activity by absorption on activated charcoal. Clinical results have shown that measurement of the regional distribution of alveolar volume, ventilation and perfusion is valuable in establishing whether pulmonary resection can be tolerated. In several cases conventional pulmonary function tests suggested that the patient would be unable to tolerate this surgical procedure but Xe-133 studies demonstrated that the disability was due primarily to the pathology of the portion to be resected.

Fig. 19. Washout of a single breath of $C^{15}O_2$ from the lungs of a normal dog

(4) The function of homotransplanted dog lungs was investigated by studies using Xe-133 as a gas or dissolved in saline as well as the short-lived cyclotron produced gases, $^{13}\text{N}_2$, $^{15}\text{O}_2$ and Cl^{50} . Results showed an immediate decline to very minimal levels of both ventilation and perfusion in the transplanted lung in which pneumonia had developed. The decline in immuno-suppressed dogs was gradual.

(5) The department of Physical Medicine at Brompton Hospital, London, England advocated lower costal expansion as a technique for preferential ventilation of the lower lung fields (1). The effectiveness of this maneuver has been tested using radioactive gases. To date, preliminary studies have shown the desired increase in concentration of the tracer on the appropriate side in four out of six subjects.

Significance and Present State of Knowledge

(1) These preliminary baseline studies on normal dogs are the first step in a series of experiments to measure and evaluate respiratory dynamics. Since the early work in London (2) in the late 50's and early 60's, the rate of pulmonary clearance of O-15 labeled oxygen has been considered an index of regional oxygen consumption; the clearance of carbon dioxide, a measure of regional pulmonary blood flow; and the clearance of carbon monoxide, a parameter of regional membrane diffusion capacity.

(2) Despite wide recognition that radiation therapy of pulmonary lesions is frequently followed by a typical clinical pneumonitis and occasional respiratory insufficiency, there has been little clarification of the physiologic course of events (3, 4). This has not been carefully documented to date because of the difficulty and discomfort of conventional studies of pulmonary function. However, the gas radionuclide procedures are simple and non-traumatic. Pathologic analysis of acutely irradiated lungs, i.e., alveolar transudate and septal edema, as well as conventional pulmonary tests demonstrating severe arterial hypoxemia suggest that pulmonary irradiation results in the formation of arteriolar-venular shunts.

(3) Information obtained from Xe-133 ventilation and perfusion studies has been valuable in assessing preoperative lung cancer patients in whom conventional physiological tests would suggest inadequate lung reserve to permit surgery. However, radionuclide studies have demonstrated that the abnormality was largely confined to the portion being considered for resection and surgery in most cases has been successfully performed.

(4) Preliminary animal results indicate that it may be possible by means of radionuclide techniques to distinguish the pulmonary rejection phenomenon, suggested by the gradual decline in function exhibited by one immuno-suppressed dog, from the pneumonia suggested by the prompt decline in function in two other immuno-suppressed dogs. It is of course unquestionable that a great step forward in the treatment of infiltrative and primary lung disease would be made when a consistently viable lung transplant is achieved. A method of documenting the course of pulmonary rejection would be a significant advance toward this end.

(5) It is of importance to confirm the effectiveness of lower costal expansion as a technique for preferential ventilation of the lower lung field since this maneuver is commonly used to prevent or combat post operative atelectasis.

Future Investigations

(1) An attempt will be made to isolate or identify the factors which contribute to the variability in the rate of uptake of labeled gases. Emphasis will be on the uptake of $^{15}\text{O}_2$ but studies will also be performed on the uptake of carbon monoxide labeled with ^{15}O . Among parameters to be varied will be the frequency of controlled respirations, the influence of spontaneous respiration, the concentration of inspired oxygen before administration of the nuclide, the percentage of carrier O-16 with O-15, the dog's heart rate, and arterial blood gas tension.

In addition, the effect on respiratory gas transport of a new chemotherapeutic agent, Bleomycin, which uniformly produces pulmonary fibrosis if an adequate dose is given, will be assessed. Aside from determination of the rates of uptake of O-15 labeled oxygen, carbon monoxide, and carbon dioxide, efforts will be made to dissolve nitrogen-13 in saline or water to evaluate the distribution of pulmonary blood flow from the vascular side of the alveolar-capillary membrane.

(2) The present work in dogs will be continued using 2,000 and 3,000 rads from a Co-60 source delivered as a single dose. Serial studies to measure the clearance rates of oxygen, carbon dioxide and carbon monoxide labeled with O-15 will be performed at intervals of one or two weeks. The study will be extended to humans with serial evaluations during and after therapeutic irradiation of 3,000 to 4,500 rads to the mediastinal and supraclavicular portal in the treatment of Hodgkin's disease as well as to other patients with pulmonary or intra-thoracic lesions, who have a sufficiently good prognosis to expect long term follow-up studies.

2

(3) Evaluation of regional alveolar volume, ventilation, and perfusion by means of inhaled and intravenously injected Xe-133 has been helpful in the clinical management of neoplastic disease involving the lungs. This benefit is appreciated not only in the pre-operative assessment of patients with bronchogenic carcinoma, but also in a variety of patients with infiltrative pulmonary disorders. Extension of this project is anticipated.

(4) The continuation of our work to evaluate homologous lung transplants in dogs will depend upon the availability of these animals, which at the present time is minimal.

(5) Although results are encouraging, additional studies will be necessary and must be carried out before quantitative conclusions concerning the efficacy of lower costal expansion can be drawn.

References

1. Physiotherapy for medical and surgical thoracic conditions; Pamphlet published by Physiotherapy Dept. Brompton Hospital, London, England, 1960, Third Revision, 1967.
2. West, J.B., Studies of pulmonary and cardiac function using short lived isotopes oxygen-15, nitrogen-13, and carbon-11, Chapter 2 in Progress in Atomic Medicine, (Lawrence, J.H., Ed.), p. 39-64, 1968.
3. Emirgol, C., and Heinemann, H.O., Effects of irradiation of chest on pulmonary function in man. J. Appl. Physiol. 16:331, 1961.
4. German, P.A., and Brady, L.W., Physiologic changes before and after radiation treatment for carcinoma of the lung. JAMA 206:809, 1968.

3. Erythropoietic and Phagocytic Function Studies

- a) The evaluation and quantification of reticulo-endothelial organ function with Fe-52 and Fe-59, both as ferrous citrate and Tc-99m sulfur colloid

Objectives

To evaluate and compare erythropoietic and phagocytic functional sites following tracer doses of Fe-52, Fe-59 and Tc-99m sulfur colloid in order to quantify reticuloendothelial organ function in patients with anemias and various neoplastic processes.

Research Accomplishments

Thirty scanning studies were made on patients following separate administrations of Tc-99m sulfur colloid and/or Fe-59 ferrous citrate or Fe-52 ferrous citrate during 1967 and 1968. During the past three years 11 additional patients have been scanned with Tc-99m sulfur colloid, 12 with Fe-52 and 9 with Fe-59. Since August 1969, all of the Fe-52 for patient studies has been supplied by the SKI cyclotron. Iron-52 ($T_{1/2} = 8.2$ hr) was administered to patients with acute lymphocytic leukemia, chronic myelogenous leukemia, polycythemia rubra vera, myelofibrosis, lymphosarcoma, primary hemochromatosis and severe anemia with carcinomatosis. Patients were scanned 12-24 hours after receiving 85-300 μ Ci of this positron emitter. Scans of patients who received 10 mCi of Tc-99m sulfur colloid to determine sites of phagocytosis were initiated 15-30 minutes post-injection while the patients who received 50-100 μ Ci of Fe-59 were scanned 17-25 hours after injection.

Two detector systems were employed. Rectilinear scanning was done on an Ohio-Nuclear Scanner (ONS) or the recently re-designed High Energy Gamma Ray Scanner (HEG). The ONS (3" x 5" dual probe scintillation detector) stores data on magnetic tapes which are subsequently computer-analyzed. The HEG scanner system, containing two 4" x 4" sodium iodide crystals, has been converted from punched paper tape to magnetic tape output. A new program specifically designed for the quantitative analysis of data has been written so that scans may be processed and evaluated with a minimum time lapse.

Significance and Present State of Knowledge

Myelocytic as well as lymphocytic leukemias showed a striking extension of phagocytic activity as demonstrated by Tc-99m sulfur colloid scans. The localization of Fe-52 and Fe-59 demonstrated extramedullary hematopoiesis in many cases of myelofibrosis (Figure 20). The results of patients studied with both Fe-52 and Tc-99m sulfur colloid were correlated to attain a more precise hematologic picture (Figure 21).

One case of chronic myelogenous leukemia exhibited inadequate phagocytic marrow activity with increased colloid phagocytosis by liver and spleen. Considerable iron is seen in the vertebrae, pelvis, distal ends of the femora and proximal ends of the tibiae. Summation of counts from scans employing ACD damaged red cells tagged with Cr-51 indicated a greater splenic sequestration than normal. These changes indicate greater red cell activity on the basis of iron uptake in the marrow with a corresponding decrease in functioning granulocytic activity. Results therefore were indicative of marrow aplasia with a reduced "myeloid erythroid" ratio.

H.M.; σ ; Fe⁵² 68.9 μ c
L and R distal femora-proximal tibiae
Level spacing: 4 σ

Fig. 20. Computer-analyzed scan showing uptake of Fe-52, injected as ferrous citrate, at a site of extra-medullary hematopoiesis in the area of the left proximal tibia. The HEG scanning system was used with a 1-in. single-hole, cylindrical-bore collimator. Count-rate levels are separated by four standard deviation intervals with the maximum being designated by the letter D.

G.S. σ_1 Tc-99m S Colloid 10mc	A	0-8	c/5 sec	Data sampling increment: $\frac{3}{8}^{\circ}/5$ sec
L and R distal femora-proximal tibiae	B	8-24	"	Collimator: $\frac{1}{2}^{\circ}$ Cylindrical bore
Level spacing: 4 σ	C	24-48	"	
	D	48-58	"	

[illegible]

Fig. 21. Computer-analyzed scan of the distal femora and proximal tibiae using Tc-99m sulfide colloid. The HEG scanning system was used with a 1/2-in. single-hole, cylindrical-bore collimator. Count-rate levels are separated by intervals of four standard deviations with the maximum being designated by the letter D.

A patient with an 18 year history of polycythemia rubra vera was investigated with iron, technetium and chromium tracers. Previous evidence suggested that a conversion to myeloid metaplasia had transpired. Results indicated an absence of erythropoiesis, phagocytosis and an increased red cell destruction.

A patient with lymphosarcoma was studied with Fe-52 and Tc-99m sulfur colloid to determine his hematologic status following massive radiation therapy. Both parameters indicated decreased uptake in irradiated areas with an unexpected area of activity in the chest. These studies were of significant help in the patient's clinical management since they indicated the presence of enough functioning bone marrow to withstand additional radiation therapy.

A 19-year old patient, with a five-year history of acute lymphocytic leukemia, was scanned with Tc-99m sulfur colloid and Fe-52 ferrous citrate in an effort to clarify her clinical status. Bone marrow aspirates of the right and left posterior iliac crests (PIC) gave strikingly different results as to the number of blasts present. If the patient was indeed in relapse as the increased blasts on the right seemed to indicate, further antimetabolite therapy would be required. Judging by the left PIC reports, the patient was thought to be in remission. Technetium-99m sulfur colloid and Fe-52 similarly demonstrated an asymmetric concentration. In some areas, this was more apparent with the iron than with the colloid. Increased activity was seen in the left shoulder area and midshaft of the right femur. There was a major increase in the left pelvis as compared to the right. The ratio of left/right, as calculated from the raw data matrix of the TOKIM, was 2.1 for both the Tc-99m sulfur colloid and Fe-52 scans. Therefore, the marrow areas which were predominated by blasts showed an absence of uptake.

The lack of uptake in blastic areas may indicate poor vascularization of the leukemic marrow or an absence of reticuloendothelial function by a marrow impacted with leukemic cells. Further work to clarify these points is contemplated.

Future Investigations

It is planned to incorporate this Fe-52 and Tc-99m sulfur colloid project with the ACD damaged Cr-51 labeled red cell study under the composite title Clinical Appli-

cations of Absolute Quantitative Organ Scanning. Since both are basically hematologic projects it is felt that a combined regimen will facilitate the accumulation and evaluation of significant data. The determination of the actual contribution of one organ, such as the spleen, to erythropoiesis in a given individual remains a goal. With quantitation the contribution of an extramedullary site to the entire erythron could be ascertained. The quantitative evaluation of the effect that radiation therapy has on the erythropoietic and phagocytic systems remains to be determined.

b) Clinical applications of absolute quantitative
organ scanning

Objectives

To evaluate acid-citrate dextrose (ACD) as a red cell damaging agent to delineate the spleen and then to quantitate the extent of phagocytic function in patients with various clinical entities.

Research Accomplishments

It has previously been shown by us that when the proportion of acid-citrate dextrose (ACD), (normally used to preserve blood), to blood is significantly increased, red cell survival will be definitely impaired (1). Splenic erythrocyte sequestration by one day post-injection occurs when there are, by volume, 2 parts of blood to one of ACD. Based on these observations, Sharma, using dogs, denatured Cr-51 labeled red cells with excess ACD and demonstrated uptake by the spleen (2). We have altered the ratio of blood to ACD and changed the incubation conditions in accordance with our experience with humans.

Five ml. of blood, freshly drawn by venipuncture, are immediately added to an equal volume of the "special" acid citrate dextrose (ACD) solution. This solution is commercially available through Abbott Laboratories, and is the same material recommended for routine labeling of red cells with sodium chromate. It consists of citric acid 8 mg., sodium citrate (anhydrous) 25 mg., and dextrose 12 mg. per 1.0 ml. water. It is isotonic and has a pH of 5.0. To this mixture, 300-400 microcuries of Cr-51 sodium chromate are added. The material we used contained less than 2 micrograms sodium chromate in less than 0.5 ml. physiological saline. The mixture is carefully swirled and incubated for one hour at 37°C in a water bath with occasional intermittent agitation by hand.

At the conclusion of the incubation period, the mixture is centrifuged at 2,000 RPM for 15 minutes at 20°C. The supernatant is discarded and the red cells are resuspended to the original volume in physiologic saline.

A weighed quantity of the preparation is injected into the patient. Fifteen minute, 2 and 4 hour, and one day blood samples are drawn by vacutainer into heparinized tubes. Two, one-milliliter samples are counted to a minimum of 10,000 counts. In each study, the patient's blood volume is determined from the radioactivity of the 15-minute sample as against the total number of counts

injected. The resulting calculation is compared with the blood volume estimated from the patient's body weight. This rules out a "collapse" survival within the first 15 minutes.

In the localization studies, patients were scanned 24 hours after injection using the dual headed High Energy Gamma (HEG) scanning system with 4" x 4" sodium iodide crystals. Results indicated all except one patient had cleared their circulating blood sufficiently to permit localization in sites of sequestration with minimum background levels. In each of the scans, the liver, spleen (Figure 22) and bone marrow were clearly defined. Increased splenic uptake in patients with polycythemia rubra vera was noted (3).

Initially a collimator with a 9 cm. focus was used for the scans. The punched paper tape output was computer analyzed yielding an alphabetic mapping of the area with letters signifying levels whose midpoints are separated by any desired number of standard deviations ('). The single focus collimators have now been replaced by dual foci depth independent collimators designed for absolute organ uptake measurement. The HEG scanner has recently been modernized so that magnetic tape output is now analyzed on the IBM 1800 computer. Work in determining the relative acidity of liver, spleen and bone marrow for denatured red cells led to an expansion of scope to arrive at a method of absolute organ quantification.

Significance and Present State of Knowledge

There are many established methods for spleen visualization (1-5). Although scout films of the abdomen are simple to obtain and give some helpful information, procedures using radionuclides have provided more valuable information. The phagocytic function of the spleen has been utilized by tagging cellular and colloidal particles with appropriate nuclides. One such approach utilizes Cr-51 chromate labeled red cells which have been "heat" treated. This technique depends upon a critical temperature at which red cells are sufficiently damaged to be phagocytized after injection without immediate in-vitro or intravascular hemolysis. The effective temperature range, 49-50°C, is very narrow. If the incubation temperature is too low, the red cells will survive well and radioactivity will not be concentrated in the spleen. When the temperature is too high, they will hemolyze before they are injected (5).

P.D. 350 μ Ci ^{51}Cr ACD RBC's Summed Detector Image
Level spacing 2σ

Fig. 22. Spleen scan following injection of 350 μ Ci of Cr-^{51} ACD damaged erythrocytes. Letters are separated by two standard deviation intervals.

The inherent drawbacks of these methods led to our use of ACD damaged chromated red cells to determine absolute organ quantification. The procedures used for quantitating organ activity were described previously in detail in section IV-3 of this document. In these clinical studies, patients who were to undergo splenectomy were injected with ACD damaged Cr-51 labeled autologous cells on the day prior to surgery. The splenic area was scanned within 3 hours prior to splenectomy, and the in-vivo uptake was calculated. Coordinates corresponding to the splenic area on the scan printout were taken and the magnetic tape was rerun through the computer to integrate the counts corresponding to a specified contour. The 30-50% contour based on the maximum count rate was applied in all instances. Conversion factors, based on the series of phantom measurements, were then applied to these values to arrive at the percent of injected dose and microcuries in the organ. The true chromium-51 content was determined by in-vitro radioassay of the homogenized, excised spleen.

In the 12 patients who underwent splenectomy, the percentage of Cr-51 activity calculated in-vivo compared very favorably with the in-vitro measurement, Table IV. As expected, patients with chronic myelogenous leukemia (CML) have a high uptake. R.M. was the only patient in whom a histologic diagnosis of Hodgkin's disease (HD) in the spleen could be made. Although the spleen was of normal size and weighed 160 grams, the two patients with systemic lupus erythematosus (SLE) and thrombocytopenia had a low uptake.

Table IV represents a summary of the patients studied thus far. In-vivo determinations of splenic uptake have been corrected for surrounding tissue activity. Background correction is expressed as a percent of the splenic uptake in μCi before background was subtracted. The first three patients were done on the HEG scanner before it was updated, so that a different series of conversion factors was used.

Future Investigations

The patient data indicates that it is possible to obtain absolute organ quantitation which is essential for determining the contribution made by an organ such as the spleen to cell destruction or production. This will permit a more informed judgement with respect to splenectomy. The quantitative determination of what portion of cells is produced at an extramedullary site

Patient Summary of Absolute Quantitation of Splenic Uptake

<u>Patient</u>	<u>Diagnosis</u>	<u>$\mu\text{Ci } ^{51}\text{Cr}$ Injected</u>	<u>C.F.</u>	<u>Background Correction</u>	<u>Splenic % of Injected Dose</u>	
					<u>In Vivo</u>	<u>In Vitro</u>
A.G.*	CML	348	1.12		43%	50%
A.A.*	CML	360	1.14		16%	17%
R.H.*	HD	420	1.05		26%	21%
J.L.**	HD	310	27/31.5	2.8%	10%	12%
D.F.	HD	311	22.3	2.2%	7%	8%
V.Z.	HD	294	34	9.3%	9%	11%
L.V.	SLE	434	30.5	17.9%	4%	3%
P.D.***	Waldenstrom's macroglobulinemia	352	22.0	7.5 Ci	100 μCi	84 μCi
C.B.	SLE	331	21.9	4.4%	7%	5%
S.P.	HD	307	29.4	12.3%	5%	5%
D.C.	HD	344	30.5	8.6%	5%	4%
E.B.	HD	345	31.0	6.5%	7%	7%
R.W.	HD	300	32.0	4.1%	16%	26%

*Patients done under old system

**Anterior and posterior scans were obtained separately and were averaged to obtain % dose, therefore, C.F.'s represent Ant./Post.

***Results in microcuries since patient had residual ^{51}Cr from previous injections

is also significant to the understanding of the mechanism of anemia. Much work remains to be done to correct for over and underlying active tissue including the scatter from active liver tissue. What is needed is a method for determining the true image profile of an organ without interference from bone marrow and liver uptake. The reduction of image degradation by application of the modulation transfer function has been proposed as a means of correcting the scan data, thereby facilitating studies in clinical cases such as low splenic uptake and hyperfunctioning bone marrow.

References

1. Mayer, K., Ley, A.B., and D'Amaro, J., Impairment of red cell viability by exposure to "excess" acid-citrate dextrose (ACD). Blood 28:513-523, 1966.
2. Sharma, S.M., Patel, M.D., Ramanthau, P., Ganatra, R.D., and Blau, M., Improved technique to denature red cells for spleen scanning. J. Nuc. Med. 9:348, 1968.
3. Mayer, K., Dwyer, A., and Laughlin, J.S., Spleen scanning using ACD-damaged red cells tagged with Cr-51. J. Nuc. Med. 11:455-458, 1970.
4. Laughlin, J.S., Ritter, F.W., Dwyer, A., Mayer, K., Greenberg, E.J., Dimich, A.B., Hasan, S., Rothschild, E., and Myers, W.P.L., Development and applications of quantitative and computer-analyzed counting and scanning. Cancer 25:395-405, 1970.
5. Kyle, R.W., Gotshall, E., and Johnston, G.S., Spleen scanning with red cells simultaneously Cr-51 tagged and heat treated. J. of Nuc. Med. 10:480-482, 1969.

c) In-vitro analysis of Cr-48 as a red cell tag

Objectives

To test the red cell tagging efficiency of Cr-48 sodium chromate and the elution properties of its daughter V-48.

Research Accomplishments

It was observed by in-vitro counting that both Cr-48 and V-48 tagged the red cell to a degree of 80% and 50% respectively as compared to 91% for Cr-51. Data analysis over a period of 14 days showed that V-48 did not remain within the red cell. It was evident that the escaping V-48 was re-incorporated by freshly added red cells with a half-time of 16 days. This was the case when the V-48 tag resulted from Cr-48 decay as well as when it was directly introduced into the system.

Significance and Present State of Knowledge

Since 1954, in-vivo surface counting studies with Cr-51 labeled red cells have been employed for identification of sites of red cell destruction. Analysis of such studies have been complicated, however, owing to its physical characteristics, i.e., Cr-51 decays primarily by electron capture with only 9% gamma emission. With the maximum activity that can be administered, limited mainly by dose considerations, poor statistics were encountered. With the advent of the cyclotron, shorter lived nuclides with suitable gamma rays for scanning were investigated for their red cell tagging properties. Cr-48 was considered for short term studies and its daughter product V-48 for an extended study if after decay the daughter product would remain within the red cell.

Future Investigations

Chromium-48 was found to have a high red cell tagging efficiency but the subsequent elution and reincorporation of its daughter V-48 make it an unsuitable agent for cell survival studies. Areas of phagocytic activity could still be localized by in-vivo scanning techniques using this nuclide as a damaged red cell tag. The cyclotron Cr-48 production procedure results in a 20% (average) contamination of Cr-51 at the end of bombardment. This is undesirable since the amount of Cr-48 administered would have to be reduced to combat the elevation of patient exposure from long-lived Cr-51.

d) Dynamic function studies in auxiliary liver transplanted dogs

Objectives

An auxiliary liver graft may be an attractive mode of therapy for various types of end stage liver disease, such as hepatic cirrhosis. It might also function as a means of temporary hepatic support in acute hepatic failure, when recovery of the host liver is expected to occur. The purpose of this study was to monitor auxiliary liver graft function, or rejection, and to measure liver blood flow without involving complicated vascular catheterizations.

Research Accomplishments

The procedure used to evaluate these dogs was the measurement of the uptake of 0.5 millicuries of intravenously administered Tc-99m labeled sulfur colloid. The apparatus was the TOKIM system. We compared the ratios of uptake doubling time and total accumulated activity for the auxiliary liver to the host liver.

In order to evaluate our technique we performed sixty trials in five normal dogs over a period of three months. The weighted mean doubling time for the accumulation of radiocolloid in the liver for these runs was 1.12 ± 0.04 minutes. This 3.6% standard deviation indicates a remarkable degree of reproducibility, considering that the uptake doubling time is affected by: (1) the particle size spectrum of the colloid and (2) the cardiac output of the dog.

Some of the dogs with auxiliary livers previously had a host bile duct ligation, so that their serum bilirubin levels could be correlated with the measured doubling times. We were able in most cases to predict rejection, as indicated by the increase in serum bilirubin level, from the change in the uptake doubling time (Figure 23). Also, the ratio of the saturation values for uptake of colloid over the two livers seems to be a measure of their relative blood flow. An unexpected result was the large variability, of the order of 10 to 30%, in the uptake doubling times for the host and auxiliary liver.

Significance and Present State of Knowledge

The relatively large changes in the radiocolloid uptake doubling times might indicate that these measurements are more sensitive to physiologic changes than are the serum bilirubin levels. On the other hand, the uptake doubling times indicate rejection prior to the increased bilirubin level in only 30% of the dogs studied. There are no future investigations planned for this research.

Fig. 23. Serum bilirubin concentration and radiocolloid doubling time plotted as a function of days after transplantation. The increase in bilirubin concentration indicates rejection of the auxiliary liver.

4. Radioisotope Studies of Parathyroid Localization

Objectives

To develop and evaluate radioisotope localization techniques for hyperfunctioning parathyroid tissue in man.

Research Accomplishments

a) Uptake and Retention of Se-75 by Various Tissues in Man Following Intravenous Injection of Se-75-Selenomethionine

For evaluation of early uptake of the neck and superior mediastinal tissues in man, patients were given 50 μ Ci of Se-75-selenomethionine intravenously at the beginning of exploration of the neck for hyperparathyroidism. At the precise time of removal of the parathyroid adenoma, this tissue and other surrounding tissue samples were obtained. The tissues examined included thyroid, muscle, fat, skin, and blood. Se-75 activity was determined in a well-cup counter. Concentration of Se-75 as percent of dose per gram of tissue was calculated. Ratios of the parathyroid to other tissues are shown in Table V in three such studies done within the first two hours following injection. Parathyroid uptake is consistently greater than that of all other tissues measured, ranging from approximately one and one-half to over twenty-five times as active. Since perfusing blood in the periods immediately following intravenous injection was thought to be a major factor in interfering with the separation of parathyroid activity from background activity, detailed blood studies were done on several patients. A typical study of the early blood radioactivity is shown in Figure 24. These studies demonstrated that the injected labeled amino acid disappeared rapidly from the blood so that by approximately one hour almost all activity in the blood was associated with protein. The low point of total blood activity occurred at approximately thirty minutes following injection. The protein bound or incorporated radioactivity rose gradually from approximately thirty minutes to a peak at about five hours after injection. Red cell activity was low during the early hours following injection. In-vivo measurements over the neck in a number of patients confirmed these blood studies. As shown in Figure 25, total activity over the neck and superior mediastinum falls rapidly during the first ten minutes following injection and seems to reach a low point at about thirty minutes after injection and then gradually rises over the next two hours.

b) Evaluation of Rectilinear Scanning Versus Gamma Camera Imaging

During the course of these studies three instruments were used for

TABLE V

EARLY ^{75}Se RATIOS OF PARATHYROID TO
OTHER TISSUES IN MAN

PARATHYROID ^{75}Se RATIOS OTHER TISSUE		1	2	3
Patient				
Time postinjection		29'	90'	120'
Thyroid		4.8	1.5	5.6
Muscle		7.3	5.7	11.8
Fat		15.5	26.3	22.7
Skin		5.7	6.1	16.4
Whole blood		5.6	4.5	11.3
Plasma		7.1	3.2	8.1

Fig. 24. Se⁷⁵ activity in blood following intravenous injection.

Fig. 25. Sequential changes of Selenium-75 Selenomethionine in the neck area of 7 patients

parathyroid scanning. These instruments were the High Energy Gamma (HEG) scanner, the Multi-Functioning Scanner (MFS), and the gamma camera (Chicago Nuclear). Characteristics of these instruments and their collimators are shown in Table VI. While all three instruments were capable of localizing some parathyroid adenomas, the gamma camera has proven to be consistently the most valuable instrument. Part of the reason for this may lie in the fact that particularly in the early phases of scanning, rectilinear scanning is seeing more blood activity in one part of the scan than in another. Instrumentation problems were also approached from the point of view of attempting to compare the relative value of photoscanning (analog) versus computer analyzed digital information. Digital information was obtained from all three instruments and appropriate computer programs were prepared. Scans were analyzed both by decade analysis and two standard deviations. The overall results of these studies indicate that while only approximately twenty percent of the tumors are visible on photoscan under optimal conditions, approximately sixty percent can be defined by computer analysis. Nonuniformity of the crystal-photomultiplier tube configuration of the gamma camera initially gave difficulty. Uniformity floods of the instrument and subsequent correction of the scans by the computer for nonuniformity resolved this problem. Phantom studies were also performed to define the practicality of localization of anticipated parathyroid to other tissue concentrations in a variety of target sizes. A phantom 12 cm in diameter was prepared with a target placed 3 cm deep within it. The scanning head or gamma camera head was located 8 cm above the target. It was possible to localize the targets as small as 0.5 cm with a 10:1 activity ratio and targets of 1 cm with a 5:1 ratio.

c) Problems of Confirmation of Localization

In order to relate focal areas of Se-75 uptake to anatomically significant landmarks, several approaches were employed. In all patients, Co-57 point sources were placed at the midline of the angle of Louis, sternal notch, and thyroid cartilage. These were then imaged and used to relate Se-75 uptake. At the time of surgery in all patients, measurements were made of the location of the tumor relative to these anatomical landmarks. In some patients a silver clip was placed at the site of the adenoma. Post operatively the patient was placed in the scanning position and an x-ray taken to localize the clip. These methods provided a check on operative localization relative to the landmarks noted above. Since the major anatomical structure related to parathyroid tumors is the thyroid gland, it was hoped that this organ could be imaged successfully at the time of parathyroid scanning. Initial studies with I-131 indicated that there was significant scatter radiation seen at the Se-75 peak (260 keV) to preclude the use of this nuclide. With the recent availability of technetium-99m pertechnetate, studies have been initiated to determine the usefulness of this isotope. The thyroid gland in these patients (see below) is suppressed with triiodothyronine prior to selenium scanning to decrease the uptake of the labeled amino acid by the thyroid. Prior technetium studies had been done by others indicating that technetium

TABLE VI

	HEG	MFS	CAMERA
<u>BASELINE</u>	220 Kev	220 Kev	R = 260
<u>WINDOW</u>	100 Kev	100 Kev	W = 25%
<u>COLLIMATOR</u>	W 19 Hole Focus	Pb 32 Hole Focus	Pb 1000 Hole Parallel
<u>CRYSTAL</u>	4 x 4"	5 x 3"	10 1/2 x 1/2"
<u>STEPS</u>	3/8 x 3/8"	0.2 x 0.2"	
<u>SPEED</u>	2 1/4"/min	4"/min	

was taken up adequately for imaging by the unsuppressed thyroid. Studies were performed in our patients by giving them 2 μ Ci of technetium-99m during the process of parathyroid scanning. One-half hour after injection, the technetium peak (140 keV) was scanned. In all patients sufficient uptake was obtained in the thyroid gland (despite prior suppression) to permit anatomical correlation of Se-75 uptake relative to the thyroid anatomy. The emission characteristics of Tc-99m are such that there is no interference with the Se-75 photo peak. This procedure has now been adopted as part of routine parathyroid scanning.

d) Studies of Preoperative Localization

The currently employed procedure for parathyroid scanning is as follows. Patients are given triiodothyronine 75 μ g/day (25 μ g t.i.d.) for a minimum of five days prior to scanning to suppress the thyroid uptake. The patient is placed in the supine position with the neck hyperextended and the head is held in place with a sandbag. Cobalt-57 markers are placed in the midline at the angle of Louis, sternal notch, and thyroid cartilage, and using these markers the patients are centered appropriately in the gamma camera field. Three hundred μ Ci of Se-75-selenomethionine are injected intravenously. Beginning five minutes after injection, four, thirty-minute scans of the neck and superior mediastinum are obtained sequentially. Each scan is recorded as a photo-scan on x-ray film and polaroid film. In addition, digital information is stored on magnetic tape. At the beginning of the final Se-75 scan, 2 μ Ci of Tc-99m pertechnetate are injected intravenously. Following the final parathyroid scan, the technetium photo peak is scanned for thyroid imaging. Photoscans are examined individually and by superimposition of x-ray films for focal persistent areas of Se-75 uptake. Digital information, corrected for nonuniformity of the gamma camera, is analyzed by both decade analysis and two standard deviation levels. Areas of persistent focal activity are noted.

A total of fifty-five patients have been scanned to date in thirty-six of whom we have complete surgical information. Of these thirty-six patients, localization with a single dominant focal area was achieved in twenty-two, or sixty-one percent. An additional six patients (sixteen percent) had two focal areas; one of which localized the tumor. Eight scans were negative (twenty-two percent); three of these eight had negative explorations and four were found to have adenomas not seen on the scan (false negatives). False positives numbering seven were found including the six mentioned above. One interesting false positive finding was in a patient with metastatic breast carcinoma in whom a persistent focus of Se-75-methionine was localized in a metastatically involved lymph node. In only five of twenty-two studies was the tumor identified by photoscan. This represents one-fourth of those identified by digital analysis. One patient in the series had four gland hyperplasia correctly identified and localized preoperatively. This patient had a history of chronic renal disease. Another patient had recurrent parathyroid carcinoma which was localized preoperatively in the superior

mediastinum. Two patients that had previous negative explorations were correctly localized by this procedure. One patient had a recurrent parathyroid adenoma located deep in the scar tissue of previous surgery ten years earlier.

Severity of hyperparathyroidism, as judged by the degree of elevation of serum calcium, failed to correlate with preoperative localization. In addition, at surgery, the tumor was weighed and/or measured accurately in most patients. The weight or volume of the tumor failed to correlate with preoperative localization. Localized adenomas range in weight from 160 mg to 8.1 grams. Seven of twelve tumors accurately weighed and preoperatively localized weighed less than 1 gram.

e) Long-Term Studies and Dosimetry

A total of twelve patients were studied by obtaining multiple tissue samples at times of surgery or subsequent post mortem at times up to one year following injection. In addition, six patients were followed to a maximum of two years by total body retention measurements. Studies indicated that total body retention measurements correlated well with measurements obtained by excretion analysis. A wide variety of tissues were sampled in these patients. Collaborative work with others using these basic data has provided the basis for calculating various bio-radiological parameters. Calculation of biological half-life indicates that the isotope follows at least a three exponential fall off. Approximately thirteen percent has a biological half-life of 0.55 days, forty-four percent - 46.0 days, and forty-two percent - 220.0 days. Fifty percent of the injected Se-75 is excreted by seventy days. Taking physical decay into consideration, the administered activity retained in the body is reduced to fifty percent at forty-seven days. These total body and tissue measurements have permitted estimated dose calculations. The organ receiving the largest dose is the liver; 300 μ Ci giving approximately 8.4 rads plus or minus fifty percent. Whole body radiation is calculated to be 2.4 rads plus or minus fifty percent.

f) Studies of Se-75-Selenomethionine in the Rat Thyroid

To evaluate the thyroid handling of this labeled amino acid and possible means of thyroid suppression for scanning, the following animal studies were done. Groups of rats were given 4-20 μ Ci of Se-75-selenomethionine intravenously or interperitoneally and exsanguinated at time intervals from five minutes to twenty-four hours after injection. The entire thyroid and aliquots of plasma, liver, and muscle were weighed and counted and the amount of Se-75 calculated as percent dose per mg of wet tissue. Following injection, thyroid uptake rose to a peak level of approximately $8 \times 10^{-4}\%$ dose/mg wet weight by sixty minutes and remained stable over the next twenty-four hours. Following an initial fall after injection, Se-75-selenomethionine rose slowly in plasma from thirty minutes to a peak concentration at two hours of $1.5 \times 10^{-3}\%$ dose/ml with a slight fall noted by twenty-four hours.

Other groups of rats were given TSH (thyroid stimulating hormone) at intervals from 0-95 hours prior to Se-75-selenomethionine, and sacrificed one hour after the tracer with similar tissue analyses performed. TSH pretreatment led to a peak two to three fold increase in thyroidal nuclide concentration at six hours. Increased uptake was seen at four hours but not at one hour after TSH, and the effect was diminishing at twelve hours and gone by eighteen hours after TSH. Liver uptake similarly increased two to three fold by six hours after TSH, but tended to remain 1.5-2 times higher than controls over the next seventy-two hours.

Groups of rats were given triiodothyronine (T3) 2 µg daily subcutaneously or propylthiouracil (PTU) 0.1% in their drinking water, or both, for six days. Control animals were untreated. On the fifth day each animal received 20 µCi Se-75-selenomethionine. Four to six animals were sacrificed two hours after injection and the remaining animals sacrificed twenty-four hours after injection. Propylthiouracil (PTU) treatment led to an increase in the weight of the thyroid, this increase being reversed by the concomitant T3 administration. PTU caused an increase in isotope uptake by the thyroid at two hours and twenty-four hours which was reversed by T3. Triiodothyronine (T3) alone caused a significant decrease in thyroid uptake at two hours but not at twenty-four hours. Triiodothyronine also led to increased hepatic uptake at two hours. The maximum suppression of thyroidal uptake was seen at two hours after T3 alone.

g) Carbon-11 Labeled Toluidine-Blue-O

Reports of visible staining of the parathyroid following intravenous infusion of toluidine-blue-o, a quinone dye, led to studies of the preparation and localization of a C-11 toluidine-blue-o. Studies of its distribution in the dog were undertaken to evaluate its potential use as a scanning agent for the parathyroids in man.

Approximately 200 mg of toluidine-blue-o (Allied Chemical Corp.) was irradiated for varying periods of time from fifteen to thirty minutes at several beam currents of helium-3 particles in our cyclotron. A specially constructed target holder, 1" diameter x 0.020" thick, was employed. At the end of irradiation, the material was removed from the holder, ground in a mortar and pestle with 20 ml water, and centrifuged. The supernatant was adsorbed onto a 2.5 x 40 cm alumina column and eluted with isotonic saline. The first (clear) and last (light blue) fractions were discarded. The dark blue middle fraction was filtered through two millipore filters (0.8 and 0.22 micropore size), and the filtrate radioactivity measured in a gamma ionization chamber. Table VII shows the results of the first eight cyclotron production runs. The isotopically labeled dye from the last three runs was infused over a fifteen to twenty minute period into anesthetized mongrel dogs in whom the thyroid, parathyroids, and abdominal organs had been exposed. Tissue samples including

TABLE VII

<u>Run #</u>	<u>Beam Current</u> (μ A)	<u>Irradiation Time</u> (min.)	<u>Time Lapse for Chemical Separation</u> (min.) <u>*</u>	<u>Final Solution</u> (μ Ci)
1	7	15	40	112
2	20	30	40	60**
3	5	20	35	500
4	5	20	40	500
5	5	20	44	130
6	10	20	47	330
7 ‡	5	20	35	650
8 ‡	7	20	27	910

* from end of irradiation to measurement of solution for injection

‡ beam spread in these two runs

** marked thermal effect noted (charring)

blood, thyroid, parathyroid and pancreas were taken at varying times during and after infusion and counted in an automatic well-cup counter. A computer program was designed and used to correct for physical decay and to calculate dose distribution in the tissues. The whole blood radioactivity rose during the infusion to levels of 2.8 and $6.0 \times 10^{-3}\%$ dose/ml, with an apparent exponential fall over the next thirty minutes to 1.2 and $1.7 \times 10^{-3}\%$ dose/ml, in two dogs. In the third study, which was technically most satisfactory, all tissues except muscle showed a decrease in decay-corrected radioactivity with time from the end of infusion. In one dog parathyroid/thyroid ratio four minutes after the end of infusion was approximately $5.3/1$. In another animal tissue data one to fifteen minutes after the end of infusion showed liver and parathyroid to have had concentrations of approximately $7-8 \times 10^{-3}\%$ dose/gm, pancreas $4.5 \times 10^{-3}\%$ dose/gm, thyroid $3.2 \times 10^{-3}\%$ dose/gm, and muscle $1.4 \times 10^{-3}\%$ dose/gm. The dye visibly stained the parathyroids and pancreas blue at the time of these tissue samples and it was also visible in urine and bile.

Significance and Present State of Knowledge

We have demonstrated that Se-75-selenomethionine is a useful agent for preoperative scan localization of hyperfunctioning parathyroid tissue in man. As such, it deserves a place in the preoperative management of patients scheduled for surgery for hyperparathyroidism. However, it has been demonstrated by these studies that photoscan imaging alone, produces a positive yield significantly lower by approximately one-third than that of computer analyzed digital scanning. In view of this, it is recommended that these scans only be done under circumstances where such computer analysis is available. We have further demonstrated that the addition of Tc-99m pertechnetate thyroid imaging is a practical procedure and provides significant information regarding localization of hyperfunctioning parathyroid tissue in relationship to the thyroid. Tissue uptake and retention measurements coupled with phantom studies have provided a model for investigation of similar nuclear medical problems. Difficulties related to nonuniformity responses of the gamma camera have been resolved by computer correction. Long-term studies including the studies of total body retention by external counting as well as tissue analyses have provided basic information to enable calculation of biological half-lives and dosimetry. The radiation dose delivered by $300 \mu\text{Ci}$ as used in this procedure is in an acceptable range. However, since the long-term effects of low level radiation delivered over long periods of time are not completely known, this isotope preparation should probably not be given to children or young adults unless previous surgery has been unsuccessful and reexploration has been indicated.

Studies of the rat thyroid metabolism of this labeled amino acid have provided information to support the pretreatment of patients with triiodothyronine to suppress background thyroid uptake. In addition, these studies suggest that this labeled amino acid may be useful in the

further study of thyroidal protein metabolism under various conditions.

Reports of the visible staining of parathyroids by toluidine-blue-o suggested that this dye, if labeled with a suitable gamma emitting isotope, might be useful for external parathyroid localization. Radiochemical procedures were developed to prepare a C-11 labeled material in sufficient amounts for study in the dog. These studies indicated that the differential concentration of the dye in parathyroid tissue was not sufficient to permit external imaging in man. These studies taken as a group provide a firm basis for a procedure of major clinical significance in the management of a large number of patients.

VI. METABOLIC AND ORGAN SCANNING STUDIES (OSSEOUS)

AEC support for research in various aspects of bone metabolism dates from 1950 when a grant was made for support of a study of the effects of external radiation on mouse bone. Many other projects have been added to the program since then, the major emphasis having been on mineral metabolism. Several of these have been joint studies with members of other divisions of Memorial Center. Two Ph.D. theses have been written in the field. The work was closely associated with technical and mathematical developments of the scanning systems, report of which will be made in other sections.

1. Effects of External Irradiation on Animal Bone

Objectives

This program was started in Nov., 1949 under AEC Contract AT (30-1)-799 and shifted to Contract AT(30-1)-910 as of June, 1953.

The initial object of the work was to obtain a quantitative comparison between the biological damage caused by X-rays of different qualities and the energy absorbed by appropriate living soft tissue elements in bone. The test object was the leg bones of the adolescent albino mouse, and the critical tissue was the region which elaborated alkaline phosphatase. The initial objective was achieved by 1959, but, in the course of the work a number of unexpected findings had been made on the time course and late results of radiation injury in bone. Hence emphasis was changed to a study of the mechanisms of radiation effects. This involved kinetic studies with bone-seeking radionuclides, some of which will be reported in a later section.

Research Accomplishments

a) Relative biological effectiveness (RBE) of X-rays in altering alkaline phosphatase elaboration in mouse bone.

The results of the RBE work were described in detail in the 15 year report. In summary, it was shown that the observed depression of alkaline phosphatase activity in mouse bone caused by external irradiation varied in a cyclic manner with time. Evidence of injury persisted for at least one year, or one half the normal life span. If the phosphatase loss was integrated over a 100 day period the result was a straight line function of dose between 780 and 3260 rads. Based on the 100 day integration of phosphatase loss the RBE of X-rays generated at 100 keV, 180 keV, 1000 keV or 22.5 MeV, and of

15 MeV electrons was unity within less than $\pm 5\%$.

b) Mechanism of radiation changes in bone.

The early results of this work were described in the 15 year report; considerable progress has been made since then.

The early observation that, despite the depression of the anabolic enzyme alkaline phosphatase which is caused by external irradiation, irradiated mouse bones had a higher mineral concentration than their controls led to the hypothesis that anabolic and catabolic processes in bone had separate radiosensitivities. This hypothesis was tested by measuring the net gain in length and wet and ash weights, and the uptake and loss of Sr-89 in irradiated and control mouse tibias, and the uptake of Ca-47 and loss of Sr-85 in irradiated and control leg bones of dogs. The overall rates of bone anabolism and catabolism in the mice were modified by the use of estrogenic, thyroid, or adrenocortical hormones, and the local metabolism was modified by fracture before or after irradiation.

Both the absolute and relative effects of radiation on bone anabolism and catabolism were found to vary with dose, with time after exposure and with the metabolic activity at the time of exposure. Determinations of Sr-89 retention at a single time after irradiation or injection had little value in predicting long term mineral changes in bone, but the results of serial determinations of the 4 hour uptake and 44 hour loss of Sr-89 correlated well with net gain or loss of mineral. These results underline the importance of the serial scans in the human patient which are reported elsewhere. Quantitative measurement of radiation injury showed that the status of the bones was still changing at 10 months after exposure in the dog and 4 months in the mouse, but the irradiated mouse bones appeared to have stabilized at one year. This would suggest corresponding periods of about 7 and 30 years in man. Radiation-induced stunting of bone growth appeared to be a sigmoid function of dose, and to be independent of age provided evaluation was based on increments in length rather than on total length.

Related to this experimental work have been studies of the composition of human bone and of clinical evidence of late radiation injury. The results of the animal work have clarified the well-known clinical observation that irradiated bone may be either more or less dense than normal. A disturbing clinical observation is that the induction of osteogenic sarcoma by external irradiation of human bone appears

to be much more frequent when exposure occurs before the age of 6 years than in older children and adults.

Significance and Present State of Knowledge

The results obtained in this work make it clear that apparent differences in the RBE of X-rays in the ranges from 100 keV to 22.5 MeV for injury to the mineralized portions of bone are due to differences in the quantity of energy absorbed by the soft tissue elements in the bone, and not to any qualitative difference in biological effect. Results also show that a large fraction of the normal life span of the species is necessary for evaluation of radiation injury to bone, and that the net changes produced vary with dose and with time after exposure, and with both the absolute and relative rates of the different metabolic processes involved.

Future Investigations

No further experimental work on effects of external irradiation on animal bone is planned. The results of past animal work will be used in evaluating studies being made in the human.

2. Local Kinetics of Bone-Seeking Tracers

Objectives

The work reported in the previous section made extensive use of radionuclide kinetics in bone, but only as an aid in evaluating effects of external irradiation. In this section various studies will be reported in which the primary objective was the study of the kinetics of different elements which are constituents of normal and abnormal bone.

Research Accomplishments

a) Comparative kinetics of calcium and fluoride in rabbit bone.

Most of the extensive literature on bone kinetics deals with reactions taking place on a time scale of days to years. Hence a study was made of the comparative kinetics of Ca-47 and F-18 in rabbit bone during the neglected period of the first 4 hours after a pulse label, when radionuclide uptake is primarily by surface exchange. The uptake of both nuclides was greater in the epiphyses and metaphyses than in the diaphyses. The uptake of F-18 was always greater than that of Ca-47, but the relative uptake of the two nuclides was the same in different parts of the bone. The ratio of the sizes of the exchangeable pools of the two elements was comparable to the ratio of their natural abundances in bone. In cortical bone the uptake of nuclide was related linearly to a part of the water content. Treatment with parathyroid hormone resulted in a 60% increase in uptake of F-18 and a 25% increase in the size of the exchangeable pool of fluoride. Nearly half of the Ca-47 activity recorded by external counting over the intact leg during the first 4 hours was in soft tissue, while the soft tissue component of F-18 was small after one hour. It was shown that the depletion of nuclide from blood on each passage through bone decreased with time after a pulse label.

b) Study of primary malignant tumors of bone with Sr-85

A study of the uptake of Sr-85 in the tissue of primary malignant tumors of bone, especially osteogenic sarcoma, was undertaken for the combined purposes of clarifying the metabolic patterns of these tumors and evaluating the usefulness of external scanning in their clinical management. External scanning was found to be useful in outlining the extent of bone tumors at their primary sites, but was not reliable in detecting early metastases in the lungs. Exam-

nation of tumor tissue obtained at surgery showed that tumors which were growing mainly as chondrosarcoma or fibrosarcoma took up little activity but the adjacent non-tumor reactive bone had a high uptake. All specimens of osteogenic sarcoma tissue which contained osteoid, whether or not they had calcified, had a high selective uptake of Sr-85.

Three of the patients studied were children aged 10, 10 and 11 years who were in good health after surgical removal of osteogenic sarcoma. The whole-body retention of Sr-85 of these children was studied by external counting for 6 to 8 months. There was a 3-fold range in the retention of strontium by these 3 children in the same age range.

A study of the Sr-90 from fallout in the long bones of patients with sarcoma, done in collaboration with the N.Y. Operations Office of the AEC, showed no evidence of unusual internal irradiation which might have been a factor in the induction of the tumors. The data yielded useful information on the Sr-90 retention in different parts of the same bone and in corresponding bones of people of different ages. An interesting observation was that, although the mean Sr-90 concentration was much higher in the bones of children than adults, the rate of remodeling of the children's bones was so rapid that the difference between nuclide content of the ends and shafts, which is seen regularly in adult bone, was almost lacking.

Significance and Present State of Knowledge

The studies with F-18 and Ca-47 have shown that the early uptake of bone-seeking elements within a few days after a pulse label depends on the area of bone surfaces available for exchange with blood. Observations made a few days after a pulse label of Sr-85 have shown that the level of uptake by primary bone tumors corresponds to the amount of bone-forming elements which they contain and points up the distinction between uptake by tumor within bone and bone around tumor. The observation of Sr-85 during months after a pulse label and of Sr-90 during years of chronic intake from fall-out have given vital information on strontium metabolism in children.

Future Investigations

The projects described above have all been completed and the information obtained is being used in various clinical studies. Some new kinetic studies involving neutron activation of calcium are being planned (See Proposal #3 for 1972, Section C3).

c) Comparison of short and long-lived bone-seeking radionuclides (Ca-47, Sr-85, Sr-87m, F-18 and Ga-68)

Objectives

The objective has been to investigate the patterns of local kinetics of bone-seeking radionuclides suitable for bone scanning during the first few hours following the intravenous injection of tracer doses and to compare and contrast nuclides of different elements with short and long half-lives, and to establish their relative usefulness for the diagnosis and follow-up of the clinical course of bone lesions.

Research Accomplishments

A comparison of the distribution and kinetics of Ca-47, Sr-85, Sr-87m and F-18 in man and of these same bone-seeking radionuclides, in addition to Ga-68 in rats, has been carried out.

Studies of eleven patients with radiographic evidence of localized cancer involving bone were made. Two nuclides were administered to each patient intravenously: six received F-18 and Ca-47, three received F-18 and Sr-85 and two received Sr-87m and Sr-85. Blood and urine samples were taken at selected intervals. Local uptake and point-count measurements over both normal and lesion-involved bones were carried out at intervals of one-half hour for the first hour, and then hourly for five hours. In addition, measurements were carried out at twenty-four hours and five days with the longer lived radionuclides Ca-47 and Sr-85.

Each of the nuclides studied in man was also studied in Sprague-Dawley rats. In addition, Ga-68 citrate, both with and without carrier, was also studied in rats. In the first group of twenty rats (five for each of the nuclides Ca-47, Sr-85, F-18 and Ga-68), distribution characteristics between tissue, normal bone, and repairing bone (fractured tibia) were studied, while in the second series of fifteen rats (five for each of the nuclides Ca-47, Sr-85, F-18), different regions of the normal and fractured tibiae were evaluated. In the third group of forty-two rats, the nuclides were administered simultaneously (twenty-four with Ca-47 and Sr-85, eighteen with F-18 and Ca-47) in order to make an absolute comparison in terms of the fraction of the total dose taken up in bone and soft tissue, and also the kinetics of uptake and turnover of each radionuclide. All tracer studies were carried out eleven days after the fracture of a single tibia, with sacrifice five hours post injection. The results of our investigations led us to the following conclusions.

If we exclude such considerations as the availability of a source of production of the materials used, our human and animal studies indicate that of the short-lived bone-seeking radionuclides, F-18 is the most suitable for bone scans performed within five hours of injection of the isotope.

In man, F-18 was found to disappear more rapidly from the plasma than did Ca-47, Sr-85 and Sr-87m. The urine excretion of F-18 was consistently greater than that of the other nuclides. Within experimental variations, urine excretion of Sr-85 and Sr-87m was the same. Ca-47 showed the lowest five hour excretion in urine. Uptake over normal compact bone was the greatest per unit dose for Ca-47, less for Sr-85, and least for F-18. The same results were observed for external point counts recorded over lesion-involved bone. The specificity of F-18 to the bone lesion at five hours is similar to that for Ca-47, and is probably greater than that for Sr-85 or Sr-87m due to plasma concentration at this time. F-18 demonstrates the highest lesion-to-normal bone ratio at three to five hours after injection. Sr-87m duplicated the findings seen with Sr-85 with better count-rate statistics. The low radiation dose of F-18 provides good count-rate statistics, and combined with the rapid plasma clearance, gives better scans of the skeleton. Its rapid decay also permits sequential studies. Ca-47 and Sr-85 frequently show higher diseased-to-normal bone ratios at five days and are therefore useful in the cases where early results are equivocal. Ga-68 has to be given in the form of citrate, the preparation of which is tedious and necessitates the addition of stable carrier in order to increase the bone uptake. This raises the problem of toxicity. These difficulties, added to the finding of low ratios of diseased-to-normal bone and bone-to-muscle, indicated that this particular nuclide offers absolutely no advantage for the purpose of bone scanning. Except for its short half-life, Sr-87m kinetics were identical to those of Sr-85.

A study of the distribution of Ca-47, Sr-85 and F-18 in various portions of bone and fracture callus taken as a model lesion was also carried out in a group of rats in addition to the above reported work.

Three groups of five rats with a tibia fracture of eleven days duration were sacrificed five hours after injection of each of the above-mentioned radionuclides. The tibiae of each animal were dissected free of all soft tissue, and then were sectioned as shown in Figure 26. Each bone yielded a spongy part (upper end), a compact part of the shaft,

Fig. 26. Sectioning of rat tibiae (fractured and normal) after all soft tissue has been removed.

and a fracture callus or a corresponding part of the contralateral normal bone. The lower end was discarded. In addition, a piece of muscle was obtained from each animal. Thus, a total of seven tissue specimens were obtained from each animal. The radioactivity of each specimen was measured in a scintillation detector, and was expressed as a percent of the administered dose of the radionuclide for each total sample and per gram of sample.

In addition, the tissue specimens of two rats in the Ca-47 group and of two in the F-18 groups were ashed, and the calcium contents were determined. This then served to calculate the specific activity of these two radionuclides relative to stable calcium in each tissue sample.

Since one of the objectives of this work was to apply our findings to bone scanning methods in vivo, a number of ratios of radioactivity were calculated. These are summarized in Table VIII.

1. The ratio of the radioactivity per gram of callus to muscle was highest for F-18: ten times greater than for Ca-47, and six times greater than for Sr-85. The ratio of intact bone to muscle was likewise greatest for F-18.

These ratios indicate one of the advantages of F-18 for bone scanning, i.e., a better resolution due to higher bone/muscle ratio. This value is enhanced over the lesion, thus theoretically improving its detection by external counting.

2. Since an external detector usually sees a whole long bone lesion or its major part, we calculated the ratio of the radioactivity in the whole callus and whole contralateral bone shaft fragment to 1 gram of muscle. This ratio was also highest for F-18, being ten times higher than Sr-85 when callus was compared to muscle. This ratio was much smaller for intact contralateral bone, although also higher for F-18 than for the other nuclides.

3. The ratio of callus to intact bone radioactivity was then calculated for the total specimen and per unit weight of tissue.

The ratio for total bones was the same for the three nuclides, about ten, and much smaller per unit weight of tissue (1.6 to 2.1). This indicated that the size of the lesion largely contributed to the difference between it and the intact contralateral bone, although more nuclide was also deposited per unit weight of tissue.

4. The specific activity of Ca-47 and F-18 relative to calcium was calculated for muscle, callus and normal corresponding portions of the contralateral tibia. It is considerably lower in muscle for F-18 than for Ca-47.

TABLE VIII

<u>Ratio of Activity per Tissue</u> <u>Mass Indicated</u>	Ca-47	Sr-85	F-18
Gm Callus/Gm muscle	100	166	1000
Gm intact bone/Gm muscle	60	80	400
Total Callus/Gm muscle	64	107	660
Total intact bone/Gm muscle	7	10	55
Total Callus/Total intact bone	9.2	10	10.2
Gm Callus/Gm intact bone	1.8	2.1	1.6
<u>Specific Activity</u> <u>(% Dose/mgCa)</u>			
Muscle	0.35		0.017
Callus	0.074		0.054
Intact bone	0.015		0.0072

reflecting a rapid clearance from the extracellular compartment and soft tissues. When callus and normal bone are considered the ratio between them is higher for F-18 than for Ca-47. The relatively high specific activity in muscle compared to bones is due to the higher amount of stable calcium in bone tissue.

5. The percent dose per gram of tissue in spongy bone (upper end) for each nuclide in the fractured bone was not significantly different from that in the intact bone. It was slightly higher for Ca-47 than for F-18 and Sr-85 for both bones. Comparison of the upper end to the compact bone of the shaft was not significantly different for each nuclide. In general, the shaft of the fractured bone (not including callus) had a higher activity than the intact bone for each of the three nuclides.

These results indicate that our animal preparation was a good model in which to study a bone lesion for comparison to normal bone and parts of each bone and soft tissue.

The distribution of the bone-seeking radionuclides utilized in this study measured five hours after their intravenous administration indicated that, taking into account soft tissue levels of these agents as well as levels in normal bones and bone lesions, F-18 appears to be the agent of choice for bone scanning done a few hours after injection.

Considering the higher excretion of F-18 we have previously observed, and the low soft tissue levels indicating a low retention of this nuclide compared to Ca-47 and Sr-85, it appears that F-18 has a much greater relative affinity for bone tissue than the other two nuclides.

The actual measurements of the levels of bone-seeking radionuclides in bone and soft tissue samples may be of use for comparison with external counting findings, providing it may be shown that levels in man would be similar to those found in the rat.

Significance and Present State of Knowledge

At present, F-18, in contrast to the other nuclides investigated:

- (1) Demonstrates the highest lesion to normal bone differential between three and five hours after injection.
- (2) Shows the most rapid plasma clearance and the greatest short term excretion, giving better scans of the skeleton.
- (3) Gives low radiation dose to the patient, while providing good count-rate statistics.
- (4) Localizes rapidly at bony sites.
- (5) Decays rapidly to permit sequential studies.
- (6) Decays by positron emission, thus providing unique physical detection characteristics.

The combined observations of the distribution of various bone-seeking radionuclides in-vivo in man and in total animals, as well as in portions of soft tissues, normal bones and bone lesions has led to:

(1) A better understanding of the factors which influence this distribution.

(2) Correlation of external measurements with actual tissue measurements.

(3) An optimal diagnostic program for the detection and follow-up of malignant bone lesions by the use of a combination of bone-seeking nuclides such as F-18 and Ca-47 or Sr-85 with scheduled early measurements at approximately five hours and five days.

d) Effect of Radiation Therapy on the Local Kinetics of Ca-47
and Sr-85 in Bone Lesions

Objectives

To investigate the changes in mineral deposition and removal which eventually lead to recalcification of destructive bone lesions.

Research Accomplishments

Patients received a single labeling dose of Sr-85 (100 μ Ci) intravenously, usually fourteen days prior to starting radiation therapy. Approximately one week later, a 50 μ Ci tracer dose of Ca-47 was given and repeated at several intervals after completion of radiation therapy. A total of thirty-six kinetic studies have been carried out on twelve patients. Ten of these were with Sr-85 and twenty-six with Ca-47. Three patients were dropped from study because of their rapidly worsening condition. During the past year, eleven Ca-47 and three Sr-85 studies were carried out among five patients. Presently, one patient remains on study while the others either expired or have required some other form of systemic therapy. Of the nine patients studied at length, eight had bone lesions from metastatic breast carcinoma and one from reticulum cell sarcoma. Daily determination of in-vivo total body retention and uptake measurements over diseased and normal bone were carried out for both these radionuclides using the Institute's dual probe High Energy Gamma Ray Scanner (HEG).

Although we have carried out our diagnostic bone scans with F-18 in over one-hundred patients, this radionuclide was not considered suitable for this study because its short half-life would not allow daily measurements after a single dose.

After radiation therapy was instituted, Sr-85 total body retention (TBR), as well as uptake over the diseased bone and normal bony landmarks were followed weekly, monthly, and finally up to two months after completion of the treatment.

Calcium-47 tracer doses were administered repeatedly at intervals of one to three months after completion of radiation therapy and occasionally earlier. Each time, the point counts were repeated daily for seven to fourteen days, and the TBR was measured whenever possible. Measurements were sometimes difficult to carry out as a result of the clinical condition of the patient.

Radiographs taken at intervals of one to four months were used to follow the changes in the bone lesions subsequent to radiation therapy, and the clinical evaluation of

symptoms, usually pain, was also noted.

Seven patients with metastatic breast cancer showed improvement both in symptoms and on x-ray films. One showed improvement only by x-ray, but expired shortly thereafter from other complications of her cancer. The patient with RCS experienced considerable decrease in pain and swelling of the area involved in the lower left femur but little x-ray changes.

A comparison of the local kinetics in diseased and normal bone during measured periods of one to two weeks at intervals for up to eight months emphasized the differences in the behavior of these two areas. Figure 27 shows high uptake over a lesion, rising for several days and more marked after radiation therapy but never returning to normal, even after eight months.

Figure 28 summarizes the general trend in Ca-47 uptake over bone lesions in relation to radiation therapy. All the patients with breast cancer metastatic to bone who were followed for over one month after radiation therapy showed a rise in the uptake of this radionuclide within thirty to sixty days following completion of treatment. The extent of this rise was variable. One patient with an ischial lesion showed no rise, possibly due in part to difficulty in counting the lesion.

The fall observed in RCS may indicate a different radiosensitivity and bone reaction to tumor, but this will require more observations.

The sequential retention of Sr-85 measured over lesion and normal bone was plotted and showed an uptake in the lesion higher than normal, and a gradual fall in both during and following radiation therapy. When plotted as percent of dose retained in the body as measured by TBR, we obtain an indication of the distribution of this radionuclide in the bone relative to the body contents. It appears that the dose retained in normal bone relative to the whole body is about constant. This is interpreted as meaning that after the first few days, the Sr-85 excreted is that lost from the bone which is its major reservoir. On the other hand, the rising retention in the lesion means that whatever Sr-85 was deposited there is either not remobilized and excreted or is removed at a much slower rate than from normal bone and, therefore, relative to a diminishing total body retention, the retention in these lesions increases.

In one patient, Figure 29, we observed a relative rise in lesion retention during and following radiation therapy, followed by a fall from fifty to one hundred and fifty days post radiation therapy. The rise then started again.

If this is an effect of radiation therapy, it may indicate that during this period of relatively decreasing retention, the Sr-85 is mobilized

Fig. 27. Uptake over lesion and comparable normal bone from sequential Ca-47 studies and after radiation therapy.

Fig. 28. Summary of maximum Ca-^{47} uptake over lesion on all patients in relation to days pre and post end of radiation therapy.

⁸⁵Sr IN LESION AND NORMAL BONE AS % OF BODY RETENTION

SW NO 26-75-28 PRIMARY: BREAST CANCER

⁸⁵Sr INJECTION 94 μ Ci

Fig. 29. Local uptake of Sr-85 over lesion and comparable normal bone expressed as a percentage of dose retained in the body and plotted as a function of time.

from the lesion and excreted at a greater rate than from the rest of the skeleton and then the retention is stabilized, and while the gradual loss from the rest of the skeleton continues, the relative retention in diseased irradiated bone increases again.

An interesting observation is the relative rise in the retention of normal bone at the last measurement. At about that time, pain has developed in that area and it is suspected that a lesion is present although not confirmed by x-ray.

Significance and Present State of Knowledge

An increase in the already elevated calcium-47 deposition was observed after radiation therapy to bone invaded by metastatic breast cancer. A relative increase of Sr-85 retention reflecting retention of bone mineral was also observed, and these two processes lead to a recalcification of previously destructive bone lesions. This apparent contrast with previous reports is the result of sequential measurements carried out at intervals after radiation therapy.

The occurrence of these changes and their degree when they occur may reflect the radiation sensitivity of the malignant process and the type of bone reaction it elicits.

Our observations on tumor-invaded bone are unlike those reported by Finston and Woodard on normal irradiated bone in the dog, where a net loss of mineral is observed for similar radiation doses.

The resulting gain in mineral which is inferred by our observations is probably due to a combined effect of bone healing after tumor destruction.

Future Investigations

It has become increasingly difficult to obtain cases where treatment can be limited to radiation therapy. The study protocol has therefore been modified as outlined in the proposal for 1972 to include patients undergoing systemic therapy.

e) Detection of Occult Metastases

Objectives

To demonstrate the extent to which radionuclide (Ca-47/Sr-85) scanning and radiography complement one another in the diagnosis of neoplastic lesions of the skeleton. In particular, the study was designed to establish the value of bone scans in the diagnosis of lesions not yet apparent radiographically.

Research Accomplishments

During 1965 and 1966, a total of thirty patients were studied with Ca-47/Sr-85. All the patients had bone pain, and the skeletal surveys revealed either no lesions or not more than one or two discrete lesions, remote from the symptomatic area. Stationary external counting was done over selected parts of the skeleton at five hours, twenty-four hours, and five days after the intravenous injection of 100 μ Ci of Ca-47 or Sr-85. Twenty-seven patients had positive tracer findings. Three were equivocal for metastatic disease. Nine were equivocal, due to the presence of osteoarthritis in the area scanned. Five patients had one or two distinct lesions in locations other than the areas scanned. Abnormal scans were obtained, both over the known and suspected lesions. At the end of the study, ten were confirmed by histology or serial x-ray films, ten had not confirmation, five expired without autopsy, and two patients had equivocal findings on follow-up.

Significance and Present State of Knowledge

Calcium-47 or Sr-85 bone scanning offers an excellent diagnostic tool for detecting bone lesions. The advantage of performing multiple digital scans after a single tracer dose was demonstrated. Early equivocal findings can be confirmed by observing an increasing differential uptake between diseased and normal bone as time progresses. The significance of this investigation is that bone scans, repeated at intervals in asymptomatic patients having a high risk of bone metastases, may reveal early lesions previously undetected by x-ray, despite careful scrutiny by a radiologist. Bone lesions were detected by scanning thirty-four to one hundred and forty-six days prior to their appearance on radiographs in this study.

Differentials in uptake of as low as twenty percent between vertebrae, corresponding to approximately 2 mg of calcium, can easily be measured in a few minutes counting time at twenty-four hours following intravenous injection of 100 μ Ci of Ca-47. A differential gain or loss of 2 mg calcium per day between adjacent vertebrae would require a minimum of eighty-five days to reach the 170 mg differential detectable by the most sensitive radiographic methods employing densitometry, or five hundred

days by standard radiographic techniques. Therefore, it would appear that the sensitivity of the radionuclide test for determining changes in bone mineral content is two orders of magnitude greater than that of radiographic methods.

f) Radiocalcium uptake in the fingers

Objective

To study the uptake of Ca-47 in the fingers as an index of local bone kinetics; particularly in true and pseudo-hyperparathyroidism.

Research Accomplishments

In four patients receiving injections of Ca-47 for the study of bone metabolism, finger uptake measurements were made daily, for up to 14 days after injection. Because of the counting geometry, only a relative measurement of activity was obtained.

Significance and Present State of Knowledge

The normocalcemic and hyperparathyroid patients studied showed a relatively constant or declining level of ⁴⁷Ca activity (corrected for decay) in the fingers over the period studied. The pseudohyperparathyroid patient, however, exhibited an increasing level of uptake. These observations are at variance with previous trends observed at other bone sites in true versus pseudo-hyperparathyroid patients.

Future Investigations

We are considering doing further ⁴⁷Ca finger uptake measurements in patients, employing a counting apparatus which would enable absolute determination of activity.

3. Total Body Kinetics of Bone-Seeking Tracers

a) Kinetics of the hypercalcemic state

Objectives

The objectives of this work have been to study the kinetics of calcium metabolism in the hypercalcemic state of cancer (patients with and without bone metastases) and to compare these kinetics with those of patients with hyperparathyroidism and with normocalcemic cancer patients.

Research Accomplishments

Twenty-nine patients have been studied. (a) Five patients with cancer, bone metastases and normocalcemia were found to have normal accretion and resorption rates of calcium, and slightly lower than normal exchangeable calcium pools. The turnover rate of calcium was slightly decreased owing to decreased excretion. One patient had breast cancer, bone metastases and mild hypocalcemia. She had decreased accretion and resorption rates of calcium.

(b) The development of hypercalcemia in eight patients with cancer and bone metastases was attended by a two-fold increase in the rate of skeletal accretion of calcium, and a more than three-fold increase in the resorption rate. The calcium turnover rate was increased and the miscible pool size rose by about thirty percent.

(c) Five patients with cancer hypercalcemia and no bone metastases (pseudohyperparathyroidism - syndrome resembling hyperparathyroidism, clinically and biochemically) were studied. Four patients had normal rates of skeletal accretion of calcium, a two-fold increase in resorption rates, and an increased exchangeable calcium pool. The turnover rate was moderately increased, due primarily to an increase in calcium excretion. One patient however, had two and one-half times elevated accretion rate of calcium and a high resorption rate.

(d) Ten patients with true hyperparathyroidism were studied. Seven of the ten patients had elevated rates of calcium accretion and resorption. The exchangeable pool was elevated in six patients. A summary of these studies to date is shown in Table IX.

(e) Post treatment studies were done in some of the above hypercalcemic groups, and successful treatment was, in general, attended by a return of the calcium kinetics to normal. There was usually a proportionately greater decrease in the resorption rate as compared to the accretion rate. Total body retention of calcium (as measured by the High Energy Gamma Scanner) increased with successful treatment of the hypercalcemic state.

TABLE IX

Pseudohyperparathyroidism

<u>Primary Site of Neoplasm</u>	<u>A (gm/day)</u>	<u>E (gm)</u>	<u>R (gm/day)</u>	<u>TBR (%dose retained)</u>	<u>(days)</u>
1. Lung (A.G.)	0.57	5.51	1.05	31.2, 28.8	7, 11
2. Kidney (K.B.)	0.37	5.85	0.68	69.8, 57.8	7, 13
3. Lung (C.M.)	0.34	6.43	0.86	49.8	7
4. Penis (L.R.)	0.53	9.4	1.65	51.6	6
5. Lung (W.L.)	1.07	7.47	1.66	62.5	10

True Hyperparathyroidism

<u>Patient</u>					
1. R.C.	1.20	4.82	-	72.5, 56.9	5, 12
2. M.D.	0.94	7.33	1.03	74.9	9
3. B.S.	0.71	4.97	1.10	67.7, 57.4	5, 10
4. H.B.	1.23	7.54	1.51	69.8	6
5. S.H.	0.49	5.4	0.47	65.2	9
6. A.M.	1.03	6.4	1.59	79.7	8
7. I.B.	0.86	6.99	0.71	72.5	14
8. A.E.	0.34	5.70	0.42	71.1	14
9. E.G.	0.47	6.23	0.60	50.7	14
10. A.M.	1.39	14.6	1.47	80.9	14

Note: A - Accretion rate of calcium (mean normal 0.40 gm/day)
E - Exchangeable calcium pool (mean normal 5 gm)
R - Resorption rate of calcium (mean normal 0.40 gm/day)
TBR - Total body retention of calcium tracer

Significance and Present State of Knowledge

(a) The data suggest that the primary defect in the hypercalcemia of cancer is an enhanced rate of resorption of calcium, which is associated with an increased rate of accretion (of lesser magnitude) in patients with skeletal metastases, and a normal rate of accretion in most of those without skeletal metastases. Treatment, if it is to be successful, must therefore be directed against this defect.

(b) Earlier experience indicated that there was a difference in the calcium kinetics between hyperparathyroidism and the cancer syndrome resembling hyperparathyroidism. This would be of clinical importance because the two entities are extremely difficult to differentiate and treatment for each is totally different. However, the experience gained in the past two years suggests that these earlier differences may, in fact, not pertain. Thus, we have now observed some patients with true hyperparathyroidism who have normal accretion rates of radiocalcium and, in contrast, we have seen one patient (W.L.) with the syndrome of pseudo-hyperparathyroidism who had an elevated accretion rate. Further observations will have to be made to understand what factors underlie the differences in kinetics within each respective group.

Future Investigations

One important area of study will be in the field of local bone kinetics. Although some of our data thus far indicate that the local kinetics in the hypercalcemic disorders noted above may be different, there is insufficient information to date. A demonstrated difference in local kinetics would have considerable importance so far as clinical application is concerned, since the present time-consuming metabolic studies necessary for the analyses cited are not practical for general clinical studies. Another area of future investigation is that concerned with the isolation and chemical characterization of the bone resorbing material apparently synthesized by certain tumors. A third area of future study concerns patients who demonstrate changes in serum phosphorus in association with hypercalcemia. Some of our previous studies have suggested that hypophosphatemic and normophosphatemic cancer patients may have different calcium kinetics, despite the same degree of hypercalcemia.

b) Effect of phosphate administration on Ca-47 kinetics

1. Studies in Man

Objectives

To study the effect of phosphate therapy on calcium metabolism and calcium kinetics in patients with primary hyperparathyroidism and patients with cancer

Research Accomplishments

a) The effectiveness of intravenous phosphate in the emergency treatment of patients with cancer hypercalcemia was studied. Thirty-six infusions of varying doses of phosphate (Inphos - sodium-potassium phosphate) were given to twenty-two hypercalcemic patients. Intravenous phosphate was compared with sodium sulfate and hydrocortisone infusions. Phosphate was found to be more effective in reducing serum calcium than either sulfate or hydrocortisone and the degree of the calcium-lowering effect was found to be dose related. It can be relatively safely given with predictable results and is therefore the treatment of choice among these three compounds in acute hypercalcemia.

b) The mechanism by which inorganic phosphate administration lowers serum calcium is not clearly understood. The calcium-lowering effect of phosphate is not due to increased urinary or fecal calcium losses since the renal excretion of calcium decreases during phosphate treatment and fecal calcium does not change. The following factors may be involved: (1) increased bone accretion, (2) diminished bone resorption, or (3) deposition of calcium-phosphate salts into soft tissues or bone. To investigate these factors, the effect of oral phosphate administration has been studied in a total of five patients to date; four patients with hypercalcemia due to primary hyperparathyroidism and one patient with mild hypocalcemia (serum calcium 8.7 mg%) associated with carcinoma of the breast with metastases to bone. Techniques employed have included metabolic balance, radiocalcium kinetics, and urinary hydroxyproline excretion. Complete metabolic balances of calcium, phosphorus, and nitrogen lasted six weeks. Phosphate (2-3 gm orally/day) led to a sustained reduction in serum and urinary calcium in all four hypercalcemic patients with primary hyperparathyroidism, but did not change the serum calcium in the patient with breast cancer although the urinary calcium decreased in this patient during treatment. Metabolic balance studies in the hyperparathyroid patients showed a shift to a more positive calcium balance, the change from pretreatment balance being in the range of +60 to +234 mg calcium/day. However, in the patient with breast cancer there was a shift towards less positive calcium balance. Phosphate therapy did not change nitrogen balance,

which remained positive in all patients. The phosphorus balances which were positive in two patients and negative in three before phosphate administration became markedly positive in all patients during phosphate therapy.

Calcium-47 kinetic studies during the pretreatment and treatment phases demonstrated a fall in bone resorption rate during treatment in the hypercalcemic patients and an increase in the bone resorption rate in the hypocalcemic patient with cancer. The accretion rate and total exchangeable calcium pool did not change significantly in any patient. Urinary hydroxyproline excretion which was measured in three hypercalcemic patients, decreased in all three patients following phosphate therapy. External radioactivity measurements obtained over several bony sites revealed that relative uptake of Ca-47 (expressed as percentage of the retained dose of Ca-47) was the same before and during phosphate therapy. This indicates that the proportion of Ca-47 moving into bone compared to the amount retained in the body at any time is not altered by phosphate as already shown by accretion rate measurements.

Significance and Present State of Knowledge

(1) Studies with intravenous phosphate demonstrate the superiority of phosphate as compared to sulfate and hydrocortisone infusions in the treatment of hypercalcemia and suggest guidelines for dose selection.

(2) Oral phosphate given to patients with hypercalcemia causes a sustained reduction in serum calcium, a positive calcium balance, a fall in urinary hydroxyproline and reduction in bone resorption rate. The significance of these studies is that the mechanism of action of phosphate on hypercalcemia appears to reflect its inhibition of bone resorption.

The findings in the patient with breast cancer in the preliminary study suggest that at least in this mildly hypocalcemic patient with bone metastases, the effect of phosphate on bone resorption rate was different. It is possible that the effect of phosphate is different in hypercalcemic, normocalcemic, and hypocalcemic patients.

Future Investigations

We plan to confirm our recent findings on the effect of oral phosphate therapy and to investigate further the effect of phosphate on calcium kinetics in hypercalcemic patients with cancer (with and without bone metastases) and in patients with bone metastases with normal or low levels of serum calcium.

The purpose of these studies is to investigate the mechanism of action of phosphate on calcium homeostasis and also to determine whether the effect of phosphate is different depending on serum calcium level. In addition to these human studies, rat experiments will be carried out to elucidate further the mechanism of action of phosphate.

2. Animal Studies

Objectives

To study the effect of phosphate administration on bone and soft tissue uptake of Ca-47 in the rat.

Research Accomplishments

Groups of rats have been treated at three different dosage levels of phosphate added to their drinking water: (1) 10 mg/day, (2) 150-190 mg/day, (3) 230-270 mg/day. Rats were raised in the laboratory with careful measurement of food and water intake and body weight. Experiments ran for a period of up to six weeks. Following treatment, phosphate treated and control rats were given tracer amounts of Ca-47 and sacrificed twenty-four hours later. Blood, soft tissue, and bone radioactivity were measured in all animals. Results were expressed as the ratio of percent of administered dose per gram of tissue (wet weight) divided by percent of dose per gram of plasma. The level of significance of difference of tissue uptake between the phosphate-treated and control groups was estimated by use of the "t" test and the non-parametric rank-sum test. In addition, kidneys were examined in some animals for metastatic calcification using von Kossa stain.

Rats were able to tolerate the first two dosage regimens but a majority of rats failed to survive the higher dose. In animals treated with the lowest dose, no significant difference in the distribution of tracer uptake was observed between the treated and control groups. Results obtained in rats treated with 150-190 mg/day are summarized in Table X.

Increased uptake of Ca-47 in the kidney and stomach was noted at a high level of confidence. In the rats treated with 230-270 mg of phosphate/day, of those that survived, significantly increased uptake of Ca-47 was demonstrated also in kidney and stomach. In addition, there was histological evidence of calcium deposition in the kidneys of those animals.

Significance and Present State of Knowledge

The data obtained from animal studies so far indicate that the action of phosphate to lower extracellular calcium concentrations is probably partly mediated by increased soft tissue uptake of calcium. An enhanced osseous uptake of calcium does not appear to be involved in phosphate action.

TABLE X
Percent Dose Per Gram of Tissue
Percent Dose Per Gram of Plasma

TISSUE	MEAN $\pm$ S.E. MEAN				LEVEL OF CONFIDENCE	
	No.	Experimental	No.	Control	T-Test	Rank-Sum Test
Brain	22	.850 $\pm$.058	21	.762 $\pm$.023		
Heart	22	.339 $\pm$.017	22	.304 $\pm$.012		
Large Intestine	22	1.263 $\pm$.134	23	1.062 $\pm$.114		
Small Intestine	21	1.031 $\pm$.078	23	1.057 $\pm$.098		
Kidney	22	.700 $\pm$.038	23	.604 $\pm$.009	99%	99.88%
Liver	22	.402 $\pm$.028	23	.362 $\pm$.015		
Lung	21	.823 $\pm$.067	22	.778 $\pm$.022		
Muscle	22	.708 $\pm$.060	23	.640 $\pm$.012		
Pancreas	22	1.088 $\pm$.076	23	.956 $\pm$.049		
Spleen	22	.402 $\pm$.016	23	.374 $\pm$.013		
Stomach	22	.815 $\pm$.056	23	.691 $\pm$.015	98%	97%
Cortical Bone	22	189 $\pm$ 20	23	200 $\pm$ 20		
Skull	22	141 $\pm$ 18	22	112 $\pm$ 6		
Tibia	22	222 $\pm$ 25	23	196 $\pm$ 13		

Future Investigations

It is planned to study the effect of phosphate on bone resorption by prelabeling rat skeletons with Sr-85, initiating phosphate administration at the level of 150-190 mg of phosphorus per day in the drinking water one week later, sacrificing experimental and control rats at various intervals up to eight weeks afterwards, and determining the amount of Sr-85 retained in bone. Total body retention measurements will also be made at one week intervals throughout the study.

c) Tetracycline and Ca-47 kinetics in bone disease

Objectives

To obtain simultaneously information about the dynamic state of the mineral and the organic phases of bone tissue, about the changes which occur in diseases affecting bone and calcium homeostasis, and about the effect of treatment.

We have emphasized the following aspects of this investigation:

(1) The study of the pseudohyperparathyroid syndrome associated with some soft tissue cancers without bone involvement: 2 patients (3 studies).

(2) The study of true hyperparathyroidism and comparison of this data with (1): 3 patients (4 studies).

(3) The study of conditions associated with rapid turnover rate of calcium and diffuse bone metastases where the tetracycline (Tc) kinetics would help define whether the process is associated with net rapid increase with a decreased bone tissue formation: 4 patients (4 studies).

Ten simultaneous Tc and Ca-47 studies were carried out on 8 patients. One additional patient was studied with Tc and a Sr-85 tracer was given at another institution but the data was obtained and recalculated in our laboratory. This brought our total experience to 38 Tc tests conducted on 31 patients. In 5 of these (in 3 patients) Ca-47 kinetics were not determined simultaneously.

Research Accomplishments

The patients have been kept under conditions of metabolic balance. The Ca-47 kinetics were calculated after the intravenous injection of a tracer dose of this radionuclide amounting to 50-100 μ c, and based upon the serum specific activity and the fecal and urinary excretion measured sequentially over a period of 2 weeks according to the method of Bauer et al (1). Values were obtained for the rate of accretion of calcium (a) and the exchangeable calcium pool (e).

The Tc kinetics were also based on the sequential measurements of the concentration of this drug in the serum and its fecal and urinary excretion over a 3 day period. The slope Ktc of the tetracycline retention curve on serum was taken to be an index of its rate of deposition in bone, probably by binding to newly forming bone matrix. This assumption is based on the reports indicating that tetracycline fluorescence in bone is located in the growing portions of the bone and the newly formed parts of individual osteons.

1. Reproducibility - Table XI

In each of 3 patients with inactive bone disease, the

TABLE XI

REPRODUCIBILITY OF TETRACYCLINE STUDIES

PATIENT	DIAGNOSIS	TREATMENT	Ktc	a (gm/day)
D. D.	Breast Ca Arrested bone Metastases post hypox	1 - Cortisone 50mg/day Thyroid replacement	0.053	
		2 - Same	0.051	
B. D.	Breast Ca with bone metastases arrested post hypox	1 - Cortisone 50mg/day Thyroid replacement	0.055	0.28
		2 - Same	0.057	0.25
B. T.	Paget's and osteogenic sarcoma - Recent head surgery	1 - None	0.045	2.12
		2 - None	0.044	

Tc test was carried twice, at an interval of approximately 1 week. In 3 of them, the Ktc varied by less than 5%. The values obtained for Ktc have been taken to represent our "normal" results. However, definite conclusions will have to await results to be obtained in patients without bone disease.

The accretion rate of calcium was similarly reproducible in one patient noted in this Table, and on an additional one not reported herein, on whom only Ca-47 kinetics studies were carried out twice.

2. Hyperparathyroidism - Table XII

Two patients were studied before and after excision of a parathyroid adenoma. The following results were obtained:

- a) Preoperatively: high accretion rate of calcium "a"
normal or low "Ktc"
elevated calcium excretion, hypercalcemia
- b) Postoperatively: fall in "a"
rise in "Ktc"
fall in calcium excretion with increased body retention

Four additional studies were carried out in the uncorrected hyperparathyroid state only:

S.M.: The Ktc was low in the presence of marked hypercalcemia, marked elevated calcium excretion and multiple bone cysts. The presence of a parathyroid adenoma was confirmed at surgery. No Ca-47 kinetics were done.

A.M.: "a" was elevated
"Ktc" was low
Severe hypercalcemia and elevated calcium excretion. The patient had a large recurrent carcinoma of the urethra. Parathyroid adenoma was found at post-mortem examination.

S.H.: Both "a" and "Ktc" were normal in the presence of mild hypercalcemia and mild hypercalciuria of long standing. Parathyroid adenoma was confirmed at surgery. A set of postoperative studies has been carried out and the results are currently being analyzed.

S.C.: Both "a" and "Ktc" were low in the presence of mild hypercalcemia, hypophosphatemia, and low urinary calcium excretion. She did not undergo surgery.

These results indicate that active hyperparathyroidism causing severe hypercalcemia and hypercalciuria is associated with a high accretion rate of calcium, but low retention and low or

TABLE XII

TETRACYCLINE AND ^{47}Ca STUDIES IN HYPERPARATHYROIDISM

PATIENT	DIAGNOSIS PROVEN	TREATMENT	Ktc	a (gm/day)
M. D.	At surgery	1 - None	0.011	0.95
		2 - Post excision of parathyroid adenoma	0.024	0.65
H. B.	At surgery	1 - None	0.053	1.20
		2 - Post excision of parathyroid adenoma	0.071	1.01
S. M.	At surgery	1 - None	0.031	
A. M.	At p.m. examination	1 - None	0.027	0.96
S. H.	At surgery	1 - None	0.057	0.49
S. C.	Serum Ca^{++} and P^- only	1 - None	0.030	0.30

normal bone matrix formation. These conditions are reversed by excision of the adenoma. This emphasizes the fact that a high calcium accretion rate does not imply a high rate of bone tissue formation, and suggests that the tetracycline test will become an important method for the complete evaluation of the effects of this metabolic disturbance on bone.

When a parathyroid adenoma caused minimal disturbance of serum and urinary calcium (S.H.), the accretion rate of calcium and "Ktc" were not disturbed, indicating that a state of near equilibrium existed. Postoperative changes have yet to be evaluated.

In patient S.C., diagnosis of hyperparathyroidism has been entertained but has not been confirmed by surgery. Both "a" and "Ktc" were found to be low. It is possible, therefore, to conclude that in this case, the disturbance in calcium homeostasis is different in nature or in degree from the cases described above.

3. Cancer Hypercalcemia: pseudohyperparathyroidism (Table XIII)

Two patients were studied in the hypercalcemic state and again during a period of partial or complete correction of the hypercalcemia as a result of treatment.

In both, the accretion rate of calcium "a" and the "Ktc" were normal.

In patient C.M., treatment with mithramycin resulted in a fall in serum and urine calcium. It had no effect on the growth of the pulmonary malignancy, and it produced a fall in both "a" and "Ktc."

In patient L.R., the surgical removal of a large recurrent penile carcinoma resulted in a return of the serum calcium to normal, a fall in urine calcium and a rise in both "a" and "Ktc."

A third patient with soft-tissue malignancy but no hypercalcemia, was also treated with mithramycin, which caused a fall in serum and urine calcium as well as a fall in both "a" and "Ktc."

This group of studies suggests:

a) that the accretion rate of calcium is not elevated in pseudohyperparathyroidism, unlike true hyperparathyroidism.

b) that removal of the malignancy removes the source of a humoral factor which affects bone tissue, and when this condition is corrected, bone tissue formation is increased as manifested by increases in both the accretion rate of calcium and the rate of Tc deposition in bone presumably by binding to bone matrix.

c) that mithramycin effects a fall in serum calcium which is not necessarily associated with tumor growth suppression and which does not result in increased bone tissue formation and may actually slow it down.

TABLE XIII

TETRACYCLINE AND ^{47}Ca STUDIES IN HYPERCALCEMIA

PATIENT	DIAGNOSIS	TREATMENT	Ktc	a (gm/day)
C. M.	Lung Ca	1 - None	0.063	0.43
		2 - Mithramycin: serum Ca^{++} ↓	0.031	0.41
G. F.	Embryonal Cell Ca of testis (normocalcemic)	1 - None	0.073	0.69
		2 - Mithramycin: serum Ca^{++} ↓	0.042	0.59
L. R.	Ca of Penis	1 - None	0.054	8.8 mg/kg/day
		2 - Hemipelvectomy for recurrent tumor: serum Ca^{++} ↓ to normal	0.066	12.5 mg/kg/day

4. Extensive Bone Metastases - Table XIV

Three patients with metastatic breast cancer were treated with cortisone in pharmacological doses. In two of them, (T.K. and B.D.), an improvement in their clinical condition was associated with a fall in the accretion rate of calcium "a" and a rise in "Ktc," indicating increased bone tissue formation.

One patient continued to experience a progression of her malignancy, and we observed a rise in "a" and no change in "Ktc" indicating lack of bone repair.

5. Other Bone Diseases - Table XV

Patients with a variety of diffuse bone diseases as listed below were studied:

	2 Osteogenic sarcoma
(high "a")	3 Paget's disease
	2 Osteogenesis imperfecta
	1 Metastatic carcinoma of the prostate
(normal "a")	
(low "a")	1 Multiple myeloma
	1 Idiopathic osteoporosis

One patient with osteogenic sarcoma (C.M.) and a high "a" value, showed a rise in "Ktc" during a week interval between two studies. This may indicate an increasing rate of bone tumor formation in the involved portion of his pelvis. The other patient (J.D.) with osteogenic sarcoma metastatic in lung and pleura, showed an elevated "a" value. Changes in "Ktc" in time cannot be evaluated, and comparison to patients with a normal skeleton will be necessary for the proper interpretation of this isolated study.

The three patients with Paget's disease (J.B., B.T., and A.W.) all had high "a" values with normal or low "Ktc" values. A tentative conclusion is that the several-fold increase in the accretion rate of calcium is not associated with any elevation of "Ktc" and in one case (A.W.), the "Ktc" is actually low, indicating that this condition is associated with a high turnover rate of mineral but no proportional increase in newly-formed bone tissue.

The two sisters with osteogenesis imperfecta (Co. R. and Ca. R.) showed elevations in both "a" and "Ktc," suggesting a rapid rate of new bone formation.

The patient (C. McG.) with extensive osteoblastic bone metastases from carcinoma of the prostate and high serum alkaline phosphatase levels, revealed markedly elevated "a" and "Ktc." This may indicate a high rate of bone formation, although possible binding of tetracycline to metastatic tumor renders the interpretation difficult.

TABLE XIV

EFFECT OF TREATMENT ON THE TETRACYCLINE AND ^{47}Ca IN PATIENTS WITH BONE METASTASES

PATIENT	DIAGNOSIS	TREATMENT	RESULT	Ktc	a (gm/day)
T. K.	Breast Ca Progressive bone metastases, status post breast adrena- lect. and oophorect.	1 - Cortisone 50mg/day		0.020	1.16
		2 - Cortisone 150mg/ day	subjective improve- ment. Fall in urine Ca^{++}	0.054	0.53
A. T.	Breast Ca Progressive bone metastases, Hypercalcemia	1 - None		0.048	0.74
		2 - Cortisone 150mg/ day	No improvement	0.048	1.39
B. D.	Breast Ca	1.- Cortisone 50mg/day Thyroid replace- ment		0.035	0.31
		2 - Cortisone 150mg/ day. Thyroid replacement	Some relief of symptoms	0.053	0.22

TABLE XV

TETRACYCLINE AND ^{47}Ca STUDIES IN BONE DISEASES

PATIENT	DIAGNOSIS	Ktc	a (gm/day)
C. M.	Osteogenic sarcoma	0.044 0.050	1.22
J. D.	Osteogenic sarcoma	0.054	2.29
J. B.	Paget's disease	0.040	8.50
B. T. **	Paget's disease	0.045 0.044	2.12
A. W.	Paget's disease	0.029	1.19
Co. R.	Osteogenesis imperfecta	0.085	5.90
Ca. R.	"	0.147	2.28
J. N.	Multiple myeloma	0.065	0.47
A. L.	Idiopathic osteoporosis	0.031	0.19
C. McG.	Metastatic Prostate Carcinoma	0.082	6.62

*Two studies at 1 week interval - Progressive disease

**Reported in Table I

The patient with idiopathic osteoporosis (A.L.) reveals a low "a" and "Ktc," indicating a low total skeletal turnover rate of calcium and/or new bone formation.

The results in multiple myeloma (J.N.) are difficult to interpret. Binding of tetracycline to myeloma tissue may be the cause for the elevated "Ktc." The results indicate that elevated accretion rates of calcium occur in processes which are mainly bone-destructive as well as bone-proliferative, and the "Ktc" obtained through the use of the Tc tests helps to amplify the interpretation of the process taking place in bone-tissue.

Significance and Present State of Knowledge

Ca-47 kinetic studies yield information pertaining essentially to the mineral phase of bone-tissue. Increased rates of calcium deposition are seen in processes characterized essentially by either bone-tissue destruction or by bone-tissue proliferation.

The rate of tetracycline binding to bone calculated from the tetracycline (Tc) test is presumed to be an indicator of bone matrix formation. As such, it adds information about the organic phase of bone-tissue for a more complete evaluation of the effects of disease and treatment on the skeleton as a whole.

Patterns of calcium and Tc kinetics are beginning to evolve from this investigation, and their possible application to the differential diagnosis of hypercalcemic states has been described in the results. Such patterns will also help in differentiating the non-specific effect of treatment of hypercalcemia as a chemical disturbance from its effect on the source of the substance which initiates this process.

The possible simultaneous binding of tetracycline to bone and tumor requires further investigation for better interpretation of our observations.

d) Metabolism of scandium-47

Objective

To study the metabolic pathways of Sc-47, the radioactive daughter of Ca-47, a frequently used bone-seeking nuclide.

Research Accomplishments

Experimental and data-processing techniques have been developed which enable the simultaneous radioassay of parent and daughter activities in-vitro, and simultaneous scintigraphic studies in-vivo. To date, in 18 patients receiving injections of Ca-47 for the study of bone metabolism, specimens of serum, urine and feces have been assayed for Sc-47 as well as Ca-47 content. In addition, scans and stationary counts have been performed over various bones and organs.

Significance and Present State of Knowledge

In general, Sc-47 activity in serum, expressed as per cent of existing Sc-47 activity per liter, was observed to be several times that of Ca-47 at one hour after injection, falling to levels comparable with that of Ca-47 at 14 days after injection.

Overall excretion of Sc-47 in urine and feces was considerably lower than that for Ca-47, with the exception of the first (24 hour) collection period, in which large portions of the injected Sc-47 often appeared in the urine. For the remainder, the urine represented an almost insignificant pathway for the in-vivo produced Sc-47, most of which was retained.

Since it is well established that the distribution of injected scandium is a function of its chemical state, it seems probable that the qualitative difference in renal clearance of injected versus in-vivo decay produced Sc-47 reflects the difference in chemical form of the two. This conclusion is reinforced by what appears to be a strong dependence of the percent of injected Sc-47 excreted on the particular shipment of Ca-47 employed.

In agreement with previous reports of colloid formation by scandium in the plasma, we observed excellent imaging of the liver with Sc-47 at one and 10 days after injection.

The ratio of Sc-47 to Ca-47 counts over various bone sites was seen to increase with time after injection, indi-

cating that a considerable portion of Sc-47 produced by decay of Ca-47 in bone remains there. This, coupled with the inherently greater count rates available from the daughter suggest the possibility of extending the precision and duration of present studies of bone metabolism.

Future Investigations

It is planned to investigate the metabolic pathways of Sc-47 in the rat, to determine: what proportion of Sc-47 generated in bone remains there, what proportion undergoes colloid formation and uptake by the reticuloendothelial system, the excretion of injected scandium as a function of pH of the injection solution, and the uptake, if any, in bone of injected scandium.

4. Studies of Bone Mineralization by Gamma Ray Absorption

Objectives

To evaluate and apply a method of measuring the state of mineralization of bone in-vivo. To combine such measurements of the index of mineralization with other studies of calcium metabolism and kinetic state in-vivo would lead to a better understanding of the disturbances of calcium homeostasis in diseased states.

Research Accomplishments

Studies of calcium metabolism until recently have been carried out by means of two major methods:

(1) Metabolic balance which gives the net gain or loss of calcium from the whole body and

(2) Kinetics of radioactive calcium which is supposed to give the rate of deposition of calcium in the skeleton as well as the size of the miscible calcium pool.

In combination, both methods yield also a rate of removal of calcium from the body, presumably from the skeleton. Bone-seeking radionuclides have also yielded information about their local deposition by external measurement, as well as rates of removal from parts of the skeleton, reflecting the kinetics of bone mineral.

A considerable amount of variation exists, however, among normal individuals based essentially on sex, age, size of the skeleton, and to some extent on normal differences. Since the weight of the skeleton seems to be proportional to the lean body weight, expressing the calcium kinetic data as a function of estimated lean body weight lessens the variability of results among normal individuals of the same anatomical grouping.

However, when one is concerned with patients affected by diseases causing a generalized increase or decrease in skeletal mineralization and density, such as osteoporosis, hyperparathyroidism, generalized Paget's disease, or wide-spread metastases to bones, the relationship between the skeletal weight and lean body weight is disrupted. The interpretation of results in comparison with normal values then becomes invalid.

For example, we studied an osteoporotic patient with extreme demineralization of the skeleton. His weight was stable over a period of several years. The accretion rate of calcium based on Ca-47 kinetics was .26 gm/24 hrs. His skeleton on x-ray films looked at best twenty-five percent mineralized. It is then estimated that the accretion rate per unit weight of mineralized tissue or better, per unit weight of bone mineral would have been four times the calculated value. We have had until now, of course,

no way of expressing calcium kinetics as a function of weight of bone or bone mineral. The latter would be the most valuable.

Bone radiographs can give only a limited evaluation of changes in bone mineralization occurring during disease states and under treatment. Semiquantitative evaluation using radiographs are based on the appearance of deformities, particularly in the vertebrae, measurement of cortical thickness, a study of the trabecular pattern as well as the light transmission through the film indicating greater or lesser degrees of mineralization. Even with those parameters, a thirty to fifty percent change in mineral must take place in order to appreciate the change.

The use of Cameron's technique which is based on the absorption by bone of a finely collimated single energy x-ray emission from I-125 is a highly accurate method of measurement of the bone mineral content. This method and its evaluation are described separately by Dr. Zeitz. Previously published data have indicated that measurements of the relative bone mineralization index (RBMI) across the forearm indeed reflects the state of mineralization of the rest of the skeleton. While such changes may be more profound in some bones, such as the vertebrae, than the others, they all vary in the same direction. The calcium kinetic data may then be expressed relative to the RBMI rather than to body weight or to the individual patient.

Presently, RBMI determinations are being carried out on normal individuals as well as on patients with cancer and metabolic disorders affecting the skeleton and calcium homeostasis. At the same time, we are collecting bones from amputation specimens. Accurately measured segments are cut after defatting and drying the bone. The RBMI of such segments is determined and the bone sample is then ashed for chemical analysis of calcium content. It is hoped that this information may then lead to express the RBMI as grams of calcium per centimeter of bone.

To date RBMI determinations were obtained in seventy-one normal individuals and forty-eight patients. The latter include the following diagnosis: postmenopausal and senile osteoporosis, hyperparathyroidism, pseudohyperparathyroidism associated with malignancy, osteomalacia, metastatic breast cancer with normal serum calcium, metastatic breast cancer with hypocalcemia, osteolytic bone lesions of undetermined etiology.

The number of normal values and the distribution of those values among the various age groups and according to sex is being undertaken, but is too small at present to permit the establishment of significant values for each group. The recruiting of volunteers among the hospital staff and in the medical school is underway.

The data on patients are being analyzed for the present time by comparing the values to the normal ones available in the larger series of Cameron et al. Various methods of reporting the data are under evaluation. In three cases a combination of metabolic

balance of calcium, Ca-47 kinetics and RBMI were carried out simultaneously.

Patient L.U. had osteomalacia secondary to previous subtotal gastrectomy. Patient M.B. had osteoporosis due to a combination of pelvic exenteration, wet colostomy with reimplantation of the ureters into the large intestine and causing chronic hyperchloremic acidosis. Her primary disease had been carcinoma of the cervix. Patient M.A. had breast cancer with extensive osteoblastic metastases, had undergone bilateral oophorectomy and developed severe prolonged hypocalcemia. The preliminary data indicate the following:

Patient L.U.:

1. Accretion rate of calcium	0.92 gm/24 hrs. (average normal 0.40 gm)
2. RBMI	7.8
3. Normal premenopausal RBMI (Cameron)	23.0
4. Mineralization ratio: $\frac{\text{RBMI patient}}{\text{RBMI normal}}$	$\frac{7.8}{23.0} = 0.34$
5. Accretion rate relative to state of mineralization	$\frac{0.92}{0.34} = 2.7 \text{ gm/24 hrs/unit mineralization ratio}$

Formulation: This patient with a severely hypomineralized skeleton has an accretion rate (1) which is about twice the normal average value. When corrected for the fact that her RBMI is one-third of normal (2) the accretion rate per unit mineralization ratio is six times normal.

Patient M.B.:

1. Accretion rate	0.26 gm/24 hrs (average normal 0.40 gm/day)
2. RBMI	9.5
3. Normal premenopausal RBMI (Cameron)	23.0
4. Mineralization ratio	$\frac{9.5}{23.0} = 0.41$
5. Accretion relative to mineralization state	$\frac{0.26}{0.41} = 0.63 \text{ gm/24 hrs/unit mineralization ratio}$

Formulation: This patient with hypomineralized bone has an accretion rate of calcium which is about two-thirds of the average normal. When corrected for state of mineralization, the accretion rate is found to be within normal range, (0.4 to 0.6 gm/24 hrs.)

Patient M.A.:

1. Accretion rate	2.7 gm/24 hrs
2. RBMI	16.7
3. Normal premenopausal RBMI (Cameron)	23.0
4. Mineralization ratio	$\frac{16.7}{23.0} = 0.73$

Formulation: Patient with osteoblastic metastases causing very rapid deposition of calcium in those diseased portions of the skeleton to the point of causing hypocalcemia, has an accretion rate approximately six times the average normal value. The uninvolved parts of her bones are hypomineralized while the diseased parts are overmineralized.

The data cannot be interpreted in the same manner as the two previous cases because different phenomena are operative simultaneously. It may be that calcium is mobilized from normal bone and deposited in the osteoblastic lesions. Serial RBMI measurements during the course of disease would help in evaluating this possibility.

High serum calcium concentration generally suppresses parathyroid hormone production and stimulates secretion of thyrocalcitonin. If, as reported, thyrocalcitonin inhibits and parathyroid hormone enhances the resorption of bone, calcium infusion should lead to an increase in bone mineral content (1). It is reported that osteoporotic patients treated with calcium infusion showed clinical improvement which persisted for months after infusions were stopped (1) and evaluation of bone biopsies by microradiography shows an increase in bone formation area and a decrease in bone resorption area with treatment (2). It should be possible to verify this hypothesis by direct bone mineral content measurements with the photon absorptiometric technique.

An osteoporotic patient was given calcium gluconate in 12 approximately equally spaced I.V. infusions over a period of three weeks from 11/17/70 to 12/14/70. The total infusion resulted in a dose of 15 mg calcium/kg body weight. Scans of the forearm bones were carried out on 10/27/70, 12/10/70 and 12/18/70. There was no significant difference found in the integral values of the radius or ulna bones for the three scans. Studies of Ca-47 uptake for this same individual showed no appreciable increase in calcium accretion rate as a result of the treatment. Metabolic studies showed the patient remained in calcium balance.

Significance and Present State of Knowledge

The above three studies illustrate some of the contribution of the RBMI to the overall understanding of calcium homeostasis in bone. The data are not fully analyzed yet and this type of

combined study will be further extended. The significance as related to individual studies is noted above.

Future Investigations

The accumulation of data on normal individuals of both sexes in all age groups will continue and will serve as standards for comparison. Studies of calcium metabolism will now routinely be combined with RBMI determinations and the data will be analyzed and interpreted in the light of this new parameter.

References

1. Pak, C.Y.C., Zisman, E., Evans, R., Jowsey, J., Delea, C.S., and Bartler, F.C., The treatment of osteoporosis with calcium infusion - clinical studies. Am. Jour. of Med. 47:7-16, 1969.
2. Jowsey, J., Hoyer, R.C., Pak, C.Y. and Bartler, F.C., The treatment of osteoporosis with calcium infusions - evaluation of bone biopsies. Am. Jour. of Med. 47:17-22, 1969.

5. Evaluation of F-18 as a Routine Scanning Agent

Objectives

To evaluate the usefulness of F-18 bone scans in the management of a variety of malignant diseases.

Research Accomplishments

Sodium fluoride F-18 is produced in the Sloan-Kettering Institute isochronous cyclotron. The preparation is according to an FDA approved protocol (IND #5373), and checks for freedom from pyrogens and contaminants are made. Patients receive 1.0 mCi intravenously and are scanned on one of two dual 5 x 3 inch crystal rectilinear scanners. Extensive computer analysis is carried out on most of the scans.

We have performed 611 bone scans in over 500 patients to the date of this report. Many lesions are detected which were not seen on the radiograph, amounting to 30 percent of cases with lymphomas recently surveyed. The fluorine-18 scan is superior to other available bone-seeking nuclides in that data is available 3 hours after injection, the radiation exposure to the patient is low, and good bone to soft tissue ratios are obtained at the time of scanning. The lesion to normal bone ratios are adequate for diagnosis in most instances, but may not be quite as high at these times as the ratios would be at 5 days after Sr-85. Some false negative bone scans may have been due to radiation or chemotherapeutic treatment of patients prior to the scan.

Significance and Present State of Knowledge

The speed, convenience and low bone radiation dose of the F-18 bone scan make this the agent of choice in bone imaging at this time, where it is available. The occurrence of some false negative scans, possibly secondary to treatment, requires the simultaneous use of the radiograph. Many lesions missed on the radiograph are detected in the F-18 bone scan.

Future Investigations

Computer analysis of our data during the past year (see Section B5) confirms the superiority of quantitative scanning over photoscanning in detecting bone lesions. We believe that an increased amount of F-18 per injection (3 mCi in adults with malignant disease) will further improve the statistical reliability of the computer analysis. Some of the suspicious areas which counted 2 or 3 standard deviations above the contralateral bone would probably count 4 standard deviations higher with a larger dose without increasing the

scan time (40-50 minutes). We will evaluate this effect (providing the FDA approves our amendment) and compare it to the simultaneous change in the photoscan quality.

We are analyzing the role of F-18 bone scans in the management of lymphomatous diseases. Preliminary results show that nearly 1/3 of our patients have positive scans in areas not abnormal on radiographs (1). These and additional cases will be followed and reviewed to discover the optimum time for bone scans in the course of each of the 3 types of lymphoma (Hodgkin's disease, reticulum cell sarcoma and lymphosarcoma) and the contribution of the scan to the management of the patients. We will continue to evaluate the F-18 bone scans in other malignant diseases, and extend our studies to benign diseases where possible.

Collaboration with investigators at other institutions including Columbia Presbyterian Hospital, the Hospital for Special Surgery, Overbrook Hospital and Massachusetts General Hospital will be extended.

Reference

1. Benua, R.S., Laughlin, J.S., Lee, B.J. and Tilbury, R.S.
Use of sodium fluoride F-18 bone scans to determine the
extent of disease in lymphoma. J. Nucl. Med. 12:340, 1971.
(Abstract)

6. Metabolism of Sulfur-35 Sulfate by Cartilage Tumors

Objectives and Accomplishments

In a field related to the metabolism of various radionuclides in the mineral structure of bone we have studied the metabolism by cartilage tumors and normal soft tissues of ^{35}S administered as sodium sulfate. Our results have confirmed and extended the findings of previous investigators that both the uptake and the retention of ^{35}S by chondrosarcoma tissue are high and that, while the initial uptake by marrow also is high, the subsequent loss is so rapid that the total radiation dose is moderate. A new finding is that the selective uptake by chordoma tissue is as great as that by chondrosarcoma. We have also been able to locate superficial tumors containing ^{35}S by external scanning of the associated bremsstrahlen. The method is not sufficiently sensitive for use in diagnosis, but it does make possible a semiquantitative determination of the retention half-time of the nuclide in the tumor. Evidence from serial biopsies suggests that the major biological half-time of ^{35}S in chondrosarcoma tissue is about 30 days, and that there are two half-times of about 0.5 and 6 days in bone marrow, the shorter one accounting for 95% of the total uptake. Radioautographs of the hair of one patient showed that the hair bulb was heavily labeled one week after a therapeutic dose, and that the growth of the hair could be followed by areas of activity in the shaft corresponding to successive doses.

Significance and Present State of Knowledge

The magnitude of uptake of ^{35}S sodium sulfate by cartilage tumors and various normal tissues has been measured and the half-times of sulfur in chondrosarcoma and bone marrow have been estimated. It has been shown that the ^{35}S uptake of chordoma is comparable to that of chondrosarcoma.

Future Investigations

This work will be continued as a part of the study of treatment of malignant cartilage tumors with ^{35}S which is described in detail in another section.

VII. -CYCLOTRON OPERATION AND RADIONUCLIDE PRODUCTION

A. Introduction

This report summarizes the progress of the SKI cyclotron group from the time of acceptance of the machine in December 1967 to the present (May 31, 1971). More complete annual progress reports are available (report Nos. NYO 910-72, -124, -150).

B. Radiochemistry

1. Radioactive Gases

The radioactive gases $^{15}\text{O}_2$, C^{15}O , C^{15}O_2 , $^{13}\text{N}_2$, ^{11}CO and $^{11}\text{CO}_2$ have been produced routinely on Wednesday and Friday mornings² for studying the lung function of dogs with lung transplants, and to gain operating experience with the Institute's dual head coincidence gamma camera.

Some alternative methods of producing radioactive gases have been investigated. Various target material (mainly gases) were irradiated with deuterons, protons or helium-3 particles, and the radioactive products were analyzed by gas chromatography. A radioactivity detector was connected to the effluent of the gas chromatograph so that the radioactivity of the separated gases could be measured. The radioactivity detector was an internal gas flow proportional counter (Nuclear Chicago Model 4998) connected to a ratemeter the output of which was connected to the same two pen strip chart recorder as the output of the thermal conductivity detector of the gas chromatograph. Thus two simultaneous traces were obtained corresponding to the chemical composition and the radioactive content of each constituent of the gas. The results are shown in Table I. The total activity was measured by a 500 cc flow-through ion chamber connected to an electrometer, and is expressed as microcuries per liter at the indicated beam current in microamperes. We are at present limited to 20 μA as a maximum beam current we can get through the foil window without puncturing it. The following columns were used in the gas chromatograph. A column of 2' x 1/4" molecular sieve 5A programmed from 35°C to 250°C at 30°C per minute, using helium carrier gas at a flow rate of 50 ml/min, was used to separate O_2 , N_2 , CO , N_2O and CO_2 . CO_2 labeled with ^{15}O could not be detected with this column since the ^{15}O of the CO_2 was found to exchange with water on the column, and no radioactive CO_2 was obtained as effluent from the column. For this reason, C^{15}O was detected using a 18" x 1/4" column of activated charcoal at 40°C with a helium flow of 115 ml/min.

Thus it is possible to make $^{15}\text{O}_2$ either by deuterons on 5% O_2 plus N_2 or by ^3He on O_2 . Also, ^3He on nitrogen proved to be a more convenient method to make $^{13}\text{N}_2$ and gave a higher yield than deuterons on carbon. In the irradiation of N_2 with

Table I
YIELDS OF RADIOACTIVE GASES

<u>Target</u>	<u>Bombarding particle & energy (MeV)</u>	<u>Products (%)</u>	<u>Total activity (μCi/liter)</u>
5% O ₂ +N ₂	d(7.5)	¹⁵ O ₂ (99)	10000/15 μ A
O ₂	³ He(21)	¹⁵ O ₂ (99+) ¹¹ CO ₂ (0.01)	300/2 μ A
5% O ₂ +N ₂	d(7.5)	C ¹⁵ O(97) ¹³ N ₂ (3)	3000/15 μ A
5% O ₂ +N ₂	d(7.5)	C ¹⁵ O ₂	480/8 μ A
5% O ₂ +N ₂	³ He(21)	¹⁵ O ₂ (90) ¹³ N ₂ (10) CO) N ₂ O) trace CO ₂)	6500/5 μ A
Graphite	d(7.5)	¹³ N ₂ (97) ¹³ N ₂ O(2) ¹¹ CO(1)	16/20 μ A
N ₂	³ He(21)	¹³ N ₂ (97) *O ₂ (3) N ₂ O) trace CO ₂)	1000/5 μ A
N ₂	p(14)	¹³ N ₂ (44) ¹¹ CO ₂ (50) ¹¹ CO(5) ¹⁴ O ₂ (1)	6000/4 μ A
O ₂	p(14)	¹³ N ₂ (50) ¹³ N ₂ O(50) ¹⁵ O ₂ trace	31/9 μ A

...continued

...continued from preceding page

<u>Target</u>	<u>Bombarding particle & energy (MeV)</u>	<u>Products (%)</u>	<u>Total activity (μCi/liter)</u>
CO_2	$^3\text{He}(21)$	$^{11}\text{CO}_2(60)$ $^{13}\text{N}_2(34)$ $^{15}\text{O}_2(1)$ CO) trace N_2O)	4800/5 μ A
B_2O_3	d(8)	^{11}CO $^{11}\text{CO}_2$	100/15 μ A
B_2O_3	p(10)	^{11}CO $^{11}\text{CO}_2$	1300/15 μ A

protons, 50% $^{11}\text{CO}_2$ is obtained. This must be due to reaction of ^{11}C hot atoms with O_2 impurities in the nitrogen, and provides an alternative method of making $^{11}\text{CO}_2$ to the one using deuterons or protons on B_2O_3 . The $^{13}\text{N}_2$ is made by (p, pn) reaction on ^{14}N , and since this reaction has a Q value of 10.6 MeV, it may be eliminated by reducing the proton energy to 10 MeV. It is useful to have alternative methods for the production of the radioactive gases since it sometimes saves switching bombarding particles and helps the efficient scheduling of the cyclotron's use time.

2. Fluorine-18

High purity carrier free fluorine-18 in isotonic saline solution has been regularly prepared for clinical investigative use. The procedure has been previously given (p. 21, prog. rep. NYO 910-72). The Radioactive Isotope Service in Memorial Hospital performed over 300 patient scans using fluorine-18 produced with the SKI cyclotron. These were carried out under an investigative protocol and are described in the report of Proposal #3. In addition clinical studies under a more extensive investigative protocol have been carried out comparing bone uptake of F-18 with calcium-47, as well as three phantom studies with fluorine-18.

In order to measure any gamma-ray emitting impurities, samples of F-18 solution were measured for periods up to 67 days from the end of bombardment, on a 3 x 3 inch NaI (Tl) crystal coupled to a photomultiplier tube and a 400 channel pulse height analyzer. The gamma-ray spectrometer was calibrated by means of a set of point source gamma-ray standards obtained from the International Atomic Energy Agency, Vienna, Austria. V-48 was detected in runs 1 and 2 at a concentration of 10^{-3} microcuries/ml or 1 part per million parts of the F-18 formed at end of bombardment. The V-48 was presumably formed by (^3He , p) reaction on Ti-46, or (^3He , n) reaction on Ti-46 to produce Cr-48 which then decayed with a half-life of 23 hours to V-48. V-48 has a half-life of 16 days. Cr-48 was not detected in the F-18 product solution, because the F-18 activity masked any Cr-48 activity that might have been present. If all the V-48 activity had been produced by decay of Cr-48, the amount of Cr-48 present at end of bombardment would have been 0.02 $\mu\text{Ci/ml}$.

When using 0.001" tungsten foil in place of the titanium foil in the liquid target chamber, the only long-lived impurity in the F-18 product solution was Be-7 in an amount of 10^{-3} $\mu\text{Ci/ml}$. This was presumed to be formed by the reaction $^{16}\text{O}(^3\text{He}, ^7\text{Be})^{12}\text{C}$ which has a Q value of -5.57 MeV. Be-7 was subsequently found in the F-18 product solution at about the same activity/ml from runs using titanium foils, when the V-48 had decayed sufficiently.

An aged sample of F-18 product solution No. 1 was measured from time to time by liquid scintillation counting over a 1000-day period, in order to set an upper limit for tritium or other soft beta-emitting impurities. A component with half-life of 330 days

was detected, which was attributed to vanadium-49. This amounted to $1.4 \times 10^{-6}\%$ of the F-18 at end of irradiation, or 7×10^{-4} μCi . No tritium was detected but an upper limit for its content was set at $4 \times 10^{-7}\%$ of the F-18 or 2×10^{-4} μCi in the whole batch.

Chemical Form of F-18

The chemical form of the F-18 in the irradiated water and in the final saline solution was investigated by ion exchange chromatography following the method of Nielson (1). An ion exchange column 7 cm long and 1 cm in diameter, of Dowex - 1 x 8, 200-400 mesh, was made, converted to the acetate form, and washed with water. Aliquots of the F-18 product solution with and without fluoride carrier (5 mg NaF) were stepwise eluted with 20 ml 0.13 M sodium acetate followed by 20 ml of 0.27 M sodium acetate. No significant difference was observed in the elution characteristics indicating that the F-18 in the irradiated water was present as fluoride ion. The presence of carrier fluoride ion in each fraction was measured calorimetrically using the zirconium-alizarin red complex which changes to yellow in the presence of fluoride ion (2). Similar results were obtained with the ^{18}F -saline solution. To test the effect of complex fluoride ion formation on the elution characteristics, 1 mg of aluminum chloride and 5 mg of zirconyl-chloride in 0.1 N HCl solution were added to aliquots of the irradiated water and these were eluted in the same way. The elution characteristics of these solutions were significantly different from the untreated irradiated water. We conclude that our product solution contains F-18 as fluoride ion and not as complex metal fluorides. BF_4 -labeled with F-18 also followed the elution curves of AlF_6^{3-} , and ZrF_6^{2-} .

Fluorine-18 Fluoroborate

A method for the preparation of fluorine-18 labeled potassium fluoroborate has been developed. Samples have been prepared for pyrogen and sterility testing prior to its being made available for clinical investigational purposes.

Fluorine-18 labeled potassium fluoroborate has been used for localization of intracranial lesions (3, 4) and has been suggested as being potentially useful for scanning of the stomach (5).

10 mCi of labeled potassium fluoroborate has been prepared in a form suitable for clinical use. The labeling procedure can be completed within 90 minutes of the end of bombardment and the efficiency of the procedure is about 35%. The final solution has a specific activity of about 2 mCi/ml of solution containing 8 mg KBF_4/ml (i.e., 250 $\mu\text{Ci}/\text{mg}$ KBF_4).

Preparation

Fluorine-18 is prepared on the cyclotron by irradiation

of pyrogen free water with ^3He particles. The yields are about 6 mCi/ml at the end of bombardment with 25 μA but this can, if necessary, be increased to 60 mCi/ml. In order to prepare 2 mCi of F-18 labeled fluoroborate for injection, it is necessary to start with about 18 mCi or 3 ml of solution as prepared at present. This allows 2-3 hours before administration and a labeling efficiency of 30%.

This activity is transferred to a round bottomed centrifuge tube containing 0.5 ml of 3M HCl and 1 ml KBF_4 solution in water for injection (40 mg/ml). If the volume of solution exceeds 4 ml then it is necessary to reduce the volume. This is best achieved by using a rotary evaporator and reduced pressure. The tube is then placed in a water bath at 100°C for 30 minutes, water for injection being added at intervals to prevent evaporation to dryness. The solution is then neutralized with 1.5 ml 1M sodium bicarbonate solution and then passed through an alumina column to remove free fluoride ions. The solution is sterilized by filtration through a 0.22 μ Millipore filter and collected in a sterile vial.

The alumina column is prepared in a 2.5 ml syringe barrel fitted with a sintered polyethylene support. Chromatographic grade alumina is washed with strong HCl and then repeatedly with water for injection until the washings are neutral. The alumina is then slurried onto the column. Care is necessary to exclude air from the column and the Dynaflex pump tubing should be filled with water for injection before the active solution is pumped. This water is pumped to waste via a 2-way tap and only the fluoroborate solution passed through the Millipore filter into the sterile vial. A flow rate of 1-2 ml/minute is suitable.

3. Iodine-123 and Iodine-121

A series of experiments have been performed to measure the yields of I-121, I-123, and I-124 in the He-3 irradiation of antimony. About 2 grams of antimony metal powder were irradiated in a Faraday cup, by compressing the powder between 2 pieces of aluminum foil. The powder was evenly distributed over a circle of 1.8 inches diameter, and irradiated for periods of 10 minutes at beam currents of 5 μA . After irradiation, the iodine was separated by dissolving the antimony in 10 ml of concentrated nitric acid plus 10 ml of saturated tartaric acid, and the iodine was distilled in a stream of nitrogen and collected in 1N NaOH solution. About 1 mg of sodium iodide was used as carrier in these experiments. The distilled iodine was repurified by solvent extraction into carbon tetrachloride and back extracted into 0.1N NaOH solution. Aliquots were taken for gamma-ray spectrometry on stainless steel planchets, a drop of dilute nitric acid and of dilute silver nitrate added, and the sample evaporated to dryness under an infra-red lamp. Corrections were made for chemical yield when required. I-121 was measured by its gamma-ray of energy 210 Kev, (90% abundant), I-123 by its gamma-ray of energy 160 Kev (83% abundant), and I-124 by the combined

605 and 644 Kev gamma-rays, with a combined abundance of 79%. Decay curves were plotted for each of these gamma-rays, and the correct half-lives established before accepting the photopeak areas as representing the given radionuclide. Photopeak detection efficiencies were experimentally determined by use of a set of point-source gamma-ray standards supplied by the International Atomic Energy Agency, Vienna, Austria. Activity measurements were made at a distance of 6 cm or greater (to avoid errors due to coincident summing) from a 3 x 3 inch sodium iodide crystal coupled to a photomultiplier tube and 400 channel pulse height analyser.

Irradiations were performed with increasing thicknesses of aluminum foil (0.0007 inches thick) to determine the effect of particle energy on the relative yields. For these experiments, no chemical separations were performed, since the iodine radioactivities could be measured in the unseparated antimony-aluminum sandwich. These results show that about 4 times as much I-121 as I-123 is produced by 22 MeV He-3 on natural antimony, and that the yield of I-121 decreases relative to that of I-123 with decreasing bombarding energy.

The I-124 yield at end of bombardment was about 5% of the I-123 at 22 MeV, and increased with decreasing bombarding energy. Thus, the I-124 content in I-123 could not be reduced by lowering the bombarding energy.

Enriched isotopes of Sb-121 and Sb-123 having abundances were irradiated with 22 MeV He-3 particles. The gamma-ray spectra obtained from each of these samples are shown in Figures 9 and 10. The sample enriched to 98% in Sb-121 produced I-121 almost exclusively. I-123 was detected in the sample only after a decay of 22 hours, and amounted to 1.7% of the I-121 at end of bombardment.

The sample enriched to 98% in Sb-123 produced mainly I-123 and 6% I-124. The relative yield of I-123 produced by He-3 particles on Sb-121 and Sb-123 stable isotopes was calculated from the above 2 experiments to be 1:38. Thus, I-123 is produced almost exclusively by $^{123}\text{Sb}(\text{He-3}, \text{n})$ I-123 reaction, and only 2.6% by Sb-121 (He-3, n) I-123 reaction. Since I-124 is produced by (He-3, 2n) reaction on Sb-123, it is not possible to significantly reduce the I-124 contamination by irradiation of enriched isotopes of antimony instead of natural antimony. However, I-121 could be produced with less than 1% I-123 and other activities by irradiation of enriched Sb-121.

The thick target yield of I-123 was measured by irradiating a 1/8 inch thick piece of antimony metal (roughly 1" x 1") covered with a 0.0007" thick aluminum foil. The antimony and foil were measured together, and the yield of I-123 was found to be 24 $\mu\text{Ci}/\mu\text{A}$. The yield of I-123 using 22 MeV He-3 particles on antimony is lower than other methods of production.

After two years of experimentation with various methods

of producing I-123, it is possible to make a critical evaluation of each individual production method and determine the most feasible method. Several reactions were studied, as well as target form, and the post irradiation chemical processing. The determining factors are the nuclear reaction and the target form. The first experiments involved the irradiation of antimony with 23 MeV $^3\text{He}^{++}$ utilizing the $^{123}\text{Sb}(^3\text{He}^{++}, 3n)^{123}\text{I}$ reaction. Although the yield of I-123 by this method is high enough (24 $\mu\text{Ci}/\mu\text{Ah}$) to make this a feasible method of producing I-123, the I-124 content is 5% at end of bombardment. Also, it was shown last year that the use of enriched isotopes of antimony did not give a lower I-124 content.

An alternative method of producing I-123 is by the $^3\text{He}^{++}$ bombardment of tellurium (6), either natural or enriched, to produce Xe-123 which decays via electron capture and positron emission to I-123. The nuclear reaction sequence is

$^{122}\text{Te}(^3\text{He}^{++}, 2n)^{123}\text{Xe}_{\text{EC}}^{+} \rightarrow ^{123}\text{I}$. In theory it should be possible to

irradiate tellurium, separate the Xe-123 formed, and allow decay to generate I-123. This method produces the purest I-123 possible. Nine attempts were made to run generator-targets at beam currents up to 10 μA . The highest yield of I-123 obtained was 1.2 μCi , too small to be useful. Successful operation of this system requires that mutually exclusive conditions be simultaneously met. First, the tellurium target must be efficiently and effectively cooled to prevent volatilization. If the Te volatilizes, it does not remain in the beam path and is not irradiated. Only the stainless steel mesh is irradiated. The second requirement is that the tellurium be hot enough to allow the hot xenon atoms to recoil out and be carried away by a carrier gas stream. This is difficult to achieve in practice. There is also the possibility of the Xe-123 recoiling out of one particle and becoming trapped in the next. This will prevent the Xe-123 from being swept out by the carrier gas. The Te may be allowed to heat up during the irradiation to drive off the Xe-123; however, this was attempted and was unsuccessful. The tellurium vaporized and was found throughout the system. An experiment in which the Te was fused before irradiation, kept very cool during irradiation and then melted in a furnace to drive off the xenon-123 failed; the Xe-123 remained in the tellurium. In summary, the method is very good on paper but useless in our practice.

When 30 mg of Te-122 (enriched to 73.3%) covering an area one inch in diameter was irradiated, and the I-124 content was 0.37%, the ratio of I-123 formed from Xe-123 decay to the I-123 formed by other routes (e.g., $^{123}\text{Te}(^3\text{He}^{++}, p2n)^{123}\text{I}$) was 0.5. The thick target yield of I-123 by ^3He on natural tellurium was 5 $\mu\text{Ci}/\mu\text{Ah}$. Thus, the theoretical thick target yield of I-123 by the generator method from 73% enriched Te-122 is 74 $\mu\text{Ci}/\mu\text{Ah}$.

Experiments with the method of Hupf, Eldridge and Beaver (7) as used at Oak Ridge National Laboratories to produce

I-123 by the $^{123}\text{Te}(p, n)^{123}\text{I}$ reaction have shown this to be the best, if not the only, method open to SKI if I-123 is to be produced routinely in useful quantities. Several irradiations of natural tellurium were made using 15 MeV protons as the bombarding particle.

The iodine is separated from the acid solution of the tellurium target by distillation into a sodium hydroxide solution. Te-123 is only 0.87% abundant in nature.

A target has been designed which will allow irradiation of tellurium powder at currents up to at least 10 amps. This will produce about 50 millicuries of I-123 per hour, which means that 100 to 200 mCi batches are possible if 77% enriched tellurium is used. This product will contain only 0.8% I-124. The biggest disadvantage of producing I-123 by this method is the high initial cost of the 77% enriched tellurium: \$13.50 per milligram. The target will require 600 mgs of tellurium minimum which means a total outlay of \$8,100. The tellurium is recoverable by precipitation with SO_2 after neutralization of the target solution.

4. Chromium-48 Sodium Chromate

At the completion of five experiments investigating the production of 23 hour chromium-48 by the 23 MeV $^3\text{He}^{++}$ bombardment of titanium, an evaluation of the procedure and product is necessary to determine if further work is justifiable.

The target configuration required will be titanium metal, plasma sprayed on copper discs, for use on the internal probe. This requirement is a consequence of two factors, the most important of which is the low target yield of 1.7 μCi chromium-48 per μAh . To produce 500 μCi of Cr-48 with the extracted $^3\text{He}^{++}$ beam which has a maximum intensity of 50 μA requires a six hour irradiation. This can be reduced to three hours by using the internal probe. The second factor dictating target choice is the relatively low thermal conductivity of titanium. The combination of a very thin layer (10-20 mils) of titanium sprayed on 1/8" of copper overcomes this difficulty. Target failure was the result of the use of solid 1/8" titanium plate. The failure was due to localized heating on the uncooled target edges which resulted in seal failure. During the initial external irradiations the lower beam intensity made cooling less difficult and eliminated heating problems. (No rubber O ring present.)

After irradiation of the copper backed target, the titanium is dissolved with 50% H_2SO_4 . The copper backing does not dissolve in 50% H_2SO_4 . When the titanium is dissolved, the solution is diluted to about 5% H_2SO_4 concentration, transferred to a 250 ml separatory funnel and cooled in ice. Cold 6% aqueous cupferron solution is added until a slight excess is indicated by a white precipitate. The bright yellow titanium

cupferrate is extracted with 25 ml aliquots of chloroform.

The organic layer, containing gross impurities as the cupferrates, is discarded. One milliliter of a solution of 10 mg Fe^{+++} /ml is added to the aqueous phase. Then 5 ml 95% ethanol is added and the solution boiled for 5 minutes. The solution is removed from the hot plate and 8M NaOH is immediately added to the hot solution until the precipitation of hydrated iron III oxide is complete. The Fe^{+++} acts as a non-isotopic carrier and collects the Cr^{+++} on the very flocculent precipitate. The mixture is heated until the precipitate is well coagulated, then filtered through a 0.22 μ Millipore filter. The precipitate is then washed from the filter with 12 M HCl and passed through an anion exchange column which has been pre-equilibrated with 12 M HCl. The column is Bio-Rad AG 1-X8 100-200 mesh, RCl form, Ca 1.0 x 8 cm. The column is washed with 10 ml 12 M HCl and the initial 12 M HCl fraction containing pure Cr-48 is evaporated to dryness under an air jet. The iron carrier remains on the anion exchange column. The residue is taken up in 0.31 M NaOH, a few drops of 50% H_2O_2 added to oxidize the Cr^{+++} to Cr^{VI} , the solution boiled to expel excess peroxide, and neutralized to pH=6.5 to 7.5 with 0.31 M HCl. The resulting solution is carrier-free 48-chromium-sodium chromate in isotonic saline. Filtration through a 0.22 μ Millipore filter yields a sterile solution. The chemical processing, from the beginning of target dissolution to final product, requires approximately eight working hours.

In addition to Cr-48, the product solution contains the decay product of Cr-48, 16 day V-48 in an amount which increases to 0.05 μCi V-48 per μCi Cr-48 initially present, 133 hours after separation. Chromium-51 produced by the reactions $^{49}\text{Ti}(^3\text{He}^{++}, n)^{51}\text{Cr}$ and $^{50}\text{Ti}(^3\text{He}^{++}, 2n)^{51}\text{Cr}$ is present in the amount of 25% of the Cr-48 on a millicurie basis at end of bombardment. The only other radiochemical impurities present in the final product are scandium activities.

Among the radiochemicals currently under investigation with the SKI cyclotron, Cr-48 sodium chromate is one of the most difficult to make and uses the available facilities least efficiently. The lengthy irradiation requirement and the lengthy radiochemical processing also make Cr-48 one of the least economical isotopes to produce. Additional disadvantages are the high Cr-51 content and the relatively high radiochemical impurities. Additional steps could reduce the level of scandium impurities, but would also extend the processing time. The high Cr-51 content cannot be overcome except by the use of isotopically enriched target material. The difficulty of fabricating targets prohibits the use of enriched titanium.

5. Iron-52

An alternative method of processing the irradiated chromium target to recover the Fe-52 has been developed and com-

Table II

YIELDS OF RADIONUCLIDES WITH THE S.K.I. CYCLOTRON

Radio Nuclide	Half Life	Decay γ -Energy (Kev)	Target (1)	Production Reaction	Yield at E.O.B. (2)		Impurities (%) (4)
					$\mu\text{Ci}/\mu\text{A}\cdot\text{h}$	$\mu\text{Ci}/\mu\text{A}$ at sat n(3)	
N-13	10 m	511	H ₂ O	p, α	6×10^4	6.1×10^4	0.2 ¹⁸ F
F-18	110 m	511	H ₂ O	³ He, p	6×10^3	14×10^3	Negligible
P-30	2.5 m	511, 2240	CaSi	³ He, np	1.6×10^4	1.6×10^4	
S-37	5.1 m	β^-	Ar(5)	n, α	0.3	0.3	
K-43	22 h	β^- , 370+	Ar(5)	³ He, n	10	321	
Cr-48	23 h	300, 120	Ti	³ He, 2n	1.5	50	25 ⁵¹ Cr
Cr-51	28 d	320	V	p, n	340	3.3×10^5	
Fe-52	8 h	165	Cr	³ He, 3n	0.7	8.4	Negligible
Se-73	7.1 h	360	Ge	³ He, 2n	250	2350	
Rb-81	4.7 h	190, 450	NaBr	³ He, 3n	35	255	
Rb-82m	6.3 h	780, 620	NaBr	³ He, 2n	70	670	
In-111	2.8 d	173, 247	Cd	p, n	420	4.1×10^4	3. In ^{114m}
I-121	2.0 h	210	Sb	³ He, 3n	110	390	2. ¹²³ I
I-123	13 h	160	Te	³ He, pn	5.0	96	
I-123	13 h	160	Te(5)	p, n	46	886	
I-123	13 h	160	¹²² Te(80%)	³ He	80	1.5×10^3	0.5 ¹²⁴ I
I-123	13 h	160	Sb	³ He, 3n	24	462	5. ¹²⁴ I
Xe-123	2.0 h	150	¹²² Te(80%)	³ He, 2n	270	922	
Cs-127	6.2 h	410, 125	NaI	³ He	43	407	

Footnotes:

- (1) Target is "thick" and of natural isotopic abundance unless otherwise indicated
- (2) E.O.B. = end of bombardment
- (3) For a saturation bombardment
- (4) As a percentage of the given radionuclide at E.O.B.
- (5) Not a thick target

pared to the previous ion exchange method. Additionally, the ion exchange procedure has been investigated to determine the elution curve with the purpose of increasing the purity of the product and reliability of the yield. Improvements include dissolving the copper from the chromium rather than etching the chromium from the copper as was previously done, and eliminating several washes from the ion exchange procedure. These improvements resulted in an overall reduction of impurities in the product, with the greatest improvement being a reduction of the zinc level by a factor of 4 for elemental zinc and a factor of over 100 for zinc-65. Data from radiochemical analysis of the ion exchange procedure used during the last reporting period and reported at that time are given to provide a convenient comparison. The overall reduction in non-radioactive impurities is due primarily to the removal of the copper backing by stripping with nitric acid. Considerable levels of impurities were introduced by the amount of copper removed during electrolytic etching. This is also believed to be the source of the Co-57. The Co-57 is not present in products prepared using copper stripping. The most probable source of the zinc present in the solvent extraction product is the isopropyl ether, although no effort has been made to find the source of the zinc. The elution curve for the easily identifiable radionuclides in the Fe-52 ion exchange procedure was determined using a fraction collector, the 3 x 3 NaI (Tl) well crystal and 1024 multichannel analyser, and the "YIELD" computer program in conjunction with the IBM 1800 computer. Two ml fractions were collected. The gamma ray spectrum of each fraction was examined and analyses made of each nuclide identified through detection of its photopeak. It may be seen from examination of the elution curve that the 9M, 6M, 4M and the 3M washes could be eliminated from the procedure without affecting the radionuclidic purity of the final product, though causing a saving in time. However, the solvent extraction process, as given in Figure 1, provides a product which is not significantly different from the ion exchange product in about half the time. An amendment to our IND to allow use of the solvent extraction procedure has been submitted to the F.D.A.

6. Other Radionuclides

Table II summarizes the yields of radionuclides obtained so far with the SKI cyclotron. This table does not include data on the radioactive gases which are produced in a continuous flow and are discussed elsewhere. The yields are for thick targets of natural isotopic abundance unless otherwise indicated. The energies of the bombarding particles are those available from the SKI cyclotron which are: protons - 15 MeV, helium-3 - 23 MeV, and deuterons - 8 MeV. The yields are in microcuries per microampere of beam current for a one-hour irradiation, and for irradiation to saturation, both at end of bombardment. Some of the yields presented in this table are the result of only one or two irradiations and so should not be taken as final. When water was irradiated with protons (using our F-18 target chamber), the irradiated water was tested for gaseous activities and none was found. So presumably all the N-13 activity was present as nitrite or nitrate ions. The S-37 production was by fast neutrons on argon gas. No S-38 was detected.

7. N-13 Labeled Ammonia

N-13 labeled ammonia has been shown to be a valuable new radiopharmaceutical for scanning the myocardium and for liver function test. It has been produced previously by irradiating metallic carbides, particularly aluminum carbide, with 7 MeV deuterons, when N-13 is produced by (d, n) nuclear reaction on carbon-12. On dissolving the aluminum carbide in either acid or alkali, ammonia labeled with N-13 is produced which is recovered by distillation. A disadvantage of this method is that Al-28 is also produced in the irradiation, which, with its 2.4 minute half-life, and high energy beta and gamma radiation (1.78 MeV) constitutes a considerable radiation hazard in handling and processing the target. About sixteen times as much Al-28 is produced as N-13 at the end of irradiation.

We have made $^{13}\text{NH}_3$ by irradiating methane gas with 8 MeV deuterons. The target chamber is lined with a pyrex glass tube and the out-gas is bubbled through water or isotonic saline in which the ammonia dissolves. By irradiating at 5 μA for ten minutes, and allowing the methane to flow at 1 cu. ft. per hour for an additional 5 minutes, 20 mCi of $^{13}\text{NH}_3$ activity can be collected in the bubbler. A decay study of the radioactivity in the bubbler showed a pure 10.0 minute half-life from 5 to 95 minutes after the end of irradiation.

Analysis of the solution in the bubbler by radioactive gas chromatography showed that the radioactive products were 95% $^{13}\text{NH}_3$, 4% CH_3NH_2 , 0.2% $\text{C}_2\text{H}_5\text{NH}_2$. Less than 3% HCl^{13}N was detected in the solution and no $\text{CH}_3\text{C}^{13}\text{N}$ was detected. No significant differences in the product composition were found by adding hydrogen gas to the methane in the range 0 to 50% H_2 in CH_4 . No noticeable differences in product composition were noted when methane was irradiated at 5, 1, 0.2 μA of beam current for the same length of time.

Gas chromatography using three different column packings was used to analyze the product for both its chemical and radioactive content. A column of Poropak QS (8 ft. x 1/4 in. stainless steel) was used to analyze for NH_3 , H_2O , HCN , CH_3NH_2 , $\text{C}_2\text{H}_5\text{NH}_2$, CH_3CN and $(\text{CH}_3)_2\text{NH}$. The column was operated isothermally at 70°C until the H_2O came off the column and then the temperature was increased to 250°C at the rate of 20°C/min., and held at 250°C for an additional 10 minutes. The chromatogram showed radioactivity peaks due to NH_3 , and CH_3NH_2 , and thermal conductivity peaks due to NH_3 , H_2O , CH_3NH_2 , $\text{C}_2\text{H}_5\text{NH}_2$ and CH_3CN . HCN elutes at the same position as CH_3NH_2 , so a second column was used to measure radioactive HCN content. This was 30% triacetin on Chromosorb P (10 ft. x 1/4 inch) isothermal at 70°C. No HCN was detected, and an upper limit for its content was set at 5%. Analysis of the undissolved gas on a 2 ft. column of molecular sieve 5A showed the presence of small amounts (11%) of N-13 labeled nitrogen and nitrous oxide but no radioactive methane.

Confirmation of these results was obtained by adding a few drops of the $^{13}\text{NH}_3$ -water solution, containing 1 mg of NH_4OH as carrier to an excess of Nessler's reagent, a specific test for ammonia. The characteristic brown precipitate of $\text{Hg}_2(\text{NH}_2)\text{I}_3$ was found, after centrifugation and washing, to contain 95% of the N-13 activity. A small amount of N-13 labeled cyanide was detected by adding carriers of NaCN and NH_4NO_3 and precipitating silver cyanide from a solution made acid with HNO_3 . The precipitate was centrifuged off, washed with water and redissolved in concentrated ammonium hydroxide solution. Silver cyanide was then reprecipitated by making acid with nitric acid. The AgCN precipitate was found to contain 2.6% of the total activity in the N-13 solution.

The presence of a very small amount of methane in the solution was not objectionable, as methane is non-toxic, odorless, and has a low solubility. The methylamine ^{13}N impurity is present in sufficiently small quantities as to present no interference with the $^{13}\text{NH}_3$. Both are carrier free radiochemicals. The aluminum carbide method also produces trace impurities of methylamine.

8. P-30 Labeled Phosphate

We have made P-30 by irradiating 0.004 inch thick aluminum foils with 14 MeV alpha particles from the Sloan-Kettering Institute cyclotron. The yield was 10 mCi/ μA at saturation and by irradiating at 10 μA for 10 minutes we made 100 mCi at the end of irradiation. The aluminum foil was removed from the target and dissolved in 5N hydrochloric acid in a 200 ml distillation flask shown in Figure 5. The hydrochloric acid was preheated to 90°C . The aluminum dissolved and hydrogen and carrier free phosphine gas labeled with P-30 was evolved. The radioactive gas was swept out of the distilling flask through a trap (to collect water vapor) and into one side of a Tescom welding torch (Model 11-1101). The other side of the torch was supplied with oxygen from a cylinder and the hydrogen and oxygen gases were burnt in a small flame (1 inch long) which was ignited by an adjacent gas burner. The H_2 - O_2 flame was directed downwards onto the surface of 10 ml of isotonic saline in a 50 ml beaker immersed in ice water. Phosphine was oxidized in the flame to P_2O_5 phosphorus pentoxide which dissolved in water to give phosphate ion PO_4^{3-} . The size of the orifice of the welding torch was chosen to be 0.020 in. so that the flame did not overheat the saline. The distillation flask and the Tescom torch were placed in separate fume hoods to minimize the possibility of a hydrogen-oxygen explosion.

The whole apparatus was set up before the irradiation, the hydrogen and oxygen flows started and the flame ignited and adjusted, while pointing away from the saline solution. The aluminum was then irradiated and about 20 seconds after the irradiation the ground glass joint A was opened, the aluminum dropped into the acid, the flask resealed and the flame re-ignited. When radioactivity was detected in the trap, the flame was directed

downward towards the saline, and when radioactivity in the trap fell off the preparation was complete.

The saline solution usually contained between 5 and 10 mCi of activity 4 minutes after the end of irradiation. About 75% efficiency was achieved in converting P-30 in Al to P-30 labeled PO_4 .

The $^{30}\text{PO}_4$ has been injected in dogs and the isotope distribution studied with a gamma camera. Phosphine gas labeled with P-30 has also been collected and used in breathing experiments on dogs.

9. Carbon-11 Labeled Amino Acids

The synthesis of C-11 labeled amino acids requires a rapid procedure for mixing the reactants and separating the reaction products. Although most reports describing the Strecker synthesis of amino acids involve techniques of several days in duration, we have been successful in demonstrating the synthesis and isolation of C-11 labeled amino acids within 2 hours.

In our procedure, gas mixtures of 5% hydrogen and 95% nitrogen, which had been passed over a catalyst to combine any O_2 impurities with H_2 , and dried, are bombarded with protons at a current of 10-15 μamps at a temperature of 300°C in a target chamber made of Vycor glass. 50-100 millicuries of H^{11}CN (identified by gas chromatography and by precipitation as silver cyanide) are generated during a typical run. The ^{11}C hydrogen cyanide is bubbled continuously through a solution containing the appropriate aldehyde and ammonia during the period of irradiation (40 minutes).

Millicurie quantities of carrier-free racemic alanine labeled with ^{11}C have been produced by reacting H^{11}CN with acetaldehyde and ammonia and hydrolyzing the resulting aminonitrile with hydrobromic acid. The solution is boiled down to a paste, stirred at -40°C with methyl alcohol and filtered. The filtrate is absorbed onto a Dowex-50 column and labeled alanine is recovered from the column after washing.

10. Target Chambers

A number of target chambers have been designed and constructed for the irradiation of various target materials. An internal target probe was made to fit in the internal beam monitor probe and is used for irradiation of solid discs of copper, titanium or electrodeposits of zinc, chromium, cadmium, and antimony. Target chambers have been made for use with the external beam, for irradiating water (for F-18 production), gases (for producing O-15, N-13 or C-11 labeled radioactive gases), and solids (for irradiating powders or volatile solids which can not be irradiated in vacuum). A water cooled, air-cooled foil holder has been made for bringing the beam out into the air, and

a spinning target has been made for irradiating twelve targets simultaneously under the same conditions. Two glass lined target chambers have been made for the production of hydrogen cyanide labeled with C-11 and for the production of ammonia labeled with N-13.

C. Fast Neutron Studies

1. Neutron Dosimetry

During the current report period, the research on neutron dosimetry using the small isochronous cyclotron at Sloan-Kettering Institute has been concentrated on:

a) Investigation of the characteristics of fast neutron beams using various charged particles available from this cyclotron (protons, deuterons, helium-3, and alpha particles) and for various target materials (principally beryllium and heavy water).

b) Dosimetry measurements necessary for research on the biological response of mammalian cell cultures and sea urchin sperm to fast neutrons.

Biological studies indicating a decrease in relative biological effectiveness (RBE) with a depth for neutrons from the $^9\text{Be}(^3\text{He}, n)$ reaction have been correlated with measurements of the dose fraction as a function of LET.

This work, done principally by A. Pinkerton, has been fully described in the AEC contract AT(30-1) 3510 report for the period July 1, 1969 - June 30, 1970 (document NYO 3510-15).

2. Biological Effects of Fast Neutron Beams

Fast neutrons (modal energy 6 MeV) from the reaction $^9\text{Be}(^3\text{He}, n)$ have been employed to determine the RBE of fast neutrons on tissue-equivalent material. Cultured HeLa cells were used for these studies. This work, undertaken principally by R.G. Evans and B. Djordjevic, is fully described in the progress report for Proposal #2 of this contract (document NYO 910-148).

3. RBE of Fast Neutrons on Sea Urchin Eggs

Studies are being carried out to determine the relative biological effectiveness of different types of radiation, with varying values of linear energy transfer (LET), in producing radio-induced division delay in the developing fertilized sea urchin egg. Division delay caused by the fertilization with irradiated sperm has been measured with three types of radiation of

nearly equal LET, 95 KV, ^{60}Co gamma-rays, and 3 MeV betatron produced electrons. The same measurement is now being carried out with radiation of considerably higher LET, i.e., fast neutrons produced by our small isochronous cyclotron, using protons (15 MeV) in the reaction $^9\text{Be}(p, n)$.

VIII.

BIOPHYSICS GRADUATE STUDENTS TRAINED

Sloan-Kettering Division

School of Medical Sciences

Cornell University Medical College

<u>NAME</u>	<u>DATE</u>	<u>DEGREE RECEIVED</u>
Schulz, Robert Jack	9/12/55-6/4/58	M.S.
Wood, Robert	9/12/55-6/7/61	Ph.D.
Cohen, Beverly Singer	9/11/56-6/6/61	M.S.
Hands, Joan Myrtle	9/9/57-6/1/60	M.S.
Vickers, Shirley Danson	9/13/57-6/7/58	M.S.
Milvy, Paul	9/14/59-2/6/62	M.S.
Finston, Roland Alan	9/14/59-3/4/65	Ph.D.
King, Barrie	2/2/61-6/5/63	M.S.
Lo, Hing Har	9/7/61-2/5/64	M.S.
Milvy, Paul	2/6/62-10/31/65	Ph.D.
Wallace, Susan	2/5/62-5/27/65	Ph.D.
Henke, John Wm., Jr.	9/10/63-6/29/67	M.S.
Costeas, Aristides	9/15/64-6/28/68	Ph.D.

IX. Division of Medical Research
AEC - Supported Bibliography

1968-1971

1. Beattie, J.W., Monahan, W.G., and Laughlin, J.S., Digital anger camera with three-dimensional resolution for positron emitters. AAPM Bull 3:27, 1969.
2. Benua, R.S., Laughlin, J.S., Lee, B.J., and Tilbury, R.S., Use of F-18 Sodium Fluoride Bone Scans to Determine the Extent of Disease in Lymphoma. J. Nucl. Med. 12:340, 1971.
3. Benua, R.S., Weber, D.A., Kenny, P.J., and Laughlin, J.S., Digital scanning compared with photo-scanning in liver examination. J. Nucl. Med. 9:135, 1968.
4. Chabrow, F., and Pullman, I., Simulated first-order lorentizism ESR hyperfine derivative spectra including modulation and time constant effects. Indiana University Quantum Chemistry Program Exchange, Catalog and Procedures, 1968.
5. Chu, F.C.H., Greenberg, E.J., Dwyer, A.J., Dimich, A.B., and Laughlin, J.S., Effects of radiation therapy on bone lesions as measured by Ca-47 and Sr-85 local kinetics. J. Nucl. Med. (in press).
6. Clarkson, B.D., Fried, J., and Ogawa, M., Magnitude of Proliferating Fraction and Rate of Proliferation of Populations of Leukemic Cells in Man, in Recent Results in Cancer Research 17:175-185, 1969.
7. Clarkson, B.D., Sakai, Y., Kimura, T., Ohkita, A., and Fried, J., Studies of cellular proliferation in human leukemia. II. Variability in rates of growth and cellular differentiation in acute myelomonoblastic leukemia and effects of treatment, in The Proliferation and Spread of Neoplastic Cells, pp. 295-330, 1968.
8. Costeas, A., Woodard, H.Q., and Laughlin, J.S., Depletion of F-18 from Blood Flowing Through Bone. J. Nuc. Med. 11: 43-45, 1970.
9. Dahler, A., Baker, A.S., and Laughlin, J.S., Comprehensive electron-beam treatment planning. Annals of the New York Academy of Sciences 161:198-213, 1969.
10. Djordjevic, B., Sensitization of BUdR and repair of radiation damage in synchronized HeLa cells. Proc. IVth, Inter. Congress of Radiation Research, Evian, 1970.
11. Djordjevic, B., and Evans, R.G., Spontaneous uptake of thymidine

into the DNA of G1 cells, *Nature* 224:803-804, 1969.

12. Djordjevic, B., and Kim, J.H., Different lethal effects of mitomycin C and actinomycin D during the division cycle of HeLa cells. *J. Cell Biology* 38:477-482, 1968.
13. Djordjevic, B., and Kim, J.H., Modification of radiation response in synchronized HeLa cells by metabolic inhibitors: Effects of inhibitors of DNA and protein synthesis. *Rad. Rec.* 435-450, 1969.
14. Djordjevic, B., and Perez, A., Repair of damage in irradiated HeLa cells. *Radiation Research* 43:270-271, 1970.
15. Evans, R.G., and Djordjevic, B., Effect of non-toxic concentrations of acriflavine on unscheduled DNA synthesis and survival of synchronized HeLa cells exposed to ultraviolet light. *Inter. J. of Rad. Biol.* 15:239-244, 1969.
16. Evans, R.G., Djordjevic, B., and Perez, A.G., Temperature Dependence of Thymidine Uptake and Stability of Thymidine Incorporated during Unscheduled DNA synthesis in Irradiated and Unirradiated HeLa cells. *Inter. J. of Rad. Biol.* 18:277-280, 1970.
17. Evans, R.G., Pinkerton, A., Djordjevic, B., Mamacos, J., and Laughlin, J.S., Changes in biological effectiveness of a fast neutron beam with depth in tissue-equivalent material. *Radiation Research* 45:235-243, 1971.
18. Eyster, E., Mayer, K., and McKenzie, S., Traumatic hemolysis with iron deficiency anemia in patients with aortic valve lesions. *Annals of Internal Medicine* 68:995-1004, 1968.
19. Fried, J., Compartmental analysis of kinetic processes in multicellular systems: A necessary condition. *Physics in Medicine and Biology* 13:31-43, 1968.
20. Fried, J., Correction for threshold error in the determination of generation times by the grain-count halving method. *J. Theoret. Biol.* 24:108-120, 1969.
21. Fried, J., Mean, geometric mean, or median grain count in cell cycle studies. *Exptl. Cell Research* 59:447-451, 1970.
22. Furlan, M., and Moroson, H., Effects of Polycation on Tumor Cells, *Radiation Protection and Sensitization, Proceedings*, pp. 233-242, 1970.
23. Gelbard, A.S., Perez, A.G., Kim, J.H., and Djordjevic, B., The Effect of X-irradiation on Thymidine Kinase Activity in Synchronous Populations of HeLa Cells. *Radiation Research* 46:334-342, 1971.

24. Gelbard, A.S., Kim, J.H., and Perez, A.G., Fluctuations in deoxycytidine monophosphate deaminase activity during the cell cycle in synchronous populations of HeLa cells. *Biochim. Biophys. Acta* 182:564-566, 1969.
25. Greenberg, E.J., Weber, D.A., Pochaczewsky, R., Kenny, P.J., Myers, W.P.L., and Laughlin, J.S., Detection of neoplastic bone lesions by quantitative scanning and radiography. *Journal of Nuclear Medicine* 9:613-620, 1968.
26. Kenny, P.J., Myers, M.J., Lundy, A., Ritter, F., and Laughlin, J.S., Digital anger camera with electronic focusing for positron emitters, in *Fundamental Problems in Scanning*, Chapter 16, pp. 226-228, 1968.
27. Kessarlis, N.D., Absorbed dose and cavity ionization for high energy electron beams in water and carbon, *Radiation Research* 43:No. 2, 281-287, 1970.
28. Kessarlis, N.D., Diffusion of oxygen in cells after high intensity pulsed irradiations. To be submitted to *Radiation Research*.
29. Kessarlis, N.D., Relation Between Absorbed Dose and Cavity Ionization for High Energy Electron Beams in Water, *Annals, N.Y. Acad. of Sci.* 161:24-26, 1969.
30. Kessarlis, N.D., Spectra of high energy electron beams in water, *Radiation Research* 43:No. 2, 288-301, 1970.
31. Kim, J.H., Gelbard, A.S., Djordjevic, B., Kim, S.H., and Perez, A.G., Action of daunomycin on the nucleic acid metabolism and viability of HeLa cells. *Cancer Research* 28:2437-2442, 1968.
32. Kim, J.H., and Kim, S.H., Modification of radiation response in synchronized HeLa cells by metabolic inhibitors. I. Effect of an inhibitor of RNA-synthesis, puromycin amino-nucleoside. *Int. J. Radiat. Biol.* 14:1-7, 1968.
33. Kim, J.H., Perez, A.G., and Djordjevic, B., Studies on unbalanced growth in synchronized HeLa cells. *Cancer Research* 28:2443-2447, 1968.
34. Kim, J.H., Pinkerton, A., and Laughlin, J.S., Dosimetry and biological parameters in studies of the relative biological effectiveness of high energy electron beams. *High Energy Radiation Therapy Dosimetry. Annals of the N.Y. Academy of Sciences* 161, 1969.
35. Kim, J.H., Pinkerton, A., and Laughlin, J.S., Studies on the biological effectiveness of high energy electron beams at different depths in tissue absorbing material. *Radiation Research* 33:419-425, 1968.

36. Kim, S.H., Kim, J.H., and Djordjevic, B., Effects of X-irradiation on RNA and protein synthesis in HeLa cells. Radiation Research 42:577-589, 1970.
37. Laughlin, J.S., Computer-aided measurement of the distribution of radionuclide tracers in vivo. Proc. for Conference on the Use of Computers in Radiology. (October 20-23, 1966), Center for Continuing Education, University of Chicago, Chicago, 1968, p. E70-90.
38. Laughlin, J.S., The Cyclotron in Nuclear Medicine, in Nuclear Medicine, 2nd Edition, W. Blahd, M.D., Editor, McGraw-Hill, Inc., New York, N.Y., pp. 677-692, 1971.
39. Laughlin, J.S., Electron Beams, Radiation Dosimetry 3: 91-149, 1969.
40. Laughlin, J.S., High energy electron treatment planning and dosimetry: Past experience and future development, in Frontiers of Radiation Therapy and Oncology 2:87-103, 1968.
41. Laughlin, J.S., Quantitative and Computer Analyzed Scanning, in Nuclear Medicine, 2nd Edition, W. Blahd, M.D., Editor, McGraw-Hill, Inc., New York, N.Y., pp. 47-53, 1971.
42. Laughlin, J.S., Realistic treatment planning, Cancer 22: 716-729, 1968.
43. Laughlin, J.S., Gelbard, A.S., and Perez, A.G., Inhibition of DNA synthesis by actinomycin C and cycloheximide in synchronized HeLa cells. Experimental Cell Research 53: 478-487, 1968.
44. Laughlin, J.S., Kenny, P.J., Weber, D.A., Dwyer, A.J., Mayer, K., Greenberg, E.J., Dimich, A.B., and Hasan, S., Methods and applications of quantitative computer-analyzed scanning. Medical Radioisotope Scintigraphy 1:633-652, 1969.
45. Laughlin, J.S., Mamacos, J.P., Tilbury, R.S., Isochronous cyclotron installation for radionuclide production, Radiology 93:2, 331-337, 1969.
46. Laughlin, J.S., Ritter, F.W., Dwyer, A.J., Mayer, K., Greenberg, E.J., Dimich, A.B., Hasan, S., Rothschild, E., and Myers, W.F.L., Development and applications of quantitative and computer-analyzed counting and scanning. Cancer 25:395-405, 1970.
47. Laughlin, J.S., Weber, D.A., Benua, R.S., Kenny, P.J., and Ritter, F., Quantitative storage, analysis and display in digital scanning, in Fundamental Problems in Scanning, Chapter 22, pp. 267-274, 1968.

48. Laughlin, J.S., Vacirca, S.J., and Duplissey, J.F., Exposure of embalmers and physicians by radioactive cadavers. *Health Physics* 15:451-455, 1968.
49. Mayer, K., Bettigole, R.E., Harris, J.P., and Tegoli, J., Test in vivo to determine donor blood compatibility. *Transfusion* 8:28-32, 1968.
50. Mayer, K., Dwyer, A.J., and Laughlin, J.S., Spleen scanning using ACD damaged red blood cells with Cr-51. *J. Nucl. Med.* 11:455-458, 1970.
51. Mayer, K., and Mink, B., Slide rule for platelet determinations with the Coulter Counter. *Am. J. Clin. Path.* 48:453-454, 1968.
52. Milvy, P., and Pullman, I., ESR studies of spin transfer in irradiated nucleic acid - cysteamine systems. *Radiation Research* 34:265-286, 1968.
53. Monahan, W.G., Data Analysis and Display for Digital Anger Camera System, AEC Conference on Computer Applications in Nuclear Medicine, Oak Ridge, Tennessee, April, 1971 (in press).
54. Moroson, H., Radiosensitizers for Mammalian Cells. *Proceedings, IVth International Congress of Radiation Research*, 1970.
55. Moroson, H., and Furlan, M., An Improvement in alkaline density gradient sedimentation of mammalian cell DNA. *Radiation Research* (in press).
56. Moroson, H., and Furlan, M., Methyl Hydrazine Radiosensitized Ehrlich Ascites Tumor Cells, pp. 257-263 *in* *Radiation Protection and Sensitization* (Moroson, H., and Quintiliani, M., Editors), 1970.
57. Moroson, H., and Furlan, M., Radiosensitization of Anoxic and Aerobic tumor cells, *Physiol. Chem. Phys.* 1:368-376, 1969.
58. Moroson, H., and Furlan, M., Single Strand breaks in DNA of Ehrlich ascites tumor cells produced by methyl hydrazine, *Radiation Research* 40:351-365, 1969.
59. Ogawa, M., Fried, J., Sakai, Y., Strife, A., and Clarkson, B., Studies of cellular proliferation in human leukemia. VI. The proliferative activity, generation time, and emergence time of neutrophilic granulocytes. *Cancer* 25:1031-1049, 1970.
60. Okumura, Y., The Application of the Modulation Transfer Function to Reduce Unsharpness in Scintigrams, *International Journal Appl. Radiat. and Isot.* 22:49-52, 1971.
61. Perez, A.G., Evans, R.G., and Djordjevic, B., Spontaneous unscheduled DNA synthesis in HeLa cells. *Radiation Research* 43:254, 1970.

62. Pinkerton, A., Mamacos, J.P., and Laughlin, J.S., Dosimetry of Fast Neutron Beams from a Small Medical Cyclotron, *Radiology* 96:131-136, 1970.
63. Saito, S., Zeitz, L., Bush, I.M., Lee, R., and Whitmore, W.F., Zinc uptake and EDTA-deprivation in canine or rat spermatozoa. *Amer. J. of Physiology*, 217:1039-1043, 1969.
64. Tilbury, R.S., Dahl, J.R., and Laughlin, J.S., Cyclotron Production of Radioactive Gases. *J. Nucl. Med.* 12:468, 1971.
65. Tilbury, R.S., Dahl, J.R., Mamacos, J.P., and Laughlin, J.S., Fluorine-18 Production for Medical Use by Helium-3 Bombardment of Water, *Int. J. Appl. Rad. & Isot.* 21:277-281, 1970.
66. Tilbury, R.S., Mamacos, J.P., and Laughlin, J.S., Initial Experience with a 30-inch Isochronous Cyclotron for Medical Use. *Uses of Cyclotrons in Chemistry, Metallurgy and Biology*, Edited by C.B. Amphlett, pp. 117-124, Butterworth, London, 1970.
67. Watson, I.A., The production of Rb-81 and Rb-82m for Medical Use, *Radiochem. Radioanal. Letters* 4/1/7-14, 1970.
68. Watson, I.A., and Tilbury, R.S., Cyclotron Production of the Radionuclides Rb-81, Rb-82m and Cs-127, *J. Nucl. Med.* 11:6, 373, 1970.
69. Weber, D.A., Greenberg, E.J., Dimich, A., Kenny, P.J., Rothschild, E.O., Myers, W.P.L., and Laughlin, J.S., Detection of neoplastic bone lesions by quantitative scanning and radiography. *J. of Nuc. Med.* 9:613-620, 1968.
70. Weber, D.A., Greenberg, E.J., Dimich, A., Kenny, P.J., Rothschild, E.O., Myers, W.P.L., and Laughlin, J.S., Kinetics of radionuclides used for bone studies. *J. Nucl. Med.* 10: 8-17, 1969.
71. Wood, R., Chabrow, F., and Pullman, I., A method for determining hyperfine coupling constants from unresolved ESR spectra of hydrocarbon ions. *J. Magn. Resonance* 2:88-98, 1970.
72. Woodard, H.Q., The biological effects of tritium. U.S. Atomic Energy Commission Report, HASL-229, May 18, 1970.
73. Woodard, H.Q., Effects of external irradiation on the bones of mice and humans of different ages. *Proceedings of Symposium on "The Radiation Biology of the Fetal and Juvenile Mammal," Ninth Annual Hanford Biology Symposium, Richland, Washington.*
74. Woodard, H.Q., The influence of hormones on the uptake and loss of strontium-89 in mouse bone, *in Monographs on Nuclear Medicine and Biology*, No. 2, Diagnosis and Treatment of

Deposited Radionuclides.

75. Woodard, H.Q., and Corey, K.C., The uptake of Sr-89 and Zn-65 by mouse melanoma. *Health Physics* 20:643-644, 1971.
76. Woodard, H.Q., and Marcove, R.C., A comparison of the chemistry in blood from bone and from peripheral veins. *Clinical Orthopaedics* 66:254-264, 1969.
77. Zeitz, L., X-ray emission analysis in biological specimens, in *Progress in Analytical Chemistry 3:X-ray and Electron Probe Analysis in Biomedical Research*. Plenum Press, N.Y. 35-73, 1969.
78. Zeitz, L., Ferguson, R., and Garfinkel, E., Incorporation of ^3H -TdR and ^3H -BUdR into sperm DNA of *Arbacia punctulata* after coelomic injection. *Experimental Cell Research* 56: 159-163, 1969.
79. Zeitz, L., Ferguson, R., and Garfinkel, E., Radiation-induced effects on DNA synthesis in developing sea urchin eggs. *Radiation Research* 34:300-308, 1968.
80. Zeitz, L., and Freed, B., Design and calibration of Sloan-Kettering Institute osteodensitometer. USAEC Report CONF-700515, 1970.
81. Zeitz, L., and Lee, R., Element analysis in labeled DNA by X-ray fluorescence. *Anal. Biochem.* 23:442-453, 1968.
82. Zeitz, L., Lee, R., and Rothschild, E.O., Element analysis in hair by x-ray fluorescence. *Analytical Biochemistry* 31:123-132, 1969.

Division of Biophysics

AEC - Supported Bibliography

1950-1968

1. BALNER, H., SIMMEL, E.B. and CLARKE, D.A., Proliferation and rejection of transplanted tritium-labeled bone marrow cells in mice, Transplant. Bull. 29: No. 4, 1962.
2. BARR, N.F., GEISSELSODES, J. and LAUGHLIN, J.S., Use of the Fricke Dosimeter to measure photoelectric absorption, Radiation Res. 14:291, 1961.
3. BARR, N.F., HANDS, J. and LAUGHLIN, J.S., Dosimetry with small silver-activated glass rods, Health Physics 7:48, 1961.
4. BARR, N.F., STARK, M. and LAUGHLIN, J.S., A comparison of two fluorometers designed to measure the radiation-induced fluorescence of silver-activated glass rods, Radiology 76:113, 1961.
5. BARR, N.F., STARK, M.B. and LAUGHLIN, J.S., Calibration of the absorbed dose produced in water by betatron electrons with the benzoic acid dosimeter, Radiology 78:625, 1962.
6. BARRY, M.C., EIDINOFF, M.L., DOBRINER, K. and GALLACHER, T.F., The fate of C^{14} -testosterone and C^{14} -progesterone in mice and rats, Endocrinology 50:587, 1952.
7. BEATTIE, J.W., TSIEN, K.C., OVADIA, J. and LAUGHLIN, J.S., Production and properties of high energy electrons for therapy, Amer. J. Roentgenol. 88:235, 1962.
8. BENUA, R.S., WEBER, D.A., KENNY, P. and LAUGHLIN, J.S., Digital scanning compared with photoscanning in liver examinations, Journal of Nuclear Medicine 9:135-139, 1968.
9. BERMAN, M., LAUGHLIN, J.S., YONEMITSU, M. and VACIRCA, S., Automatic isodose recorder, Rev. of Sci. Insts. 26:328, 1955.
10. BERMAN, M. and SCHOENFELD, R., Invariants in experimental data on linear kinetics and the formulation of models, J. App. Phys. 27:1361, 1956.
11. BERMAN, M. and SCHOENFELD, R., Information content of tracer data with respect to steady state systems, Symp. on Information Theory in Biology, October 1956, Ed. Quastler and Yockey, New York, Pergamon Press, 1958, p. 181.
12. BERMAN, M., RALL, J.E. and HESLIN, J., Some physical considerations for governing the choice of internally administered radioisotopes for therapy, Phys. in Med. and Biol. 1:243, 1957.

13. BERMAN, M., BECKER, D.V. and BENUA, R.S., The use of I-133 in the treatment of Graves' disease, J. Clin. Endoc. and Med. 17:1222, 1957.
14. BEYER, H.G., MURRIN, J. and EIDINOFF, M.L., Changes in ultra-violet absorption spectra after ultrasonic irradiation of some compounds related to the nucleic acids, Radiation Res. 3:214, 1955.
15. BLUM, P., An electron scintillation spectrometry method, Radiation Res. 16:626, 1962.
16. BIRNBAUM, D. and HALL, T., An electroejaculation technique for rats, Anatomical Record 140:49, 1961.
17. BIRNBAUM, D., HALL, T. and LEE, R., Zinc content of rat sperm cells from ejaculate, vas, epididymis and testis, Proc. Soc. Exptl. Biol. and Med. 108:321, 1961.
18. BRASFIELD, R.D., LAUGHLIN, J.S. and SHERMAN, R.S., Thermography in the management of cancer: a preliminary report, Ann. N.Y. Acad. Sci. 121:235, 1964.
19. CHEONG, L., PERRI, G.C. and SHARPE, L.M., Assay of radioiron and radio-silver in biological samples, Anal. Chem. 26:242, 1954.
20. CHEONG, L., RICH, M.A. and EIDINOFF, M.L., Introduction of the 5-halogenated uracil moiety into deoxyribonucleic acid of mammalian cells in culture, J. Biol. Chem. 235:1441, 1960.
21. CHEONG, L., RICH, M.A. and EIDINOFF, M.L., Mechanism of growth inhibition on H. Ep. #1 cells by 5-fluorodeoxycytidine and 5-fluorodeoxyuridine, Cancer Res. 20:1602, 1960.
22. CHU, W.C.H., NISCE, L. and LAUGHLIN, J.S., Treatment of breast cancer with high-energy electrons produced by 24-Mev Betatron, Radiology 81:871, 1963.
23. COOPER, G.W., VAN DYKE, J.G., NICKSON, J.J. and LAUGHLIN, J.S., Relative biological efficiency of 20 Mev electrons and 250-Kev x-rays as measured in the 12-day-old chick embryo, Radiation Res. 16:686, 1962.
24. COREY, K.R., KENNY, P., GREENBERG, E.J., PAZIANOS, A., PEARSON, O.H. and LAUGHLIN, J.S., The use of calcium-47 in diagnostic studies of patients with bone lesions, Am. J. Roentgenol. 85:955, 1961.
25. COREY, K.R., KENNY, P., GREENBERG, E. and LAUGHLIN, J.S., Detection of bone metastases in scanning studies with calcium-47 and strontium-85, J. Nucl. Medicine 1:454, 1962.
26. COREY, K.R., KENNY, P., GREENBERG, E. and LAUGHLIN, J.S., Methods of localization of calcium-47 and strontium-85, pp. 25-28; results of kinetic analysis and calcium balance studies in malignancy, pp. 80-85;

- The use of Ca-47 and Sr-85 in diagnosis of bone tumors, pp. 95-102; Medical uses of Ca-47, Technical Reports Series #10, International Atomic Energy Agency, Vienna, 1962.
27. COREY, K.R., Results on kinetic analyses and external counts; medical uses of Ca-47, Technical Reports Series #32, International Atomic Energy Agency, Vienna, 1964, p. 110.
28. COREY, K.R., The turnover of Ca-47 and Sr-85 in bone lesions; medical uses of Ca-47, Technical Reports Series #32, International Atomic Energy Agency, Vienna, 1964, p. 148.
29. COREY, K.R., WEBER, D., MERLINO, M., GREENBERG, E., KENNY, P. and LAUGHLIN, J.S., Calcium turnover in man. in Kniseley, Ralph, M. and Tauxe, W. Nelson (eds.), Symposium on Dynamic Clinical Studies with Radioisotopes, Oak Ridge, Tennessee, Oak Ridge Institute of Nuclear Studies, TID 7678, 1964, pp. 519-536.
30. COREY, K.R., GREENBERG, E., KENNY, P., LAUGHLIN, J.S., MERLINO, M. and WEBER, D., Developments in technique. in Technical Report Series No. 32. Symposium on Medical uses of Ca-47: Second Panel Report, International Atomic Energy Agency, STI/DOC/10/32, Vienna, 1964, pp. 1-3.
31. COREY, K.R., GREENBERG, E., KENNY, P., LAUGHLIN, J.S., MERLINO, M. and WEBER, D., The use of the HEG-Scanner for whole body counting. in Technical Report Series No. 32. Symposium on Medical Uses of Ca-47: Second Panel Report, International Atomic Energy Agency, STI/DOC/10/32, Vienna, 1964, pp. 5-9.
32. COSTEAS, A., WOODARD, H.Q. and LAUGHLIN, J.S., Comparative kinetics of calcium and fluoride in rabbit bone. (Thesis), 1968. Sloan-Kettering Institute Division of the Cornell University Graduate School of Medical Sciences.
33. DANZKER, M., KESSARIS, M.D. and LAUGHLIN, J.S., Absorbed dose and linear energy transfer in radiation experiments, Radiology 72:51, 1959.
34. DELIHAS, N., The ability of irradiated bacteriophage T2 to initiate the synthesis of deoxycytidylate hydroxymethylase in Escherichia coli, Virology 13:242, 1961.
35. DELIHAS, N., RICH, M.A. and EIDINOFF, M.L., Radiosensitization of a mammalian cell line with 5-bromodeoxyuridine, Radiation Res. 17:479, 1962.
36. DJORDJEVIC, B. and KIM, J.H., Lethal effect of phleomycin in different stages of the division cycle of HeLa cells, Cancer Research 27:2255-2260, 1967.
37. DJORDJEVIC, B. and KIM, J.H., Differential effects of mitomycin C and actinomycin D during the division cycle of HeLa cells, J. Cell Biology 38:477-484, 1968.

38. DJORDJEVIC, B., KIM, J.H. and KIM, S.H., Modification of radiation response in synchronized HeLa cells by metabolic inhibitors. I. Effect of an inhibitor of RNA synthesis, puromycin amino-nucleoside, *Int. J. Radiat. Biol.* (in press).
39. DJORDJEVIC, B. and KIM, J.H., Modification of radiation response in synchronized HeLa cells by metabolic inhibitors. II. Effects of inhibitors of DNA and protein synthesis. (submitted to *Radiation Research*).
40. DUPLISSEY, J.F., VACIERCA, S.J. and LAUGHLIN, J.S., Exposure of embalmers and physicians by radioactive cadavers, *Health Physics* (in press).
41. EIDINOFF, M.L. and KNOLL, J.E., Measurement of radioactive hydrogen in solid samples - comparison with gas counting, *Science* 112:250, 1950.
42. EIDINOFF, M.L., FITZGERALD, P.J., SIMMEL, E.B. and KNOLL, J.E., Intracellular localization of compounds labeled with tritium, H³ by radioautography, *Proc. Soc. Exptl. Biol. and Med.* 77:225, 1951.
43. EIDINOFF, M.L., KNOLL, J.E., FUKUSHIMA, D.K. and GALLACHER, T.F., Equilibrium between protium, deuterium and tritium in the system; hydrogenacetic acid; isotopic fractionation factors in a catalytic hydrogenation, *J. Amer. Chem. Soc.* 74:5280, 1952.
44. EIDINOFF, M.L., REILLY, H.C., KNOLL, J.E. and MARRIAN, D.H., Hydrolysis products of nucleic acids labeled with tritium preparation by biosynthesis, *J. Biol. Chem.* 199:511, 1952.
45. EIDINOFF, M.L. PERRI, G.C., KNOLL, J.E., MARANO, B.J. and ARNHEIM, J., The fractionation of hydrogen isotopes in biological systems, *J. Amer. Chem. Soc.* 75:248, 1953.
46. EIDINOFF, M.L. and KNOLL, J.E., The introduction of isotopic hydrogen into purine ring systems by catalytic exchange, *J. Amer. Chem. Soc.* 75:1992, 1953.
47. EIDINOFF, M.L., Some fractionation effects involving the isotopes of hydrogen, *N.Y. Acad. Sci.* 16: 76, 1953.
48. EIDINOFF, M.L., ROSENFELD, R.S., KNOLL, J.E., MARANO, B.J. and HELLMAN, L., The biosynthesis of squalene from acetate in man, *J. Clin. Invest.* 33: 333, 1954.
49. EIDINOFF, M.L., KNOLL, J.E. and KLEIN, D., Effect of 5-fluorouracil on the incorporation of precursors into nucleic acid pyrimidines, *Arch. Biochem. Biophys.* 71:274, 1957.
50. EIDINOFF, M.L., KNOLL, J.E., MARANO, B.J. and CHEONG, L., Pyrimidine studies. I. Effect of DON (6-diazo-5-oxo-L-Norleucine) on incorporation of precursors into nucleic acid pyrimidines, *Cancer Res.* 18:105, 1958.

51. EIDINOFF, M.L., MARANO, B.J., KNOLL, J.E., KVAMME, E., ROSENFELD, R.S. and HELLMAN, L., Cholesterol biosynthesis - studies related to the metabolic role of squalene, *J. Clin. Invest.* 37:655, 1958.
52. EIDINOFF, M.L. and RICH, M.A., Growth inhibition of a human tumor cell strain by 5-fluoro-2'-deoxyuridine: Time parameters for subsequent reversal by thymidine, *Cancer Research* 19:521, 1959.
53. EIDINOFF, M.L., CHEONG, L., GAMBETTA CURPIDE, E., BENUA, R.S. and ELLISON, R.R., Incorporation of 5-iodouracil labeled with iodine-131 into the deoxyribonucleic acid of human leukemic leucocytes following *in vivo* administration of 5-iododeoxyuridine labeled with iodine-131, *Nature* 183:1686, 1959.
54. EIDINOFF, M.L., CHEONG, L. and RICH, M.A., Incorporation of unnatural pyrimidine bases into deoxyribonucleic acid of mammalian cells, *Science* 129:1550, 1959.
55. EIDINOFF, M.L., KNOLL, J.E., MARANO, B.J. and KLEIN, D., Pyrimidine studies. II. Effect of 5-bromodeoxyuridine and related nucleosides on incorporation of precursors into nucleic acid pyrimidines, *Cancer Res.* 19:738, 1959.
56. EIDINOFF, M.L., RICH, M.A. and PEREZ, A.G., Growth inhibition of a human tumor cell strain by 5-fluorocytidine and 5-fluoro-2'-deoxycytidine: Reversal studies, *Cancer Research* 19:638, 1959.
57. EIDINOFF, M.L., KNOLL, J.E., MARANO, B.J. and KLEIN, D., Pyrimidine studies. III. Effect of several compounds with anti-tumor activity on utilization of precursors for synthesis of nucleic acid pyrimidines, *Cancer Research* 21:1377, 1961.
58. EIDINOFF, M.L., Tritium in biochemical studies, *Advances in Tracer Meth.* 1:222, 1963.
59. EPP, E.R., WOODARD, H.Q. and WEISS, H., Energy absorption by the bone marrow of the mouse receiving whole-body irradiation with 250-KV x-rays or Cobalt-60 gamma-rays, *Radiation Res.* 11:184, 1959.
60. EPP, E.R., WEISS, H. and LAUGHLIN, J.S., Measurement of bone marrow and gonadal dose from the chest x-ray examination as a function of field size, field alignment, tube kilovoltage, and added filtration, *Brit. J. Radiol.* 34: 85, 1961.
61. EPP, E.R. and WEISS, H., Investigation of narrow well K X-ray in NaIOTl for reduction of iodine spectra with collimated photon beams, *Rev. Sci. Instr.* 32:651, 1961.
62. EPP, E.R., HESLIN, J.M., WEISS, H., LAUGHLIN, J.S. and SHERMAN, R.S., Measurement of bone marrow and gonadal dose from x-ray examination of the pelvis, hip, and spine as a function of field size, tube kilovoltage, and added filtration, *Brit. J. Radiol.* 36:247, 1963.

63. EPP, E.R., WEISS, H., SINOWITZ, N.R. and LAUGHLIN, J.S., Measurement of the ovarian dose in a human phantom subjected to an irradiation technique for female sterility, Amer. J. Roent., Rad. Ther. and Nucl. Med. (in press).
64. EPP, E.R., WEISS, H., SANTOMASSO, A., The oxygen effect in bacterial cells irradiated with high intensity pulsed electrons, Radiation Res. 34:320-325, 1968.
65. FINSTON, R.A., WOODARD, H.Q. and LAUGHLIN, J.S., Effects of external irradiation on the bones of adult dogs, Clin. Orthopaedics 46:183-201, 1966.
66. FITZGERALD, P.J., Erythroblastemia, Cancer 3:504, 1950.
67. FITZGERALD, P.J., The zone of localization of antibodies. VI. The rate of localization of anti-mouse-kidney serum, J. Immunol 64:281, 1950.
68. FITZGERALD, P.J., Cancer of the thyroid in children, a report of 28 cases, J. Clin. Endocrinol. 10:1296, 1950.
69. FITZGERALD, P.J., An investigation of the ultraviolet absorption of colloid and thyroid epithelium in various types of thyroid carcinoma, Proc. Fifth Internat. Cancer Cong., p. 194, 1950.
70. FITZGERALD, P.J., The zone of localization of antibodies. X. The use of radioactive sulfur-35 as a label for anti-kidney serum, J. Immunol. 65:559, 1950.
71. FITZGERALD, P.J., Staining of radioautographs with metanil yellow and iron hematoxylin, Stain Tech. 26:25, 1951.
72. FITZGERALD, P.J., Thyroid cancer in childhood and adolescence, Cancer 3:1018, 1950.
73. FITZGERALD, P.J., EIDINOFF, M.L., KNOLL, J.E. and SIMMEL, E.G., Tritium in radioautography, Science 114:494, 1951.
74. FITZGERALD, P.J., Radioautography in cancer - a review, Cancer 5:166, 1952.
75. FITZGERALD, P.J., The use of ultraviolet microscopy, x-ray absorption, and radioautographic techniques in the study of neoplastic diseases - a discussion, Cancer 5:643, 1952.
76. FITZGERALD, P.J., BEYER, H.G., WILLIAMS, N.T. and SHERMAN, H., The determination of the mass of histologic structures by x-ray absorption, Amer. J. Path. 28:533, 1952.
77. FITZGERALD, P.J., The use of radioautographic technic in pathology, Bull. N.Y. Acad. Med. 28:680, 1952.

78. FITZGERALD, P.J. and ALVIZOURI, M., Rapid restitution of rat pancreas following acinar cell necrosis subsequent to ethionine, *Nature* 170:929, 1952.
79. FITZGERALD, P.J., SIMMEL, E.G., WEINSTEIN, J. and MARTIN, C., Radioautography: theory, technic and applications, *Lab. Invest.* 2:181, 1953.
80. FITZGERALD, P.J., HELLMAN, L., WEINSTEIN, J., SCHIMMEL, R., The concentration, distribution, and excretion of radioethionine (S-35) in the rat on stock and protein depleted diets - determined by radioactivity counting and radioautography, *Amer. J. Path.* 30:619, 1954.
81. FITZGERALD, P.J. and HELLMAN, L., The organ concentration, cellular distribution, and excretion of S-35 administered as S³⁵-ethionine, *Lab. Invest.* 10:1, 1961.
82. FOX, J.J., VAN PRAAG, D., WEMPEN, I., DOERR, I.L., CHEONG, L., KNOLL, J.E., EIDINOFF, M.L. and BENDICH, A., Synthesis of 5-methylcytosine deoxyriboside and related pyrimidine nucleosides, *Fed. Proc.* 17:222, 1958.
83. FOX, J.J., VAN PRAAG, D., WEMPEN, I., DOERR, I.L., CHEONG, L., KNOLL, J.E., EIDINOFF, M.L., BENDICH, A. and BROWN, G.B., Thiation of nucleosides. II. Synthesis of 5-methyl-2'-deoxy-cytidine and related pyrimidine nucleosides, *J. Amer. Chem. Soc.* 81:178, 1959.
84. FUKUSHIMA, D.K., KRITCHOVASKY, T.H., EIDINOFF, M.L. and GALLAGHER, T.F., Synthesis of radioactive cortisone, *J. Amer. Chem. Soc.* 74:487, 1952.
85. FUKUSHIMA, D.K., BRADLOW, H.L., HELLMAN, L., GALLAGHER, T.F., Peripheral metabolism of hormones on congenital adrenal hyperplasia, *Proc. Conf. on Human Adrenal Cortex*, Glasgow, 1960.
86. FUKUSHIMA, D.K., BRADLOW, H.L., HELLMAN, L., ZUMOFF, B. and GALLAGHER, T.F., Metabolic transformation of hydrocortisone-4-C¹⁴ in normal man, *J. Biol. Chem.* 235:2246, 1960.
87. GALLAGHER, T.F., HELLMAN, L., BRADLOW, H.L., ZUMOFF, B. and FUKUSHIMA, D.K., The effects of thyroid hormones on the metabolism of steroids, *Ann. N.Y. Acad. Sci.* 86:605, 1960.
88. GARRETT, R., TAMUZZI, O., MEURK, M.L., LAUGHLIN, J.S. and SHERMAN, R.S., Dose measurement technics for diagnostic procedures, *Radiology* 71:216, 1958.
89. GARRETT, R. and LAUGHLIN, J.S., A diagnostic x-ray exposure dose chamber, *Health Physics* 2:189, 1959.
90. GEISSELSODER, J., KINGKADE, M.J. and LAUGHLIN, J.S., The radiation chemistry of aqueous ferrous sulphate-benzoic acid solutions, *Radiation Res.* 20:263, 1963.

91. GEISSELSODER, J., KOEPKE, K. and LAUGHLIN, J.S., Calorimetric determination of absorbed dose and $G_{\text{Fe}^{+++}}$ of the Fricke Dosimeter with 10 Mev and 20 Mev electrons, *Radiation Res.* 20:423, 1963.
92. GELBARD, A.S., KIM, J.H. and PEREZ, A.G., Fluctuations in deoxycytidine monophosphate deaminase activity during the cell cycle in synchronous populations of HeLa cells (submitted to *Bioch. Biophys. Acta*).
93. GOODWIN, J.T., et al., Pathological effects and radioautographic localization of I-131 in humans: postmortem study in nine patients, *Cancer* 4:936, 1961.
94. GREENBERG, E.J., PAZIANOS, A., COREY, K.R., KENNY, P., LAUGHLIN, J.S. and PEARSON, O.H., Ca-47 tracer studies in patients with bone lesions, *Cancer, Proc. Conf. on Research on Radiotherapy of Cancer*, p. 158, 1961.
95. GREENBERG, E., WEBER, D.A., KENNY, P.J., POCHACZEWSKY, R., MYERS, W.P.L. and LAUGHLIN, J.S., Detection of neoplastic bone lesions by quantitative scanning and radiography, *Journal of Nuclear Medicine* (in press).
96. HALL, T., Interpretation of exponential dose-effect curves, *Bull. Math. Biophys.* 15:43, 1953.
97. HALL, T., Chemical analysis of radioactive mixtures in biological materials, *Nucleonics* 12:34, 1954.
98. HALL, T., et al., Production of I-24 by the deuteron bombardment of tellurium, *Phys. Rev.* 95:1208, 1954.
99. HALL, T., X-ray spectroscopy, YOE and KOCH, *Trace Analysis*, New York, John Wiley and Sons, Inc., 1957, Chapter 17.
100. HALL, T., X-ray fluoresanalysis in biology, *Science* 134:449, 1961.
101. HAMPTON, A., HAMPTON, E.G., EIDINOFF, M.L., Nucleotides. III. Synthesis and effects with cells in culture of I-131-labeled 5-Iodo-2'-deoxyuridine 5'-phosphate, *Biochem. Pharmacol.* 11:115, 1962.
102. HAMPTON, E.G., RICH, M.A. and EIDINOFF, M.L., Introduction of the 5-iodouracil moiety into DNA of mammalian cells, *J. Biol. Chem.* 235:3562, 1960.
103. Hampton, E.G. and EIDINOFF, M.L., Administration of 5-iodo-deoxyuridine-I-131 in the mouse and rat, *Cancer Res.* 21:345, 1961.
104. HAMPTON, E.G. and EIDINOFF, M.L., Transformation of chick embryo cells in culture by Rous sarcoma virus histochemical studies, Abstract appears in *Fed. Proc.* 20:153, 1961. Presentation before Amer. Assoc. Biol. Chem., April, 1961.

105. HAMPTON, E.G. and EIDINOFF, M.L., Transformation of chick embryo cells in culture by Rous sarcoma virus - cytochemical studies, *Cancer Res.* 22:1061, 1962.
106. HELLER, I.M., KOPAC, J.J. and SHARPE, L.M., Cytochemical reactions of normal and starving amoeba proteus. I. Localization of thiols, DNA, RNA and other organic phosphates, Congress of the International Society for Cell Biology, Leyden (Netherlands), 1954.
107. HELLER, I.M., et al., Cytochemical localization in normal and starving amoeba proteus, Congress of the International Society for Cell Biology, Leyden (Netherlands), 1954.
108. HELLER, I.M. and SIMMEL, E.B., Alkaloid B-72 as a protective film between specimen and emulsion in radioautography.
109. HELLMAN, L. and EIDINOFF, M.L., Dynamic aspects of carbon dioxide and urea metabolism in the human studied with radioactive carbon, *J. Clin. Invest.* 31:638, 1952.
110. HELLMAN, L., ROSENFELD, R., FUKUSHIMA, D., EIDINOFF, M.L., BRUNSWIG, A. and GALLAGHER, T.F., The major pathways of steroid metabolism in man, *J. Clin. Invest.* 32:557, 1953.
111. HELLMAN, L., FRAZELL, E.L. and ROSENFELD, R.S., Direct measurement of cholesterol absorption via the thoracic duct in man, *J. Clin. Invest.* 39:1288, 1960.
112. HELLMAN, L., BRADLOW, H.L., ZUMOFF, B., FUKUSHIMA, D.K. and GALLACHER, T.F., The influence of thyroid on steroid metabolism. A "thyromimetic" effect of androsterone, Proc. Fourth International Goitre Conference, London, 1960.
113. HELLMAN, L., BRADLOW, H.L., ZUMOFF, B., FUKUSHIMA, D.K. and GALLACHER, T.F., Thyroid-androgen interrelations and the hypocholesteremic effect of androsterone, Proc. First Internat. Cong. Endocrin, Copenhagen, 1960.
114. HEMS, G. and EIDINOFF, M.L., Effect of x-radiation on aqueous solutions of adenosine diphosphate, *Rad. Res.* 9:305, 1958.
115. HENSCHKE, U. and BRASFIELD, R.D., Intravascular irradiation of the internal mammary lymph nodes in breast cancer, *Amer. J. Roentgenol.* 85:849, 1961.
116. HENSCHKE, U. and COLE, D.R., Local cancer chemotherapy, collective review, *Internat. Abst. Surg.* 112:513, 1961, (Bound with Surg., Gynec., and Obst.)

117. HENSCHKE, U. and BRASFIELD, R.D., New technique for treating internal mammary node metastases in breast cancer, N.Y.S.J. Med. 61:3817, 1961.
118. HOLODNY, E., LECHTMAN, H. and LAUGHLIN, J.S., Bone marrow dose produced by radioactive isotopes, Radiology, 77:1, 1961.
119. HOLODNY, E.I., RAGAZZONI, G.D., BRONSTEIN, E.L. and LAUGHLIN, J.S., Patient effective thickness contour measurement, Presented at 49th Ann. Meeting Radiation Society of North America, 1963, Radiology, 82:131, 1964.
120. KABAT, E.A., BERG, D., RITTENBERG, D., PONTECORVO, L., EIDINOFF, M.L. and HELLMAN, L., Precipitation of C^{14} -labeled dextran by human antidextran, J. Amer. Chem. Soc. 76:564, 1954.
121. KARNOSFSKY, D.A. and SIMMEL, E.B., Effects of growth inhibiting chemicals on the sand dollar embryo, Echinarachnium parma, Progr. Exptl. Tumor, Res. 3:254, 1963.
122. KESSARIS, N.D., Calculated absorbed dose for electrons, Radiation Res. 23:630-640, 1964.
123. KESSARIS, N.D., Penetration of high energy electron beams in water, Physical Review, 145:164-174, 1966.
124. KESSARIS, N.D., LAUGHLIN, J.S., Relation between absorbed dose and cavity ionization for high energy electron beams in water and carbon (to be published).
125. KENNY, P.J., LUNDY, A. and LAUGHLIN, J.S. Analysis of digital Anger camera with three dimensional resolution for positron emitters. Trans - N.Y. Acad. Sci. 28:628-634, 1966.
126. KENNY, P.J., LAUGHLIN, J.S., WEBER, D.A., COREY, K.R. and GREENBERG, E. High-energy, gamma-ray scanner in Kniselye, R.M., Andrews, G.A., and Harris, C., (eds.) Symposium on Progress in Med, Radioisotope Scanning, Oak Ridge Institute of Nuc. Studies, TID 7673, 236-250, 1962.
127. KIM, J.H., EIDINOFF, M.L. and LAUGHLIN, J.S., The effect of 5-bromodeoxyuridine on the recovery phenomenon in sub-lethally irradiated cells, Exptl. Cell Res. 36:411, 1964.
128. KIM, J.H. and STAMBUK, B.D., Synchronization of HeLa cells by vinblastine sulfate, Exptl. Cell Research 44:631-634, 1966.
129. KIM, J.H., GELBARD, A.S. and PEREZ, A.G., Inhibition of DNA synthesis by actinomycin D and cycloheximide in synchronized HeLa cells, Cell Research, (in press).
130. KIM, S.H. and KIM, H.H., Effects of X-irradiation on RNA and protein synthesis in HeLa cells (in preparation).

131. KIM, J.H., GELBARD, A.S., PEREZ, A.G. and EIDINOFF, M.L., Effect of 5-bromodeoxyuridine on nucleic acid and protein synthesis and viability in HeLa cells, *Biochem. Biophys. Acta* 134:388-394, 1967.
132. KIM, J.H., GELBARD, A.S. and PEREZ, A.G., Action of hydroxyurea on the nucleic acid metabolism and viability of HeLa cells, *Cancer Research* 27:1301-1305, 1967.
133. KIM, J.H., GELBARD, A.S., DJORDJEVIC, B., KIM, S.H. and PEREZ, A.G., Action of daunomycin on the nucleic acid metabolism and viability of HeLa cells, *Cancer Research* (in press).
134. KIM, J.H. and EIDINOFF, M.L., Action of 1- β -d-arabinofuranosylcytosine on the nucleic acid metabolism and viability of HeLa cells, *Cancer Research* 25:698-702, 1965.
135. KIM, J.H., EIDINOFF, M.L. and FOX, J.J., Action of 1- β -d-arabino-furanosylcytosine 5-fluorocytosine on the nucleic acid metabolism and viability of HeLa cells, *Cancer Research* 26:1661-1664, 1966.
136. KIM, J.S., KIM, S.H. and EIDINOFF, M.L., Cell viability and nucleic acid metabolism after exposure of HeLa cells to express thymidine and deoxyadenosine, *Biochem. Pharmacol.* 14:1821-1829, 1965.
137. KIM, J.H., PEREZ, A.G. and DJORDJEVIC, B., Studies on unbalanced growth in synchronized HeLa cells, *Cancer Research* (in press).
138. KIM, J.H., EIDINOFF, M.L. and LAUGHLIN, J.S., Recovery from sub-lethal X-ray damage of synchronized HeLa cells during inhibition of protein synthesis, *Int. J. Rad. Biol.* 11:No. 5, 509-511, 1966.
139. KIM, J.H., PINKERTON, A. and LAUGHLIN, J.S. Studies on the biological effectiveness of high-energy electron beams at different depths in bone-absorbing material, *Rad. Res.* 33:419-425, 1968.
140. KIM, J.H., BOYSE, E.A., OLD, L.J. and CAMPBELL, R.A., Inhibition of HeLa cell cultures by preparation of *E. coli* L-asparaginase, *Biochem. Biophys. Acta* 158:476-479, 1968.
141. KIM, J.H., EIDINOFF, M.L. and LAUGHLIN, J.S., Recovery from sub-lethal x-ray damage of mammalian cells during inhibition of DNA synthesis, *Nature* 204:598, 1964.
142. KRITCHEVSKY, D., McCANDLESS, R.F.J., KNOLL, J.E. and EIDINOFF, M.L., Triclein-H₃, *J. Amer. Chem. Soc.* 77:6655, 1955.
143. LAUGHLIN, J.S., MEURK, M.L., PULLMAN, I. and SHERMAN, R.S., Bone, skin and gonadal doses in routine diagnostic procedures, *Amer. J. Roent. Rad. Ther. and Nuc. Med.* 78:961, 1957.

144. LAUGHLIN, J.S. and SHERMAN, R.S., Radiation exposure incidental to medical practice, Bulletin of the Atomic Scientists 14:41, 1958.
145. LAUGHLIN, J.S., Dosage and biological effects of external radiation sources, A.M.A. Arch. Indus. Health 18:102, 1958.
146. LAUGHLIN, J.S., Medical applications of radioactive isotopes, Medical Tech. Bull. 10:151, 1959.
147. LAUGHLIN, J.S., BEATTIE, J.W., COREY, K., ISAACSON, A. and KENNY, P.J., Total body scanner for high energy gamma-rays, Radiology 74:108, 1960.
148. LAUGHLIN, J.S., Ionization chambers for diagnostic x-ray exposure studies in: Janower, M.L., (ed.), Technological Needs for Reduction of Patient Dosage from Diagnostic Radiology, Springfield, Illinois, Charles, C. Thomas, 1963, p. 11.
149. LAUGHLIN, J.S., SILER, W.S., HOLODNY, E.I. and RITTER, F.W., A dose description system for interstitial radiation therapy: I. seed implants, Amer. J. Roentgenol. 89:470, 1963.
150. LAUGHLIN, J.S., WEBER, D.A., KENNY, P.J., COREY, K.R. and GREENBERG, E., Total body scanning, Brit. J. Radiology, 37:286-296, 1964.
151. LAUGHLIN, J.S., Accelerators: High Energy; betatron, Medical Physics (in press).
152. LAUGHLIN, J.S., KENNY, P.J., COREY, K.R., GREENBERG, E., and WEBER, D.A., Localization and total body high-energy gamma-ray scanning studies in cancer patients, Symp. on Med. Radioisotope Scanning, Proc. 1: IAEA, STI/PUB/82, Vienna, 253-272, 1964.
153. LAUGHLIN, J.S., KENNY, P.J., WEBER, D.A., DWYER, A.J., MAYER, K., GREENBERG, E., DIMICH, A. and HASAN, S., Methods and applications of quantitative computer-analyzed scanning, Proc. of a Symp. on Med. Radioisotope Scintigraphy, International Atomic Energy Agency, Salzburg, Austria, August 6-15, 1968 (to be published).
154. LAUGHLIN, J.S., WEBER, D.A., BENUA, R.S., KENNY, P.J. and RITTER, F., Quantitative storage, analysis and display in digital scanning, Proc. of Symp. on Fundamental Problems in Scanning, Society of Nuc. Med. Midwestern Chapter, Chicago, Illinois, May, 1965, (in press).
155. LAUGHLIN, J.S., Physical aspects of high energy electron therapy, Amer. J. of Roentgenol. 99:915-923, 1967.
156. LEWALLEN, C.G., RALL, J.R., BERMAN, M. and HAMEL, H., The effects of thyroid hormone on the distribution kinetics and degradation of iodo-albumin, J. Clin. Invest. 34:949, 1955.

157. LEY, A. and SHARPE, L.M., The absorption of vitamin B-12 after gastrectomy, Clin. Res. Proc. 2:31, 1954.
158. MILVY, P., BARR, N.F., GEISSELSODER, J. and LAUGHLIN, J.S., The calorimetric determination of local absorbed dose, LXth Internat. Cong. Radiol, Munich, II:1348, 1961.
159. MOOKERJEE, A., VAN DYKE, J.G. and LAUGHLIN, J.S., The relative biological efficiency of 20 Mev electrons and 250 kvp x-rays in *Saccharomyces cerevisiae* inactivation, Rad. Res. 22:431, 1964.
160. MOROSON, H. and ALEXANDER, P., Crosslinking of deoxyribonucleic acid to protein following ultraviolet irradiation of different cells, Nature 194:882, 1962.
161. MOROSON, H., A chemically produced radiation lesion, crosslinking of DNA by hypochlorite, Proc. III Internat. Cong. Chemotherapy, Stuttgart, Germany, George Thieme Verlag, 1964.
162. MOROSON, H. and ALEXANDER, P., Crosslinking of DNA in irradiated cells in Ebert, M. and Howard, A., (eds.), Radiation Effects in Physics, Chemistry and Biology, Amsterdam, Holland, North Holland, Pub. Co., 1963.
163. MOROSON, H. and LAUGHLIN, J.S., Fluorescence intensity studies with the benzoic acid dosimeter, in Ebert, M., and Howard, A., (eds.), Radiation Effects in Physics, Chemistry and Biology, Amsterdam, Holland, North Holland Pub. Co., 1963.
164. MOROSON, H. and GREGORIADES, A., A sensitive chemical actinometer for ultraviolet radiation, Nature 204:676, 1964.
165. MOROSON, H., A chemically produced radiation lesion - crosslinking of DNA by hypochlorite, Proc. of IIIrd Internat. Cong. Chemotherapy II, George Thieme Verlag, 1964.
166. MOROSON, H. and FURLAN, M., Can N-ethylmaleimide preferentially radiosensitize hypoxic tumor cells in vivo? Int. J. of Rad. Biol. (in press).
167. MOROSON, H., SCHMIDT, M. and FURLAN, M., Enhancement of murine ascites tumor cell killing with X-irradiation by thiol binding agents, Rad. Res. (in press).
168. MOROSON, H. and FURLAN, M., Effects on DNA strand breakage and repair in vitro by the radiosensitizer methyl hydrazine (to be submitted to Science).
169. MYERS, M.J., KENNY, P.J., LUNDY, A. and LAUGHLIN, J.S., Quantitative analysis of scintillation camera data, Nucleonics 24:58-60, 1966.

170. OETTGEN, H.F., OLD, L.J., BOYSE, E.A., CAMPBELL, H.A., PHILIPS, F.S., CLARKSON, B.D., TALLAL, L., LEEPER, R.D., SCHWARTZ, M.K. and KIM, J.H., Inhibition of Leukemias in man by L-asparaginase, Cancer Research, 27:2619-2631, December, 1967.
171. OWEN, D., et al., Some observations on the distribution of radioactive zinc in prostatic and other human tissues, Urology 28:271, 1956.
172. PULLMAN, I., Experimental Methods, Chapter 1.1 of section 1 in Reactor Physics in the Reactor Handbook, 1-Physics, 1st ed. U.S. Atomic Energy Commission, AEC 3645, 1955 (Also published by McGraw Hill).
173. TERMINE, J.D., PULLMAN, I. and POSNER, A., Electron spin resonance study of irradiated bone and its constituents, Arch. of Biochem. and Biophys. 122:318-330, 1967.
174. MILVY, P. and PULLMAN, I., ESR studies of spin transfer in irradiated nucleic acid-cysteamine systems, Rad. Res. 34:265-285, 1968.
175. RACHELE, J.R., KUCHINSKAS, E.J., KNOLL, J.E. and EIDINOFF, M.L., Isotopic selection in neogenesis of labile methyl groups from monodeuterio-, monotrityl-, C-14 labeled methanol, J. Amer. Chem. Soc. 76:4342, 1954.
176. RACHELE, J.R., KUCHINSKAS, E.J., KNOLL, J.E. and EIDINOFF, M.L., Hydrogen isotope effects in the *in vivo* utilization of formate, Arch. Biochem. Biophys. 81:55, 1959.
177. RICH, M.A., BOLAFFI, J.L., KNOLL, J.E., CHEONG, L. and EIDINOFF, M.L., Growth inhibition of a human tumor cell strain by 5-fluorouracil, 5-fluorouridine, and 5-fluoro-2'-deoxyuridine - Reversal studies, Cancer Res. 18:730, 1958.
178. RICH, M.A., STONEHILL, E.H. and EIDINOFF, M.L., Growth inhibition of chick embryo cells by some 5-fluorinated pyrimidines - Effect on Rous sarcoma virus infection, J. Nat. Cancer Inst. 25:1355, 1960.
179. RICH, M.A., PEREZ, A.G. and EIDINOFF, M.L., Effects of several nucleic acid analogs on chick embryo fibroblasts and cells of human tumor origin, Bacteriologist Proc. p. 154, 1964 (abstract) Presentation before Amer. Soc. Bacteriologists, 1961.
180. RICH, M.A. and EIDINOFF, M.L., Effects of several purine analogs on the growth of mammalian cells in culture, Pathologie-Biologie 9:600, 1961.
181. RICH, M.A., PEREZ, A.O. and EIDINOFF, M.L., Effect of 5-fluorodeoxyuridine on the proliferation of Rous sarcoma virus 16:98, 1962, Virology.
182. RICH, M.A., PEREZ, A.G. and EIDINOFF, M.L., The effects of some purine analogs on the growth of H. Ep. #1 and chick embryo fibroblast cells, Cancer Res. 22:3, 1962.

183. ROSOFF, M., BATURAY, G., EIDINOFF, M.L. and CAVALIERI, L.F., A study of the x-irradiation of deoxypentose nucleic acid solutions, *Rad. Res.* 3:592, 1956 (abstract).
184. SCHOENFELD, R. and BERMAN, M., An electrical network analogy for isotope kinetics, Presentation to the Medical Electronics Session, IRE Nat. Conv., 1957, Publ. in the Conv. Record, 1957, Part 4, 84, IRE, New York.
185. SHARPE, L.M., KRISHNAN, P.S., KLEIN, J.R., Uptake by duck erythrocytes of iron added to blood, *Arch. Biochem. Biophys.* 35:409, 1952.
186. SHARPE, L.M. and SIMMEL, E.B., Radioautographic demonstration iron-59 in duck reticulocytes.
187. SILER, W.M. and LAUGHLIN, J.S., A computer method for radiation treatment planning, Communication of the Assoc. for Computing Machinery, 5:407, 1962.
188. SILER, W.M., SNYDER, R.E., GARRETT, R., LAUGHLIN, J.S. and SHERMAN, R.S., Development and use of a mammographic technique, *Amer. J. Roentgenol.* 91:910, 1964.
189. SILER, W.M., RITTER, F. and LAUGHLIN, J.S., Patient stimulation in x-ray therapy, *Ann. N.Y. Acad. Sci.* 115:1038, 1964.
190. SIMMEL, E.B., The use of a fast, coarse-grain stripping film for radioautography, *Stain Tech.* 32:299, 1957.
191. SIMMEL, E.B. and KARNOFSKY, D.A., Observations on the uptake of tritiated thymidine in the pronuclei of fertilized sand dollar embryos, *J. Biophys. Biochem. Cytol.* 10:59, 1961.
192. VAN DYKE, J.G., The inactivating effects of x-rays and incorporated radioactive phosphorus and their modification by oxygen and other substances, *Radiology* 78:91, 1962.
193. VERLY, W.G. and EIDINOFF, M.L., KNOLL, J.E., A test of tritium as a labeling device in a biological study, *J. Amer. Chem. Soc.* 74:5941, 1952.
194. WEBER, D.A., KENNY, P.J., POCHACZEWSKY, R., LAUGHLIN, J.S. and CORY, K.R., Liver scans with digital readout, *J. of Nuc. Med.* 6: 528-530, July, 1965.
195. WEBER, D.A., GREENBERG, E.J., DIMICH, A., KENNY, P.J., ROTHSCHILD, E.O., MYERS, W.P.L. and LAUGHLIN, J.S., Kinetics of radionuclides used for bone scanning, *J. of Nuc. Med.* (in press).
196. WOODARD, H.Q. and SPIERS, F.W., The effect of x-rays of different qualities on the alkaline phosphatase of living mouse bone, *Brit. J. of Radiol.* 26:38, 1953.

197. WOODARD, H.Q., Some effects of roentgen rays on mouse bone, Rad. Res. 1:567, 1954.
198. WOODARD, H.Q., Comparison of the loss of alkaline phosphatase produced in mouse bone by x-rays of lower voltages, Rad. Res. 3:359, 1955.
199. WOODARD, H.Q. and PHILLIPS, R.F., The mineral content of bone tumors and its influence on radiation tumor dose, Amer. J. Roent. Rad. and Nucl. Med. 78:109, 1957.
200. WOODARD, H.Q., Some effects of x-rays on bone, Clin. Orthopaed 9:118, 1957.
201. WOODARD, H.Q. and LAUGHLIN, J.S., The effect of x-rays of different qualities on the alkaline phosphatase of living mouse bone, II, Effects of 22.5 Mev x-rays, Rad. Res. 7:236, 1957.
202. WOODARD, H.Q., Time relationships in the appearance of radiation damage in mouse bone, Radiation Res. 9:204, 1958.
203. WOODARD, H.Q. and GLICKSMAN, A.S., The effect of 3,5, 3' L-tri-iodothyronine (3-3) on the response of mouse bone to x-radiation, Rad. Res. 11:478, 1959.
204. DZIEWIATKOWSKI, D.D. and WOODARD, H.Q., Effect of irradiation with x-rays on the uptake of S-35 sulfate by the epiphyseal cartilage of mice, Lab. Invest. 8:205, 1959.
205. WOODARD, H.Q. and HOLODNY, E., A summary of the data on mechanic on the distribution of human bone marrow, Physics in Med. and Biol. 5:57, 1960.
206. WOODARD, H.Q. and HILJINBOTHAM, N.L., Osteogenic sarcoma developing in a radium dial painter 37 years after the end of exposure, Amer. J. Med. 32:96, 1962.
207. WOODARD, H.Q., The effects of x-rays on the composition of mouse bone, Second International Cong. Rad. Res., 1962, p. 216 (abstract)
Manuscript available at the Atomic Energy Documentation Center.
208. WOODARD, H.Q., The elementary composition of human cortical bone, Health Physics 8:513, 1962.
209. WOODARD, H.Q., A discussion of the hazards of ingested strontium-90, Proc. Cong. Scientists on Survival, 1963, New York.
210. WOODARD, H.Q., The composition of human cortical bone, II. Effect of age and of some abnormalities, Clin, Orth. 37:187, 1964.
211. WOODARD, H.Q. and HARLEY, J.H., Strontium-90 in the long bones of patients with sarcoma, Health Physics 10:596, 1964.
212. ZEITZ, L. and LEE, R., Bromine analysis in 5-bromouracil-labeled DNA by x-ray flourescence, Science 142:1670-1673, 1963.

Summary of Activities 1950 - 1971

related to

AEC Contract AT(30-1)910

(Proposal #5)

CHEMICAL AND BIOLOGICAL STUDIES ON NUCLEIC ACIDS AND DERIVATIVES

Co-investigators: George B. Brown, Ph.D. Section 5701
Aaron Bendich, Ph.D. Section 7701

and

(Proposal #6)

PHYSICAL-CHEMICAL STUDIES ON NUCLEIC ACIDS AND DERIVATIVES

Co-investigators: Mary L. Petermann, Ph.D. Section 5702
Liebe F. Cavalieri, Ph.D. Section 6601

I. DIVISIONAL SUMMARY

Main Research Accomplishments

1950-1971, Division 5700, Biological Chemistry

George B. Brown, Ph.D., Head

(Proposal #5, Drs. Brown, Bendich)

(Proposal #6, Drs. Petermann, Cavalieri)

The following is a condensed summary of the lines of investigation by members of Division 5700 - Biological Chemistry, formerly Nucleoprotein Chemistry, who received support from AEC Contract AT(30-1)910 during the period 1950-1971 and draws heavily upon previous summary reports. The Divisions which were formed with former personnel of 5700 (Division 6600, Genetics, Dr. L.F. Cavalieri and Division 7700, Cell Biochemistry, Dr. A. Bendich) each summarize their activities since those divisions were initiated.

Our experiments with ^{15}N -adenine (26, 72) provided the first demonstration of the incorporation of an organic compound into nucleic acids. During several years a systematic survey was made of the metabolism of many related purines, pyrimidines, their nucleotides and nucleosides. These were accompanied by comparisons of the utilizations and interconversions of purines by various tissues and by numerous species. Studies with ^{15}N were followed by others with ^{14}C and ^{32}P , and with double and triple labeling (112, 124, 125, 144). The demonstrations that the purine ring is incorporated intact, and that pyrimidine ribosyl derivatives are utilized, and are reduced to deoxyribosyl derivatives without cleavage of the glycosyl linkage, furnished the background for many metabolic studies and chemotherapeutic trials. Most of those results are now "textbook" information.

The "chloromercuri method" for the synthesis (66) of purine nucleosides (U.S. Patent 2,719,843, assigned to the AEC) (134) was developed, and extended to pyrimidine nucleosides (141). The method has been used in many laboratories, and several recent improvements with newer aprotic dipolar solvents, silazanes, etc., still retain the use of a mercury salt. It permitted the incorporation of isotopes, and syntheses of many naturally occurring compounds, and of analogs for chemotherapeutic trials. Such work is still being carried on by Division 6-00 (Dr. J.J. Fox) with primary support by the National Cancer Institute.

The knowledge of the metabolism of purines and certain of their analogs and of the pharmacological effects of 2,6-diamino-purine (30) and of other purine derivatives stimulated the synthesis of hundreds of purine analogs in scores of laboratories for chemotherapeutic approaches to cancer control. Among those, 6-mercaptopurine and thioguanine (synthesized by the Wellcome

Laboratories) are still the only ones in wide clinical use for the treatment of leukemia. The chemotherapeutic possibilities of purine analogs have been repeatedly reviewed, most recently in "Antagonists of Nucleic Acids", by M.E. Balis. Metabolic studies in microorganisms, resistant mutants and mammals, have revealed much information about the mechanisms of action in vivo of purine analogs. Such work continues in Division 5800 (Dr. M.E. Balis) with primary support by the National Cancer Institute.

Syntheses of mononucleotides of purine (173), of 6-mercaptopurine, of 6-chloropurine, and thioguanine (245) and 6-succinomeraptopurine (the S analog of succinoadenylic acid) enabled demonstrations that one of the in vivo effects of 6-mercaptopurine involve the formation of a nucleotide and then inhibitions of two steps of adenylic acid synthesis, and one of guanylic acid synthesis. The succinomeraptopurine nucleotide is not only an inhibitor of adenylosuccinase but is also cleaved by it, which supports the possibility that purine analogs may be metabolized into "active intermediates" more complex than mononucleotides.

Organic chemical techniques for nucleoside syntheses (66, 81, 106, 141, 198, 199), isopropylidene derivatives (172), thiations of nucleosides (181, 207, 268), and nucleotide syntheses (173, 259) have been derived from these works. Many new compounds synthesized in the course of such investigations have proven useful in this and other laboratories for studies of inhibitory activities, enzyme susceptibilities, identifications, spectra (33, 56, 113), etc. Proof of structure by synthesis has been offered for several natural nucleosides (70, 78, 106, 110, 141, 170, 207), and several nucleosides first synthesized here as potential antimetabolites were subsequently found in nature, most notably, all of the major methylated nucleosides of the tRNA's.

Studies of the relationship of structure to biological or pharmacodynamic activities and chemical properties received major attention. These have included an explanation of the toxicity of adenine (51) and its monohydroxy derivatives. The structural features of adenosine analogs, which are necessary for vasodepressor activity, were explored (81, 92), and it was also found that 2-substitutions which greatly enhance it also prevent the action of adenosine deaminase. The marked toxicity of ribosylpurine (deaminoadenosine) in contrast to the parent purine was investigated and a preferential anabolism to nucleotides which may antagonize AMP, ADP and ATP was found (146, 147, 174, 186). A previously unrecognized lability in alkali of the imidazole ring of purine nucleosides which do not bear ionizable substituents on the pyrimidine ring was investigated in detail (171, 172, 173).

Early results with ¹⁵N labeled yeast nucleic acid suggested that pyrimidine nucleotides were metabolized differently from free pyrimidines, and with double and triple labels it was shown (196, 143, 144, 163) that the cytidine unit is utilized without cleavage of the glycosyl bond and is converted directly to deoxyribosyl derivatives.

The use of cytidine-2-¹⁴C-ribosyl-H³ (199) has shown that carbonyl or unsaturated derivatives cannot be intermediates (286), and 2,2'-anhydro(cyclo)-cytidine was considered as a possible intermediate. Studies of blocking agents useful in its synthesis reached the stage of a 5'-benzyloxycarbonyl-2'-tosylcytidine, but the synthesis is not readily adaptable to isotope incorporation, and enzyme studies by others (Reichard, Blakely) have not tended to implicate such an intermediate.

Differences in the incorporations of adenine and smaller precursors under various conditions suggested that the theoretically necessary inhomogeneity of nucleic acids could be experimentally approached (88, 100, 194). Fractionations were achieved and the necessary characterizations involved isotopes and biological (transforming) activities as labels. Modern techniques have gone far beyond those initial fractionations. Improvements in the handling of biologically active DNA preparations (261, 346, 382) led to the demonstration of active DNA from the Stewart-Eddy polyoma virus (233). Current approaches to the chemical basis of such activities and to the transfer of information between cells (307, 404) continue in Division 7700 (Dr. A. Bendich).

During the 1950's a series of studies were made of the physical chemical properties of nucleic acids. Dye-binding studies (63, 76, 154) suggested triple H-bonding between G and C units. An apparatus for flow-dichroism was designed, and the use of shear forces for the cleavage of DNA molecules was introduced (227, 224, 240). Other physicochemical techniques were instituted (247). These have recently been applied to correlate the physical with the biological structure. Evidence suggesting a direct replication of the double-helix of DNA, (248, 249, 250, 253, 258) and of RNA acting as a template for DNA-synthetase was reported (318, 361). Studies of the enzymatic activities of fractions (337, 353, 426) obtainable from DNA-synthetase are reported by Division 6600 (Dr. L.F. Cavalieri).

The cytoplasmic ribonucleoprotein particles from mammalian tissue, as they were first called, or the "ribosomes", have been extensively studied. The major and minor components have been carefully characterized, both by physico-chemical methods and for retention of biological properties. Recombined "ribosomes" active in protein synthesis are now possible. Studies of their proteins and nucleic acids under various chemical and physiological conditions have been undertaken. These are reviewed in "The Ribosomes" by M.L. Petermann. Recent developments are summarized in Proposal #6 (Section 5702, Dr. M.L. Petermann).

Initial approaches to the synthesis of pseudouridine, (ψ), the chemistry of which is no doubt important to its unelucidated structural role in tRNA's, have resulted in a "lactone approach" to the synthesis of 5-hydroxyalkyl-pyrimidines (330). That was extended to 5-(2'-hydroxycyclopentyl)- (378) and to 5-(2', 3'-dihydroxycyclopentyl)-pyrimidines (411). Successful conversion of a 2-deoxysugar lactone to an α -formyl derivative and studies of carbohydrate derivatives which could lead to the necessary 2-deoxyheptulonolactone show promise of leading to 5-glycosylpyrimidines analogous to pseudouridine, if not to the appropriate isomerism for pseudouridine itself.

Studies of the infrared spectra of the several 5-hydroxyalkyluracils revealed that extensive intramolecular hydrogen bonding occurs in all the compounds studied. The hypothesis has been advanced that in compounds such as 5(2'-hydroxyethyl)- and 5(2'-hydroxycyclopentyl) uracil the hydrogen bond occurs between the side chain and the C-4 carbonyl of the pyrimidine ring, while in compounds like 5-hydroxymethyluracil the pyrimidine π -electrons or the C-5, C-6 double bond acts as the hydrogen bond receptor site. This hypothesis can account for the differences in the ultraviolet absorption properties observed in the above two groups of 5-substituted uracils (411, Chambers).

To further establish the extent to which the inductive effect of individual groups influences the hydrogen bonding properties of 5-hydroxyalkylpyrimidines, the 5(2'-hydroxytetrahydrofuranyl)- and 5-tetrahydrofuranyl derivatives of uracil, and 5'-hydroxyalkylpyrimidines lacking the 4-carbonyl have been prepared. The natural 5'-carboxyethyluridine has been synthesized (507).

Explorations of the chemistry of adenine led (191, 192) to an N-1-oxide with many unusual chemical behaviors. Several facets of purine N-oxide chemistry have since been investigated. These were reviewed in "Progress in Nucleic Acids" (434). In brief, studies have been reported on the effect of other substituents on the stability and reactivity (273), on the activating effect of an N-oxide on other substituents (301), on reactions with acetic anhydride (236), and on several oxides of adenine nucleotides (212), azaadenines (238), and others (239).

Cytosine and cytidine were oxidized to 3-N-oxides; these undergo a facile rearrangement at room temperature to a 2-hydroxy-4-hydroxylamino-pyrimidine (360). A rearrangement of amino-¹⁵N-cytosine, wherein the exocyclic amino group equilibrated with the N-3 of the ring in hot acetic anhydride, was also found (341). Counterparts of these "Dimroth-like" rearrangements were also encountered as a second reaction pathway in syntheses of 1-hydroxyxanthine from 1-hydroxyisoguanine (400). The pyrimidine ring of adenine 1-oxide is readily opened (192) and reclosure with various reagents permits the synthesis of several 2-substituted adenine 1-oxides (301). Deamination of adenine 1-oxide to 1-hydroxyhypoxanthine can be accomplished (373). Guanine was not oxidized until trifluoroacetic acid (in which it is solubilized) was used with H₂O₂ (344, 368). That product was hydrolyzed to a xanthine N-oxide. These were first termed X-N-oxides (344), later, erroneously, 7-N-oxides (368), and were finally proven, by degradation (468) and synthesis (342, 468), to be 3-N-oxides. Details of their chemistry (469, 470) are outlined in the Recent Developments section of this report.

In the course of studies of total syntheses (474, 475, 478) of purine N-oxides, a general synthesis for N-hydroxy- α -aminoacids was devised (392, 393, 420).

Attention was first focused on the purine N-oxides as antimetabolites since the O substitution on the N represented a new blocking group on a potential binding site. No significant tumor inhibitory activity was observed with over 30 derivatives over a period of several years.

Since most purine N-oxides are less toxic than the parent purine, and since it was found that the N-oxide function was reduced in vivo, attempts were made to prepare N-oxides of therapeutically useful purines, in hopes they would be less toxic "reservoir doses" and would be converted to the active agent in vivo. Slight success was obtained with 6-methylpurine-1-oxide (273) and with 6-mercaptopurine 3-N-oxide (343). The 6-mercaptopurine-3-oxide was obtained by an indirect synthesis (305) in which the N-oxide function was introduced into 7-aminothiazolo[5,4-d]pyrimidine, the thiazole then opened and an imidazole closed. (U.S. Patent 3,312,702, April 4, 1967, to the AEC with approval of the USPHS).

Administered (343) intraperitoneally to mice at 500 mg/kg (daily for one week), it was 10-fold less toxic than 6-mercaptopurine (343); long-term chronic toxicities (399, 434) show a difference of nearly 20-fold. Despite the lower toxicity when parenterally administered, the oral toxicities are similar; the different relative parenteral and oral toxicities are associated with different metabolic fates by the two routes.

The tumor inhibitory effects on S180 transplants during a week of treatment with 6-MP-3-oxide intraperitoneally administered (500 to 12.5 mg/kg/day) are identical with those of 10-fold lower doses (50 to 1.5 mg) of 6-MP. The rate of recovery (complete regressions) was higher (69% vs. 52%) with the N-oxide.

Repeated injections of 500 or 250 mg/kg/day of 6-MP-3-oxide produce a partial to complete inhibition of growth of 18 of 21 solid mouse or rat tumors, results quite similar to those obtained with 30 mg levels of 6-MP, and support the assumption that the antitumor activity is the result of the conversion of some of it to 6-MP (344, 399, and K. Sugiura, Cancer Chemother. Repts. 1, 38 (1968)). In tests by the Cancer Chemotherapy National Service Center, 6-MP-3-N-oxide, like 6-MP, was highly effective against Walker Carcinoma 256 but at 10-fold the dosage, with 90% "cures" of Adenocarcinoma 755 and up to 150% prolongation of life with lymphoid Leukemia L 1210, but in the 6-MP resistant strains of these two tumors it was ineffective.

Although up to 2 gm/day were administered intravenously to man without apparent toxicity, the large dosage necessary by a parenteral route, together with the greater ease of oral administration of 6-MP to man, discouraged further clinical trial (D.A. Karnofsky and I.H. Krakoff, unpublished).

In 1965 (344) it was found that 3-hydroxyxanthine and 3-hydroxyguanine induced tumors in rats, and since then attention has focused on the oncogenicity of these purine derivatives.

Thus far 3-hydroxyxanthine and 3-hydroxyguanine are the most potent (399, 494); adenine 1-oxide and 1-hydroxyxanthine (400) require about 100-fold larger doses. A recent assay, with smaller and more frequent doses (494.5), of 3-hydroxyxanthine resulted in a 65% tumor incidence at the site of injection (intrascapular) of 660 µg per rat, a potency in the range of the most oncogenic arylamines and hydrocarbons.

After subcutaneous or intraperitoneal administration of 3-hydroxyxanthine (479) or 3-hydroxyguanine (476), each is rapidly metabolized: 80% within 8 hours as urinary metabolites. Most of the products are the results of oxidations or reductions catalyzed by xanthine oxidase (206, 477, 479, 476).

Xanthine oxidase catalyzes oxidation at C-8 to yield 8-hydroxyguanine 3-oxide, and of 3-hydroxyxanthine to the analogous 3-hydroxyuric acid. Xanthine oxidase also catalyzes reduction of the N-oxide function (477). This has been demonstrated in vitro under anaerobic conditions for numerous purine N-oxides. Both guanine and its usual metabolites, and 8-hydroxyguanine, plus some of the 3-N-oxides of each, appear in the urine (479). 3-Hydroxyxanthine leads to an analogous series of products in the urine (479), as did an earlier study of adenine 1-oxide (213).

The major urinary metabolites do not now appear to be related to the process of oncogenicity, but are considered to be detoxification products.

Quantitatively, the xanthine oxidase induced metabolites represent 90 to 95% of the excretion products. Two additional metabolites of 3-hydroxyxanthine have been identified as 8-chloroxanthine, a few tenths of a % of the total, and 8-methylmercaptioxanthine, about 1 to 2% of the total (498). Following administration of guanine 3-oxide, 8-methylmercaptoguanine has also been identified.

The latter metabolites arise through an unusual chemical characteristic of these 3-N-oxides. The acetylation of 3-hydroxyxanthine leads to uric acid and that of guanine 3-oxide leads to 8-hydroxyguanine (469). That reaction is partially clarified by the behavior of the intermediate, 3-acetoxyxanthine, which can be prepared under mild acetylating conditions. This is a highly reactive compound which can react in several ways in water, at room temperature, and at physiological pH. A portion reverts by hydrolysis to 3-hydroxyxanthine. Another portion reacts with water to yield uric acid with the loss of the acetoxo group from the 3-position. Many other nucleophiles, including chloride ion or methionine, react at the 8-position even more readily than does water, and this reaction leads to products such as 8-chloroxanthine and 8-methylmercaptioxanthine (470).

The correlation of the reaction involving nucleophilic substitution of 3-acetoxyxanthine at C-8 in vitro and the production of 8-chloro- and 8-methylmercaptioxanthine in vivo from 3-hydroxyxanthine is explained by the fact that 3-hydroxyxanthine can be converted in vivo to an ester with a reactivity comparable to that of the chemical model. Enzymatic activations with rat liver supernatant protein and appropriate co-factors furnished evidence for the intermediary formation of phosphate and sulfate esters (498).

Either ester, formed in vivo, can interact with nucleophilic groups in macromolecules, and may be considered to be possible proximate oncogens. Reaction has been demonstrated with proteins, in vivo and in vitro, including with the protein fraction furnishing the activating enzymes just mentioned (476, 498). About 20% of the radioactivity so bound to protein is released, by heating, as 8-methylmercaptioxanthine. Other nucleophilic groups of proteins are also involved; there are at least four other unidentified ¹⁴C-labeled products from protein hydrolysates. Little binding to RNA (476) or DNA has been detected. This metabolic activation to a chemically reactive compound has considerable similarity to results with the oncogenic N-hydroxy-N-acetylaminofluorene and other aryl amines.

II: CHEMICAL AND BIOLOGICAL STUDIES ON NUCLEIC ACIDS AND DERIVATIVES

RECENT DEVELOPMENTS RELATED TO PROPOSAL #5

Section 5701

George B. Brown, Ph.D., Head

Findings which furnish the basis for recent photochemical studies and the current proposals, but are not yet described in print, are outlined here in some detail.

The action of acetic anhydride on 3-hydroxyxanthine is an apparent rearrangement of oxygen from N-3 to C-8, or is effectively a reduction at N-3 accompanied by substitution, or an oxidation, at C-8. When other nucleophiles react at C-8 an intermolecular reaction is obviously involved, effectively an S_N1 substitution with rearrangement. We have termed it the "3-acyloxyxanthine 8-substitution reaction" and studied its mechanism in some detail with water alone, to avoid the complications of a competing nucleophile. In water at room temperature the reaction proceeds slowly fairly below pH 2 and is greatly accelerated between pH 3 and 7, concomitant with the initiation of some reduction to xanthine. The beginning of the fast reaction coincides with the initial ionization of the imidazole hydrogen of 3-acetoxypurine. It is deduced that the slow reaction (path a, Scheme A) involves a heterolytic cleavage of the 3-acetoxy group of the neutral or protonated molecule to yield a nitrenium ion, which undergoes an allylic shift to a carbonium ion at C-8, followed by nucleophilic substitution at that position. That is also the probable mechanism in polar aprotic solvents. The fast reaction (path b, Scheme A) results from a similar, but more facile, heterolytic cleavage of the 3-acetoxy group from the 3-acetoxypurine anion, in this case to yield dehydroxanthine, which can protonate to the same C-8 carbonium ion. The reduction of xanthine has the characteristics of a free-radical reaction in that it is essentially independent of temperature or solvent polarity, but it occurs only in conjunction with the fast 8-substitution reaction and exhibits a similar pH dependence. It is proposed that it results from a homolytic cleavage of the 3-acetoxy anion to yield a radical-ion which then abstracts hydrogen from water to yield xanthine (Scheme B).

Scheme A

The rate of the reaction of 3-acetoxyxanthine extrapolates to seconds or less at a physiological pH of 7.3, and the extent of reaction via the free-radical mechanism is at least 35 to 40% at that pH (A). (For lettered refs. see In Press section of Bibliography).

Scheme B

In support of the participation of the 7-H (imidazole proton) in the reaction, the 7-methyl-3-acetoxyxanthine (B) does not undergo the reaction, while the 1-methyl-3-acetoxyxanthine (B) does so.

Recently 7-hydroxyxanthine has been synthesized (C) and its acetoxyderivative undergoes the same facile nucleophilic substitutions that 3-acetoxyxanthine does (D). Presumably this involves ionization of its proton from N-3 and departure of the acetoxy group from N-7 to yield the same dehydroxanthine and carbonium ion intermediates (Scheme C), and lends support to the proposed dehydroxanthine intermediate.

Scheme C

-In association with this reaction mechanism study, all of the mono- and two di-methyl derivatives of 3-hydroxyguanine and guanine 3-oxide were synthesized (E). Through studies of their spectra and pK_a 's, the tautomeric forms of every ionic species of each parent compound were deduced (F). For guanine 3-oxide there are two coexistent neutral species, one the unexpected 3-hydroxy- form in which the 1-H is tautomerized to the 3-oxygen. The existence of that tautomer is essential to the correlation of the 3-acyloxypurine 8-substitution reaction mechanism with the metabolism observed in vivo. That group of compounds with each mobile hydrogen selectively blocked by a methyl is available for studies of the details of the chemical reactivities, photochemical susceptibilities, and radical formation of these compounds.

In support of the postulated free-radical, 3-hydroxyxanthine and 3-acetoxypurine can, in the solid state, be converted by photochemical energy to a free-radical (G). In the dry state this radical is infinitely stable. In water it yields xanthine, and in this respect its behavior parallels the free-radical mechanism suggested for the formation of xanthine in one of the reactions of 3-acetoxypurine in water. In an earlier study (321, 322) of the photochemical products from adenine-1-oxide, deoxygenation was observed, in addition to rearrangement of the oxygen to the adjacent C-2.

Free-radicals have been repeatedly suggested to be oncogenic, and with two modes of formation of reactive intermediates, the ionic and the radical, it is necessary to consider which reaction mechanism is responsible for the initiation of the cancer process by these purine derivatives.

Recent photochemical studies are reported in detail in the 1971 progress report.

RESEARCH DEVELOPMENTS RELATED TO PROPOSAL #5

Division 7700, Cell Biochemistry
Aaron Bendich, Ph.D., Chief

Main Research Accomplishments, 1950-1968

These are included in the "Summary of Activities of Division 5700" outlined under Proposal No. 5.

Recent Research Accomplishments, 1969-1971

The formation of the Division of Cell Biochemistry on January 1, 1969 facilitated a more comprehensive study of the biochemical and genetic basis of cancer.

The exposure of normal mammalian cells to various environmental factors leads to their malignant transformation. This alteration has the attributes of heritable change since the ensuing properties of unlimited growth are passed on to offspring cells indefinitely (4B). At the molecular level, the change could arise from a) acquisition of new genetic determinants, or b), derepression of pre-existing ones. These possibilities were studied, by the methods given below, with the following results.

a) Acquisition of new genetic determinants

When mouse Ehrlich ascites tumor cells (EA) and Chinese hamster (CH) cells were grown together in culture, adhesions and bridges developed between some of them. The bridges originated from the mouse cells as evidenced by indirect immunofluorescence techniques employing specific antisera. When the cells were labeled with thymidine-³H, an occasional passage of DNA through a bridge from EA to CH, but not the reverse, was revealed by autoradiography. Such observations were made during the first few days of co-culture. After about two months of co-culture, new cellular forms appeared resembling CH cells morphologically but which reacted as if they contained murine antigens when examined by the immunofluorescent method using anti-EA antiserum (obtained in the rabbit). Three lines of such cells, isolated by cloning, have been maintained in culture for over a year. They show a typical CH karyotype. Extracts of these cells produced a precipitin line of identity with extracts of EA cells when examined by the Ouchterlony immunodiffusion technique in agar using anti-EA rabbit antiserum. When inoculated into the cheek pouch of cortisone-conditioned Syrian hamsters, the cells produced malignant tumors which could be passed on to other animals (4B).

We regard this as an example of the induction of genetic and malignant change presumably resulting from the transfer of DNA from tumor to heterologous normal cells when these are allowed to interact in culture. If an effect of this kind is operative in animals which carry malignant tumors, it might constitute a possible mechanism in metastases.

Chinese hamster (CH) cells were exposed to DNA from mouse Ehrlich ascites tumor (EA) cells, and new forms appeared at low frequency which showed murine antigens by immunofluorescence tests. After screening a few hundred thousand colonies, five pure clones of cells so affected were isolated. They maintained the new property on long-term passage in culture, showing a typical CH karyotype; some grew as malignant tumors in conditioned Syrian hamsters. Proteins were extracted and examined by the Ouchterlony technique. The extracts gave a precipitin line of identity when compared with extracts of EA cells, employing anti-EA rabbit antisera. Results of mixed hemagglutination tests with mouse red blood cells and anti-EA antisera were also consistent with the presence of murine antigen on the surface of these cultured cells. On the other hand, CH cells transformed to malignancy by chemical cancerogens or oncogenic viruses showed none of these antigenic properties (8B, 9B).

Attempts were made to increase both the frequency of this DNA-mediated change and the efficiency of selection of altered cells. A promising technique involves treatment of cells, suspended in a dilute solution of DNA, with DEAE-dextran (a basic polymer which inhibits nuclease action and enhances pinocytosis). This results in the precipitation of fibers of DNA enmeshing cells therein. When plated onto glass surfaces and cultured for 2 to 3 days, only those CH cells in intimate contact with fibrous DNA from EA cells showed a positive immunofluorescence when tested with anti-EA rabbit antiserum. The fibers themselves were negative. The DNA fibers with cells still attached can be removed from the bulk of the suspended cells and then plated. The immunofluorescence reaction is observed after 48 hours in the adhering CH cells when the DNA is of EA origin, but not when derived from hamster, calf or human cells (9B).

Thus the results of the cell-cell and DNA-cell interaction experiments support the view that malignancy may arise as a consequence of the acquisition of new genetic determinants. Another view is obtained by the studies in b), below.

b) Derepression of genetic activity

Extracts of 88 murine tumor and leukemic tissues of chemical, viral, radiologic and spontaneous origins, all contained embryonal antigens. Except for skin, these antigens were undetectable in all normal adult tissues of 11 strains of mice examined by Ouchterlony immunodiffusion. Normal skin extracts cross-reacted with the absorbed anti-embryo sera (6B). However, the adult skin antigens were easily distinguished from those of embryos and tumors by chromatographic analysis. From the Sephadex G-200 elution profiles, the molecular weights of the latter two were estimated to be 67,000 and those of skin 15,000 (14B).

Attempts were made to identify and uncover the biological functions of these antigens. Chromatographically purified saline extracts of Ehrlich ascites tumor cells have yielded a water-soluble protein preparation (antigen 76) which was chromatographically, electrophoretically, and immunologically indistinguishable from components found in a similarly

purified embryo extract (antigen 81). The former contained two distinct electrophoretic components, whereas antigen 81 contained these two, plus a third. Preliminary attempts to immunize mice with either antigen 76 or 81 produced neither detectable precipitating antibodies nor protection against challenge injections of Ehrlich ascites cells. However almost all mice injected with antigens 76 or 81 displayed the peculiar response of loss of whiskers; those receiving higher doses lost large patches of body hair (17B).

Fractionation of the rabbit antisera has yielded a gamma globulin concentrate which is useful for quantitation of the antigens in various extracts. In embryos the pertinent antigens were present, in order of decreasing concentration, in intestine, plasma, red blood cells, bone, heart, lung, kidney, liver and peritoneal membrane. Antigen persists until the fifth week of neonatal life. Little or no antigen was detected in gonads, muscle, brain and spleen, at any stage of development.

It is significant that spleen extracts were consistently negative, since this is a reservoir for the group-specific (gs) antigen of murine leukemias. That the gs antigen is not related to the embryo-tumor system was revealed by specific immunologic analyses with purified reagents.

The universal appearance of embryonal gene products in murine tumors supports the hypothesis of retrogenetic expression as a significant aspect of the cancer cell's biochemical development. Retrogenetic expression may be an example of a more general turning-on of dormant gene functions, as for example the appearance of hormone activities in non-metastatic human cancers in tissues which do not ordinarily have the relevant endocrine function. It is not yet known if the turning-off of ectopic activities or antigens would lead to tumor inhibition; however, if turning-off could be achieved, significant information will have been gained regarding the mechanisms which regulate cell genetic expression. The inherent principle could then be applied to the key problem: the control of tumor growth.

c) Synthetic antimetabolites

Synthesis of compounds related to several experimental anti-tumor agents previously made in this program was continued. Many were structural analogs of the heterocyclic bases in the nucleic acids. Testing was performed in the pre-clinical and clinical departments of the Institute.

The promising anti-leukemic and anti-tumor agent 6-hydroxylamino-9- β -ribosylpurine was found to induce methemoglobinemia in clinical trials. To circumvent this toxic side reaction, attributable to release of hydroxylamine by adenosine deaminase, the arabinosyl analog was synthesized (2B). Despite the subtle change in structure (inversion of the 2'-hydroxyl group), the latter compound was devoid of toxicity and of antagonistic activity in mouse leukemias, and was unaffected by the deaminase (2B, 18B). Related compounds were studied in the hope that anti-tumor activity, inherent in several hydroxylaminopurines, can be retained with minimal toxicity. An

interesting example of this has been observed with 2,6-dihydroxylaminopurine (DHAP). There was a considerable enhancement of survival of mice with leukemia L1210, and with leukemia P815; in both instances lower dosages of DHAP (1.3 to 18 mgs/kg) were much more effective than those several times higher. A similar effect has again been seen with the new potent agent, 2-hydroxylamino-6-mercaptopurine, but not with "standard" anti-tumor agents, not with another effective new synthetic, 2-fluoro-6-hydroxylaminopurine; the 9- β -D-ribose derivative of the latter was devoid of anti-leukemia activity, perhaps because of its intrinsic instability. Only traditional dose-responses were seen when the compounds were tested for toxicity against normal and tumor cells in culture. Several other candidate analogs were synthesized and are undergoing evaluation; these include 2-hydroxylamino-6-N-methylhydroxylaminopurine, 2-methylamino-6-mercaptopurine, 2,6-bis(N-methylhydroxylaminopurine), 2-fluoromercaptopurine, and the 3-oxides of 6-selenopurine, purine-6-sulfonyl fluoride, purine-6-sulfonamide, 8-carboxyadenine, 6-methylpurine 3-oxide and 9- β -D-arabinofuranosyl-6-aminopurine 3-oxide (5B, 7B, 10B, 11B, 13B, 16B, 19B, 20B).

Since the toxicity of certain candidate anti-tumor purine derivatives may be decreased by conversion to their 3-oxides, 6-hydroxylaminopurine 3-oxide was synthesized (1B, 2B). The compound showed a marked activity against mouse leukemia P815. However, it proved to be carcinogenic in 100% of rats tested; the compound is structurally related to the potent carcinogen and mutagen 4-hydroxylaminoquinoline 1-N-oxide.

III. PHYSICAL-CHEMICAL STUDIES ON NUCLEIC ACIDS AND DERIVATIVES

RECENT RESEARCH DEVELOPMENTS RELATED TO PROPOSAL #6

Section 5702

Mary L. Petermann, Ph.D., Head

This Section has continued to study ribosomes, the subcellular particles in which protein synthesis occurs. A detailed knowledge of their structure is essential to an understanding of normal growth, differentiation, and malignant transformation.

Unfolded subunits. The 35-42S particles obtained by treating the large subunits of rat liver ribosomes with 5 M urea or 9 M formamide still had molecular weights of about 3×10^6 daltons, and contained 28S RNA. The only components lost were the 5S RNA and a protein associated with it as a nucleoprotein complex. Formamide gave the best yield of this material (5S RNP) (P-75). It has been purified by fractionation on G-200 Sephadex, where its elution volume indicated a molecular weight of about 80,000 daltons. In a CsCl gradient it banded at a density of 1.568 g/ml, corresponding to an RNA content of 48.5%. Combining this value with the molecular weight of the 5S RNA, 40,000, gave 83,000 daltons for the complex. Since the protein had a molecular weight of 41,000 (measured by SDS gel electrophoresis) the complex must contain only one molecule of protein. The small subunits, 24-27S, still contained 18S RNA, but their protein content was 20 to 25% less than that of the active small subunits described below.

Active Subunits from Rat Liver Ribosomes (P-71, 74, 77, 78, 81). To decrease the RNase content of the liver adult male rats were given phenobarbital for seven days before sacrifice. The isolated ribosomes were dissociated by removing half their bound magnesium and adding urea, in the presence of a reducing agent, dithiothreitol. The urea was removed by rapid dialysis against a low-magnesium buffer, and the subunits were fractionated by sucrose gradient centrifugation in a zonal rotor. When 2 M urea was used the small-subunit (40S) fraction was free of large ones, but the large-subunit (59S) fraction contained about 15% of small subunits (which had dimerized to 60S). Large subunits almost free of small ones could, however, be obtained by increasing the urea concentration to 2.7 M. Since this converted all the small subunits to an inactive 28S form, the large subunits were stored at -20° until fresh 40S had been isolated in a separate experiment. The isolated 40S was immediately mixed with 59S, the magnesium concentration was increased to 3 mM, and the reconstituted ribosomes were concentrated by centrifugation. The isolated subunits could also be concentrated and stored separately.

The incorporation of ^{14}C -phenylalanine, endogenously or in the presence of polyuridylic acid, was measured initially by Dr. I.B. Weinstein of Columbia University (P-71), as described by Weinstein, Ochoa and Friedman (Biochemistry 5:3332, 1966). It is now being measured in this laboratory.

The endogenous activity was lost on dissociation. With polyuridylic acid the separate subunit fractions had little or no activity, but the reconstituted ribosomes were 80% as active as the controls.

For the 40S subunit Dr. Hamilton found a molecular weight of 1.5×10^6 daltons, of which 850,000 were protein (P-81); the active subunit thus contained about 250,000 daltons of extra proteins that were missing from the 30S particle described previously. On electrophoresis in 6 M urea at pH 4.6 the 40S pattern showed additional amounts of several components, plus one extra slow-moving protein. In SDS gels, however, the 30S and 40S patterns showed only minor quantitative differences, so the extra 40S proteins do not have unique sizes.

The 40S subunit also contained about 50,000 daltons of "rapidly labeled" RNA. Extraction with EDTA alone removed this RNA, but only 1/3 of the extra protein; the remainder may be attached to the stable proteins by disulfide bonds, since reducing agents increased the amount of protein extracted. The active 40S subunit obtained by dissociation with KCl (Martin, Wool, and Castles, Meth. Enzymol. 20:433, 1971) had a molecular weight and gross protein content like those of the urea 40S subunit, but was much less stable (P-81).

The 59S subunit had about the same molecular weight as the 50S subunit described previously, 3.1×10^6 daltons, and a similar gross protein content, 45% (P-81). Although its frictional ratio was high, 1.86, electron micrographs showed a compact particle with an axial ratio of only 1.18 (P-74). Thus the hydration of this subunit appeared to be unusually high, 3.3 g of water per g of dry nucleoprotein. Since the small subunit, with a similar chemical composition, had a hydration of only 1.7 g/g, we suggested that the large subunit might contain a solvent-filled groove or channel (P-68, 74). The 59S subunit contained the theoretical amount of 5S RNA, one strand per subunit (P-78).

On SDS gel electrophoresis the proteins of whole ribosomes showed a slow band, of molecular weight about 70,000 daltons, that was not found in either the 40S or 59S subunit.

At a lower concentration, 6.6 M, formamide dissociated the ribosomes to 55S and 40S subunits which retained most of their ability to synthesize polyphenylalanine.

Yeast ribosomes (P-73). Most of the RNase was removed from yeast ribosomes by DEAE-cellulose chromatography. The molecular weights of the whole ribosome, large subunit and small subunit were 3.6, 2.6, and 1.0×10^6 daltons.

RECENT RESEARCH DEVELOPMENTS RELATED TO PROPOSAL #6

Section 6601

Liebe F. Cavalieri, Ph.D., Head

Recent research accomplishments are included in the 1970-71
Progress Report of Division 6600.

IV. BIOLOGICAL CHEMISTRY GRADUATE STUDENTS TRAINED

Sloan-Kettering Division

School of Medical Sciences

Cornell University

<u>NAME</u>	<u>DATE</u>	<u>DEGREE RECEIVED</u>
Giner-Sorolla, Alfredo	9/7/54 - 10/1/58	Ph.D.
Hamilton, Mary G.	10/1/56 - 9/20/60	Ph.D.
Lowy, Bertram A.	10/1/51 - 8/15/53	Ph.D.
Mazelis, Anne	9/1/64 - 5/20/70	Ph.D.
Myles, Arthur	9/1/64 - 9/23/68	Ph.D.
Rosenberg, Barbara	9/16/57 - 5/28/62	Ph.D.
Rosenkranz, Herbert	10/15/56 - 12/11/58	Ph.D.
Schwartz, Edith	9/1/61 - 10/2/64	Ph.D.
Stone, Audrey	10/21/52 - 12/9/58	Ph.D.

V. BIBLIOGRAPHY

Biological Chemistry Division

George B. Brown, Ph.D. - Section 5701
Aaron Bendich, Ph.D. - Section 5703 (7701 - 12/30/68)
Liebe F. Cavalieri, Ph.D. - Section 5704 (6601 - 1/1/68)

AEC-supported Bibliography

1950 - 1971

- Our ref.
2b THE UTILIZATION OF ADENINE FOR NUCLEIC ACID SYNTHESIS AND AS A
(not AEC PRECURSOR OF GUANINE.
supported) G. B. Brown, P. M. Roll, A. A. Plentl and L. F. Cavalieri,
J. Biol. Chem., 172, 469 (1948).
- # 52 ON THE ROLE OF 2,6-DIAMINOPURINE IN THE BIOSYNTHESIS OF
NUCLEIC ACID GUANINE.
A. Bendich, S. S. Furst and G. B. Brown,
J. Biol. Chem., 185, 423 (1950).
- # 53 A STUDY OF THE METABOLISM OF 2,4-DIAMINOPYRIMIDINE.
A. Bendich, W. Geren & G. B. Brown,
J. Biol. Chem., 185, 435 (1950).
- # 57 BIOSYNTHESIS OF NUCLEIC ACIDS IN THE MAMMAL.
G. B. Brown,
Federation Proc., 9, 517 (1950).
- # 58c ADENINE INTOXICATION IN RELATION TO IN VIVO FORMATION AND
DEPOSITION OF 2;8-DIOXYADENINE IN RENAL TUBULES.
F. S. Philips, J. B. Thiersch and A. Bendich,
J. Pharmacol. Exptl. Therap., 104, 20 (1952).
Abstr: Federation Proc.; 9, 308 (1950).
- # 63c STUDIES ON THE STRUCTURE OF NUCLEIC ACIDS. I. INTERACTION
OF ROSANILINE WITH DESOXYPENTOSE NUCLEIC ACID.
L. F. Cavalieri & A. Angelos,
J. Am. Chem. Soc., 72, 4686 (1950).
- # 64 ON THE UTILIZATION OF PURINES AND THEIR RIBOSE DERIVATIVES BY
YEAST.
S. E. Kerr, K. Seraidarian and G. B. Brown,
J. Biol. Chem., 188, 207 (1951).
- # 65 ON THE INTERCONVERSION OF PURINES BY LACTOBACILLUS CASEI.
M. E. Salis, G. B. Brown, G. B. Elion, G. H. Hitchings and
H. VanderWerff, J. Biol. Chem., 188, 217 (1951).

Our ref.

- # 66c A NEW SYNTHESIS OF PURINE NUCLEOSIDES. THE SYNTHESIS OF ADENOSINE, GUANOSINE AND 2,6-DIAMINO-9- β -D-RIBOFURANOSYL-PURINE.
J. Davoll and B. A. Lowy
J. Am. Chem. Soc., 73, 1650 (1951).
- # 67 PURINE INCORPORATION INTO PENTOSE NUCLEOTIDES OF THE RAT.
D. H. Marrian, V. L. Spicer, M. E. Balis and G. B. Brown,
J. Biol. Chem., 189, 533 (1951).
Abstr: 118th Meeting Am. Chem. Soc., Chicago, 1950, p. 56C.
- #68c STUDIES ON THE STRUCTURE OF NUCLEIC ACIDS. II. INVESTIGATION OF PENTOSE NUCLEIC ACID AND ENZYME-RESISTANT RESIDUES.
L. F. Cavalieri, S. E. Kerr and A. Angelos,
J. Am. Chem. Soc., 73, 2567 (1951).
- # 71 ON THE UTILIZATION OF GUANINE BY THE RAT.
M. E. Balis, D. H. Marrian and G. B. Brown,
J. Am. Chem. Soc., 73, 3319 (1951).
Abstr: 119th Meeting Am. Chem. Soc., Boston, April 1951, p. 12C.
- # 72 ON THE ROLE OF GLYCINE AND ADENINE AS PRECURSORS OF NUCLEIC ACID PURINES.
S. S. Furst and G. B. Brown,
J. Biol. Chem., 191, 239 (1951).
- # 73 PRECURSORS OF NUCLEIC ACID.
G. B. Brown,
Biology Conference, "Current Problems in Nucleic Acid Chemistry",
Oak Ridge National Laboratory, April 1950, J. Cellular Comp. Physiol., 38, 121 (July 1951)
- # 74 NUCLEIC ACID METABOLISM CHEMOTHERAPEUTIC ASPECTS
G. B. Brown, 120th Meeting Amer. Chem. Soc., N. Y., Sept. 1951, p. 1L.
- # 75c STUDIES ON THE STRUCTURE OF NUCLEIC ACIDS. III. KINETICS OF THE ALKALINE HYDROLYSIS OF YEAST PENTOSE NUCLEIC ACID.
L. F. Cavalieri and L. P. Hammett,
J. Am. Chem. Soc., 73, 4899 (1951).
Abstr: Federation Proc., 10, 170 (1951).
- # 76c STUDIES ON THE STRUCTURE OF NUCLEIC ACIDS. IV. INVESTIGATION OF DYE INTERACTIONS BY PARTITION ANALYSIS.
L. F. Cavalieri, A. Angelos and M. E. Balis,
J. Am. Chem. Soc., 73, 4902 (1951).
- # 77 STUDIES WITH ORGANIC AND BIOSYNTHETIC NUCLEOSIDES AND NUCLEOTIDES.
G. B. Brown, in "Isotopes in Biochemistry", A CIBA Foundation Conference, Churchill, London, 1951, page 164-174.

Our ref.

- # 78 A NEW SYNTHESIS OF 1-GLYCOSYLBENZIMIDAZOLES.
J. Davoll and G. B. Brown,
J. Am. Chem. Soc., 73, 5781 (1951).

- # 79c A POLAROGRAPHIC STUDY OF PYRIMIDINES.
L. F. Cavalieri and B. A. Lowy,
Arch. Biochem. Biophys., 35, 83 (1952).

- # 80c STUDIES OF THE STRUCTURE OF NUCLEIC ACIDS. V. ON THE
MECHANISM OF METAL AND ENZYMES INTERACTIONS.
L. F. Cavalieri, J. Am. Chem. Soc., 74, 1242 (1952).

- # 81c SOME SYNTHETIC ANALOGS OF THE NATURAL PURINE NUCLEOSIDES.
J. Davoll and B. A. Lowy,
J. Am. Chem. Soc., 74, 1563 (1952).

- # 82 THE INCORPORATION OF EXOGENOUS PURINES INTO PENTOSE NUCLEIC
ACID BY LACTOBACILLUS CASEI.
M. E. Balis, D. H. Levin, G. B. Brown, G. B. Elion,
H. VanderWerff and G. H. Hitchings,
J. Biol. Chem., 196, 729 (1952).
Abstr: XII International Cong. Pure & Applied Chem., New York,
September 1951.

- # 83c EFFECTS OF A FOLIC ACID ANTAGONIST ON NUCLEIC ACID METABOLISM.
D. Goldthwait and A. Bendich,
J. Biol. Chem., 196, 841 (1952).
Abstr: Federation Proc., 10, 190 (1951).

- # 85 THE UTILIZATION OF PURINE NUCLEOSIDES FOR NUCLEIC ACID
SYNTHESIS IN THE RAT.
B. A. Lowy, J. Davoll and G. B. Brown,
J. Biol. Chem., 197, 591 (1952).

- # 86 AN UNUSUAL TRANSFORMATION OF ISOMERS OF TETRAACETYLRIBOSE.
J. Davoll, G. B. Brown and D. Visser,
Nature, 170, 64 (1952).

- # 87 THE INCORPORATION OF FORMATE INTO VARIOUS ORGANS OF THE RAT.
P. Drochmans, D. H. Marrian and G. B. Brown,
Arch. Biochem. & Biophys., 39, 310 (1952).
Abstr: XII International Cong. Pure & Applied Chem., New York,
September 1951, p. 93.

- # 88c STUDIES ON THE METABOLISM OF THE NUCLEIC ACIDS.
A. Bendich,
Conference on Chemistry and Physiology of the Cell,
Brookhaven National Laboratory, August 1951;
Exptl. Cell Res., 3, Suppl. 2, 181 (1952);
119th Meeting Am. Chem. Soc., Boston, 1951, p. 12C.

Our ref.

- # 89c EFFECT OF NITROGEN MUSTARD ON NUCLEIC ACID METABOLISM.
D. A. Goldthwait,
Proc. Soc. Exptl. Biol. Med., 80, 503 (1952).
- # 91 UTILIZATION OF SOME PURINE RIBOSE DERIVATIVES BY
LACTOBACILLUS CASEI.
M. E. Balis, D. H. Levin, G. B. Brown, G. B. Elion,
H. Vander Werff and G. H. Hitchings,
J. Biol. Chem., 199, 227 (1952).
- # 93c THE IDENTIFICATION OF CYTIDYLIC ACIDS a and b.
L. F. Cavalieri, J. Am. Chem. Soc., 74, 5804 (1952).
- # 94c HYDROLYSIS PRODUCTS OF NUCLEIC ACIDS LABELED WITH TRITIUM;
PREPARATION BY BIOSYNTHESIS.
M. L. Eidinoff, H. C. Reilly, J. E. Knoll and D. H. Marrian,
J. Biol. Chem., 199, 511 (1952).
- # 95 METABOLIC AND CHEMOTHERAPEUTIC STUDIES WITH PURINE DERIVATIVES
G. B. Brown, in Texas Reports on Biology and Medicine, vol. 10,
1052, p. 961.
- # 96 BIOSYNTHESIS OF NUCLEIC ACIDS.
G. B. Brown, P. M. Roll and H. Weinfeld,
in: Phosphorus Metabolism II, Johns Hopkins Press (1952),
p. 385-405.
Abstr: Federation Proc., 10, 238 (1951);
" " 12, 287 (1953).
- # 97 THE UTILIZATION OF PURINE RIBOTIDES BY L. CASEI.
M. E. Balis, D. H. Levin, G. B. Brown, G. B. Elion,
H. Vander Werff and G. H. Hitchings,
J. Biol. Chem., 200, 1 (1953).
Abstr: 2nd International Cong. Biochem., Paris, 1952, p. 75;
Federation Proc., 11, 83 (1952).
- # 98 PURINE METABOLISM OF A DIAMINOPURINE-RESISTANT STRAIN OF
L. CASEI.
G. B. Elion, H. Vander Werff, G. H. Hitchings, M. E. Balis,
D. H. Levin and G. B. Brown,
J. Biol. Chem., 200, 7 (1953).
Abstr: Federation Proc., 11, 207 (1952).
- # 99c STUDIES ON THE STRUCTURE OF NUCLEIC ACIDS. VI. THE KINETICS
OF DEOXYRIBONUCLEASE ACTION.
L. P. Cavalieri and B. Hatch,
J. Am. Chem. Soc., 75, 1110 (1953).
Abstr: 2nd International Cong. Biochem., Paris, 1952, p. 253.
- # 100 ON THE HETEROGENEITY OF THE DESOXYRIBONUCLEIC ACIDS.
A. Bendich, P. J. Russell Jr. and G. B. Brown,
J. Biol. Chem., 203, 305 (1953).
Abstr: Federation Proc., 12, 176 (1953).

Our ref.

- #101 EFFECTS OF PURINE ANTAGONISTS ON A DIAMINOPURINE-RESISTANT STRAIN OF L. CASEI.
G. B. Ellison, S. Singer, G. H. Hitchings, M. E. Balis and G. B. Brown, J. Biol. Chem., 202, 647 (1953).
Page 19
- #102 NUCLEIC ACIDS, PURINES AND PYRIMIDINES
G. B. Brown, Annual Reviews of Biochemistry, 22, 141 (1953).
- #103 SYNTHESIS OF ETHYL ETHOXYBROMOACETATE AND ETHYL ETHOXYCYANO-ACETATE.
A. Bendich and G. C. Clements,
J. Am. Chem. Soc., 75, 4075 (1953).
- #104c THE IDENTIFICATION OF CYTIDYLIC ACIDS a (2') AND b (3') BY SPECTROPHOTOMETRIC METHODS.
J. J. Fox, L. F. Cavalieri and N. Cheng,
J. Am. Chem. Soc., 75, 4315 (1953).
- #106 THE SYNTHESIS OF 9- β -D-RIBOPURANOSYLPURINE AND THE IDENTITY OF NEBULARINE.
G. B. Brown and V. S. Weliky,
J. Biol. Chem., 204, 1019 (1953).
- #109c STUDIES ON THE STRUCTURE OF NUCLEIC ACIDS. VII. ON THE IDENTIFICATION OF THE ISOMERIC CYTIDYLIC AND ADENYLIC ACIDS.
L. F. Cavalieri,
J. Am. Chem. Soc., 75, 5268 (1953).
- #110c REVISION OF THE STRUCTURAL FORMULATION OF VICINE AND ITS PYRIMIDINE AGLUCONE, DIVICINE.
A. Bendich and G. C. Clements,
Biochim. Biophys. Acta, 12, 462 (1953).
Abstr: Trans. N. Y. Acad. Sci., 15, 58 (1952).
- #112 THE METABOLISM IN THE RAT OF GUANYLIC ACID LABELLED WITH ³²P.
P. M. Roll, H. Weinfeld and G. B. Brown,
Biochim. Biophys. Acta, 13, 141 (1954) (short communication).
- #115c THE REUTILIZATION OF NUCLEIC ACID CATABOLITES.
J. Dancis and M. E. Balis,
J. Biol. Chem., 207, 367 (1954).
- #119c THE SYNTHESIS AND PROPERTIES OF 6-CHLOROPURINE AND PURINE.
A. Bendich, P. J. Russell Jr. and J. J. Fox,
J. Am. Chem. Soc., 76, 6073 (1954).
- #120 THE BIOSYNTHESIS OF NUCLEIC ACIDS AS A BASIS FOR AN APPROACH TO CHEMOTHERAPY.
G. B. Brown,
6-Mercaptopurine Conf., Ann. N. Y. Acad. Sci., 60, 2, 185 (1954).

Our ref.

- #122c NUCLEOTIDE UTILIZATION BY E. COLI.
M. E. Balis, C. T. Lark and D. Luzzati,
J. Biol. Chem., 212, 641 (1955).
Abstr: Federation Proc., 13, 178 (1954).
- #123c A POSSIBLE MECHANISM FOR DISTURBANCE IN TYROSINE METABOLISM
IN PHENYLPYRUVIC OLIGOPHRENIA.
J. Dancis and M. E. Balis,
Pediatrics, 15, 63 (1955).
- #124c THE UTILIZATION OF NUCLEOTIDES BY THE MAMMAL. II.
N15-LABELED PURINE NUCLEOTIDES.
P. M. Roll and I. Weliky,
J. Biol. Chem., 213, 509 (1955).
- #125 THE UTILIZATION OF NUCLEOTIDES BY THE MAMMAL. III.
ISOMERIC PURINE NUCLEOTIDES LABELED WITH C14.
H. Weinfeld, P. M. Roll and G. B. Brown,
J. Biol. Chem., 213, 523 (1955).
Abstr: Federation Proc., 13, 282 (1954)
and See Page 19
- #126c CHEMISTRY OF PURINES AND PYRIMIDINES
A. Bendich, in E. Chargaff and J. N. Davidson eds., The Nucleic
Acids. Chemistry and Biology, vol. 1, New York, Academic Press,
1955, p. 81.
- #127 BIOSYNTHESIS OF NUCLEIC ACIDS
G. B. Brown and P. M. Roll, in E. Chargaff and J. N. Davidson,
eds., The Nucleic Acids. Chemistry and Biology, vol. 2, New York,
Academic Press, 1955, p. 341.
- #128 THE METABOLIC BASIS FOR OPTIMISM REGARDING CHEMOTHERAPY
WITH NUCLEIC ACID DERIVATIVES.
G. B. Brown, in: C. P. Rhoads and A. D. Bass, AAAS, Monograph,
Chemotherapy of Cancer (1954), p. 285-299.
- #130c STUDIES OF A PURINE OR HISTIDINE REQUIRING MUTANT OF E. COLI.
D. Luzzati and R. Guthrie,
J. Biol. Chem., 216, 1 (1955).
Abstr: Federation Proc., 13, 256 (1954).
- #131c A PURINE-HISTINE RELATIONSHIP IN E. COLI.
M. E. Balis, D. H. Levin and D. Luzzati,
J. Biol. Chem., 216, 9 (1955).
- #132c FRACTIONATION OF DEOXYRIBONUCLEIC ACID (DNA) BY ION
EXCHANGE.
A. Bendich, J. R. Fresco, H. S. Rosenkranz,
J. Am. Chem. Soc., 77, 3671 (1955).
Abstr: 126th Meeting Am. Chem. Soc., New York, Sept. 1954, p. 52C.

Our ref:

- #133 PATHWAYS OF BIOSYNTHESIS OF NUCLEIC ACIDS.
G. B. Brown, International Conf. on the Peaceful Uses of Atomic Energy, Geneva, 1955, Proceedings, Vol. 12, p. 485 (1956).
(no fund acknowledgements)
- #134 METHOD OF SYNTHESIZING NUCLEOSIDES AND ANALOGOUS COMPOUNDS PREPARED THERAPY.
J. Davoll and G. B. Brown,
U. S. Patent #2,719,843, October 4, 1955.
(Sponsored by A.E.C.)
- #135c EFFECTS OF A-METHOPTERIN ON NUCLEIC ACID SYNTHESIS IN LEUKEMIC SPLEEN BRIES.
M. E. Balis and J. Dancis,
Cancer Res., 15, 603 (1955).
Abstr: Proc. Am. Assoc. Cancer Res., 1, 2, 3 (1954).
- #136 STUDIES ON THE METABOLISM OF HUMAN TUMORS. I. PENTOSE NUCLEIC ACID SYNTHESIS IN TUMOR BEARING HAMSTERS.
M. E. Balis, D. Van Praag and G. B. Brown,
Cancer Res., 15, 673 (1955).
Abstr: 127th Meeting Am. Chem. Soc., Cincinnati, April 1955, p. 11C
3rd International Cong. Biochem., Brussels, August 1955.
- #137 PURINE NUCLEOSIDE ANALOGS AS POTENTIAL ANTIMETABOLITES.
G. B. Brown, Discussion on "Chemistry of Antimetabolites Related to Purines", 3^e Congres Internationale de Biochimie, Brussels, Conférences et Rapports, 61 (1955-6).
(no fund acknowledgements)
- #138c HETEROGENEITY OF DEOXYRIBONUCLEIC ACID (DNA).
A. Bendich, in Hans T. Clarke Festschrift, Edited by S. Graff, Col. Press (1956), p. 14.
(no fund acknowledgements)
- #139c STUDIES ON THE STRUCTURE OF NUCLEIC ACIDS. VIII. APPARENT DISSOCIATION CONSTANTS OF DEOXYPENTOSE NUCLEIC ACID.
L. F. Cavalieri and A. Stone,
J. Am. Chem. Soc., 77, 6499 (1955).
Abstr: Federation Proc., 13, 190 (1954);
126th Meeting Am. Chem. Soc., September 1954, p. 51C.
- #141 PYRIMIDINE NUCLEOSIDES. I. A NEW ROUTE FOR THE SYNTHESIS OF THYMINE NUCLEOSIDES.
J. J. Fox, N. C. Yung, J. Davoll and G. B. Brown,
J. Am. Chem. Soc., 78, 2117 (1956).
Abstr: Federation Proc., 13, 211 (1954).
AAAS Gordon Conf., June 1955.
- #143 THE UTILIZATION OF NUCLEOTIDES BY THE MAMMAL. IV. TRIPLY LABELED PURINE NUCLEOTIDES.
P. M. Roll, H. Weinfeld, E. Carroll and G. B. Brown,
J. Biol. Chem., 220, 439 (1956).
Abstr: Federation Proc., 13, 282 (1954).

Our ref.

- #144c THE UTILIZATION OF NUCLEOTIDES BY THE MAMMAL. V. METABOLISM OF PYRIMIDINE NUCLEOTIDES.
P. M. Roll, H. Weinfeld and E. Carroll,
J. Biol. Chem., 220, 455 (1956).
- #145 THE UTILIZATION OF PURINES BY PURINELESS MUTANTS OF AEROBACTER AEROGENES.
M. E. Balis, M. S. Brooke, G. B. Brown and B. Magasanik,
J. Biol. Chem., 219, 917 (1956).
- #146 A STUDY OF THE METABOLISM OF PURINE RIBOSIDE.
M. P. Gordon and G. B. Brown,
J. Biol. Chem., 220, 927 (1955).
Abstr: Federation Proc., 14, 219 (1955);
3rd International Cong. Biochem, Brussels, 131 (1955)
- #147 G. B. Brown, Chairman of the Gordon Conference of the American Association for the Advancement of Science, on NUCLEIC ACIDS AND PROTEINS in 1965, Science, 123, 361 (1956), page 19.
- #148c STUDIES ON THE STRUCTURE OF NUCLEIC ACIDS. IX. STRUCTURAL CHANGES IN SOLUTION.
L. F. Cavalieri and A. Stone,
J. Am. Chem. Soc., 78, 353 (1956).
- #149 CHEMICAL PATHWAYS OF NUCLEIC ACID BIOSYNTHESIS.
G. B. Brown,
Federation Proc., 15, 823 (1956).
- #152c STUDIES ON THE METABOLISM OF HUMAN TUMORS. II. PENTOSENUCLEIC ACID SYNTHESIS IN TUMOR-BEARING RAT.
M. E. Balis, D. Van Praag and F. Aezen,
Cancer Res., 16, 628 (1956).
- #153 STUDIES ON THE METABOLISM OF HUMAN TUMORS. III. INFLUENCE OF REGENERATING LIVER ON PURINE METABOLISM.
M. E. Balis, D. Van Praag and G. B. Brown,
Cancer Res., 16, 632 (1956).
Abstr: Proc. Assoc. Cancer Res., 2, 2 (1956).
- #154c ON THE ANOMALOUS TITRATION OF DEOXYRIBONUCLEIC ACID.
L. F. Cavalieri and B. H. Rosenberg,
Biochim. Biophys. Acta, 21, 202 (1956).
- #155c FLOW DICHROISM AND ITS APPLICATION TO THE STUDY OF DEOXYRIBONUCLEIC ACID STRUCTURE.
L. F. Cavalieri, B. H. Rosenberg and M. Rosoff,
J. Am. Chem. Soc., 78, 5235 (1956).
- #156c STUDIES ON THE STRUCTURE OF NUCLEIC ACIDS. X. ON THE MECHANISM OF DENATURATION.
L. F. Cavalieri, M. Rosoff and B. H. Rosenberg,
J. Am. Chem. Soc., 78, 5239 (1956).
Abstr: Federation Proc., 15, 230 (1956).

Our ref.

- #157 6-FURFURYLAMINO-9- β -D-RIBOFURANOSYLPURINE: SYNTHESIS AND DIFFERENTIAL TOXICITY TO MAMMALIAN CELLS IN VITRO.
A. Hampton, J. J. Bieseke, A. E. Moore and G. B. Brown;
J. Am. Chem. Soc., 78, 5695 (1956).
- #158 NUCLEIC ACIDS AND RELATED DERIVATIVES AS TARGETS FOR CHEMOTHERAPY.
G. B. Brown and M. E. Balis,
The Leukemias: Etiology, Pathophysiology, and Treatment, 1957,
Academic Press, Inc., Publishers, New York 3, N. Y. pp. 507-515.
(Review - no fund acknowledgements)
- #159c STUDIES WITH 5-BROMOURACIL IN RODENTS AND DOGS.
J. Scholler, M. P. Gordon and S. S. Sternberg,
Proc. Soc. Exptl. Biol. Med., 93, 124 (1956).
- #160c SYNTHESIS OF 6-(1,2-DICARBOXYTHEYLAMINO)-9- β -D-RIBOFURANOSYLPURINE
AND THE STRUCTURE OF ADENYLOSUCCINIC ACID.
A. Hampton,
J. Am. Chem. Soc., 79, 503 (1957).
- #161 STRUCTURE-ACTIVITY RELATIONSHIPS.
G. B. Brown and A. Bendich,
[Reprinted from Symposium on Anti-Tumor Agents, Proceedings of the
Royal Society of Medicine, Vol. 50, No. 1, January 1957, pp. 6-7
(Section of Experimental Medicine and Therapeutics, pp. 6-7)]
(Review - no fund acknowledgements)
- #162c SPECTROPHOTOMETRIC STUDIES OF NUCLEIC ACID DERIVATIVES AND RELATED
COMPOUNDS AS A FUNCTION OF pH. IV. ON THE STRUCTURE OF OROTIDINE.
A STUDY OF N-METHYLATED OROTIC ACIDS.
J. J. Fox, N. Yung and I. Wempen,
Biochim. Biophys. Acta, 23, 295 (1957).
- #163 THE UTILIZATION OF NUCLEOTIDES BY THE MAMMAL. VI. PURINE
NUCLEOTIDES TOTALLY LABELED WITH C¹⁴ IN MICE BEARING SARCOMA 180.
H. Weinfeld, P. M. Roll, E. Carroll, G. B. Brown and C. P. Rhoads,
Cancer Res., 17, 122 (1957).
- #164 CHROMATOGRAPHIC FRACTIONATION OF E. COLI DNA CONTAINING
5-BROMOURACIL-2-C¹⁴.
A. Bendich, H. P. Pahl and G. B. Brown,
[Reprinted from the McCollum-Pratt Institute Symposium,
"The Chemical Basis of Heredity" (edited by W. D. McElroy and
B. Glass). The Johns Hopkins Press, Baltimore (1957), pp. 378-386]
- #165c CHROMATOGRAPHIC FRACTIONATION OF DEOXYRIBONUCLEIC ACIDS WITH
SPECIAL EMPHASIS ON THE TRANSFORMING FACTOR OF PNEUMOCOCCUS.
A. Bendich, H. B. Pahl and S. M. Beiser,
[Reprinted from Cold Spring Harbor Symposia on Quantitative Biology,
Volume XXI, 1956, pp. 31-48]
Abstr: Federation Proc., 16, 230 (1957).

Our ref.

- #166c SYNTHESIS AND PROPERTIES OF PURINES OF POTENTIAL BIOLOGICAL INTEREST.
A. Bendich, A. Giner-Sorolla and J. J. Fox,
[Reprinted from Ciba Foundation Symposium on The Chemistry and
Biology of Purines, 1957, pp. 3-16]
- #167 CHEMICAL AND BIOLOGICAL BEHAVIOURS OF 9- β -D-RIBOFURANOSYLPURINE.
G. B. Brown, M. P. Gordon, D. I. Magrath and A. Hampton,
[Reprinted from Ciba Foundation Symposium on The Chemistry and
Biology of Purines, 1957, pp. 192-201]
- #171 A STUDY OF THE ACTION OF ACID AND ALKALI ON CERTAIN PURINES AND
PURINE NUCLEOSIDES.
M. P. Gordon, V. S. Weliky and G. B. Brown,
J. Am. Chem. Soc., 79, 3245 (1957).
- #172c SYNTHESIS OF AN ISOPROPYLIDENE DERIVATIVE OF AN ALKALI-LABILE
NUCLEOSIDE: 2',3'-O-ISOPROPYLIDENE-9- β -D-RIBOFURANOSYLPURINE.
A. Hampton and D. I. Magrath,
J. Am. Chem. Soc., 79, 3250 (1957).
- #173 THE SYNTHESIS OF 9- β -D-RIBOFURANOSYLPURINE-5'-PHOSPHATE AND ITS
BEHAVIOR TOWARD AQUEOUS ALKALI.
D. I. Magrath and G. B. Brown,
J. Am. Chem. Soc., 79, 3252 (1957).
(Am. Cancer Soc. and N. C. I. supported)
- #174 THE IDENTIFICATION OF PHOSPHORYLATED METABOLITES OF 9- β -D-
-RIBOFURANOSYLPURINE.
M. P. Gordon, D. I. Magrath and G. B. Brown,
J. Am. Chem. Soc., 79, 3256 (1957).
- #176 PAUCIDISPERSE DEOXYRIBONUCLEIC ACID AND ITS USE IN THE STUDY OF
GENETIC DETERMINANTS.
L. F. Cavalieri,
J. Am. Chem. Soc., 79, 5319 (1957).
- #178 THE EFFECTS OF 6-MERCAPTOPURINE ON LACTOBACILLUS CASEI.
M. E. Balis, D. H. Levin and G. B. Brown, G. B. Elion, H. C. Nathan
and G. H. Hitchings,
Arch. Biochem. Biophys., 71, 358 (1957).
- #179c STUDIES ON THE STRUCTURE OF NUCLEIC ACIDS. XI. THE ROLES OF HEAT
AND ACID IN DEOXYRIBONUCLEIC ACID DENATURATION.
L. F. Cavalieri and B. H. Rosenberg,
J. Am. Chem. Soc., 79, 5352 (1957).
Abstr: Federation Proc., 16, 163 (1957).

Our ref.

- #180 POSSIBILITIES OF METABOLIC INTERFERENCES WITH NUCLEIC ACIDS.
G. B. Brown,
Cellular Biology, Nucleic Acids and Viruses.
Reprinted from Special Publications of the New York Academy of
Sciences, V, 277 (1957).
Abstr: Proc. Round Table Conference on Chemoprophylaxis and
Chemotherapy Viruses, National Foundation Infantile Paralysis (1936).
- #182c ADENINE-8-C¹⁴.
M. E. Balis,
Biochemical Preparations, Vol. V, 62 (1957).
(no fund acknowledgements)
- #186 ON THE METABOLISM OF PURINE.
M. P. Gordon, O. M. Intrieri and G. B. Brown,
J. Biol. Chem., 229, 641 (1957).
Abstr: Federation Proc., 16, 187 (1957).
- #187c ANION EXCHANGE CHROMATOGRAPHY AND MOLECULAR SIZE OF DEOXYRIBONUCLEIC
ACID.
M. Rosoff, G. diMayorca and A. Bendich,
Nature, 180, 1355 (1957).
- #188 2-CHLOROADENINE AND 2-CHLOROADENOSINE.
G. B. Brown and V. S. Weliky,
J. Org. Chem., 23, 125 (1958).
- #189c METABOLISM OF RESISTANT MUTANTS OF STREPTOCOCCUS FAECALIS. II.
INCORPORATION OF EXOGENOUS PURINES.
M. E. Balis, V. Hylin, M. K. Coultas and D. J. Hutchison,
Cancer Res., 18, 220 (1958).
Abstr: Bact. Proc., p. 135 (1955).
- #190 METABOLISM OF RESISTANT MUTANTS OF STREPTOCOCCUS FAECALIS. III.
THE ACTION OF 6-MERCAPTOPURINE.
M. E. Balis, V. Hylin, M. K. Coultas and D. J. Hutchison,
Cancer Res., 18, 440 (1958).
Abstr: Federation Proc., 16, 149 (1957).
- #191 PURINE N-OXIDES. I. MONO-OXIDES OF AMINOPURINES.
M. A. Stevens, D. I. Magrath, H. W. Smith and G. B. Brown,
J. Am. Chem. Soc., 80, 2755 (1958).
- #192 PURINE N-OXIDES. II. THE STRUCTURE OF ADENINE N-OXIDE.
M. A. Stevens and G. B. Brown,
J. Am. Chem. Soc., 80, 2759 (1958).
- #193c SYNTHESIS AND PROPERTIES OF SOME 6-SUBSTITUTED PURINES.
A. Giner-Sorolla and A. Bendich,
J. Am. Chem. Soc., 80, 3932 (1958).
Abstr: Proc. Roy. Soc. Med., 50, 6 (1957).

- #194c FRACTIONATION OF DEOXYRIBONUCLEIC ACIDS ON COLUMNS OF ANION EXCHANGERS; METHODOLOGY.
A. Bendich, H. B. Pahl, G. C. Korngold, H. S. Rosenkranz and J. R. Fresco,
J. Am. Chem. Soc., 80, 3949 (1958).
Abstr: Cold Spring Harbor Symp. Quant. Biol., 21, 31 (1956);
Soc. Exptl. Biol. Symp., 12, 31 (1958);
Federation Proc., 17, 299 (1958).
- #195c RIBONUCLEASE AND DEOXYRIBONUCLEASE ACTIVITIES DURING NORMAL AND NEOPLASTIC GROWTH.
S. Brody and M. E. Balis,
Nature, 182, 940 (1958).
- #196c THE INTERPRETATION OF BACTERIAL TRANSFORMATION DATA.
L. F. Cavalieri and B. H. Rosenberg,
Proc. Nat'l. Acad. Sci., 44, 853 (1958).
Abstr: 4th International Cong. Biochem., Vienna, p. 189 (1958).
- #198 NUCLEOSIDES LABELED WITH TRITIUM IN THE RIBOSYL GROUP.
M. P. Gordon, O. M. Intrieri and G. B. Brown,
J. Am. Chem. Soc., 80, 5161 (1958).
- #199 THE SYNTHESIS OF CYTIDINE-2-C¹⁴-RIBOSYL-c.
J. F. Codington, R. Facher, M. H. Maguire, R. Y. Thomson and G. B. Brown,
J. Am. Chem. Soc., 80, 5164 (1958).
Abstr: Federation Proc., 17, 202 (1958).
- #200c DEOXYRIBONUCLEASE ACTIVITY AND DEOXYRIBONUCLEIC ACID SYNTHESIS IN NORMAL, REGENERATING, PRECANCEROUS AND CANCEROUS RAT LIVER.
S. Brody,
Nature, 182, 1386 (1958).
- #201c STUDIES OF DEOXYRIBONUCLEIC ACIDS WITH THE AID OF ANION EXCHANGERS.
A. Bendich, H. B. Pahl, H. S. Rosenkranz and M. Rosoff,
[Reprinted from the Symposia of the Society for Experimental Biology, XII, The Biological Replication of Macromolecules, 1958] pp. 31-48.
- #202c FLUORINE-CONTAINING PYRIMIDINES AND PURINES: SYNTHESIS AND PROPERTIES OF TRIFLUOROMETHYL PYRIMIDINES AND PURINES.
A. Giner-Sorolla and A. Bendich,
J. Am. Chem. Soc., 80, 5744 (1958).
Also: A. Bendich, A. Giner-Sorolla and J. J. Fox in "The Chemistry and Biology of Purines", Wolstenholme and O'Connor editors, J. and A. Churchill, London, 1957, p. 3; a Ciba Fd. Symposium.
- #203c ON THE NATURE OF THE DEOXYRIBONUCLEIC ACID-METHYL GREEN REACTION.
H. S. Rosenkranz and A. Bendich,
J. Biophys. Biochem. Cytology, 4, 663 (1958).

Our ref.

- #204c ROLE OF THE RIBONUCLEOPROTEIN PARTICLE IN PROTEIN SYNTHESIS AND THE EFFECTS OF GROWTH HORMONE.
M. E. Balis, K. D. Samarth, M. G. Hamilton and M. L. Petermann,
J. Biol. Chem., 233, 1152 (1958).
Abstr: Federation Proc., 17, 185 (1958).
- #205 PURINE N-OXIDES. IV. ACTION OF XANTHINE OXIDASE ON ADENINE 1-N-OXIDE.
G. B. Brown, M. A. Stevens and H. W. Smith,
J. Biol. Chem., 233, 1513 (1958).
- #206 PURINE N-OXIDES. III. SOME BIOLOGICAL ACTIVITIES OF ADENINE 1-N-OXIDE DERIVATIVES.
G. B. Brown, D. A. Clarke, J. J. Bieseke, L. Kaplan and M. A. Stevens,
J. Biol. Chem., 233, 1509 (1958).
- #207 THIATION OF NUCLEOSIDES. II. SYNTHESIS OF 5-METHYL-2'-DEOXYCYTIDINE AND RELATED PYRIMIDINE NUCLEOSIDES.
J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich and G. B. Brown,
J. Am. Chem. Soc., 81, 178 (1959).
Abstr: Federation Proc., 17, 222 (1958).
(Supported by N.C.I.)
- #209c ON THE SIZE OF GENETIC DETERMINANTS IN PNEUMOCOCCUS AND THE NATURE OF THE VARIABLES INVOLVED IN TRANSFORMATION.
B. H. Rosenberg, F. M. Sirotnak and L. F. Cavalieri,
Proc. Nat'l. Acad. Sci., 45, 144 (1959).
Abstr: 132nd Meeting Am. Chem. Soc., New York, September 1957, p 13T.
- #210c PHYSICOCHEMICAL CHARACTERIZATION OF DEOXYRIBONUCLEIC ACIDS FROM HUMAN LEUKEMIC LEUKOCYTES.
E. E. Polli, M. Rosoff, D. diMayorca and L. F. Cavalieri,
Cancer Res., 19, 159 (1959).
- #211c SOME ASPECTS OF THE METABOLISM OF 5-BROMOURACIL.
H. B. Pahl, M. P. Gordon and R. R. Ellison,
Arch. Biochem. Biophys., 79, 245 (1959).
- #212 PURINE N-OXIDES. V. OXIDES OF ADENINE NUCLEOTIDES.
M. A. Stevens, H. W. Smith and G. B. Brown,
J. Am. Chem. Soc., 81, 1734 (1959).
- #213 PURINE N-OXIDES. VI. METABOLISM OF ADENINE 1-N-OXIDE IN VIVO.
D. Dunn, M. H. Maguire and G. B. Brown,
J. Biol. Chem., 234, 620 (1959).
Abstr: Federation Proc., 17, 215 (1958).
- #215c SEDIMENTATION STUDIES OF FRACTIONS OF DEOXYRIBONUCLEIC ACID.
H. S. Rosenkranz and A. Benich,
J. Am. Chem. Soc., 81, 902 (1959).
Abstr: Federation Proc., 17, 299 (1958).

- #217c SYNTHESIS OF PURINE-6-CARBOXALDEHYDE AND RELATED DERIVATIVES.
A. Giner-Sorolla, I. Zimmerman and A. Bendich,
J. Am. Chem. Soc., 81, 2515 (1959).
Abstr: 135th Meeting Am. Chem. Soc., Boston, April 1959, p. 6N.
- #218 STUDIES ON THE SEDIMENTATION BEHAVIOR OF ARTIFICIAL MIXTURES OF
DEOXYRIBONUCLEIC ACID.
H. S. Rosenkranz and A. Bendich,
J. Am. Chem. Soc., 81, 2842 (1959).
Abstr: Federation Proc., 17, 299 (1958).
- #219c TRITIUM-LABELED DEOXYRIBONUCLEIC ACID.
E. Borenfreund, H. S. Rosenkranz and A. Bendich, in Stohman, F., Jr.,
ed.
The Kinetics of Cellular Proliferation, (1959)
Grune and Stratton, Inc., New York, p. 130.
- #222c THE EFFECTS OF 5-FLUORODEOXYCYTIDINE, 5-FLUORODEOXYURIDINE, AND
RELATED COMPOUNDS ON TRANSPLANTED MOUSE LEUKEMIAS.
J. H. Burchenal, E. A. D. Holmberg, J. J. Fox, S. C. Hemphill
and J. A. Reppert,
Cancer Res., 19, 494 (1959).
Abstr: 4th International Cong. Biochem., Vienna, P. 185 (1958).
- #224c THE SUBUNIT OF DEOXYRIBONUCLEIC ACID.
L. F. Cavalieri, B. H. Rosenberg and J. P. Deutsch,
Biochem. Biophys. Res. Communications, 1, 124 (1959).
- #225c A CHROMATOGRAPHIC STUDY OF THE DEOXYRIBONUCLEIC ACIDS FROM NORMAL
AND LEUKEMIC HUMAN TISSUES.
G. diMayorca, H. S. Rosenkranz, E. E. Polli, G. C. Korngold and
A. Bendich,
J. Nat'l. Cancer Inst., 24, 1309 (1960).
Abstr: Haematologica Latina, Vol. II, p. 33 (Suppl. 20, 1959).
- #226c STUDIES ON THE FRACTIONATION OF TRANSFORMING DEOXYRIBONUCLEIC ACID
OF PNEUMOCOCCUS. I. RECOVERY AND DOSE-RESPONSE RELATIONSHIPS.
S. M. Beiser, H. B. Pahl, H. S. Rosenkranz and A. Bendich,
Biochim. Biophys. Acta, 34, 497 (1959).
Abstr: Federation Proc., 16, 230 (1957).
- #227c SHEAR DEGRADATION OF DEOXYRIBONUCLEIC ACID.
L. F. Cavalieri and B. H. Rosenberg,
J. Am. Chem. Soc., 81, 5136 (1959).
Abstr: A paper was presented at the Faraday Society Meeting, London,
March 1959.
- #228 THE CHEMISTRY AND METABOLISM OF ADENINE 1-N-OXIDE.
G. B. Brown, 4th Int'l. Cong. Biochem., XIII, 111 (1958).
Abstr: 4th International Cong. Biochem., Vienna, P. 40 (1958).
(Supported by N.C.I.)

- #229c- PYRIMIDINE NUCLEOSIDES.
J. J. Fox and I. Wempen,
Advances in Carbohydrate Chemistry, Vol. 14, Academic Press,
New York, 1959, pp. 283-380.
(No fund acknowledgements)
- #230c STUDIES ON THE EFFECT OF HEAT ON DEOXYRIBONUCLEIC ACID.
H. S. Rosenkranz and A. Bendich,
J. Am. Chem. Soc., 81, 6255 (1959).
- #231c STUDIES ON DEOXYRIBONUCLEIC ACID AFTER EXPOSURE TO TRITIUM GAS.
E. Borenfreund, H. S. Rosenkranz and A. Bendich,
J. Mol. Biol., 1, 195 (1959).
Abstr: Federation Proc., 18, 195 (1959).
- #233c ISOLATION OF INFECTIOUS DEOXYRIBONUCLEIC ACID FROM SE POLYOMA-
-INFECTED TISSUE CULTURES.
G. A. diMayorca, B. E. Eddy, S. E. Stewart, W. S. Hunter,
C. Friend and A. Bendich,
Proc. Nat'l. Acad. Sci., 45, 1805 (1959).
- #235c THE METABOLIC STABILITY OF RAT LIVER DEOXYRIBONUCLEIC ACID:
A TURNOVER STUDY.
J. R. Fresco and A. Bendich,
J. Biol. Chem., 235, 1124 (1960).
Abstr: Federation Proc., 14, 214 (1955).
- #236 PURINE N-OXIDES. VII. REACTION OF AMINOPURINE 1-N-OXIDES WITH
ACETIC ANHYDRIDE.
M. A. Stevens, H. W. Smith and G. B. Brown,
J. Am. Chem. Soc., 82, 1148 (1960).
- #238 PURINE N-OXIDES. VIII. N-OXIDES OF AZAPURINES.
M. A. Stevens, H. W. Smith and G. B. Brown,
J. Am. Chem. Soc., 82, 3189 (1960).
Also: N. Y. Section ACS Meeting-in-Miniature, March 1960.
- #239 PURINE N-OXIDES. IX. POOLAROGRAPHIC STUDIES.
F. A. McGinn and G. B. Brown,
J. Am. Chem. Soc., 82, 3193 (1960).
Abstr: 132nd Meeting Am. Chem. Soc., Chicago, April 1957, p. 15P.
- #240c PHYSICOCHEMICAL EFFECTS OF HIGH-SPEED MIXING ON DEOXYRIBONUCLEIC
ACID.
H. S. Rosenkranz and A. Bendich,
J. Am. Chem. Soc., 82, 3198 (1960).
- #242c THE METABOLISM OF PURINES BY TUMORS.
M. E. Balis,
Extrait de Acta Union Internationale Contre le Cancer,
Vol. XVI, No. 5, pp. 1037-1043 (1960).
(Presented before the 7th International Cancer Congress, London, 1958).

Our ref.

- #243c ATTEMPTS TO EXTRACT INFECTIVE NUCLEIC ACID OF ROUS SARCOMA VIRUS.
R. Weil, A. Bendich and C. M. Southam,
Proc. Soc. Exper. Biol. and Med., 104, 670 (1960).
Abstr: Proc. Nat. Acad. Sci., USA, 45, 1805 (1959).
- #244c MECHANISM OF GROWTH. II. THE INFLUENCE OF p-DIMETHYLAMINOAZO-BENZENE
ON RAT LIVER REGENERATION AFTER PARTIAL HEPATECTOMY,
S. Brody, Cancer Res., 20, 1469 (1960).
- #245c NUCLEOTIDES. I. SYNTHESIS OF 6-CHLORO-, 6-MERCAPTO-, AND 2-AMINO-6-
MERCAPTO-9- β -D-RIBOFURANOSYLPURINE 5'-PHOSPHATES,
A. Hampton and M. H. Magure, J. Am. Chem. Soc., 83, 150 (1961).
Abstr: 4th Interna. Cong. Biochem., Vienna, 1958, p. 40;
Federation Proc., 19, 310 (1960).
- #246c A STUDY OF THE PENETRATION OF MAMMALIAN CELLS BY DEOXYRIBONUCLEIC
ACIDS,
E. Borenfreund and A. Bendich, J. Biochem. & Biophys. Cytol., 2,
81 (1961).
Abstr: Xth Cong. Soc. Cell Biol., Paris, p. 170, Sept. 1960.
- #247c THE MOLECULAR WEIGHT AND AGGREGATION OF DNA,
L. F. Cavalieri, J. F. Deutsch and B. H. Rosenberg, Biophys. J.,
1, 301 (1961).
Abstr: Federation Proc., 19, 306 (1960).
- #248c THE REPLICATION OF DNA. I. TWO MOLECULAR CLASSES OF DNA,
L. F. Cavalieri and B. H. Rosenberg, Biophys. J., 1, 317 (1961).
- #249c THE REPLICATION OF DNA. II. THE NUMBER OF POLYNUCLEOTIDE STRANDS
IN THE CONSERVED UNIT OF DNA,
L. F. Cavalieri and B. H. Rosenberg,
Biophys. J., 1, 323 (1961).
- #250c THE REPLICATION OF DNA. III. CHANGES IN THE NUMBER OF STRANDS IN E.
COLI. DNA DURING ITS REPLICATION CYCLE,
L. F. Cavalieri and B. H. Rosenberg, Biophys. J., 1, 337 (1961).
- #251c NUCLEOSIDES. VIII. SYNTHESIS OF 5-NITROCYTIDINE AND RELATED NUCLEOSIDES,
J. J. Fox and D. Van Praag,
J. Org. Biochem., 26, 526 (1961).
- #252c Book Review: G. B. Brown, J. Am. Chem. Soc.,
The Neurochemistry of Nucleotides and Amino Acids.
Edited by Roscoe O. Brady, M. D., and Donald B. Tower, M.D.

Our ref.

- #253c - MULTI-STRANDED DNA AS DETERMINED BY X-IRRADIATION,
L. F. Cavalieri, R. Finston & B. H. Rosenberg,
Nature, 189, 833 (1961).
Abstr: Federation Proc., 20, 352 (1961).
- #254c PYRIMIDINE NUCLEOSIDES. IX. FACILE SYNTHESIS OF 1- β -D-LYXOFURANOSYL-
URACIL VIA 2,3'-ANHYDROLYXOSYL INTERMEDIATES,
R. Fecher, J. F. Codington and J. J. Fox,
J. Am. Chem. Soc., 83, 1889 (1961)
Abstr: 137th Meeting Am. Chem. Soc., Cleveland, Apr. 1960, p. 3D.
- #255c NUCLEOSIDES. X. ANHYDRONUCLEOSIDES AND RELATED COMPOUNDS DERIVED
FROM 2', 5'-DI-O-TRITYLURIDINE,
N. C. Yung and J. J. Fox,
J. Am. Chem. Soc., 83, 3060 (1961).
Abstr: 138th Meeting Am. Chem. Soc., New York, Sept. 1960, p. 20D.
- #256c STUDIES ON THE BIOLOGICAL ACTIVITY OF THE DEOXYRIBONUCLEIC ACIDS,
A. Bendich, Diseases of the Nervous System, Suppl. 22, 1 (1961).
Abstr: Atlantic City, May 11, 1960.
- #257c , A SIMPLE METHOD FOR SEPARATING OLIGONUCLEOTIDES FROM DEOXYRIBONUCLEASE,
H. S. Rosenkranz and A. Bendich,
Biochem. Biophys. Acta, 51, 186-188 (1961).
- #258c : FOUR-STRANDED DNA AS DETERMINED BY ELECTRON MICROSCOPY,
C. E. Hall and L. F. Cavalieri,
J. Biophysiol. & Biochem. Cytology, 10, 347-351 (1961).
Abstr: 5th Annual Meeting of the Biophysical Society, St. Louis,
1961, p. FB5.
- #259c NUCLEOTIDES. II. A NEW PROCEDURE FOR THE CONVERSION OF RIBONUCLEOSIDES
TO 2',3'-O-ISOPROPYLIDENE DERIVATIVES,
A. Hampton, J. Am. Chem. Soc., 83, 3640 (1961).
- #260c NUCLEIC ACIDS AND THE GENESIS OF CANCER,
A. Bendich, Bull. N.Y. Acad. Med., 37, 661 (1961).
- #261c ISOLATION AND PROPERTIES OF DNA FROM MAMMALIAN SPERM,
E. Borenfreund, E. C. Fitt & A. Bendich,
Nature, 191, 1375 (1961).
- #262c A CORRELATION BETWEEN THE SEDIMENTATION COEFFICIENT OF DNA AND ITS
PAPER CHROMATOGRAPHIC MIGRATION,
A. Bendich and H. S. Rosenkranz,
Arch. Biochem. Biophys., 94, 417 (1961).
- #267c SYNTHETIC REATIONS WITH OLIGO- AND POLY-DEOXYRIBONUCLEOTIDES;
NONENZYMATIC FORMATION OF PHOSPHODIESTER BONDS,
A. Bendich, H. S. Rosenkranz and P. S. Fitt,
J. de Chimie Physique, 58, 956 (1961).

Our ref.

- #272c NUCLEOTIDES. III. SYNTHESIS AND EFFECTS WITH CELLS IN CULTURE OF I-131-LABELED 5-iodo-2'-deoxyuridine-5'-phosphate
A. Hampton, E. G. Hampton, and M. L. Eidinoff,
Biochem. Pharmacol., 11, 155 (1962).
- #273 PURINE N-OXIDES. X. THE EFFECT OF SOME SUBSTITUENTS ON STABILITY AND REACTIVITY
M. A. Stevens, A. Giner-Sorolla, H. W. Smith, and G. B. Brown,
J. Org. Chem., 27, 567 (1962).
- #275c STUDIES OF THE ACTION OF ADENYLOSUCCINASE WITH 6-THIO ANALOGUES OF ADENYLOSUCCINIC ACID
A. Hampton,
J. Biol. Chem., 237, 529 (1962).
Abstr: 4th Internat. Cong. Biochem., Vienna, Supp., Pergamon Press, Ltd., London, 1958, p. 40.
- #278c OBSERVATIONS ON THE REPLICATION OF DNA
L. F. Cavalieri and B. H. Rosenberg,
in Fifteenth Annual Symposium on Fundamental Cancer Research, Houston, Texas, 1961. The Molecular Basis of Neoplasia, Austin, University of Texas Press (1962), p. 44.
- #278c-A SOME PHYSICOCHEMICAL PROPERTIES OF RIBONUCLEOPROTEIN FROM RAT LIVER MICROSOMES
M. G. Hamilton, L. F. Cavalieri, and M. L. Petermann,
J. Biol. Chem., 237, 1155 (1962).
Abstr: 138th Meeting Amer. Chem. Soc., New York, Sept. 1960, p. 53.
- #280c THE RENATURATION OF DENATURED DNA
L. F. Cavalieri, T. Small, and N. Sarkar,
Biophys. J., 2, 339 (1962).
Abstr: Biophys. Soc. Meeting, Washington, D. C., 1962.
- #281c NUCLEIC ACIDS: MOLECULAR BIOLOGY OF DNA
L. F. Cavalieri and B. H. Rosenberg,
Ann. Rev. Biochem., 31, 247 (1962).
- #282c THE PREPARATION AND PROPERTIES OF 6-HALOMETHYLPURINES
S. Cohen, E. Thom, and A. Bendich,
J. Org. Chem., 27, 3545 (1962).
Abstr: 141st Meeting Amer. Chem. Soc., Washington, D. C., March 1962, p. 23N.
- #285 THE ACTION OF XANTHINE OXIDASE ON SOME 2-SUBSTITUTED ADENINES
R. B. Scott and G. B. Brown,
J. Biol. Chem., 237, 3215 (1962).

Our ref.

- #286 ON THE CONVERSION OF CYTIDINE TO DEOXYCYTIDINE IN THE RAT
R. Y. Thomson, G. T. Scotto, and G. B. Brown,
J. Biol. Chem., 237, 3510 (1962).
- #287c AMIDES AND PEPTIDES DERIVED FROM 6-TRICHLOROMETHYLPURINE
AND AMINO ACIDS
S. Cohen, E. Thom, and A. Bendich,
Biochemistry, 2, 176 (1963).
- #289c SOME THOUGHTS ON THE DOUBLE-STRANDED MODEL OF DEOXYRIBONUCLEIC
ACID
A. Bendich and H. S. Rosenkranz,
in Davidson, J. N. and Cohn, W. E., eds., Progress in
Nucleic Acid Research, New York, Academic Press, vol. 1,
1963, p. 219.
- #291c A REIMER-TIEMANN REACTION WITH 6-TRICHLOROMETHYLPURINE
S. Cohen, E. Thom, and A. Bendich,
J. Org. Chem., 28, 1379 (1963).
- #295c REACTIONS OF RIBONUCLEOTIDE DERIVATIVES OF PURINE ANALOGUES
AT THE CATALYTIC SITE OF INOSINE 5'-PHOSPHATE DEHYDROGENASE
A. Hampton,
J. Biol. Chem., 238, 3068 (1963).
- #296c DISPLACEMENT OF THE 2-METHYLMERCAPTO GROUP IN PYRIMIDINES
BEARING A 5-NITROSO SUBSTITUENT
R. M. Cresswell and T. Strauss,
J. Org. Chem., 28, 2563 (1963).
- #297 SOME 2-SUBSTITUTED AMINO PURINES AND PURINE ANALOGS
R. M. Cresswell, T. Strauss, and G. B. Brown,
J. Med. Chem., 6, 817 (1963).
- #299c 2',3'-O-ISOPROPYLIDENE DERIVATIVES OF GUANOSINE AND OTHER
NUCLEOSIDES
A. Hampton,
in Biochemical Preparations, New York, John Wiley & Sons,
vol. 10, 1963, p. 91.
- #301 PURINE N-OXIDES. XI. AN ACTIVATING EFFECT ON SOME DISPLACE-
MENT REACTIONS
R. M. Cresswell and G. B. Brown,
J. Org. Chem., 28, 2560 (1963).
- #304c NUCLEIC ACIDS AND INFORMATION TRANSFER
L. F. Cavalieri,
J. Cellular Comp. Physiol. Supp. 62, 111 (1963).

Our ref.

- #305 SYNTHESIS OF A 6-MERCAPTOPURINE N-OXIDE
G. Levin and G. B. Brown,
J. Med. Chem., 6, 825 (1963).
- #312c POLYRIBONUCLEOTIDES AS TEMPLATES FOR POLYDEOXYRIBONUCLEOTIDES
S. Lee-Huang and L. F. Cavalieri,
Proc. Nat. Acad. Sci., 50, 11 (1963).
- #313c CHROMOSOMAL ABERRATIONS INDUCED BY HYPONITRITE AND
HYDROXYLAMINE DERIVATIVES
E. Borenfreund, M. Krim, and A. Bendich,
J. Nat. Cancer Inst., 32, 667 (1964).
- #316 A NITROGEN MUSTARD DERIVATIVE OF 6-MERCAPTOPURINE
G. Levin, K. Sugiura, and G. B. Brown,
J. Med. Chem., 7, 357 (1964).
- #318c ON THE TRANSIENT TEMPLATE FOR IN VIVO DNA SYNTHESIS
B. H. Rosenberg and L. F. Cavalieri,
Proc. Nat. Acad. Sci., 51, 826 (1964).
- #320c NUCLEIC ACID AND INFORMATION TRANSFER
L. F. Cavalieri and B. H. Rosenberg,
in Davidson, J. N. and Cohn, W. E., eds., Progress in
Nucleic Acid Research, New York, Academic Press, vol. 2,
1963, p. 1
- #321 PURINE N-OXIDES. XII. PHOTOCHEMICAL CHANGES INDUCED BY
ULTRAVIOLET RADIATION
G. B. Brown, G. Levin, and S. Murphy,
Biochemistry, 3, 880 (1964).
- #322 PURINE N-OXIDES. XIII. KINETICS OF THE PHOTOCHEMICAL
ALTERATION OF ADENINE 1-N-OXIDE
G. Levin, R. B. Setlow, and G. B. Brown,
Biochemistry, 3, 883 (1964).
- #331c. THE MODE OF INTERACTION OF ACTINOMYCID D WITH DNA
L. F. Cavalieri and R. G. Nemchin,
Biochim. Biophys. Acta, 87, 641 (1964).
- #332c AMINO ACIDS OR SMALL PEPTIDES AS PUNCTUATION IN THE
GENETIC CODE OF DNA
A. Bendich, E. Borenfreund, G. C. Korngold, M. Krim,
and M. E. Balis,
Tipografia Successori Fusi, Pavi, 1964, p. 214.

Our ref.

- #335c STUDIES ON THE TRIATION OF PURINES
A. Giner-Sorolla, E. Thom, and A. Bendich,
J. Org. Chem., 29, 3209 (1964).
Abstr: 137th Meeting Amer. Chem. Soc., Cleveland,
April 1960, p. 11N.
- #337c ISOLATION AND PROPERTIES OF A NUCLEIC ACID HYBRID POLYMERASE
S. Lee-Huang and L. F. Cavalieri,
Proc. Nat. Acad. Sci., 51, 1022 (1964).
- #342 PURINE N-OXIDES. XIV. A TOTAL SYNTHESIS OF A PYRIMIDINE
N-OXIDE, A PTERIDINE 1-N-OXIDE AND XANTHINE 3-N-OXIDE
R. M. Cresswell, H. K. Maurer, T. Strauss, and G. B. Brown,
J. Org. Chem., 30, 408 (1965).
- #343 PURINE N-OXIDES. XV. THE SYNTHESIS OF 6-MERCAPTOPURINE
3-N-OXIDE. ITS CHEMOTHERAPEUTIC POSSIBILITIES
G. B. Brown, G. Levin, S. Murphy, A. Sele, H. C. Reilly,
G. S. Tarnowski, F. A. Schmid, M. N. Teller, and C. C. Stock,
J. Med. Chem., 8, 190 (1965).
- #344 PURINE N-OXIDES. XVI. ONCOGENIC DERIVATIVES OF XANTHINE
AND GUANINE
G. B. Brown, K. Sugiura, and R. M. Cresswell,
Cancer Res., 25, 986 (1965).
Abstr: Proc. Am. Assoc. Ca. Res., vol. 6, p. 8 (1965).
- #345c EFFECTS OF MITOMYCIN C ON THE INFECTION OF CELLS BY POLYOMA
VIRUS AND ITS DNA
E. Borenfreund, M. Krim, and A. Bendich,
Virology, 25, 393 (1965).
- #346c CIRCULATING DNA AS A POSSIBLE FACTOR IN ONCOGENESIS
A. Bendich, T. Wilczok, and E. Borenfreund,
Science, 148, 374 (1965).
- #353c NUCLEIC ACID POLYMERASES: POSSIBLE SUBUNIT STRUCTURE
S. Lee-Huang and L. F. Cavalieri,
Science, 148, 1474 (1965).
- #354c O,5'-THYMIDYLYL-L-SERINE: A MODEL FOR POSSIBLE LINKERS IN DNA
W. F. Berkowitz and A. Bendich,
Biochemistry, 4, 1979 (1965).
Abstr: 148th Meeting Amer. Chem. Soc., Chicago, Aug. 1964, p. 15P.
- #360 CYTOSINE 3-N-OXIDE AND ITS REARRANGEMENT ON ACETYLATION
T. J. Delia, M. J. Olsen, and G. B. Brown,
J. Org. Chem., 30, 2766 (1965).
Abstr: Proc. Am. Assoc. Cancer Res., vol. 6 p. 8 (1965);
Royal Australian Chemical Institute Symposium on Heterocyclic
Chemistry, Melbourne, August 1965.

Our ref.-

- #361c TEMPLATE DEOXYRIBONUCLEIC ACID AND THE CONTROL OF REPLICATION
B. H. Rosenberg and L. F. Cavalieri,
Nature, 206, 999 (1965).
- #362c THE SYNTHESIS AND PROPERTIES OF 6-MERCAPTOMETHYLPURINE AND
DERIVATIVES
A. Giner-Sorolla and A. Bendich,
J. Med. Chem., 8, 667 (1965).
Abstr: 149th Meeting Amer. Chem. Soc., Detroit, April 1965, p. 7N.
- #368 PURINE N-OXIDES. XVII. THE OXIDATION OF GUANINE AT POSITION 7
T. J. Delia and G. B. Brown,
J. Org. Chem., 31, 178 (1966).
- #370c SYNTHESIS AND PROPERTIES OF 5-MERCAPTOMETHYLURACIL AND RELATED
DERIVATIVES
A. Giner-Sorolla and L. Medrek,
J. Med. Chem., 9, 97 (1966).
Abstr: 149th Meeting Amer. Chem. Soc., Detroit, April 1965, p. 7N.
- #371c SYNTHESIS AND BIOLOGICAL ACTIVITY OF 9- β -D-RIBOFURANSOYL-6-
-HYDROXYLAMINOPURINE
A. Giner-Sorolla, L. Medrek, and A. Bendich,
J. Med. Chem., 9, 143 (1966).
- #373 PURINE N-OXIDES. XVIII. DEAMINATION OF ADENINE N-OXIDE DERIVATIVES
J. C. Parham, J. Fissekis, and G. B. Brown,
J. Org. Chem., 31, 966 (1966).
- #379c THE SYNTHESIS AND PROPERTIES OF SUBSTITUTED 6-HYDROXYLAMINOPURINES
A. Giner-Sorolla, S. O'Bryant, J. H. Burchenal, and A. Bendich,
Biochemistry, 5, 3057 (1966).
- #381c ACTION OF 1- β -D-ARABINOFURANOSYL-5-FLUOROCYTOSINE ON THE NUCLEIC
ACID METABOLISM AND VIABILITY OF HELA CELLS
J. H. Kim, M. L. Eidinoff, and J. J. Fox,
Cancer Res., 26, 1661 (1966).
- #384c METAL COMPLEXES OF 2',3'-O-ISOPROPYLIDENE-6-THIOINOSINE
A. Hampton and J. C. Pratantoni,
J. Med. Chem., 9, 976 (1966).
- #390c A NOVEL REACTION OF SUBSTITUTED PURINES AND PYRIMIDINES
A. Giner-Sorolla and A. Bendich,
J. Org. Chem., 31, 4239 (1966).
Abstr: 150th Meeting Amer. Chem. Soc., Atlantic City,
Sept. 1965, p. 5P.
- #399 PURINE N-OXIDES. XIX. ON THE ONCOGENIC N-OXIDE DERIVATIVES
OF GUANINE AND XANTHINE AND A NONONCOGENIC ISOMER OF
XANTHINE N-OXIDE
K. Sugiura and G. B. Brown,
(continued next page)

Our ref.

Cancer Res., 27, 925 (1967).
Abstr: Proc. Am. Assoc. Cancer Res., 7, 69 (1966);
Ninth Int'l. Cancer Cong., Tokyo, Oct. 1966, p. 317.

- #400 PURINE N-OXIDES. XX. HYDROLYSES AND REARRANGEMENT OF PURINE
1-N-OXIDES. SYNTHESIS OF 1-HYDROXYXANTHINE
J. C. Parham, J. Fissekis, and G. B. Brown,
J. Org. Chem., 32, 1151 (1967).
- #402c STUDIES OF 6-N-HYDROXYLAMINO-9- β -D-RIBOFURANOSYLPURINE IN
MOUSE LEUKEMIA
J. H. Burchenal, M. Dollinger, J. Butterbaugh, D. Stoll,
and A. Giner-Sorolla,
Biochemical Pharmacol., 16, 423 (1967).
Abstr: Eleventh Cong. Int'l. Soc. Hematol., Sydney, Australia,
1966, p. 227.
- #410c MALIGNANT TRANSFORMATION AND INTERCELL COMMUNICATION INVOLVING DNA
A. Bendich, E. Borenfreund, A. D. Vizoso, R. G. Harris,
Subviral Carcinogenesis, Monograph First International Symposium
on Tumor Viruses, Nagoya, Japan, July 1967, pp. 99-115.
- #412 PURINE N-OXIDES. XXI. ELIMINATION OF A 5 SUBSTITUENT FROM A
SUBSTITUTED URACIL. A SYNTHESIS OF 3-HYDROXY-1-METHYLYXANTHINE
A. D. McNaught and G. B. Brown,
J. Org. Chem., 32, 3689 (1967).
- #418c THE SYNTHESIS AND PROPERTIES OF 2,6-DIHYDROXYLAMINOPURINE AND
ITS 9- β -D-RIBOFURANOSYL DERIVATIVE
A. Giner-Sorolla, C. Nanos, J. H. Burchenal, M. Dollinger,
and A. Bendich,
J. Med. Chem., 11, 52 (1968)
- #430c DARSTELLUNG UND EIGENSCHAFTEN EINIGER 6- UND 2,6-SUBSTITUIERTER
PURINE
A. Giner-Sorolla,
Chem. Ber. 101, 611 (1968).
- #433c THE SYNTHESIS AND BIOLOGICAL PROPERTIES OF HYDROXYLAMINOPURINES
AND RELATED DERIVATIVES
A. Giner-Sorolla, S. A. O'Bryant, C. Nanos, M. R. Dollinger,
A. Bendich, and J. H. Burchenal,
J. Med. Chem. 11, 521 (1968).
- #434 PURINE N-OXIDES AND CANCER
G. B. Brown, in J. N. Davidson and W. E. Cohn, Eds.,
Progress in Nucleic Acid Research and Molecular Biology,
vol. 8, New York, Academic Press, 1968, 209.

Our ref.

- #441 PURINE N-OXIDES AS ANTIMETABOLITES AND ONCOGENS
G.B. Brown, in M.E. Balis, Antagonists and Nucleic Acids, E.L. Tatum and A. Neuberger, eds., Amsterdam North-Holland Publishing Co., 1968, p. 237.
- #468 PURINE N-OXIDES. XXII. ON THE STRUCTURES OF 3-HYDROXYXANTHINE AND GUANINE 3-OXIDE
U. Wölcke and G.B. Brown, J. Org. Chem. 34, 978 (1969).
- #469 PURINE N-OXIDES. XXIII. REARRANGEMENTS OF PURINE 3-N-OXIDES ON ACYLATION AND METHYLATION
U. Wölcke, W. Pfeleiderer, T.J. Delia and G.B. Brown, J. Org. Chem. 34, 981 (1969).
- #470 PURINE N-OXIDES. XXIV. THE PREPARATION OF 8-SUBSTITUTED XANTHINES AND GUANINES BY A NUCLEOPHILIC DISPLACEMENT OF A 3- SUBSTITUENT
U. Wölcke, N.J.M. Birdsall and G.B. Brown, Tetrahedron Lett. 10, 785 (1969).
Abstr: 157th Meeting Am. Chem. Soc., Minneapolis, April 1969, p. Orgn 172; Metrochem 69 Regional Meeting Am. Chem. Soc., New York, May 1969, p. 34.
- #471C THE PRODUCT OF THE AIR OXIDATION OF URIC ACID. AN INTERMEDIATE FORMED IN THE PRESENCE OF DIMETHYLAMINE
P.H. Stahl, Biochemistry 8, 733 (1969).
- #474 PURINE N-OXIDES. XXV. 3-N-OXIDES OF ADENINE AND HYPOXANTHINE
I. Scheinfeld, J.C. Parham, S. Murphy and G.B. Brown, J. Org. Chem. 34, 2153 (1969).
- #475 PURINE N-OXIDES. XXVI. THE SYNTHESIS AND PROPERTIES OF 6-HALOGENOPURINE 3-N-OXIDES
A. Giner-Sorolla, C. Gryte, A. Bendich, G.B. Brown, J. Org. Chem. 34, 2157 (1969).
Abstr: Metrochem 69 Regional Meeting Am. Chem. Soc., New York, May 1969, p. 35.
- #478C PURINE N-OXIDES. XXIX. THE SYNTHESIS OF 6-HYDROXYLAMINOPURINE 3-OXIDE AND RELATED DERIVATIVES
A. Giner-Sorolla, J. Med. Chem. 12, 717 (1969).
- #494 A COMPARISON OF THE ONCOGENICITIES OF 3-HYDROXYXANTHINE, GUANINE 3-N-OXIDE, AND SOME RELATED COMPOUNDS
K. Sugiura, M.N. Teller, J.C. Parham and G.B. Brown, Cancer Res. 30, 184 (1970). (Purine N-Oxides XXXII)
Abstr: Proc. Am. Assoc. Cancer Res. 9, 70 (1968).

- #498 ONCOGENIC PURINE DERIVATIVES: EVIDENCE FOR A POSSIBLE
PROXIMATE ONCOGEN
G. Stöhrer and G.B. Brown
Science 167, 1622 (1970). (Purine N-Oxides XXXIII)
Abstr: 158th Meeting Am. Chem. Soc., New York City,
September 1969, p. Biol 141.
- #508C A SIMPLIFIED PROCEDURE FOR THE SYNTHESIS OF 6-METHYLTHIOPURINE
RIBONUCLEOSIDE 5'-PHOSPHATES
F. Perini and A. Hampton,
J. Heterocycl. Chem. 7, 969 (1970).
- #516C PURINE N-OXIDES. XXXIV. SYNTHESIS OF PURINE 3-OXIDE,
6-METHYLPURINE 3-OXIDE, AND RELATED DERIVATIVES
A. Giner-Sorolla, C. Gryta, M.L. Cox and J.C. Parham,
J. Org. Chem. 36, 1228 (1971).

In Press:

- A. PURINE N-OXIDES. XLI. THE 3-ACYLOXYPURINE 8-SUBSTITUTION REACTION: ON THE MECHANISM OF THE REACTION
N.J.M. Birdsall, U. Wölsche, J.C. Parham and G.B. Brown,
Tetrahedron, in press.
- B. PURINE N-OXIDES. XXXIX. N-ACETYL DERIVATIVES OF N-HYDROXYXANTHINES
N.J.M. Birdsall, T.-C. Lee, U. Wölsche,
Tetrahedron, in press.
- C. PURINE N-OXIDES. THE CYCLIZATION OF 4-AMINO-5-NITROURACIL WITH FORMALDEHYDE. PREPARATION AND PROPERTIES OF 7-HYDROXYXANTHINE
G. Zvilichovsky and G.B. Brown,
J. Org. Chem., in preparation.
- D. PURINE N-OXIDES. XL. THE 3-ACYLOXYPURINE 8-SUBSTITUTION REACTION: SCOPE: SYNTHESIS OF 8-SUBSTITUTED XANTHINES AND GUANINES
N.J.M. Birdsall, U. Wölsche, T.-C. Lee and G.B. Brown,
Tetrahedron, in press.
- E. PURINE N-OXIDES. XXXV. ALKYLATED GUANINE 3-OXIDES AND 3-HYDROXYXANTHINES
N.J.M. Birdsall, T.-C. Lee, T.J. Delia and J.C. Parham,
J. Org. Chem., in press.
- F. PURINE N-OXIDES. XXXVI. THE TAUTOMERIC STRUCTURES OF THE 3-N-OXIDES OF XANTHINE AND GUANINE
J.C. Parham, T.G. Winn, G.B. Brown,
J. Org. Chem., in press.
- G. PURINE N-OXIDES. PHOTOCHEMICALLY INDUCED FREE-RADICALS FROM ONCOGENIC PURINE N-OXIDES
J.C. Parham, I. Pullman and G.B. Brown,
in preparation.

Cell Biochemistry Division

Aaron Bendich - Section 7701
Ellen Borenfreund, Ph.D. - Section 7702

AEC-supported Bibliography

1969 - 1971

- 1B. PURINE N-OXIDES. XXVI. THE SYNTHESIS AND PROPERTIES OF
6-HALOGENOPURINE 3-N-OXIDES
A. Giner-Sorolla, C. Gryte, A. Bendich, and G.B. Brown,
J. Org. Chem., 34, 2157-2159 (1969).
Abstr: Regional Meeting of the American Chemical Society,
New York City, May 1, 1969, p. 35.
- 2B. THE SYNTHESIS AND PROPERTIES OF 9- β -D-ARABINOFURANOSYL-6-
HYDROXYLAMINOPURINE
A. Giner-Sorolla,
J. Heterocyclic Chem., 6, 405-406 (1969).
- 3B. PURINE N-OXIDES. XXIX. THE SYNTHESIS OF 6-HYDROXYLAMINOPURINE
3-OXIDE AND RELATED DERIVATIVES
A. Giner-Sorolla,
J. Med. Chem., 12, 717-719 (1969).
- 4B. CELL TRANSFORMATION AND THE GENESIS OF CANCER
A. Bendich, E. Borenfreund, Y. Honda and M. Steinglass,
Arch. Environ. Health, 19, 157-166 (1969).
- 5B. REACTIONS OF 6-HYDRAZINO-, 6-HYDROXYLAMINOPURINES AND RELATED
DERIVATIVES
A. Giner-Sorolla
J. Heterocyclic Chem., 7, 75-79 (1970).
- 6B. RETROGENETIC EXPRESSION: THE REAPPEARANCE OF EMBRYONAL ANTIGENS
IN CANCER CELLS
E.H. Stonehill and A. Bendich,
Nature, 228, 370-372 (1970).

- 7B. LOS CARCINOGENOS QUIMICOS: RETROSPECCION Y PERSPECTIVA
A. Giner-Sorolla
"Afinidad" (J. Theoret. and Applied Chem.) (Barcelona),
XXVII, #278, 438-446 (1970).
- 8B. STUDIES ON DNA-INDUCED HERITABLE ALTERATION OF MAMMALIAN CELLS
E. Borenfreund, Y. Honda, M. Steinglass and A. Bendich,
J. Exper. Med., 132, 1071-1089 (1970).
- 9B. DNA-INDUCED HERITABLE ALTERATION OF MAMMALIAN CELLS
A. Bendich, E. Borenfreund, and Y. Honda,
Proceedings of a Symposium, Uptake of Informative Molecules
by Living Cells, in press.
Abstr: Presented at Symposium, Centre d'Etude de l'Energie
Nucléaire C.E.N./S.C.K., Mol, Belgium, August 31-September 2, 1970.
- 10B. SYNTHESIS OF 6,8-DISUBSTITUTED PURINES
A. Giner-Sorolla and D.M. Brown,
J. Chem. Soc. (London) (C), pp. 126-128 (1971).
- 11B. VILSMEIER REACTION ON 6-METHYLPURINE
D.M. Brown and A. Giner-Sorolla,
J. Chem. Soc. (London) (C), pp. 128-132 (1971).
- 12B. BINDING OF [G-³H]-7,12-DIMETHYLBENZ(a)ANTHRACENE TO DNA OF
NORMAL AND OF RAPIDLY DIVIDING HEPATIC CELLS OF RATS
H. Marquardt, A. Bendich, F.S. Philips, and D. Hoffmann,
Chem.-Biol. Interactions, 3, 1-11 (1971).
- 13B. PURINE N-OXIDES. XXXIV. SYNTHESIS OF PURINE 3-OXIDE, 6-METHYL-
PURINE 3-OXIDE, AND RELATED DERIVATIVES
A. Giner-Sorolla, C. Gryte, M.L. Cox, and J.C. Parham,
J. Org. Chem., 36, 1228-1232 (1971).
- 14B. DISTINCTION OF NORMAL ADULT ANTIGENS FROM THE COINCIDENT
ANTIGENS OF TUMOR AND EMBRYO TISSUES
E.H. Stonehill, P. Higgins, and A. Bendich,
Abstr: Proc. Amer. Assoc. Cancer Res., vol. 12, p. 42 (1971).
- 15B. DNA BINDING AND SYNTHESIS IN REGENERATING LIVER OF RATS GIVEN
7,12-DIMETHYLBENZ(a)ANTHRACENE (DMBA) DURING PREREPLICATIVE
PHASE
H. Marquardt, A. Bendich, and F. Philips,
Proc. Amer. Assoc. Cancer Res., vol. 12, p. 15 (1971)

- 16B. HYDROXYLAMINOPURINES: SYNTHESIS, PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITY
A. Giner-Sorolla and J.H. Burchenal,
in The Jerusalem Symposia on Quantum Chemistry and Biochemistry,
4th Symposium on Purines
E.D. Bergmann and B. Pullman, eds., Jerusalem
Published by Israel Academy of Science and Humanities, in press.
Abstr: 4th Intl. Symp. on Quantum Chemistry and Biochemistry,
Jerusalem, Israel, April 3-9, 1971.
- 17B. ANACHRONISTIC GENETIC EXPRESSION RECOGNIZED BY THE PRODUCTION OF FETAL ANTIGENS IN CANCER CELLS
E.H. Stonehill, E. Borenfreund, and A. Bendich,
Monograph of Symposium, Fetal Antigens in Cancer, in press.
Abstr: "Fetal Antigens in Cancer" Workshop and Conference,
Oak Ridge National Laboratory, Tenn., May 23-25, 1971.
- In Press
- 18B. STUDIES ON ENZYMATIC DEAMINATION OF ADENOSINE ANALOGS
R. Dollinger, W. Kreis, J.H. Burchenal, C. Gryte, and A. Giner-Sorolla,
J. Med. Chem. (1971)
- 19B. PURINE N-OXIDES. XXXVII. DERIVATIVES FROM 6-CHLOROPURINE 3-OXIDE
A. Giner-Sorolla
J. Heterocyclic Chem. (1971)
- 20B. SUBSTITUTED HYDROXYLAMINOPURINES AND RELATED DERIVATIVES: SYNTHESIS AND SCREENING TESTS
A. Giner-Sorolla and J.H. Burchenal,
J. Med. Chem. (1971)

Biological Chemistry Division

Mary L. Petermann Ph.D. - Section 5702

AEC-supported Bibliography

1950-1971

Our ref.

- #P-1. NUCLEI FROM NORMAL AND LEUKEMIC MOUSE SPLEEN. I. THE ISOLATION OF NUCLEI IN NEUTRAL MEDIUM.
R. M. Schneider and M. L. Petermann,
Cancer Research, 10, 751 (1950).
Abstr. Federation Proc., 9, 214 (1950).
- #P-2. NUCLEI FROM NORMAL AND LEUKEMIC MOUSE SPLEEN. II. THE NUCLEIC ACID CONTENT OF NORMAL AND LEUKEMIC NUCLEI.
M. L. Petermann and R. M. Schneider,
Cancer Research, 11, 485 (1951).
- #P-3. AN ULTRACENTRIFUGAL ANALYSIS OF THE MACROMOLECULAR PARTICLES OF NORMAL AND LEUKEMIC MOUSE SPLEEN.
M. L. Petermann and M. G. Hamilton,
Cancer Research, 12, 373 (1952).
Abstr. Cancer Research, 11, 273 (1951).
- #P-4. THE BASIC PROTEINS OF ASPERGILLUS FUMIGATUS WITH TUMOR-INHIBITING PROPERTIES.
M. L. Petermann, M. G. Hamilton and H. C. Reilly,
Arch. Biochem. & Biophys., 37, 117 (1952).
- #P-5. NUCLEI FROM NORMAL AND LEUKEMIC MOUSE SPLEEN. III. THE DESOXPENTOSE NUCLEIC ACID CONTENT PER NUCLEUS CALCULATED FROM TOTAL CELL COUNTS.
N. A. Mizen and M. L. Petermann,
Cancer Research, 12, 727 (1952).
- #P-6. THE MACROMOLECULAR PARTICLES OF NORMAL AND REGENERATING RAT LIVER.
M. L. Petermann, N. A. Mizen and M. G. Hamilton,
Cancer Research, 13, 372 (1953).
Abstr. 2nd Internat. Cong. Biochem., Paris, July 1952.
- #P-7. THE CHEMISTRY OF NEOPLASTIC TISSUE.
C. J. Kensler and M. L. Petermann,
Annual Review of Biochemistry, 22, 319 (1953).
- #P-8. SEDIMENTATION OF THE THYROXINE-BINDING PROTEIN OF SERUM IN THE PARTITION CELL.
M. L. Petermann, J. Robbins and M. G. Hamilton,
J. Biol. Chem., 208, 369 (1954).

Our ref.

- #P-9. IDENTIFICATION OF THYROGLOBULIN IN HUMAN SERUM AFTER LARGE DOSES OF I^{131} . II. SEDIMENTATION OF SERUM I^{131} IN THE ULTRACENTRIFUGE.
J. Robbins, M. L. Petermann and J. E. Rall,
J. Biol. Chem., 208, 387 (1954).

- #P-10. ELECTROPHORETIC ANALYSIS OF THE MACROMOLECULAR NUCLEOPROTEIN PARTICLES OF MAMMALIAN CYTOPLASM.
M. L. Petermann, M. G. Hamilton and N. A. Mizen,
Cancer Research, 14, 360 (1954).
Abstr. Federation Proc., 12, 254 (1953).

- #P-11. CHANGES IN HUMAN PLASMA MUCOPROTEIN IN HYPERTHYROIDISM AND MYXEDEMA.
P. Mustacchi, M. L. Petermann and J. E. Rall,
J. Clin. Endocrinol. & Metab., 14, 729 (1954).

- #P-12. THE MACROMOLECULAR NUCLEOPROTEIN PARTICLES OF NORMAL RAT LIVER AND AXO-DYE INDUCED RAT LIVER TUMORS.
M. L. Petermann,
Texas Repts. Biol. & Med., 12, 921 (1954).

- #P-13. ELECTROPHORESIS OF THE SERUM THYROXINE-BINDING PROTEIN AT pH 4.5.
J. Robbins, M. L. Petermann and J. E. Rall,
J. Biol. Chem., 212, 403 (1955).
Abstr. Federation Proc., 13, 280 (1954).

- #P-14. HUMAN PLASMA LIPOPROTEINS. I. IN NORMAL WOMEN AND IN WOMEN WITH ADVANCED CARCINOMA OF THE BREAST.
M. Barclay, G. E. Cagin, G. C. Escher, R. J. Kaufman, E. D. Kidder and M. L. Petermann,
Cancer, 2, 253 (1955).
Abstr. Federation Proc., 10, 172 (1951).

- #P-15. HUMAN PLASMA LIPOPROTEINS. II. THE EFFECT OF OSSEOUS METASTASES IN PATIENTS WITH ADVANCED CARCINOMA OF THE BREAST.
R. J. Kaufman, M. Barclay, E. D. Kidder, G. C. Escher and M. L. Petermann,
Cancer, 8, 888 (1955).

- #P-16. A STABILIZING FACTOR FOR CYTOPLASMIC NUCLEOPROTEINS.
M. L. Petermann and M. G. Hamilton,
J. Biophys. Biochem. Cytol., 1, 469 (1955).

- #P-17. MULTIPLE MYELOMA PROTEINS. II. ULTRACENTRIFUGAL AND ELECTROPHORETIC ANALYSIS OF A PAUCIDISPERSE MYELOMA GLOBULIN.
M. L. Petermann, M. G. Hamilton and L. Korngold,
Cancer, 9, 193 (1956).

Our ref."

- #P-18. THE CYTOPLASMIC NUCLEOPROTEINS OF AZO DYE-INDUCED RAT LIVER TUMORS.
M. L. Petermann, N. A. Mizen and M. G. Hamilton,
Cancer Research, 16, 620 (1956).
Abstr. Proc. Am. Assoc. Cancer Res., 1 (2), 37 (1954).
- #P-19. ULTRACENTRIFUGAL STUDIES OF THE PROCESS OF THYROGLOBULIN HYDROLYSIS.
J. B. Alpers, M. L. Petermann and J. E. Rall,
Arch. Biochem. Biophys., 65, 513 (1956).
- #P-20. THE PURIFICATION AND PROPERTIES OF CYTOPLASMIC RIBONUCLEOPROTEIN FROM RAT LIVER.
M. L. Petermann and M. G. Hamilton,
J. Biol. Chem., 224, 725 (1957).
Abstr. Federation Proc., 15, 326 (1956).
- #P-21. HUMAN PLASMA LIPOPROTEINS. III. THE EFFECT OF BILATERAL OOPHORECTOMY ON THE PLASMA LIPOPROTEINS IN YOUNG WOMEN WITH ADVANCED MAMMARY CARCINOMA.
M. Barclay, R. J. Kaufman, D. W. Sved, E. D. Kidder, G. C. Escher and M. L. Petermann,
Cancer, 10, 1076 (1957).
Abstr. Proc. Am. Assoc. Cancer Res., 2, 2 (1955).
- #P-22. A CRYOGLOBULIN WITH UNUSUAL CHARACTERISTICS ASSOCIATED WITH A RARE HEMATOLOGIC DISORDER.
E. L. Crumpacker, M. L. Petermann and W. D. Irvine,
Virginia Medical Monthly, 84, 493 (1957).
- #P-23. CHANGES IN THE PLASMA LIPOPROTEINS IN WOMEN WITH CARCINOMA OF THE BREAST. IN THE LIPOPROTEINS.
F. Homburger and P. Bernfeld, eds. S. Karger, Basel, 1958.
M. L. Petermann, M. Barclay, G. Escher and R. Kaufman.
Abstr. Proc. Am. Assoc. Cancer Res., 2, 93 (1956).
- #P-24. PHYSICOCHEMICAL AND METABOLIC STUDIES ON RAT LIVER NUCLEOPROTEIN. IN MICROSOMAL PARTICLES AND PROTEIN SYNTHESIS. R. B. Roberts, Ed.,
M. L. Petermann, M. G. Hamilton, M. E. Balis, K. Samarth and P. Pecora.
Abstr. Biophysical Society, Cambridge, Mass., February 1958;
Federation Proc., 17, 288 (1958).
- #P-25. ROLE OF THE RIBONUCLEOPROTEIN PARTICLE IN PROTEIN SYNTHESIS AND THE EFFECTS OF GROWTH HORMONE.
M. E. Balis, K. D. Samarth, M. G. Hamilton and M. L. Petermann,
J. Biol. Chem., 233, 1152 (1958).
Abstr. Federation Proc., 17, 185 (1958).
- #P-26. THE INFLUENCE OF AGE, SEX, PREGNANCY, STARVATION, AND OTHER FACTORS ON THE CYTOPLASMIC RIBONUCLEOPROTEINS OF RAT LIVER.
M. L. Petermann and M. G. Hamilton,
J. Biophys. Biochem. Cytol., 4, 771 (1958).
Abstr. 3rd Internat. Cong. Biochem., Brussels, August 1955.

Our ref.

- #P-27. AN ASPECT OF NUCLEOPROTEIN METABOLISM IN TUMOR-BEARING ANIMALS.
P. Pecora, M. G. Hamilton, M. L. Petermann and M. E. Balis,
Abstr. Proc. Am. Assoc. Cancer Res., 3, 51 (1959).
- #P-28. ULTRACENTRIFUGAL STUDIES ON RIBONUCLEOPROTEIN FROM RAT LIVER
MICROSOMES.
M. G. Hamilton and M. L. Petermann,
J. Biol. Chem., 234, 1441 (1959).
- #P-29. THE AMINO ACID COMPOSITION OF PROTEINS ISOLATED FROM THE
RIBONUCLEOPROTEIN PARTICLES OF RAT LIVER.
C. F. Crampton and M. L. Petermann,
J. Biol. Chem., 234, 2642 (1959).
Abstr. Federation Proc., 16, 232 (1957);
4th Internat. Cong. Biochem., Vienna, September 1958.
- #P-30. THE RELATION BETWEEN PLASMA LIPOPROTEINS AND BREAST CARCINOMA:
EFFECT OF DEGREES OF BREAST DISEASE OF PLASMA LIPOPROTEINS
AND THE POSSIBLE ROLE OF LIPID METABOLIC ABERRATIONS.
M. Barclay, D. Calathes, J. C. DiLorenzo, A. Helper and
R. J. Kaufman,
Cancer, 12, 1163 (1959).
- #P-31. RIBONUCLEOPROTEIN FROM A RAT TUMOR, THE JENSEN SARCOMA. I.
THE EFFECT OF MAGNESIUM BINDING ON ULTRACENTRIFUGAL AND
ELECTROPHORETIC PROPERTIES
M. L. Petermann,
J. Biol. Chem., 235, 1998 (1960).
Abstr. Biophysical Society, February 1959, 1960.
- #P-32. MAGNESIUM AND PROTEIN BINDING STUDIES ON RIBONUCLEOPROTEIN
FROM RAT SARCOMA
M. L. Petermann,
Abstr. Faraday Society, March 1960; Progress in Biophysics
and Biophysical Chem., 11, 240 (1961).
- #P-33. STUDIES OF PROTEIN METABOLISM IN TUMOR-BEARING ANIMALS
P. Pecora, M. L. Petermann, and M. E. Balis,
Proc. Am. Assoc. Cancer Res., 3 (2), 141 (1960).
- #P-34. ALTERATIONS IN PLASMA PROTEIN PATTERNS IN DISEASE,
in Putnam, F. W., ed., The Plasma Proteins, New York,
Academic Press, vol. 2, 1960, p. 309., M. L. Petermann.
- #P-35. PHYSICOCHEMICAL STUDIES ON RIBONUCLEOPROTEINS FROM
MAMMALIAN CYTOPLASM, UNESCO Symposium on the Biosynthesis
of Proteins, Wassenaar, The Netherlands, 1960, in
Harris, R. J. C., ed., Protein Biosynthesis, London,
Academic Press, 1961, p. 235, M. L. Petermann and
M. G. Hamilton.

Our ref. .

- #P-36. SOME PHYSICAL CHEMICAL PROPERTIES OF MICROSOMAL RIBONUCLEOPROTEIN FROM RAT LIVER, Dissertation, Cornell University Medical College, 1960, M. G. Hamilton.
Also Abstr: 138th Meeting Amer. Chem. Soc., New York, September 1960, p. 53C.
- #P-37. PLASMA PROTEIN ABNORMALITIES IN CANCER
M. L. Petermann,
The Medical Clinics of North America, 45, 537 (1961).
- #P-38. RIBONUCLEOPROTEINS
M. L. Petermann,
Gordon Research Conferences on Proteins and Nucleic Acids, June 1958.
- #P-39. PHYSICOCHEMICAL STUDIES ON MAMMALIAN RIBONUCLEOPROTEINS
M. L. Petermann,
Fifteenth Annual Symposium on Fundamental Cancer Research, Houston, Texas, 1961, in The Molecular Basis of Neoplasia, Austin, Univ. of Texas Press, 1962, p. 564.
- #P-40. RIBONUCLEOPROTEIN FROM A RAT TUMOR, THE JENSEN SARCOMA.
II. COMPLEXES FORMED WITH HEMOGLOBIN
M. L. Petermann and A. Pavlovec,
J. Biol. Chem., 236, 3235 (1961).
- #P-41 SOME PHYSICOCHEMICAL PROPERTIES OF RIBONUCLEOPROTEIN FROM RAT LIVER MICROSOMES
M. G. Hamilton, L. F. Cavalieri, and M. L. Petermann,
J. Biol. Chem., 237, 1155 (1962).
- #P-42. RIBONUCLEOPROTEIN FROM A RAT TUMOR, THE JENSEN SARCOMA.
III. RIBOSOMES PURIFIED WITHOUT DEOXYCHOLATE BUT WITH BENTONITE AS RIBONUCLEASE INHIBITOR
M. L. Petermann and A. Pavlovec,
J. Biol. Chem., 238, 318 (1963).
- #P-43. RIBOSOMAL RNA FROM RAT LIVER CYTOPLASM
M. L. Petermann and A. Pavlovec,
Abstr: Biophys. Soc., New York, 1963.
- #P-44 STUDIES ON RIBONUCLEIC ACID FROM RAT LIVER RIBOSOMES
M. L. Petermann and A. Pavlovec,
J. Biol. Chem., 238, 3717 (1963).
- #P-45. THE RNA OF JENSEN SARCOMA RIBOSOMES AND ITS RELATION TO THE RIBOSOMAL SUBUNITS
M. L. Petermann and A. Pavlovec,
Abstr: Biophys. Soc., Chicago, 1964.

Our ref.

- #P-46. THE MOLECULAR WEIGHT OF RIBOSOMAL RNA AS DETERMINED BY EQUILIBRIUM CENTRIFUGATION
M. G. Hamilton,
Abstr: Biophys. Soc., San Francisco, 1965.
- #P-47. THE 65S FORM OF RIBONUCLEOPROTEIN (RNP) FROM THE JENSEN SARCOMA, A RAT TUMOR,
M. L. Petermann,
Abstr: Biophys. Soc., St. Louis, 1961, p. SC6.
- #P-48 THE USE OF BENTONITE IN THE PURIFICATION OF RIBONUCLEOPROTEIN,
M. L. Petermann and A. Pavlovic,
Abstr: Federation Proc., 20, 144 (1961).
- #P-49 THE PURIFICATION AND PROPERTIES OF RIBOSOMES FROM THE THYMUS NUCLEUS,
A. O. Pogo, B. G. T. Pogo, V. C. Littau, V. G. Allfrey,
A. E. Mirsky, and M. G. Hamilton,
Biochim. Biophys. Acta, 55, 849 (1962).
- #P-51. THE SUBUNITS AND STRUCTURAL RIBONUCLEIC ACIDS OF JENSEN SARCOMA RIBOSOMES,
M. L. Petermann and A. Pavlovic,
Biochim. Biophys. Acta, 144, 264 (1966).
- #P-52 MAGNESIUM BINDING AND DISSOCIATION OF RAT-LIVER RIBOSOMES AT TWO TEMPERATURES,
M. L. Petermann and A. Pavlovic,
Abstr: Biophys. Soc., Boston, 1966.
- #P-53. THE STRUCTURE OF ANIMAL RIBOSOMES,
M. L. Petermann,
Abstr: 152nd Meeting. Amer. Chem. Soc., New York,
Sept. 1966, p. 150C.
- #P-54. STUDIES OF RIBOSOME STRUCTURE: THE PROTEINS OF RAT LIVER RIBOSOMES,
M. G. Hamilton and M. E. Ruth,
Abstr: International Congress of Biophysics, Second,
Vienna, Austria, 1966.
- #P-55. THE MOLECULAR WEIGHT OF THE 30-S RNA OF JENSEN SARCOMA RIBOSOMES AS DETERMINED BY EQUILIBRIUM CENTRIFUGATION
M. G. Hamilton,
Biochim. Biophys. Acta 134:473 (1967).
- #P-56 PROTEIN-DEPLETED PARTICLES PREPARED FROM RAT-LIVER RIBOSOMES,
M. L. Petermann, A. Reboud, and M. G. Hamilton,
Abstr: Federation Proc., 26, 285 (1967).

Our ref.

- #P-57. PHYSICAL CHEMICAL STUDIES OF RAT LIVER RIBOSOMAL PROTEINS,
M. G. Hamilton and M. E. Ruth,
Seventh International Congress of Biochemistry,
Tokyo, Japan, 1967. Abstracts IV, 1967, p. 597.
- #P-58. POSSIBLE IMPORTANCE OF WATER STRUCTURE FOR RIBOSOMAL STABILITY,
M. L. Petermann and A. Pavlovic,
Seventh International Congress of Biochemistry,
Tokyo, Japan, 1967. Abstracts IV, 1967, p. 634.
- #P-59. THE EFFECT OF TEMPERATURE ON THE MAGNESIUM BINDING AND
ULTRACENTRIFUGAL PROPERTIES OF RAT LIVER RIBOSOMES,
M. L. Petermann and A. Pavlovic,
Biochemistry, 6, 2950 (1967).
- #P-60. THE PREPARATION OF PROTEIN-DEPLETED PARTICLES FROM RAT
LIVER RIBOSOMES,
A. Reboud and M. L. Petermann,
in H. J. Vogel, J. O. Lampen, and V. Bryson, eds.,
Organizational Biosynthesis, New York, Academic Press,
1967, pp. 477-479.
- #P-61. ELECTROPHORETIC CHARACTERIZATION OF THE PROTEIN OF
RAT LIVER RIBOSOMES,
M. G. Hamilton and M. E. Ruth,
Abstr: Biophys. Soc., Boston, 1966.
- #P-62. CHARACTERIZATION OF THE PROTEINS OF THE 50-S SUBUNIT OF
RAT LIVER RIBOSOMES,
M. G. Hamilton and M. E. Ruth,
Abstr: Biophys. Soc., Houston, 1967.
- #P-63. CHARACTERIZATION OF SOME OF THE PROTEINS OF THE LARGE
SUBUNIT OF RAT LIVER RIBOSOMES,
M. G. Hamilton and M. E. Ruth,
Biochemistry, 6, 2585 (1967).
- #P-64. STUDIES ON ISOLATED SUBUNITS OF RAT LIVER RIBOSOMES
M. G. Hamilton
Abstr: Biophys. J. 8, A-180 (1968).
- #P-65. AGENTS THAT AFFECT THE DISSOCIATION OF ANIMAL RIBOSOMES
M. L. Petermann and A. Pavlovic,
Abstr: Biophys. J. 8, A-181 (1968).
- #P-66. CYTOPLASMIC AND CHLOROPLAST RIBOSOMES OF CHLAMYDOMONAS:
ULTRACENTRIFUGAL CHARACTERIZATION
R. Sager and M. G. Hamilton,
Science 157, 709 (1967).

- #P-67 THE PARTIAL EXTRACTION OF PROTEINS FROM RAT LIVER RIBOSOMES,
AND THE PHYSICAL PROPERTIES OF THE RESIDUAL NUCLEOPROTEIN
PARTICLES.
A. Reboud, M. G. Hamilton, and M. L. Petermann,
Biochemistry 8, 843, (1969).
- #P-68 THE EFFECTS OF MAGNESIUM AND FORMALDEHYDE ON THE SEDIMENTATION
BEHAVIOR OF RAT LIVER RIBOSOMES.
M. L. Petermann and A. Pavlovec,
Biopolymers 7, 73 (1969).
- #P-69 THE EFFECT OF p-MERCURIBENZOATE ON THE ULTRACENTRIFUGAL BEHAVIOR
OF RAT LIVER RIBOSOMES.
G. S. Incefy and M. L. Petermann,
Biochemistry 8, 1482 (1969).
- #P-70 CHARACTERIZATION OF SOME OF THE PROTEINS OF THE LARGE SUBUNIT
OF RAT LIVER RIBOSOMES.
M. G. Hamilton and M. E. Ruth,
Biochemistry 6, 2585 (1967).
- #P-71 ACTIVE SUBUNITS OF RAT LIVER RIBOSOMES.
M. L. Petermann, A. Pavlovec and I. B. Weinstein,
Abstr. Federation Proc., 28, 725 (1969).
- #P-72 THE DISSOCIATION OF RAT LIVER RIBOSOMES BY EDTA: MOLECULAR
WEIGHTS, CHEMICAL COMPOSITION, AND BUOYANT DENSITIES OF THE
SUBUNITS.
M. G. Hamilton and M. E. Ruth,
Biochemistry 8, 851 (1969).
- #P-73 PHYSICAL-CHEMICAL PROPERTIES OF STABLE YEAST RIBOSOMES AND
RIBOSOMAL SUBUNITS.
A. G. Mazelis,
Dissertation, Cornell University, 1970.
- #P-74 ELECTRON MICROSCOPE OBSERVATIONS OF THE LARGE SUBUNIT OF THE
RAT LIVER RIBOSOMES.
J. Y. Haga, M. G. Hamilton, and M. L. Petermann,
J. Cell Biol. 47, 211 (1970).
- #P-75 A 5S RNA-PROTEIN COMPLEX FROM THE RAT LIVER RIBOSOME.
M. L. Petermann, M. G. Hamilton and A. Pavlovec,
Abstr. Federation Proc., 30, 1204 (1971).

- #P-76 THE CHARACTERIZATION OF RIBOSOMAL SUBUNITS BY ISODENSITY
EQUILIBRIUM CENTRIFUGATION.
M. G. Hamilton,
Abstr. Federation Proc., 30, 1312 (1971).
- #P-77 THE DISSOCIATION OF RAT LIVER RIBOSOMES TO ACTIVE SUBUNITS
BY UREA.
M. L. Petermann,
Methods in Enzymology, 20, 429 (1971).
- #P-78 THE DISSOCIATION OF RAT LIVER RIBOSOMES TO ACTIVE SUBUNITS
BY UREA.
M. L. Petermann and A. Pavlovec,
Biochemistry, 10, 2770 (1971).
- #P-79 HOW DOES A RIBOSOME TRANSLATE LINEAR GENETIC INFORMATION?
M. L. Petermann
Subcellular Biochemistry, 1, 67 (1971).
- #P-80 ISODENSITY EQUILIBRIUM CENTRIFUGATION OF RIBOSOMAL PARTICLES.
M. G. Hamilton,
Methods in Enzymology, 20, 512 (1971).
- #P-81 THE MOLECULAR WEIGHT, BUOYANT DENSITY, AND COMPOSITION OF ACTIVE
SUBUNITS OF RAT LIVER RIBOSOMES.
M. G. Hamilton, A. Pavlovec and M. L. Petermann,
Biochemistry, 10, 3424 (1971).
- In Press:
- #P-82 ANALYTICAL ULTRACENTRIFUGATION.
R. Trautman and M. G. Hamilton,
In Kado, C. and Agrawal, H., eds. Plant Virology: Principles and
Techniques, Van Nostrand Reinhold.

AEC-supported Bibliography1965 - 1971

68. NUCLEIC ACID POLYMERASES: POSSIBLE SUBUNIT STRUCTURE.
Lee-Huang, S., and Cavalieri, L. F.
Science, 148: 1474 (1965).
69. DNA POLYMERASES AND OTHER DNA-BOUND PROTEINS IN E. COLI.
Cavalieri, L. F., and Carroll, E.
Fed. Proc., 25: 708 (1966) Abstract 2932.
70. SYNTHESIS OF dA:dT.
Lee-Huang, S., and Cavalieri, L. F.
In: Cantoni, G. L., and Davies, D. R., eds. Procedures in Nucleic Acid Research. New York, Harper & Row, Inc., pp. 584-591 (1966).
71. VARIOUS MOLECULAR SPECIES OF DNA POLYMERASE.
Cavalieri, L. F., and Carroll, E.
Fed. Proc., 26: 867 (1967) Abstract 3425.
72. DNA POLYMERASE: MULTIPLE MOLECULAR SPECIES.
Cavalieri, L. F., and Carroll, E.
Information Exchange Group No. 7 (Nucleic Acids and the Genetic Code).
Scientific Memo No. 749 (1967).
73. PHYSICAL PROPERTIES OF INTRACELLULAR TEMPLATE DNA.
Rosenberg, B. H., and Cavalieri, L. F.
Biophys. J. Soc. Abstracts 8: WG4 (1968), 12th Annual Meeting, Pittsburgh, Pa., February 1968.
74. EVIDENCE FOR SUBUNITS OF DNA POLYMERASE.
Cavalieri, L. F., and Carroll, E.
Biophys. J. Soc. Abstracts, 8, WG5 (1968), 12th Annual Meeting, Pittsburgh, Pa., February 1968.
75. DNA METHYLASES OF E. COLI.
Wu, T. T. and Cavalieri, L. F.
Fed. Proc., 27: 806 (1968) Abstract 3331.
76. DNA POLYMERASE: EVIDENCE FOR MULTIPLE MOLECULAR SPECIES.
Cavalieri, L. F., and Carroll, E.
Proc. Natl. Acad. Sci., 59: 951-958 (1968).
77. MODELS FOR THE MECHANISM OF DNA SYNTHESIS.
Cavalieri, L. F., Rosenberg, B. H., and Wu, T. T.
Abstracts of Papers, 155th Natl. Meeting, Amer. Chem. Soc., San Francisco, 1968, Abstract K13.
78. THE BINDING OF ACTINOMYCIN D AND F TO BACTERIAL DNA.
Cavalieri, L. F., and Nemchin, R. G.
Biochem. Biophys. Acta, 166: 722-725 (1968).
79. SHEAR AND THE MELTING OF DNA: AN ESPECIALLY SENSITIVE PORTION OF THE E. COLI GENOME.
Rosenberg, B. H., and Cavalieri, L. F.
Biophys. J., 8: 1138-1145 (1968).

80. SHEAR SENSITIVITY OF THE E. COLI GENOME: MULTIPLE MEMBRANE
ATTACHMENT POINTS OF THE E. COLI DNA.
Rosenberg, B. H., and Cavalieri, L. F.
Cold Spring Harbor Symposia on Quant. Biology, 33: 65-72 (1968).
81. THE RESTRICTION AND MODIFICATION ENZYMES OF ESCHERICHIA COLI.
Wu, T. T., Matsuda, A., and Cavalieri, L. F.
Fed. Proc., 28: 465 (1969) Abstract 1156.
82. DNA POLYMERASE: EVIDENCE FOR MULTIPLE MOLECULAR SPECIES.
Cavalieri, L. F., and Carroll, E.
Year Book of Cancer, vol. 13, ed. R. W. Cumley,
The University of Texas Press (?), Houston, Tex.
83. THE NEGATIVE CONTROL MECHANISM FOR E. COLI DNA REPLICATION.
Rosenberg, B. H., Cavalieri, L. F., and Ungers, G.
Proc. Natl. Acad. Sci., 63: 1410-1417 (1969).
84. MULTIPLE FORMS OF ACTIVE DNA POLYMERASE: FURTHER EVIDENCE.
Yoshida, S., and Cavalieri, L. F.
Fed. Proc., 29: 406 (1970) Abstract 902.
85. A DNA-ACRYLAMIDE GEL COLUMN FOR ANALYZING PROTEINS THAT BIND
TO DNA, I. DNA POLYMERASE.
Cavalieri, L. F., and Carroll, E.
Proc. Natl. Acad. Sci., 67: 807-812 (1970).
86. RNA AS A TEMPLATE WITH E. COLI DNA POLYMERASE.
Cavalieri, L. F., and Carroll, E.
Biochem. Biophys. Res. Commun. 41: 1055-1060 (1970).
87. A DNA POLYMERASE-DNA COMPLEX ISOLATED FROM E. COLI.
Yoshida, S., and Cavalieri, L. F.
Bioc. Biop. Acta. 224: 647-650 (1970).
88. RNA-DEPENDENT DNA POLYMERASE FROM E. COLI: AN EFFECT PRODUCED
BY TRACES OF ADDED DNA.
Cavalieri, L. F., and Carroll, E.
Nature 232: 254-255 (1971).

AEC CONTRACT AT(30-1)910

Proposal #5

Chemical and Biological Studies on Nucleic Acids and Derivatives

Co-investigators: George B. Brown, Ph.D. Section 5701
Aaron Bendich, Ph.D. Section 7701

Opinion as to the present state of knowledge in this area of research, its significance in the fields of biology and medicine, and needed future investigations. (Dr. George B. Brown)

The actions of ionizing radiation in inducing free-radicals and in initiating cancers have long been vaguely associated. Free-radical scavengers (AET, BHT) can minimize the effects of radiation, although no direct association with preventing oncogenicity is known to me. Free-radicals in tissues vary in tumor tissues with time (N.M. Emanuel *et al.*, *Biophysika*, 11:443, 616, 704 (1966)). They can be suppressed by *n*-propylgallate (Emanuel and Lipatova, *Doklady Akad. Nauk SSSR*, 130:221 (1960)). They can be altered in character by chemical oncogens (Vithayathil, Ternberg and Commoner, *Nature*, 207:1246 (1965)); Commoner *et al.*, *Cancer Res.*, 30:2091 (1970)); Duchensen, Lion and Van de Vorst, *C.R. Acad. Sci. [D], Paris*, 296:1562 (1969). Kawazoe and co-workers have studied free-radicals produced by *h γ* or heat from the oncogen 4-NQO and mention (Araki *et al.*, *Chem. Pharm. Bull. (Tokyo)*, 17:1344 (1969) preliminary observations on radicals observed from AAF and MAB derivatives. Georgieff (*Science*, 173:537 (1971)) has noted that a variety of anticancer agents are also free-radical inhibitors.

These vague but continuing questions of the possible relationship of free-radicals to the process of oncogenesis merit further attention. Since Michaelis' original suggestion that many enzymes may operate through radical intermediates, several have been shown to do so. A foreign free-radical may well interfere with "natural" radical intermediates, as well as indiscriminately abstracting hydrogen from various cell components. Such radical reactions do not leave "footprints" as do radio-isotope labeled alkylating agents.

With a series of oncogenic agents which react, in part, as a free-radical at physiological conditions of temperature and pH, we shall attempt to correlate oncogenicities, chemical reactivities (as "alkylating agents" for tissue nucleophiles) and free-radical forming abilities (both in reactions in aqueous media and by photochemical induction) in hopes of relating one set of physical or chemical properties to one biological activity.

On the direct question of the relationship of free-radicals to radiation-induced cancer long-term feeding experiments with radical-scavengers should be coupled with oncogenic doses of radiation. Further study of the

radical-forming abilities of all types of chemical oncogens should be encouraged. Assays of the oncogenicity of known radicals and of radical-forming compounds should be initiated.

The greatly different approaches to "oncogenesis" in 5700 and 7700 are simply indicative of the breadth of the field.

Opinion as to the present state of knowledge in this area of research, its significance in the fields of biology and medicine, and needed investigations. (Dr. Aaron Bendich)

Cancer appears to involve alteration of genetic control in somatic cells, a condition about which very little is currently known. The alteration can be induced by chemical, radiologic or viral agents, and it may occur in the apparent absence of these ("spontaneous" cancer). Yet, the end-result in all instances is release from those control mechanisms which regulate normal and orderly growth. We still do not know what these controls are, in molecular biological terms, even though it is feasible to interfere with them experimentally.

We do not know why normal or tumor cells divide, why normal cells multiply only a limited number of times while cancer cells can be propagated indefinitely, and why in rare experimental cases a reversal of the malignant to the normal state can occur. Broad study of these phenomena and clarification of the molecular mechanisms involved should help in the development of methods of treatment or prevention.

AEC CONTRACT AT(30-1)910

Proposal #6

Physical-Chemical Studies on Nucleic Acids and Derivatives

Co-investigators: Mary L. Petermann, Ph.D. Section 5702
Liebe F. Cavalieri, Ph.D. Section 6601

Opinion as to the present state of knowledge in this area of research, its significance in the fields of biology and medicine, and needed future investigations. (Dr. Mary L. Petermann)

In the general field of protein synthesis and its genetic control, rapid progress is being made. The fine structure of the bacterial ribosome is being worked out, but much less is known about the animal ribosome, which is a larger, more complicated particle. In neither case do we know the mechanisms by which the transfer RNA-peptide complex moves from the acceptor site to the donor site on the large subunit, and the messenger RNA moves relative to the small subunit (the translocase reaction).

As the only known site of protein synthesis, the ribosome is important in biology. Since protein synthesis in animal cells is subject to control at the ribosomal level, a knowledge of the structure and function of ribosomes is essential to an understanding of cellular growth, differentiation, and the reverse of differentiation, the transformation of a normal cell into a cancerous one. Because many antibiotics owe their usefulness to differences in the sensitivity of bacterial and animal ribosomes, this knowledge is also important in medicine.

Future investigation of animal ribosomes should coordinate studies of structure and function. The ribosomal subunits should be dissected and reassembled, and the functions of the various proteins and the 5S RNA worked out. The reversible changes in subunit configuration should be studied further, and correlated with the various steps in polypeptide synthesis, particularly the translocase reaction.

Opinion as to the present state of knowledge in this area of research, its significance in the fields of biology and medicine, and needed future investigations. (Dr. Liebe F. Cavalieri)

Missing from this report.

AEC CONTRACT AT(30-1)910

1/1/71 - 12/31/71

<u>Section #</u>	<u>Proposal #</u>	<u>AEC Contract AT(30-1)-910</u>	<u>Institutional**</u>	<u>Non-Federal Support</u>	<u>Other Federal Support</u>	<u>Total</u>
5501	1, 2, 3A,B,D	\$ 22,311	\$132,152	-		\$154,463
5502	3A, B, and D	5,115	69,467	\$ 1,496		76,078
5503	2	38,195	114,705	7,500		160,400
5507	3A, b, and D	78,879	149,477	-		228,656
5509	4	66,022	86,541	-	-	152,563
5604	3C	46,585	92,851	-		139,436
5701	5	32,487	262,190	23,748		318,425
5702	6	35,414	106,130			141,544
6601	6	49,782	152,746	18,600		221,128
7504	1	21,254	28,013	-		49,267
7702	5	13,695	95,208			108,903

*Based on 8/31/71 operating report.

**The Institute is the recipient of National Cancer Institute Grant #CA-08748 which supports the Operating Expenditures of the Institute to the extent of 49.5% of the total Operating Expenditures. These funds are commingled with institutional funds.