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ANNUAL PROGRESS REPORT

for the period ending

June 30, 1971

UCLA #12-815
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INTRODUCTION

O. R. Lunt

Work at the Laboratory is conducted in five areas: Biochemistry, Developmental Cell Biology, Environmental Radiation, Nuclear Medicine and Radiation Biology. Within the past year, stringency in the research budget has precluded expansion of the Laboratory's overall program; however, some changes and internal shifts have taken place.

In the Radiation Biology Division, we have added Dr. Julien Van Lancker, Chairman, Department of Pathology, School of Medicine, to our staff (on a non-salaried basis). Dr. Van Lancker has had a long-term interest and research record in radiation research. Outside funding will be necessary to support the program. The program on "late effects" of radiation is being terminated in favor of a small program by Drs. Byfield and Bennett on "radiation effects on organisms". Work in this Division thus ranges from mechanisms of deposition of energy by ionizing radiation in biologically important molecules and the resulting sequence of reactions to subcellular reactions in eukaryotic cells and effects in organisms.

Work in Biochemistry, Developmental Cell Biology and Environmental Radiation remain at previous staffing levels. Within Nuclear Medicine a project is being reprogrammed to increase emphasis on radiopharmaceutical agent development.

The Biomedical Cyclotron is now functional and operating. This major research resource will provide a wide range of isotopes for research in nuclear medicine and in biology.

The relocation of most of the Nuclear Medicine Division within the Medical School space has taken place. This allows closer interaction with other Medical School staff, particularly the Department of Radiology, and places our staff closer to the clinical resources of the Medical School.

Within the past year, the Division of Developmental Cell Biology was formally organized with a nucleus of four scientists.

Twenty of our staff now have joint appointments with Medical School Departments.

Research progress by the Laboratory staff, on a wide range of fundamental and applied problems, is summarized in the body of this report. We express our appreciation to the Division of Biology and Medicine of the Atomic Energy Commission for support of this Laboratory.
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97. Poe, N.D.: Effects of Glucose-Insulin-Potassium Infusion on the
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98. MacDonald, N.S., I. Ban, Anna Mae Flesher, and M. Hackendorf:
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100. Kennady, J.C.: Rapid Assessment of Cerebral Hemodynamics. In J.P.
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101. Kennady, J.C., Barbara J. Miller, and G.H. Wilson: Advantages of
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102. Novey, H.S., A.F. Wilson, E.L. Suprenant, and L.R. Bennett: Ear19
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103. Fuenzalida Perez, S., L.R. Bennett, and Jeanne Larson: Indio-
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104. Small, R.C. and L.R. Bennett: Galio-67; Gammagrafia para Locali-
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105. Bosnjakovic, V., L. R. Bennett, W. Vincent, and Jeanne Larson:
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Dilute aqueous solutions of nucleic acids and nucleic acid derivatives were exposed to 10-100 nsec pulses (~1000 rads) of 10 mev electrons from a linear accelerator, and optical spectra of free radical products and changes in spectra with time were determined by kinetic spectroscopy. In N\textsubscript{2}O saturated solutions, in which OH\textsuperscript{-} (hydroxyl free radical) is the principal attacking species, dihydrothymine gives a broad absorption band with \( \lambda_{\text{max}} \approx 400 \text{ nm} \). This band has been assigned to the 5,6-dihydrothymin-5-yl radical which is formed when OH\textsuperscript{-} abstracts H\textsuperscript{+} from the C-5 atom of the ring. A similar absorption band is observed on addition of H\textsuperscript{+} to thymine. Reaction of e\textsuperscript{aq} with thymine and subsequent protonation gives a radical with a different spectrum. On reaction with OH\textsuperscript{-}, pyrimidine bases give four types of radicals. Their proportions depend on pH and base structure. At pH 7, OH\textsuperscript{-} adds to the 5,6 double bond of uracil, thymine, cytosine, and 5-methylcytosine to give neutral radicals whose spectra decay without change in shape (2 \( K \approx 2 \times 10^9 \text{M}^{-1} \text{sec}^{-1} \)). The spectrum of the thymine-OH\textsuperscript{-} adduct is similar to that of the thymine-H\textsuperscript{+} adduct. At pH 10.5-11.5, the spectrum obtained when OH\textsuperscript{-} adds to cytosine is initially nearly the same as at pH 7, but it changes within a few \( \mu \text{sec} \) to the spectrum observed at pH 12.6. The rate of the change is proportional to OH\textsuperscript{-} ion concentration. The change is attributed to reaction of the cytosine-OH\textsuperscript{-} adduct radical with OH\textsuperscript{-} ion to give the
singly charged anion radical formed directly at high pH by reaction of \( \cdot O^- \) with cytosine. Uracil at pH 10.5-11.5 is anionic and reacts with \( \cdot OH^- \) to give a singly charged anion radical. This radical reacts rapidly with \( \cdot OH^- \) ion to give the doubly charged anion radical which is obtained directly at pH 12.4 by reaction of \( \cdot O^- \) with uracil anion. Thymine at pH 11.4 behaves like uracil, but at pH 12.5 gives a different spectrum which is attributed to the substituted methyl radical, \( R-CH_2^- \), formed by abstraction of \( \cdot H^- \) from the 5-methyl group. 5-Methylcytosine, unlike cytosine, gives a similar spectrum. Comparative studies have been made at pH 7 with nucleotides and with native and denatured calf thymus and salmon sperm DNA.


Nanosecond Pulse Radiolysis of Nucleic Acid Constituents

L. S. Myers, Jr., L. M. Theard, F. C. Peterson and R. L. Voigt

(All pulse radiolysis work has been done at Gulf Radiation Technology, San Diego, California.) Studies of reactions between nucleic acid constituents and the products of water radiolysis (hydrogen atoms (\( \cdot H^- \)), hydroxyl free radicals (\( \cdot OH^- \)), and hydrated electrons (\( e_{aq}^- \))) are being carried out to provide a basis for understanding the mechanisms by which radiation damages nucleic acids. Previously we have reported on spectra and reactions of pyrimidine base radicals present during the period 3 to 1000 \( \mu \)sec after absorption of a short pulse of radiation by an aqueous solution. We have now used the nanosecond time
resolution capability of our pulse radiolysis system to study the rates of formation and some of the reactions of these radicals during the period 0.010 to 4 μsec after the pulse. The rate constants for reaction of \( \cdot \text{OH} \) with nucleic acid bases, nucleosides and nucleotides have been obtained by direct observation of the formation of the organic free radicals. Values are strikingly similar, ranging from \( 4.5 \times 10^9 \text{M}^{-1}\text{sec}^{-1} \) for the entire series of compounds, and are slightly larger for deoxynucleotides than for ribonucleotides. These values are significantly higher than those reported by other workers on the bases of competition kinetic methods, they show much less scatter, and are believed to be considerably more reliable. Other reactions which have been investigated include: a) reaction of \( \text{O}_2 \) with the (thymine-\( \cdot \text{OH} \)) adduct radical (\( k = 1.9 \times 10^9 \text{M}^{-1}\text{sec}^{-1} \)); b) reaction of H\( \cdot \) with cytosine, cytidine, thymine, and thymidine (\( k = 3.4, 3.4, 6.9, \) and \( 6.8 \times 10^8 \text{M}^{-1}\text{sec}^{-1} \) resp.); c) reaction of \( \text{H}_3\text{O}^+ \) with anion radicals of thymine, and thymidine, i.e. protonation of products formed by reaction of \( \text{e}^-_{\text{aq}} \) with thymine and thymidine (\( k = 6.4 \) and \( 5.2 \times 10^{10} \text{M}^{-1}\text{sec}^{-1} \) resp.); d) reaction of \( \text{T}^- \) anion radical with \( \text{H}_2\text{O} \) (\( k \leq 10^4 \text{M}^{-1}\text{sec}^{-1} \)); e) reaction of \( \text{O}_2 \) with \( \text{T}^- \) (\( k = 4.3 \times 10^9 \text{M}^{-1}\text{sec}^{-1} \)); f) rates of decay of 1) \( \cdot \text{OH} \) adduct radicals, 2) anion radicals; and g) rates of reaction of anion radicals with \( \cdot \text{OH} \) adduct radicals. The latter two investigations made use of the μsec time resolving capability of the system.

Interim Report
Sites of Attack by OH Free Radical on Nucleotides and DNA: A Pulse Radiolysis Study

L. S. Myers, Jr., M. Meyers, L. M. Theard, and F. C. Peterson

Optical absorption spectra of free radicals formed by reaction of OH with DNA and DNA constituents have been determined by pulse radiolysis of N₂O saturated aqueous solutions. Spectra of nucleotide radicals are almost identical to spectra of the corresponding bases. The extinction coefficients of the purine nucleotide radicals at wavelengths near \( \epsilon_{\text{max}} \) are larger than those of the pyrimidine radicals by a factor of as much as 5. Deoxyribose phosphate radicals have an extinction coefficient which is much smaller than that of radicals formed by any of the bases. The spectrum of free radicals formed by reaction of OH with DNA has a broad absorption band with a maximum at 310-320 nm. Its general shape resembles the deoxyribose radical spectrum, but its intensity is at least four times as large. Its shape is also similar to that of a weighted average spectrum calculated from spectra of the constituent nucleotides. The latter correlation is consistent with attack by OH on the bases in DNA in proportion to the amount of each base present, but with the radicals centered on the purine bases accounting for most of the absorption. Spectra of radicals formed by different kinds of DNA whose base compositions differ by only a few per cent, and of native and denatured DNA, have similar shapes.

EPR Studies on Trapped Species Produced in the Gamma Radiolysis of Aqueous Sugar Ices

J. D. Zimbrick and L. S. Myers, Jr.

The yields, EPR spectral characteristics, and paramagnetic relaxation of trapped electrons (e\textsuperscript{T}\textsuperscript{-}) and sugar radicals were studied in 60\textsuperscript{Co} γ-irradiated monosaccharide ices at 77°K. The e\textsuperscript{T}\textsuperscript{-} yields in the various ices ranged from 0.95 to 1.8 and were strongly dependent on solute molarity and phase of the ice matrix. In most cases the sugar radical yields were the same as the e\textsuperscript{T}\textsuperscript{-} yields. The e\textsuperscript{T}\textsuperscript{-} could be bleached with white light to form dielectrons as previously found for e\textsuperscript{T}\textsuperscript{-} in alkaline ices. The EPR line shapes, line widths, and line broadening mechanisms of e\textsuperscript{T}\textsuperscript{-} in the sugar ices were similar to the corresponding characteristics of e\textsuperscript{T}\textsuperscript{-} in alkaline ice. Spectral studies on the trapped sugar radicals indicated that two groups of radicals were present. The variation of the e\textsuperscript{T}\textsuperscript{-} and sugar radical relaxation times with radiation dose was studied in H\textsubscript{2}O and D\textsubscript{2}O matrices. The results for e\textsuperscript{T}\textsuperscript{-} were interpreted as indicating a spatially nonuniform distribution of this species; the spatial nonuniformity is not as great in the D\textsubscript{2}O matrix as in the H\textsubscript{2}O matrix. The results for the sugar radicals suggested that one group was distributed uniformly, whereas the other group was distributed nonuniformly to the same degree as the e\textsuperscript{T}\textsuperscript{-} distribution.

Free Radical Damage of Nucleic Acids and Their Components by Ionizing Radiation

L. S. Myers, Jr.

Nucleic acid free radicals are important intermediates in a chain of events leading from deposition of energy by ionizing radiation to appearance of the biological effects of radiation. They are formed by deposition of energy directly within the nucleic acid, and by reactions of nucleic acids with hydrogen atoms, hydroxyl free radicals, and hydrated electrons produced by deposition of energy in water. On irradiation of pure nucleic acids different radical species are formed under different conditions. Temperature, base composition, moisture content, protein contamination, and possibly secondary structure appear to be important parameters. The 5,6-dihydrothymine-5-yl radical is present in many irradiated DNA samples. Hydrogen atoms and hydroxyl free radicals add to the base and abstract hydrogen from the pentose moieties of nucleic acids to give free radicals localized on the moiety attacked. The yields of base radicals are approximately proportional to the relative amounts of each base in the nucleic acid. Radicals formed by nucleic acid constituents undergo rapid secondary reactions with oxygen and certain other small molecules. Proposed chemical mechanisms for the sensitization of nucleic acids to radiation by 5-bromouracil and N-ethylmaleimide, and for protection by sulfhydryl compounds are discussed.

Pulse Radiolysis Studies of Aqueous Solutions of Aminothiols and Compounds Related to Nucleic Acid

L. M. Theard, F. C. Peterson, R. L. Voigt and Lawrence S. Myers, Jr.

Radiation-induced reactions of OH with thymine and other compounds related to nucleic acid have been studied. Rate constants for these reactions were determined from the time-resolved buildup of reaction product concentration obtained by use of a pulse radiolysis apparatus with a signal detection response time of 5 nanoseconds. The aqueous solutions were saturated with N$_2$O; solute concentration ranged up to 10$^{-3}$ M and the half life for reaction with OH ranged down to about 100 nanoseconds. Values of OH reaction rate constants obtained by this method are in the range 10$^9$ - 10$^{10}$ M$^{-1}$sec$^{-1}$ and are generally higher than those reported from competition kinetics studies of the same compounds.

The absorption spectrum observed for N$_2$O saturated solutions of cysteine is pH dependent. At pH 8.5 and 11 a strongly absorbing species with an absorption maximum at about 400 nanometers is observed. At pH 5.5 a weakly absorbing species with an absorption maximum at 310 nanometers is observed. The strongly absorbing species is suggested to be RSSR$^{-}$ formed from the reaction RS + RS$^{-}$ -> RSSR$^{-}$ in analogy with the suggestions of Adams et al. for cysteamine solutions. The weakly absorbing species is suggested to be the RS radical or a complex other than RSSR$^{-}$ which contains RS. The rate of buildup of the weak absorption at pH 5.5 indicates a value of ca. 1 x 10$^{10}$ M$^{-1}$sec$^{-1}$ for the rate constant for the reaction OH + RSH -> H$_2$O + RS. A value of ca. 1 x 10$^{10}$
was also obtained for the rate constant of the reaction of OH with cysteamine at pH 5.5. In this case, a pulse radiolysis competition method was used in which thymine was selected as the reference solute because its rate constant for reaction with OH was directly determined by product buildup and the reaction appears to be a simple one-step process. Light absorption by the cysteamine reaction product is too weak for direct time resolution of its buildup. However, its absorption is not negligible compared to that of the thymine transient and must be accounted for by use of the relationship \( \frac{D_T - D_C}{D_T - D} = 1 + \frac{k_T[T]}{k_C[C]} \) where \( D_T \) is o.d. for a solution of thymine, \( D_C \) is o.d. for a solution of cysteamine, and \( D \) is o.d. observed for solutions containing both thymine and cysteamine at the concentrations \([T]\) and \([C]\), respectively.


Studied on the Chemical Basis of Cellular Radiosensitization by 5-Bromouracil Substitution in DNA

John D. Zimbrick, John F. Ward and Lawrence S. Myers, Jr.

Cells containing DNA in which 5-bromouracil (BU) is partially substituted for thymine (T) are more radiosensitive than their nonsubstituted counterparts. We have utilized pulse radiolysis and steady state radiolysis techniques in a comparative study of aqueous solutions of T, BU, thymidylic acid, 5 bromouridylic acid, E. coli DNA (DNA) and E. coli BU-DNA (BU-DNA) to investigate possible chemical mechanisms of BU sensitization.
The pulse radiolysis experiments were carried out with 10 Mev electron pulses, 50 nanoseconds or shorter in length. Transient organic radicals produced by hydrated electron (e\textsuperscript{−}\textsubscript{aq}) and hydroxyl free-radical (OH·) reactions with the solute were studied by optical kinetic spectrophotometry. Steady state radiolysis studies utilized \textsuperscript{60}Co gamma rays. Radiolytic destruction of the solute in aqueous solution at room temperature was measured by ultraviolet spectroscopy. Free radical intermediates produced from BU reactions with mobile electrons were examined in frozen aqueous glassy ice at 77\textdegree K by electron spin resonance (ESR) spectroscopy. The combined results of these studies show several important differences between the radiolysis of T and BU as well as between DNA and BU-DNA which may form the chemical basis of BU sensitization. Certain organic radicals, e\textsuperscript{−}\textsubscript{aq} and \cdot CO\textsubscript{2}\textsuperscript{−} destroy BU by reactions which yield uracil-5-yl radicals and Br\textsuperscript{−}. In contrast, these species are not important in T destruction. The uracilyl radical is a transient species having a weak optical absorption maximum at 335 nm in aqueous solution at room temperature and is stably trapped in frozen aqueous solutions at 77\textdegree K as evidenced by its characteristic ESR spectrum. This radical is very reactive, being able to abstract H· from a variety of molecules including sugars to form uracil and to propagate a chain reaction in formate-BU solutions. Further evidence that the uracilyl radical can attack sugar molecules come from measurements of phosphate release in the radiolysis of aqueous thymidylic acid and 5-bromouridylic acid solutions. These data show that twice as much phosphate is released in the
5-bromouridylic as in the thymidylic acid solution. Pulse and steady state radiolysis studies show two major differences between DNA and BU-DNA: a) on exposure of N₂-saturated solutions to low doses of ⁶⁰Co gamma rays the absorbance of the BU-DNA increases while that of DNA decreases; and b) the free radicals formed by OH⁻ attack on BU-DNA decay more rapidly than those on DNA. These differences are explained with the help of the above results by a mechanism in which the BU moieties of BU-DNA are converted to uracil (U) moieties via the uracilyl radical intermediate which may abstract H⁻ from neighboring deoxyribose moieties and thus cause the formation of single-strand breaks. A hypothesis for BU radiosensitization involving these results accounts for a) more base damage and b) more single-strand breaks in BU-DNA than in DNA; and c) formation of U in BU-DNA.


Electron Spin Resonance Studies of Free Radicals Formed from Orotic Acid

Jürgen Hüttermann, John F. Ward and L. S. Myers, Jr.

The paramagnetic species produced in orotic acid (uracil-6-carboxylic acid) by irradiation of single crystals and polycrystalline material as well as by reaction with hydroxyl free radicals in an aqueous system have been studied by electron spin resonance (ESR) spectroscopy. Three species were observed in the spectra of the crystals, two of them free radicals associated with the orotic acid molecule and the third, cupric ions resulting from radiation effects on crystal impurities. The spectra of the
latter were not investigated in full detail. One of the free radicals is formed by hydrogen addition in the 5 position of the pyrimidine ring, leaving an unpaired electron in a $2p_z$ orbital of carbon atom C\(^{(6)}\). The couplings of the two $\beta$-hydrogen atoms at the 5 position are 22.8 and 34.2 G, respectively. The principal values of the g tensor are 2.0023, 2.0066, and 2.0031. The second radical results from a hydrogen added to an oxygen of the carboxyl group, giving rise to interaction of the radical electron on carbon C\(^{(7)}\) with two hydroxyl protons. The hyperfine couplings of the latter are almost axially symmetrical with one $A_\parallel \approx 12$, $A_\perp \approx 0$ G, and the other $A_\parallel \approx 6$, $A_\perp \approx 0$ G. The g tensor of this radical has principal values of 2.0022, 2.0090, and 2.0047. The spectra obtained from irradiated polycrystalline orotic acid can be explained on the basis of the same two radicals. Hydroxyl free radicals produced by the Ti\(^{III}\)-H\(_2\)O\(_2\) reaction add to the 5 position of orotic acid in acidic aqueous solution. The unpaired electron on carbon C\(^{(6)}\) interacts with the $\beta$ proton on C\(^{(5)}\) to produce a doublet of 11.8-G spacing. Each doublet line is further split into three main lines with component spacing of 2.3 G by interaction with the \(^{14}\)N nucleus in 1 position.


ESR Studies of Free Radicals in Irradiated Single Crystals of 5-Methylcytosine


Irradiation at 77\(^{\circ}\)K followed by measurement at 130\(^{\circ}\)K shows the presence of an anion radical. Irradiation and measurement at room
temperature show the presence of two radicals: a) one with a hydrogen atom added to carbon C(6) of the pyrimidine ring, and b) one with a hydrogen atom removed from the 5-methyl group. Observation of these radicals in 5-methylcytosine confirms the assignment of spectra observed with irradiated thymine to analogous radical structures. The accumulated evidence from this and earlier work strongly suggests that the radicals, including the ones with an added hydrogen atom, have ionic precursors.

Interim Report

The Effect of Chloride Ions on the γ-Radiation-Induced Destruction of DNA, Nucleosides and Nucleotides in Aqueous Solution

J.F. Ward and I. Kuo

DNA in oxygenated aqueous solution is protected from the effects of ionizing radiation by chloride ions, as measured by chromophore destruction. The mechanism of this protection is probably a change in the mode of decay of the DNA-OH radicals and not a change in the primary attacking species. Chloride ions have no effect on the radiation-induced destruction of nucleosides and nucleotides in neutral solution. The chloride effects observed for these compounds in acid solution can be attributed to conversion of OH radicals to Cl\textsuperscript{−} ion radicals, and differences in the reaction of the latter species with solutes. There is no observable chloride effect on thymine or adenine compounds. The reaction of Cl\textsuperscript{−} with pyrimidine nucleosides and nucleotides results in destruction of the glycosidic bond and the release of undamaged base.
This effect is not observed with the purine compounds or with the deoxy-
ribose compounds. The radical which results from the reaction of Cl_2
with guanine compounds reacts efficiently to reform the parent compound.


The Effects of Radiation Modifiers on Sugar-Phosphate Bond Breakage in
Deoxynucleotides Irradiated in Aqueous Solution

John F. Ward and Irene Kuo

Deoxynucleotides are used here as a model system for examination
of the mechanisms involved in the production of single strand breaks in
irradiated nucleic acids; radiation induced release of inorganic phos-
phate from the deoxynucleotide being equivalent to the production of a
strand break. Previous work (M. Daniels, G. Scholes and J. Weiss,
J. C. S. 3771, 1956 and M. McCargo, Ph.D. thesis 1961) describes the
release of inorganic phosphate as occurring from two distinct radia-
tion products, one releasing phosphate immediately (A) while the other
releases it on alkaline hydrolysis (B). The current work has been
carried out mainly with aqueous solutions of deoxycytidine 5' monophos-
phate (dCMP) at pH 5; the yields of phosphates under various gas atmos-
pheres are 1. Oxygen G(A) = 0.16, G(B) = 0.08. 2. Nitrogen G(A) = 0.22,
G(B) = 0.03. 3. N_2O/O_2 mixture 4:1, G(A) = 0.32, G(B) = 0.26. These
results suggest that the OH radical produced in water radiolysis is the
species active in damaging the sugar molecule to produce the sugar-phos-
phate bond breaks. The presence of sulphydryl compounds in nitrogen
'saturated solutions, in concentrations too low to scavenge a significant
fraction of the OH radicals, reduces the yield for phosphate release: 
$3 \times 10^{-4}$M cysteamine in $10^{-2}$M dCMP reduces the yield from 0.25 to 0.15. Measurements of $G(A + B)$ as a function of SH concentration for various sulphhydryl compounds show that the order of efficiency in the repair reaction is cysteamine $>$ thiolactic acid $>$ cysteine. A similar system was used to examine a proposed mechanism of 5-Bromouracil (5BU) sensitization (Zimbrick, J. D., J. F. Ward and L. S. Myers, Jr., Int. J. Rad. Biol. in press). It was suggested that the uracilyl radical produced by photolysis or radiolysis of 5BU can abstract H atoms from a sugar molecule and thus cause a single strand break. Irradiation of 5BU ($5 \times 10^{-3}$M) in nitrogen saturated solution in the presence of dCMP ($2.5 \times 10^{-4}$) led to a release of phosphate, $G(A + B) = 0.035$, which is much higher than that calculated on a radical scavenging basis, $G = 0.013$. The latter yield was obtained when thymine was used in place of 5BU. The release of phosphate from 5-bromouridulic acid (Ward, J. F., and J. D. Zimbrick, unpublished) in $N_2$ saturated solution shows a marked concentration dependence which is absent in other nucleotides, suggesting that uracilyl radicals can be scavenged by reaction with sugar moieties at higher concentration. These results support the postulated mechanism. When N-ethyl maleimide (NEM) is present in $N_2$ saturated solutions of dCMP at concentrations too low to scavenge OH free radicals it reduces the yield of phosphate release. This result agrees with previous findings (Ward, J. F., I. Johansen and J. Aasen, Int. J. Rad. Biol. 15, 163 (1969)) that NEM can bind to radicals pro-
duced on nucleic acid components. In this case the binding of NEM to the sugar radical of a deoxynucleotide produces a chemically stable product which does not release phosphate.


Mechanism of Radiation Produced Single Strand Break Production in DNA

J. F. Ward

The mechanism by which sugar phosphate bonds are broken in irradiated deoxynucleotides was investigated. This bond breakage is equivalent to strand break production in DNA. The initial event is the abstraction of a hydrogen atom from the deoxyribose molecule by a radiation produced hydroxyl radical. This sugar radical can be repaired upon reaction with a sulfhydryl molecule. The extent of repair by this means is independent of dose rate showing that the sugar radical decays unimolecularly to give another radical (radicals) which is irreversibly damaged. Thus the yields of sugar phosphate bond breakage are almost independent of the presence of oxygen since oxygen reacts rapidly with the sugar radicals. The presence of impurities in the sample can markedly affect the yields; phosphate is released by bubbling oxygen through the solution in some cases. Low (10^-5 M) concentrations of ferrous ions in the presence of hydrogen peroxide also produce sugar phosphate bond breakage. Irradiation of thymidine 3'5' diphosphate in aqueous solution showed that abstraction of a hydrogen from the deoxyribose moiety resulted in the release of both phosphate groups. This mechanism for
single strand break production in DNA would predict that a base molecule (+ part of the sugar molecule) would be released as a consequence of strand breakage.


J. F. Ward and I. Kuo

Radiation produced hydroxyl free radicals react with deoxynucleotides in aqueous solution. They add on to the bases and abstract hydrogen atoms from the deoxyribose moieties. The latter reaction results in the liberation of inorganic phosphate. For the pyrimidine compounds the yield of base destruction (∼ 2.1) plus the yield of phosphate production (∼ 0.4) is equal to the total number of hydroxyl radicals reacting. The phosphate yield from thymidine 3'5' diphosphate is twice that of thymidine 5' monophosphate, while the base destruction yield is the same for both compounds. This result suggests that reaction of a hydroxyl radical with the deoxyribose moiety results in the liberation of phosphate from both 3' and 5' positions. Electrophoretic separations of radiation products from thymidine 3'5' disphosphate showed that no monophosphate compounds are formed. The radiation product resulting from the release of phosphate was isolated by column chromatography and characterized as thymine by paper chromatography.

The yield of free base from thymidine 3'5' phosphate is equal to half
the yield of phosphate. These experiments suggest that the abstraction of a hydrogen atom from a deoxyribose moiety in DNA results in the breakage of both sugar phosphate bonds and release of free base.


Mechanism of the Hydration Effect of Freeze-dried T1 Bacteriophage

Hazel L. Lewis and John F. Ward

Exposure to atmospheric moisture before ionizing irradiation increases the radiosensitivity of freeze-dried T1 bacteriophage by a factor of 2. The characteristics of the hydration effect are (1) Maximum sensitization occurs at low water vapor pressures (0.9 and 1.5 torr, depending upon the medium). (2) The presence of SH compounds during irradiation eliminates the effect. (3) The absolute sensitivities depend upon the medium from which the T1 is freeze-dried. (4) At maximum sensitization the hydrated T1 is more resistant than T1 in the corresponding liquid suspension. These observations suggest that a limited amount of water may become associated with the dried bacteriophage and raise the question of whether this water increases damage to the DNA or to the protein components of T1. Incorporation of 5 bromo-uracil (BU) into the DNA of T1 increases the radiation sensitivity in the dry state, but does not affect the magnitude of the hydration effect. Hotz and Zimmer (Int. J. Rad. Biol. 2, 75 (1964)) showed that SH compounds present during irradiation eliminate BU sensitization of T1 in liquid suspension but not of freeze-dried T1. The presence of SH com-
pounds (0.1 M cysteine) during irradiation eliminates the hydration effect in freeze-dried Tl and BU substituted Tl but does not eliminate the BU sensitization. These observations suggest that the water which attaches to dried bacteriophage does not increase the radiosensitivity due to effects on the DNA.


Repair Processes in Bacteria
J. Rudé and J. F. Ward

Several mechanisms have been characterized for repair of ultraviolet (UV) radiation induced damage to bacterial cells. In some systems the growth medium of the cells before and after irradiation can have a pronounced effect on the survival of irradiated cells while not affecting the survival of unirradiated cells. The predominant mechanism for recovery from ultraviolet induced damage in Escherichia coli strain B/r is excision-repair. Experiments have been designed to determine possible effects of different growth media on the efficiency of excision of thymine dimers. A sensitive technique for measurement of excised thymine dimers has been developed: The bacterial DNA is initially labelled with $^3$H thymine, acid soluble materials excised after irradiation are acid hydrolyzed and the dimers separated from $^3$H thymine on a charcoal column. The rate of excision of thymine dimers from UV irradiated bacteria will be measured under various growth conditions. It was hoped that the amount of other nucleotides released with the dimer could be measured, but this
is not possible here due to the persistence of a high "blank" of acid soluble radioactivity even after several "chases" with cold thymine. Attempts to specifically separate this contaminant (by ion exchange chromatography of the acid soluble material) were unsuccessful.

Interim Report

Near-Ultraviolet Absorption Bands of Tryptophan. Studies Using Indole and 3-Methylindole as Models

E. Hardin Strickland, Joseph Horwitz, and Carolyn Billups

The fine structure characteristics of tryptophanyl absorption bands are examined by using indole derivatives dissolved in nonpolar solvents. Many of the vibronic transitions of indole and 3-methylindole have been identified by using solvent perturbation to differentially shift the \( ^1L_a \) and \( ^1L_b \) electronic transitions. Perfluorinated hexane solutions give absorption spectra which are sufficiently well resolved to permit comparison with vapor-phase absorption spectra. Methylcyclohexane causes greater red shifting of the \( ^1L_a \) transitions than does perfluorinated hexane, but the individual vibronic transitions are not as well resolved. The positions of the 0-0 \( ^1L_a \) transition have been identified in the spectra of both indole and 3-methylindole. In the case of 3-methylindole dissolved in perfluorinated hexane, the 0-0 \( ^1L_a \) band (285.2 nm) is well resolved from the \( ^1L_b \) transitions (0-0 at 288 nm). The remaining \( ^1L_a \) bands of 3-methylindole cannot be identified conclusively due to overlapping \( ^1L_b \) transitions. The spectra do, however, limit the possible positions of the remaining \( ^1L_a \) bands. In indole
spectra, \( ^1L_a \) bands can be identified at 0-0, 0 + 1700, and 0 + 2450 cm\(^{-1}\). \( ^1L_b \) bands of both indole and 3-methylindole are evident at 0-0, 0 + 730, 0 + 980, and 0 - 760 cm\(^{-1}\). Additional \( ^1L_b \) bands at shorter wavelengths are unresolved. The vibronic transitions identified in 3-methylindole aid in analyzing the circular dichroism and absorption spectra of tryptophan residues in both polar and hydrophobic environments.


Near-Ultraviolet Absorption Bands of Tryptophan. Studies Using Horseradish Peroxidase Isoenzymes, Bovine and Horse Heart Cytochrome c, and N-Stearyl-L-Tryptophan n-Hexyl Ester

E. Hardin Strickland, Joseph Horwitz, Ernest Kay, Leland M. Shannon, Meir Wilchek, and Carolyn Billups

Cooling to 77°K permitted observing the long wavelength tryptophanyl absorption bands of several proteins containing a single tryptophan residue. The 0-0 \( ^1L_a \) and 0-0 \( ^1L_b \) tryptophanyl bands were resolved in the 77°K absorption spectra of horseradish peroxidase-Al, peroxidase-C, and horse and bovine heart ferri- and ferrocytochrome c. In addition, a 0 + 850 cm\(^{-1}\) \( ^1L_a \) tryptophanyl band was resolved in both peroxidase isoenzymes and in horse ferrocytochrome c. The 0-0 \( ^1L_b \) tryptophanyl absorption band was located between 288 and 290 nm in these proteins. In contrast, the position of the 0-0 \( ^1L_a \) tryptophanyl band ranged from 302 nm (apoperoxidase-C) to 292 nm (bovine ferricytochrome c). The interactions affecting the wavelength position of the \( ^1L_a \) bands were deduced from the following reference spectra: N-stearyl-L-tryptophan n-hexyl ester dissolved in methylcyclohexane, L-tryptophan in water-

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glycerol (1:1/v:v), and horse ferricytochrome c, whose 3-dimensional structure is known from X-ray analysis (Dickerson et al., 1971, J. Biol. Chem. 246, in press). The 0-0 $^{1}L_{a}$ band was not resolved when the indolyl ring was fully exposed to the water-glycerol solvent used for protein spectra at 77°K. When the ring was in a hydrocarbon environment, the 0-0 $^{1}L_{a}$ band occurred at about 289 nm. In horse ferricytochrome c the 0-0 $^{1}L_{a}$ tryptophanyl band was red shifted to 293 nm, evidently because its indolyl ring is hydrogen bonded within an otherwise nonpolar region. After ferricytochrome c was reduced to the ferro form, the $^{1}L_{a}$ bands underwent an additional 1.5-nm red shift. For peroxidase-Al and C, the 0-0 $^{1}L_{a}$ band was red shifted about 12 nm; apparently their indolyl rings occur in partially polar regions, but are not extensively exposed to the solvent.

The fine structure absorption bands of phenylalanine and tyrosine residues were also prominent in the 77°K absorption spectra of peroxidase-Al and -C. Control experiments showed that the heme moiety has only relatively broad absorption bands in the near-ultraviolet region even at 77°K.

Interim Report

Effect of Temperature Upon the Conformations of Carboxypeptidase A (Anson) $^{\gamma}$Leu, $^{\gamma}$Val, and $^{\gamma}$$^{\alpha+\beta}$

Larry Fretto and E. Hardin Strickland

The conformations of several bovine carboxypeptidase A preparations were compared using the circular dichroism (CD) spectra from 200 to
330 nm. Carboxypeptidase A Val Val α Val and A Val Val α Val (70% Val Val and 30% Val Val ) have nearly identical CD spectra. After considering the origin of the various CD bands, it is suggested that these species have similar backbone conformations and similar environments for their tryptophanyl side chains.

The CD spectrum of carboxypeptidase A (Anson) differs in one respect from that of the homogeneous species (Val Val Val γ γ and Val Val Val γ γ ). In the Anson preparation the broad, positive CD band between 255 and 270 nm is about twice as intense as that in either carboxypeptidase Val Val Val γ γ or Val Val Val γ γ . The intensification of this CD band in the Anson preparation may possibly be due to an impurity. Alternatively the conformation of the carboxypeptidase A γ molecules may be altered during their isolation from the Anson preparation.

The effect of temperature upon the conformations of carboxypeptidase A Val Val Val γ γ , A Val Val Val γ γ , A Val Val Val γ Val and A (Anson) was examined by recording CD spectra between 24 and -196°. The far ultraviolet CD bands of these carboxypeptidase preparations are not altered by cooling, which suggests that the peptide backbone conformation is relatively rigid. In contrast, the CD fine structure arising from the tryptophanyl side chains is greatly intensified upon cooling the various preparations. This behavior results because the tryptophanyl side chains of carboxypeptidase A exist in multiple conformations at 24°. Cooling shifts more side chains into the conformer having the lowest energy. Apparently some side chains of carboxypeptidase A possess appreciable motility at room temperature.

Interim Report
Use of Circular Dichroism to Study the Interactions of Carboxypeptidase A (Anson) and $A_{\alpha+\beta}$ with Substrates and Inhibitors

Larry Fretto and E. Hardin Strickland

The phenylalanyl circular dichroism (CD) bands of peptides were used to assay peptidase activity of carboxypeptidase A (Anson) and $A_{\alpha+\beta}$ ($70\% A_{\alpha}^{Leu}$ and $30\% A_{\beta}^{Val}$). Gly-Gly-L-Phe and Gly-L-Phe have a sharp, negative CD band at 267 nm, whereas L-phenylalanine (the optically active product) has positive CD. Thus the hydrolysis of these substrates may be measured from the CD change at 267 nm (change in $\Delta \varepsilon_{M}$ of about $0.04 \text{ M}^{-1}\text{cm}^{-1}$).

The addition of $\beta$-phenylpropionate to either carboxypeptidase $A_{\alpha+\beta}$ or A (Anson) makes the CD more positive in the region from 270 to 285 nm. Apparently this alteration results from the tyrosyl CD bands of carboxypeptidase A. Evidence is presented that this change arises primarily from the interaction of $\beta$-phenylpropionate with Tyr 198 (binding constant $0.5 \text{ mM}$).

Gly-L-Phe does not produce any major alteration of the tyrosyl CD bands of carboxypeptidase $A_{\alpha+\beta}$ or A (Anson). Apparently the movement of Tyr 248 into the active site does not cause any readily measurable CD alteration, even when extensive signal averaging is carried out to achieve low noise records (peak-to-peak noise less than $5 \times 10^{-6} \Delta A$).

The binding of Gly-L-Phe, Gly-D-Phe, L-phenylalanine, or D-phenylalanine shifts the wavelength positions of the tryptophanyl CD fine structure observed in carboxypeptidase A (Anson) cooled to $-196^\circ$. This effect may result from binding outside the active site pocket, possibly in the groove near Arg 71.

Interim Report
Regulation of Enzymes in a Differentiated Rat Glial Cell Line
J. de Vellis and D. Inglish

We reported previously that the brain specific induction of glycerolphosphate dehydrogenase (GPDH) by cortisol and lactate dehydrogenase (LDH) by norepinephrine has been obtained in a cloned line of astrocytes. Hormonal specificity for each induction has been studied. Corticosteroids closely related to cortisol induce GPDH; and only epinephrine and norepinephrine induce LDH. The structural requirement for induction has been determined using hormone analogs. GPDH induction is not mediated via cyclic AMP while LDH is. Theophylline, which prevents the enzymatic breakdown of 3',5' cyclic AMP potentiates the effect of exogenous cyclic AMP. The relationship of RNA and protein synthesis to the inductions was investigated. The evidence obtained suggests that hormonal control occurs both at the transcriptional and translational level. GPDH has been isolated from the brain and purified and its physico-chemical properties will be studied.

Interim Report

J. de Vellis and D. Inglish

A cell line derived from the mouse neuroblastoma tumor C-1300 contains acetylcholinesterase, choline acetyltransferase and tyrosine hydroxylase, enzymes found in neurons. When the neuroblastoma cells are grown in monolayer culture some cells develop long neurite-like processes and resemble differentiated neurons. In the present study,
the effect of thyroid hormones, growth hormone and cortisol on the differentiation of neuroblastoma cells was investigated. Numerous observations have shown that in vivo thyroid hormones, growth hormone and cortisol are required for the growth and maturation of the nervous system, including the formation of neurites. Cells were grown as monolayers in T30 Falcon plastic flasks containing 3 ml of nutrient medium. Thyroxine, growth hormone and cortisol were added in single, double or triple combinations for one week. After 5 to 7 days a 3 to 10-fold increase in the number of cells with neurites was observed in the cultures treated with all three hormones. In the presence of one or any two hormones there was a slight but not significant increase. The observed effect is blocked by high concentration of horse serum but not by fetal calf serum. The effect of the 3 hormones on cellular ultrastructure was investigated by electron microscopy. One of the main effects of the three hormones was to increase markedly the number of polysomes. The present data suggest that the action of these hormones on neuronal differentiation is coordinated. Cyclic AMP, a mediator in the action of several hormones, also affects the morphology of neuroblastoma cells.

Interim Report

Isolation and Fractionation of RNA from the Rat and the Rooster: A Comparison of RNA from Different Organs and Cultured Cells

R. B. Edwards and J. de Vellis

RNA from the rat and rooster was analyzed by polyacrylamide gel electrophoresis. Of primary interest were comparisons of the RNA profiles
of these two species and analysis of the effects of hormones on such profiles. Observations of apparent artifacts led to a more detailed study of the effects of aggregation, conformational isomerization, variations in extraction method and enzymatic degradation.

When analyzed on sucrose gradients, RNA extracted by aqueous naphthalene disulfonate and phenol at 0-8°C yields a 41S aggregate (or conformational isomer) of 28S ribosomal RNA. Denaturation with heating or dimethylsulfoxide prevents the appearance of 41S and causes changes in the relative amounts of smaller RNA molecules. Analysis by gel electrophoresis yielded no 41S RNA and showed no differences when heated.

Three different methods of extraction yielded different RNA profiles on acrylamide gels.

RNA from ribonuclease-treated liver homogenates showed differences from untreated RNA, suggesting that many of the minor RNA types may be specific degradation products.

RNA from the following sources were compared: rooster brain and liver, chick and hen liver, rat brain and liver, and cultured rat glial cells and mouse neuroblastoma cells. Definite qualitative differences were seen between rat and chicken RNA. No qualitative differences were seen between RNAs of different organs, ages or sexes of the same species, but cultured rat glial cells had two high molecular weight RNAs (5.7 and 13.0 x 10^6 daltons) not present in whole rat brain. The mouse neuroblastoma culture also contained the 5.7 x 10^6 dalton RNA. An
RNA of 88,000 daltons was common to all preparations except nuclei, suggesting that this RNA is the previously observed smooth endoplasmic reticulum RNA. A number of RNA types were observed which have not been previously reported and which may not be degradation products, therefore deserving further study.

No new or unique RNA types were induced by hormonal treatments. However, differences were noted in incorporation of uridine into RNA fractions.

All results were used to evaluate the comparative approach to RNA analysis by gel electrophoresis. It is concluded that, in general, comparisons of RNA profiles are valid only for different RNAs extracted by rigorously identical methods, and that artifacts of aggregation, conformational isomerization and degradation must be carefully ruled out before assuming that a given size of RNA is of possible biological significance.

Interim Report

Ribonucleic Acid Metabolism in Cell Division
Young C. Lee and John E. Byfield

The objective in this work was to establish an in vitro system for the study of RNA synthesis in intact macronuclei isolated from normal and synchronized cultures of Tetrahymena pyriformis GL. A nonionic detergent, Triton X-100, was used to isolate macronuclei. Examination of these macronuclei by electron microscopy showed no cytoplasmic con-
tamination. DNA-dependent RNA polymerase activity was retained in these preparations; the enzyme required all four ribonucleoside triphosphates, Mg$^{2+}$ or Mn$^{2+}$ ions, and intact DNA. No enzyme activity was observed when DNA was destroyed with deoxyribonuclease or when transcription was blocked with actinomycin D. Various factors influencing the rate of RNA synthesis in the isolated macronuclei were studied. In the presence of Mg$^{2+}$ and KCl, it was demonstrated that the macronuclear system of Tetrahymena prepared from exponentially growing cultures incorporated about 5 mpmoles of labeled UTP/mg of nuclear DNA. A stimulation of activity occurs when Mn$^{2+}$ and 200 mM ammonium sulfate are substituted for Mg$^{2+}$ and KCl. However, analysis of base composition and nearest neighbor base frequencies indicated that the product of the RNA polymerase reaction is like DNA under both conditions, and does not resemble Tetrahymena ribosomal or whole cell RNA. Studies on the kinetics of RNA synthesis in isolated macronuclei showed that the newly synthesized RNA has a high turnover rate (rapid synthesis and breakdown), and that the hydrolysis of the newly synthesized RNA occurred in direct proportion to temperature and reaction time.


Effect of Synchronizing Temperature Shifts on the Synthesis and Translation of Replication-Supporting Messengers in Tetrahymena pyriformis.

J. E. Byfield and Y. C. Lee

The effects of synchronizing hot and cold shifts on DNA metabolism have been studied in Tetrahymena pyriformis GL. Both types of temperature
shifts inhibit thymidine incorporation. Studies using Act D and cycloheximide suggest that the inhibition of replication caused by high temperature shifts is secondary to inhibition of protein synthesis. New protein synthesis is required in the G-1 phase to initiate DNA synthesis and continuous protein synthesis is required during S-phase to maintain DNA synthesis. The replication-initiating proteins synthesized in G-1 and the replication-supporting proteins synthesized during S-phase appear to be coded for by short-lived, temperature-sensitive templates. Inhibition of replication by reduction in the pool of replication-supporting messengers may lead to deficits in the late-replicating DNA in a minor fraction of the population. The latter is briefly discussed in terms of life-cycle control in *Tetrahymena*. Reference: Exptl. Cell Res. 61, 42-50, July, 1970.

**Effects of Temperature Shifts on Nucleic Acid Metabolism**

J. E. Byfield and Y. C. Lee

The effects of synchronizing temperatures on RNA metabolism in 2 strains of *Tetrahymena pyriformis* have been studied. High temperature shifts cause degradation of unstable RNA fractions, including whole cell RNA and nuclear RNA. The evidence indicates that this hydrolysis results in a net loss of unstable RNA during each temperature shift, and, indirectly, to an inhibition of uptake of extra-cellular RNA precursors. The destruction of unstable RNA appears random; no evidence for stable template pools was found. In addition, both indirect in *vivo* and direct
in vitro assays of RNA polymerase activity failed to reveal any inhibition by synchronizing heat shifts. The inhibition of precursor incorporation in vivo apparently stems from a lag in the uptake of label into the intracellular nucleotide pools. The temperature values required to induce net hydrolysis of unstable RNA correlate well with those required for synchronization for each strain.


Thermal Bone Marrow Expansion

J. E. Byfield, P. E. Byfield, J. D. Collins, and L. R. Bennett

Peripheral fatty bone marrow was shown by Huggins and his colleagues many years ago to be capable of differentiating into actively hematopoietic marrow following elevation of its ambient temperature. Since more recent experiments in several laboratories have shown that local fatty marrow cells are probably the major source of this marrow differentiation, the process of bone "warming" (here called "thermal marrow expansion") seemed a plausible means of increasing the marrow in animals and/or patients exposed to ionizing radiation. Moreover, the different anatomical location of this marrow as well as its different physiological activity suggested that its radiation properties might be different. For example, its undifferentiated cells are not dividing (i.e. are in G-0) and may be significantly more resistant to radiation. These properties suggested potential usefulness of thermal expansion in radioprotection.
To test this hypothesis we have studied several aspects of peripheral marrow metabolism in white mice. Mice were subjected to temperature increases to $33^\circ$ and to $37^\circ$. A single step increase to $37^\circ$ was found to be almost uniformly lethal, while a week's previous exposure to $33^\circ$ followed by $37^\circ$ was well-tolerated. To determine the effect of a three week exposure to $37^\circ$ on the radioresistance of the mice, we subjected groups of ten mice to various doses of Cobalt irradiation. Our preliminary results indicate that the thermally expanded mice are more resistant to sub-total body irradiation, (i.e. irradiation of the thorax and abdomen). The results obtained thus far therefore suggest that thermal expansion does in fact have a radioprotective effect.

To determine the extent of marrow expansion in thermally expanded mice we have injected the mice with Tc-99m and performed scans and direct tail counts. Both control and thermally expanded mice were studied. The results indicate thermal expansion caused by increased ambient temperature causes the formation of hematopoietic marrow including phagocytic elements and that the increase in marrow is accompanied by net bone growth.

These preliminary results suggest that Huggins' original observations that increased ambient temperature stimulates marrow transformation were correct and also suggest that thermal expansion may prove useful in radioprotection. For example, it seems quite possible that bone marrow transplantation may be avoided in selected clinical situations if thermally expanded marrow proves capable of seeding the systemic marrow sites.

In Vitro Determination of Radiosensitization

J. E. Byfield, Y. C. Lee and L. R. Bennett

A direct microradioassay for determining in vitro the ability of diverse chemotherapeutic agents to sensitize biopsied cancer cells to ionizing radiation is being developed. The assay determines directly and quantitatively the ability of individual agents to sensitize to radiation by inhibiting DNA repair replication. It is based on the uptake of $^3$H-thymidine by irradiated, hydroxyurea-treated tumor cells. The latter antibiotic almost quantitatively inhibits normal DNA synthesis but has a greatly reduced effect on repair synthesis (Cleaver, 1969).

Using this phenomenon under controlled conditions, we have studied the ability of added second drugs to inhibit the incorporation of labelled thymidine into pre-existing DNA strands (using isopycnic CsCl gradient techniques) and have also determined the inhibition of repair of single strand breaks (using alkaline sucrose, gradients) in model cell systems which have been irradiated and treated with hydroxyurea. The results indicate that net inhibition of thymidine under these circumstances is a valid measure of radiosensitization. This approach when coupled with our previously described microradioassay for nucleoside incorporation in biopsy suspensions (Byfield et al., Oncol., in press) allows a direct and immediate determination of radiosensitization by the commonly used chemotherapeutic agents. The total assay time for clinical specimens is less than 36 hours and net cell proliferations is not
required. The quantitative molecular approach to the technique will be presented and representative clinical applications illustrated.


In Vitro Radioassay of Chemosensitivity
J. E. Byfield, J. J. Stein and L. R. Bennett

The technical aspects and preliminary results of an in vitro radioassay for tumor chemosensitivity are described. A representative series of gynecologic cancers has been studied. The procedure monitors inhibition of uptake of protein and nucleic acid precursors and requires only short incubation periods and small numbers of cells. Our initial data using tritiated thymidine and a variety of common chemotherapeutic drugs indicate that significant short-term (2 h) isotope uptake takes place and inhibition by several drugs can consistently be demonstrated. However, it is also obvious that the principal effects observed relates in most cases to the specific mode of action of individual drugs on precursor pathways, rather than intrinsic cytotoxicity on each tumor strain. The limitations of the method and a partial solution to the major problems in data interpretation are briefly discussed.

Role of Lysosomes in Ischemic Cell Destruction

D. M. Rangel, J. E. Byfield, G. E. Adomian, G. H. Stevens and E. W. Fonkalsrud

The effects of hepatic ischemia on the electron microscopic appearance of hepatic cells and the release of hepatic intracellular enzymes into the general circulation were studied in monkeys. Pretreatment with phenoxybenzamine significantly retards the onset of ultrastructural cell injury when results are compared to those obtained in untreated monkeys. Of five monkeys pretreated with phenoxybenzamine, five survived chronically after two hours of hepatic ischemia, whereas no untreated monkeys and none given methylprednisolone pretreatment lived more than 96 hours. The release into the general circulation of the lysosomal enzymes of β-glucuronidase and acid phosphatase after hepatic ischemia was greatly reduced in monkeys pretreated with phenoxybenzamine. Drug pretreatment appears to be of benefit in retarding the onset and lessening the severity of hepatocellular injury after hepatic ischemia and may be of clinical benefit in preparing donors for liver transplantation.

BIOCHEMISTRY
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Culture Conditions and the Myosin Level in Rat Heart Cells

I. Harary, W. Desmond Jr., R. L. Bielesky

We have been studying the level of myosin in cultured primary rat heart cells as a model system for the study of the control of differentiation and the expression of specific gene function in eukaryotes. When these cells are incubated in Hams F 10 and serum [complete growth medium (CGM)] there occurs growth, a five-fold increase in total myosin but a decrease in the specific activity of myosin, as measured by comparing myosin and the total protein or cell number. Incubation of the cells in CGM plus colchicine leads to a cessation of cell division and growth, and also no increase in total myosin. However, under these conditions the myosin level is maintained. If on the other hand the cells are incubated on a serumless medium [minimal medium (MM)] the cells lose myosin and also do not grow or divide. The loss can be restored by the addition of serum with or without colchicine. There appears to be a serum factor which is necessary for the maintenance and increase of myosin. It also appears that cell division leads to an increase in total myosin. This has been a puzzling observation since it appears to us through the use of time lapse studies, that cell division in beating heart cells is a very rare occurrence. We now believe we have a clearer picture of the preceding observations and this concept concerns two aspects, the serum factors controlling the myosin level and the relation of cell division to myosin increase.

Final Report
Myosin Serum Factor for Rat Heart Cells
I. Harary, Judith Nuss, Delia Casanello, F. Hoover, Barbara Farley, E. Sato

Our first attempt to identify the serum factor involved in myosin maintenance concerned the study of the possible effect of hormones. Of all the hormones studied only triiodothyronine had some effect. Aldosterone, estradiol, testosterone, insulin, somatotrophin and adrenalin were without effect. Serum lipids which we have reported are capable of restoring the beating of heart cells were without effect on the myosin level.

The myosin serum factor is heat stable, capable of withstanding boiling but not ashing. It is dialyzable and can maintain the heart cells with a high level of myosin but not cell division or growth. A two-fold concentration of the factor leads to a two-fold increase in the myosin level in the cells. We are now in the process of isolating and identifying this factor. Changes in the level of myosin can be caused by changes in either the rate of synthesis or degradation. With the use of radioactive amino acids, used either in pulse or long term labeling experiment, and with protein synthesis inhibitors, we have demonstrated that there is active synthesis and degradation of myosin. In order to do this we have developed an acrylamide gel technique for the estimation and isolation of small quantities of pure radioactive myosin and have determined the total and specific radioactivity of myosin in the cultured heart cells. The half-life of myosin is about 3 days and the cells are capable of synthesizing myosin for long periods of time in the presence of serum.
Measuring the rate of synthesis and degradation separately it appears that serum is necessary both to protect myosin against degradation and for synthesis. However, the boiled serum dialized factor has no protection against degradation but a powerful stimulation of the synthesis. It appears therefore that there are multiple factors for the maintenance of heart cell function in the serum, factors for growth, beating, protection against myosin degradation and a separate factor for myosin synthesis.

Interim Report

Growth and Myosin Synthesis in Rat Heart Cells
I. Harary, Marie Joseph Masse, Barbara Farley, F. Hoover

We now have evidence that the increase of myosin, which is prevented by the inhibition of cell division, is probably the result of the division of cells with little or no myosin. This indicates that there are primitive or heart-presumptive cells which must go through a cycle of cell division before they are capable of synthesizing myosin. This has been demonstrated indirectly by the use of conditions which destroy dividing cells. Incubation with bromouridine deoxyriboside (BUDR) followed by irradiation leads to a decrease in the number of cells and a 4-to 5-fold increase in specific myosin activity. It is thought that only dividing cells incorporate BUDR and that the subsequent irradiation of the cultures kills those cells which have incorporated the BUDR not affecting the cells which do not divide. This treatment not only increases the specific myosin activity but produces a pure culture of vigorous beating heart cells with little evidence of any other type of cell and, furthermore, these cells incubated with CGM do
not grow or divide but maintain their myosin very well. Cells produced in this way lose their myosin when incubated on MM and maintain their myosin when incubated on MM plus boiled serum myosin factor. Thus it would seem that the primary culture contains presumptive heart cells or myoblasts capable of division but not of myosin synthesis. On the other hand mature heart cells capable of myosin synthesis are not capable of cell division. The serum contains a growth factor which, in its role as a cell division inducer, can lead to an increase in myosin and contains also a heat stable factor capable of stimulating myosin synthesis in the absence of cell division.

Interim Report

S-100 Protein Synthesis and Degradation in Clonal Glial Cell Cultures
H. R. Herschman

We have previously isolated two clonal cell lines which synthesize this protein; Lightbody, Pfeiffer, Kornblith, and Herschman, J. Neurobio. 1, 411 (1970); Pfeiffer, Herschman, Lightbody, and Sato, J. Cell Physiol. 75, 329 (1970). The former, a human line, accumulates the protein during all phases of growth. The latter cell line, derived from a chemically-induced rat astrocytome, does not accumulate the protein while growing, but only after becoming confluent on the culture plate.

To investigate this phenomenon further, it has been necessary to devise an analytical procedure which can measure directly the incorporation of radiolabelled precursors specifically into the S-100 protein. The requirements for such an analytical technique are twofold; 1) it must be demonstrated to be specific, and 2) quantitative recovery must be
demonstrated.

In the past year we have devised a labelling and isolation procedure for S-100 protein which combines 1) antibody precipitation (in antibody excess) of the labelled protein, 2) dissolution of the antibody-antigen complex in sodium dodecyl sulfate (SDS) solution, and 3) electrophoresis on SDS containing acrylamide gels. The gels are stained for proteins, destained, sliced, dissolved and counted in a scintillation counter. Specificity can be demonstrated by showing that control cell lines (e.g. HeLa) processed in a similar fashion do not have labelled S-100 protein. Quantitative recovery is demonstrated by showing, by a second antibody-antigen precipitation and gel electrophoresis of the supernatant remaining after the first such precipitation of labelled glial cells, that all labelled S-100 protein is removed in the first precipitation. Utilizing this technique we have measured by pulse labelling the relative rate of synthesis of total acid precipitable protein and S-100 protein during a growth cycle of the clonal human glial cells. While total protein synthesis declines with increasing density, S-100 protein synthesis appears to be constitutive. We have also measured the degradation rate of S-100 protein and total acid precipitable protein in both clonal lines of cells, and shown that, in both cell lines this protein is degraded with a half-life longer than total protein. A manuscript describing these results is in preparation.

Interim Report
Cell Surface Antigens of Clonal Glial Cells

H. R. Herschman, J. Breeding, J. Nedreud

We have previously described some of the culture-dependent surface antigens of the rat glial cells (Pfeiffer, Herschman, Lightbody, Levine and Sato, J. Cell Physiol. in press). During the past year we have also prepared antisera to the clonal human cell line. We have recently been interested in the contributions of sialic acid to the antigenic determinants of the surface membranes of mammalian cells, and have used these two clonal cultures as models for this analysis. In short, specific removal of sialic acid from either cell by enzymic digestion increases the reaction between the cultured cells and the homologous antiserum. This unexpected result implies that either 1) the antibody molecules are repelled from the cell surface prior to enzymic treatment due to the negative charge of the surface sialic acid, or 2) the sialic acid present on cell surfaces is removed by serum sialidase prior to antibody formation, exposing cryptic antigens which are masked prior to enzyme in vitro immunoassay. Studies on growth properties in relation to surface sialic acid, restoration of enzymically-removed sialic acid, etc. are currently in progress. The generality of this phenomenon in the serological analysis of carbohydrate containing antigens cannot be overemphasized. A manuscript describing these results is in preparation.

Interim Report
Antisera to Nerve Endings

H. Herschman and C. Cotman

In conjunction with Dr. Carl Cotman we have attempted to define the optimal isopycnic separation condition for synaptosomal isolation. We have subjected clonal glial cells to the various separation procedures commonly used for nerve ending particle isolation. These results [(Cotman, Herschman, and Taylor, J. Neurobiol. 2, 169 (1970)] have shown that there is much less glial contamination in the synaptosomal region of ficoll-sucrose density gradients than in the corresponding region of sucrose gradients. We have also, as a result of this technique, devised a method for the isolation of the plasma membrane of these cells.

During the past year we have prepared antisera to ficoll-sucrose isolated synaptosomes. We have employed complement-fixation to demonstrate the organ specificity of this antisera, which reacts with rat nerve ending particles but not with membrane preparations from liver, kidney, spleen or heart. Similarly the organelle specificity of the antisera has been demonstrated; no reaction is seen with nerve ending particle mitochondria, whole brain mitochondria, brain nuclei, brain soluble protein, or membranes of clonal rat glial cells. Heat stability and resistance to enzymic digestion of the antigens have been characterized. Cross reaction of a variety of vertebrate nerve ending particle preparations have been quantitated. A manuscript describing these results is in preparation. Preliminary physiological experiments have shown definite electrophysiological correlates characteristic of iontophoretic application of this antiserum (Hafemann, Costin, Herschman and Cotman, in press)

Interim Report
Fine Structural and Growth Characteristics of Cultured Rat Liver Cells.

Insulin Effects

L. E. Gerschenson, T. Okigaki, M. Allen, J. Molson and M. Davidson

A cell line (RLC) derived from rat liver has been established. Electron-microscopical studies show a close resemblance to liver parenchymal cells. Chromosomal analyses have shown the cell line to be aneuploid with a modal value of 58, several marker chromosomes have been observed. The cells are usually grown in media supplemented with 10% calf serum but can be adapted to grow in serumless media supplemented with physiological concentrations of insulin (160 μU/ml). This hormone is necessary for the adaptation and is later a growth factor for the cells. Electronmicroscope studies show the hormone to induce the formation of polyribosomes.

The cells grown in media plus sera have a typical epithelial morphology while the cells grown in serumless media have a fibroblastic morphology, which reverts to the epithelial upon the addition of serum to the media.

Cells grown in serum-free media for over a year have the same karyotypic and biochemical features as the cells grown in media plus serum.

Final Report
Regulation of the Pyruvate Kinase of an Established Rat Liver Cell Line (RLC) in Culture by Insulin, Glucose and Serum

L. E. Gerschenson and M. Allen

The pyruvate kinase of RLC cells appear to be under the influence of hormonal and nutritional regulatory mechanisms similar to those modulating the same enzyme in rat liver. The insulin effect appears to be dependent upon the novo RNA synthesis, while the serum effect is not. The stimulation of the kinase activity by glucose appears to be independent of protein synthesis. The addition of glucose of lactic acid to the culture medium inhibited the insulin effect, suggesting that the latest might be a repressor for the insulin-induced increase in enzyme activity.

Final Report

Degradation of Insulin by Cultured Rat Liver Cells (RLC)

L. E. Gerschenson and M. Davidson

When insulin was added to cultures of RLC cells it was found to disappear rapidly from the medium as measured by a double antibody method. The half-life of the hormone appeared to be 35 min. The kinetics of the disappearance suggested that 2 mechanisms may be involved in it. One of them is very early and fast, while the other is late and slow.

The second mechanism appears to be present also in cultured cells on which insulin has no biological effects. We assumed that the first mechanism is specific and involved in the binding of insulin to the cells (receptors?) through \( \text{SH} \leftrightarrow \text{S-S} \) exchange and test the enzyme insulin-glutathione-transhydrogenase might play a role in the process. The second one is non-specific and takes place through non-specific proteolysis.
Two different approaches are used to ascertain if the above mentioned hypotheses are true: a) a systematic analysis of the degradation products of the hormone and b) through the use of possible inhibitors of SH → S-S exchange and proteolysis.

Interim Report

Regulation of the Tyrosine α-Ketoglutarate Transaminase of RLC Cells

by Desamethasone and Insulin

L. E. Gerschenson and M. Allen

The enzyme tyrosine α-ketoglutarate transaminase was found to be induced by the synthetic corticosteroid dexamethasone at the transcriptional level. Insulin induces the same enzyme at the translational level, but only after the cells have been pre-treated with desamethasone. The interrelation of the hormones as well as their mechanism of action is under study.

Interim Report.
The Alpha Oxidation System of Brain Microsomes

J. F. Mead and Roberta Hare

Some time ago, in vivo experiments in which carboxy-labeled acetate was injected into pre-myelinating rats which were kept for 6 months before analysis of their brain lipids, showed that the very long-chain fatty acids of the brain sphingolipids are degraded by a one-carbon or alpha-oxidation system. The products of the system increase with aging and may be involved with disposal of certain products of myelin turnover.

In in vitro experiments, it was found that a brain microsomal system plus a variety of cofactors would convert alpha-hydroxystearic acid into heptadecanoic acid and carbon dioxide, which could be used as a measure of the extent of the reaction. The cofactors were NAD, ATP, O2, a reducing agent and the 100,000xg supernate from the microsomal preparation. Difficulties were associated with the experiments throughout the work, however, possibly because a non-enzymatic reaction giving approximately the same products, was often produced.

More recently we have prepared carboxy-labeled lignoceric and cerebronic acids and have succeeded in making a stable micellar suspension of the latter for use in these studies. This work has shown that the brain micellar fraction, oxygen and the 100,000xg supernate are the only absolute requirements readily revealed. A requirement for Fe^{2+} was shown since iron chelating agents inhibited the reaction and the inhibition was reversed by Fe^{2+}. All other metals were either inactive or inhibitory. Fe^{3+} was immediately reduced to Fe^{2+} in the mixture. By separation of
the supernate on a sephedex column it was shown that the supernate factor is ascorbic acid, which can substitute for it at a concentration of about $10^{-4} \text{M}$, the same as its concentration in the supernate. It was further shown that the function of the ascorbate is to keep the iron in the $+2$ state and that when the ascorbate becomes oxidized, $\text{Fe}^{+2}$ is oxidized to $\text{Fe}^{+3}$ and the reaction ceases. It can be renewed by further ascorbate addition.

Thus two of the components of the enzyme system are now known and an idea of their function has been gained. The complete mechanism of alpha-oxidation and its activity in brain function and aging, however, are still completely obscure and will have to await the results of further experimentation.

Interim Report

Studies on Blood Brain Barrier

G. A. Dhopeshwarkar, Carole Subramanian and J. F. Mead

Continuing the studies on the uptake of fatty acids by the adult rat brain we have now conclusively shown that essential fatty acids, linoleic acid $18:2\omega6$ and linolenic acid $18:3\omega3$, are also taken up by the brain intact and converted to polyunsaturated fatty acids, arachidonic $20:4\omega6$ and docosahexaenoic acid $22:6\omega3$. This conclusion was reached by studying the incorporation of orally administered $1^{-14}\text{C}$ labeled linoleic and linolenic acid in the brain lipids of adult rats. It was found that after administration of these carboxy-labeled essential fatty acids, their
ultimate metabolic products 20:4 and 22:6, respectively, were highly labeled and that the label distribution was as follows:

<table>
<thead>
<tr>
<th>Dose given</th>
<th>20:4</th>
<th>22:6</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>24 hr.</td>
<td>4 hr.</td>
</tr>
<tr>
<td>$^{14}$C acetate</td>
<td>85.3</td>
<td>86.3</td>
</tr>
<tr>
<td>$^{14}$C linolate</td>
<td>6.3</td>
<td>22.9</td>
</tr>
<tr>
<td>$^{14}$C linolenate</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

This distribution of the label can be expected only if both 18:2 and 18:3 were taken up directly by the brain and converted to the polyunsaturated fatty acids. If the administered fatty acids were not taken up directly across the blood brain barrier but instead oxidized to acetate which would have then contributed the chain elongation of preexisting 18:2 and 18:3, one would expect the labeling pattern shown in the first column.

Thus our studies have shown that long chain fatty acids are taken up directly by the adult rat brain and that the blood brain barrier system does not seriously restrict the uptake. As a corollary, these results show that the animals do not have to depend on the supply of essential fatty acids they received during early life or prior to myelination. They can obtain these essential nutrients from the diet even after full mature growth.

Interim Report
Wax Ester Lipases of Marine Animals

J. C. Nevenzel, Elizabeth A. Baker* and A. A. Benson*

Wax esters are major lipid constituents of many marine animals, particularly midwater pelagic animals. We have therefore examined the tissues of marine invertebrates and fishes for enzymes capable of hydrolyzing these esters. Using the release of radioactive fatty acid from a synthetic substrate as an assay, we have examined the digestive glands, liver, red muscle, and white muscle of larger fishes or whole bodies of smaller fishes and copepods. Commercial porcine pancreatic lipase served as a reference enzyme source.

The clear intermediate layer obtained by centrifugation at 27,000 x g and 2° C of dilute phosphate buffer homogenates of fresh or frozen tissues served as the crude enzyme preparation and could be partially purified by dialysis or ammonium sulfate precipitation. The pH optimum for hydrolysis of wax esters was about 7.0. Because of its availability and importance in the marine food chain, most of the work was done with epipelagic anchovy, Engraulis mordax. The pyloric caecum was the richest source of lipase activity; crude preparations were twice as active as those from liver and three times as active as those from red muscle; white muscle had no lipase activity. Similar results were obtained with a second epipelagic species, the jack mackerel, Trachurus symmetricus.

The fish lipases were also active in hydrolyzing triglycerides, the wax-ester-splitting activity being about twice that for triglyceride

*Scripps Institution of Oceanography

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hydrolysis; by contrast, the porcine pancreatic lipase in 1 hr released about 3.5 times as much fatty acid per mg protein from triglyceride as from wax ester in a single experiment. The wax ester lipase activity detected in the tissues of mesopelagic fishes was low. Only triglyceride lipase activity has so far been demonstrated in copepods.

Interim Report

γ-Ray Radiolysis of Carboxylic (Fatty) Acids in Condensed States. I. On the Mechanism of the Decarboxylation Reaction

D. R. Howton and Guey-Shuang Wu

Although it is now well established that decarboxylation (RCOOH → RH + CO₂) is a major eventuality of exposure of carboxylic acids to ionizing radiation, the mechanism by which this change occurs remains obscure. We have proposed [Howton and Wu, JACS 89, 516 (1967)] that the intercarboxy-group hydrogen-bonding which characterizes such substances in condensed (liquid or solid) states may figure importantly in this process insofar as loss of an electron from (i.e., ionization of) one molecule may result in decarboxylation of the other to which the first is H-bonded. Experimental support for this hypothesis might be provided by precise measurement (using gas-chromatographic techniques) of the ratio of hydrocarbons (i.e., decarboxylation products) obtained by radiolysis of mixtures of acids which are either self- or cross-paired. The fact that palmitic (C₁₅H₃₁COOH) and stearic (C₁₇H₃₅COOH) acids readily form a "compound", in which the acids are completely "cross-paired" [see Degerman and von Sydow, Acta Chem. Scand. 12, 1176 (1958)] provides an attractive opportunity for
such inquiry. The mole-ratio n-pentadecane:n-heptadecane obtained by
irradiating an equimolar mixture of palmitic and stearic acids would be
expected to be equal to the ratio \( \frac{G_p Z_p}{G_s Z_s} \) (whose \( G \) represents the frac-
tion of energy absorbed by the parent acid, containing \( Z \) electrons per
molecule, which results in decarboxylation - essentially equivalent to the
"C-yield" of hydrocarbon at low dose) for direct (non-"crossed") reaction,
and the inverse of this ratio for "crossed" reaction of cross-paired acids.
Exposure of such a self-paired mixture of palmitic and stearic acids to
1-Mrad of \( \gamma \)-radiation gives a penta-:heptadecane mole-ratio of 0.955,
while a sample of the cross-paired "compound" yields the two products in
a ratio of 1.035 (standard deviation 0.0033, representing reproducibility
of GLC analysis). These data indicate that about 87% of the decarboxyla-
tion reactions involve a "cross-reaction" of the sort postulated, the re-
mainder occurring via any one (or combination) of several possible "direct"
mechanisms.

Final Report (To be submitted to the J. Amer. Chem. Soc. as a
"Letter to the Editor")

\( \gamma \)-Radiolysis of Carboxylic (Fatty) Acids in Condensed States. II.
Formation of (Fatty) Aldehydes

D. R. Howton and Guey-Shuang Wu

Observations to date have been in accord with the view that the major
consequences of ionizing-irradiation of carboxylic acids are decarboxyla-
tion and dehydrogenopolymerization, in agreement with earlier work showing
that \( \text{CO}_2 \) and \( \text{H}_2 \) are prominent among gaseous products. That \( \text{CO} \) and \( \text{H}_2\text{O} \)
are also produced, however, is not explained by these reactions. Studies
of Bennett and Gale [Trans. Faraday Soc. 64, 1174 (1968)] show that electrons add readily to the carbonylic oxygen of the carboxy group, an eventuality which it seemed to us should lead to formation of water and either CO and hydrocarbon or fatty aldehyde. Since we had previously routinely removed unaltered acid as methyl ester by silicic-acid-column chromatography, the presence (or absence) of aldehydes—which have essentially identical chromatographic behavior under these conditions—had escaped notice. (At least two previous investigators had noted aldehydes among other presumably minor products identified only qualitatively.) Behavior of an authentic sample of stearaldehyde showed that this substance could be readily detected and quantitated (if present) by gas chromatographic techniques, and it was thus shown that aldehydes are indeed important products of carboxylic acid γ-radiolysis; a 10-Mrad dose delivered to crystalline stearic acid yields stearaldehyde in apparent G-yield 0.74 (uncorrected for destruction of the acid or, via secondary reaction, of the aldehyde); cf.\[^{3.08}\]_\[^{\text{hydrocarbon}}\]. That aldehyde is obtained in lower yield (0.44) at lower dose (1 Mrad) suggests that this product arises in part via secondary reaction(s).

**Interim Report**

**Biosynthesis of Unsaturated Fatty Acids by Bacilli**

A. J. Fulco

Work in the past year has shown that temperature mediates 4 apparently distinct control mechanisms affecting unsaturated fatty acid biosynthesis
in Bacillus megaterium. These include the previously mentioned processes of "hyperinduction" of the $\Delta^5$-desaturating enzyme when a culture is transferred from 35° to 20° as well as the temperature-dependent irreversible inactivation of desaturating enzyme which follows first order kinetics. In addition there is a rapid but reversible inactivation of $\Delta^5$-desaturating enzyme (when a 20° culture is transferred to 35°) which lowers the desaturation rate by approximately 1/2 every 3 min. Transfer back to 20° restores the activity to the level determined by the irreversible inactivation half-life.

A 4th temperature-mediated control appears to affect the stability of the desaturase synthesizing system. When 20° cultures are transferred to 33°, desaturating enzyme continues to be synthesized but at a constantly decreasing rate which follows 0-order kinetics. Present evidence suggests that the effect is at the DNA level, perhaps by temperature-mediated synthesis or binding of a repressor.

A mutant of B. megaterium has been found in which the $\Delta^5$-desaturase has a much shorter half-life for irreversible denaturation and also shows different substrate specificities. A $\Delta^5$-desaturase from B. licheniformis is again different in these respects from both B. megaterium enzymes, with specificities for palmitate and stearate completely reversed (palmitate preferred in B. megaterium, stearate much preferred in B. licheniformis).

Work here also proceeded on the uptake and further metabolism of exogenously supplied fatty acids. We have shown that palmitate-1-C$^{14}$ is rapidly taken up by B. megaterium (1-2 min.) and shunted to 2 distinct
pools. One pool is rapidly β-oxidized and converted to CO₂, acetate, etc. in a process that is essentially complete in 30 min. at 20°. The second pool, which is not subject to β-oxidation is utilized for desaturation and for incorporation into lipids. The flow of activity is from palmitate to activated palmitate to neutral lipids to phospholipids. Desaturation taken place at the level of activated palmitate.

Interim Report
RADIATION SAFETY AND
TECHNICAL SERVICES
Biophysical Cytology and Medical Applications
B. Cassen, T. E. Oberjat, D. Regan, R. Kvaas

(a) Precision Density Gradient Cell Separations


Refinements in the precision density gradient technique of separating leukocyte suspension into their components has enabled for the first time the separation of basophils from granulocytes. The basophils are not completely separated from lymphocytes but a method of separating these two types of leukocytes is now well under way. We have been requested by Dr. Marcel Bessis, a noted hematologist from Paris, to collaborate with him in determining the chemical composition of basophils as he had observed crystals forming in basophils in a hypotonic media.

Mr. R. Kvaas, a graduate student has evolved improved techniques for studying the effects of radiation both in vitro and pre-irradiation in vivo on separated leukocyte populations. These separated fractions are viable and can be studied in cell cultures. The techniques need slight modifications for the in vivo irradiations as these are done on rabbits while the in vitro irradiations are done on human blood.

Interim Report

(b) Electrophoretic Separations and Microholography

These promising programs are unfortunately terminated on account of severe budget cuts and unavailability of competent technicians.

Final Report

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The scanner originally developed in this laboratory is in regular use in a clinical investigation program of Dr. Paul Crandall and Dr. Ulric Batzdorf in the UCLA School of Medicine. It is now being mostly used to study changes in brain tumor size and configuration in patients undergoing chemotherapy.

Interim Report

Multielement Analysis of Biological Tissues

Geo. V. Alexander, L. T. McAnulty

The rapid emission spectrometric procedure developed at this laboratory for the determination of some 25 elements in biological tissues continues to be applied at a fairly steady rate to the analysis of plant and animal tissues. Twelve thousand samples have been analysed during the past year. This brings the number of samples analysed with results stored on disc pack for correlation studies to 25,000.

Autopsy samples of human kidney cortex, liver and spleen have been analysed and are now being correlated with data from hospital records. Correlation of kidney cortex element levels with age and with blood creatinine levels have yielded some interesting information. A plot of cadmium in kidney cortex against age shows an increase from 2 ppm dry tissue at birth to 110 ppm at age 40 through 65 then decreasing to 60 ppm by age 85. The abnormal kidney cortex contains 25% to 50% less cadmium than the 'normal' tissues. After age 20 the 'abnormal' kidney cortex contains 20% more sodium than the 'normals'. Calcium is 30% higher in 'abnormal' kidney cortex. A plot of silicon against
blood creatinine levels shows an approximately linear increase of silicon from 10 ppm dry tissue to 38 ppm as the creatinine level spans the range of 0 to 24 mg%. Copper decreases from 11.5 ppm to 6.2 ppm over this same range. These correlation studies are in a preliminary stage and will soon be concluded with a final report.

The recent acquisition of an aliquote of the National Bureau of Standards Standard Reference Material #1571 (orchard leaves) has made it possible for us to check the accuracy of analysis of plant tissues. Ten 10 mg replicates of the SRM 1571 were analysed and the mean values of P, K, Na, Ca, Mg, Fe and Cu were compared with the certified values. The spectrometric values averaged 3% higher than the certified values. This small deviation greatly increases our confidence in the use of synthetic standards as a basis for quantitative calibration of the system.

During the next few months the optical portion of the spectrometer system will be completely reworked in order to add several new elements to the array. The total number of detectors will be increased to 48 and a number of improvements will be made in the readout system in order to keep analysis time to a minimum.

Interim Report
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A Computer Program to Select Random Quadrats Within a Polygon of any Shape or Size

H. W. Kaaz

The dimensions of a polygon of any size or shape may be submitted to this program with a request for any number of random quadrats of a given size locatable within the polygon. Random locations will be returned to the user giving an X and Y coordinate to establish the lower left corner of each quadrat space. The program will also draw a graph of the sample polygon and indicate the position of each random location on the graph. It is necessary that reference positions within the polygon be established so that selected random spaces are properly placed when the program is taken to the field for use. Quadrat dimensions are given to insure that all polygon space may be represented in the random selection.

Interim Report

Spurious Thermoluminescence of Soils

H. Nishita and M. Hamilton

Spurious thermoluminescence of six soils differing widely in physical and chemical characteristics was examined. As observed by previous investigators working with other materials, oxygen adsorbed on the surface of the luminescent particles appeared to be an important causative factor. The amount of "natural" spurious thermoluminescence depended on the kind of soil. After exposure to irradiation, the volume glow of every soil examined increased considerably. The surface glow, on the other hand, increased for some soils and decreased for others. One soil (Aiken clay
loam) examined did not show detectable amounts of volume glow on radiation exposure up to 2,000 R, even though the spurious glow was produced. Except for one soil (soil No. 4FF), the volume glow of the soils exposed to Co60 photons occurred predominantly in the low temperature range (<250°C). The large amount of volume glow induced in soil No. 4FF by irradiation in the high temperature range appeared to be due to its high lime content.


Influence of Temperature During the Gamma Irradiation Period on the Subsequent Thermoluminescence of Soils and LiF and CaF$_2$ Dosimeters

H. Nishita and M. Hamilton

It is well known that soil temperature varies diurnally from day to day and season to season. It also varies widely with geographic location. At the Cs137 radiation field (Nevada Test Site) where several of our experiments on soil thermoluminescence are in progress, the ground surface temperature can range diurnally from around 20 °C to about 60 °C during the warmest period of the year. During the coldest period of the year, the ground surface temperature can range diurnally from around -14 °C to about +16 °C. In view of these diurnal and seasonal temperature variations, a question was raised as to what effect the temperature of the soil and LiF and CaF$_2$ dosimeters during its exposure to gamma irradiation had on their subsequent thermoluminescence. Laboratory experiments that have been done indicate that the thermoluminescence of these materials are influenced by their temperature during irradiation.

Interim Report
Influence of Clinoptilolite on Sr90 and Cs137 Uptake by Bean and Barley Plants

H. Nishita and R. M. Haug

With the growing use of nuclear energy, it is not improbable that certain areas of the biosphere become contaminated with radionuclides at biologically hazardous levels. In such event, it would be necessary to apply various remedial procedures, in order to minimize the transfer of hazardous radionuclides along the soil-plant-animal food chains leading to man. As an aspect of these procedures, we have been attempting to determine the effectiveness of applying clinoptilolite to contaminated soils in reducing the Sr90 and Cs137 uptake by plants.

In the experiment in which clinoptilolite was applied to the contaminated soil surface, Sr90 uptake by plants was reduced 50 to 70 per cent. The Cs137 uptake was increased or decreased to a small extent depending on the soil type. The untreated clinoptilolite, which was very high in water extractable Na, was detrimental to plant growth. The Ca-treated clinoptilolite had no adverse effect on plant growth and appeared to be more effective in reducing the Sr90 uptake.

Interim Report

Decay Characteristics of Soil Thermoluminescence

H. Nishita and M. Hamilton

In studying the thermoluminescence of soils irradiated by ionizing radiation, one of the important factors that needs to be considered is the decay or the fading of the thermoluminescent capacity of the soil with time. The results obtained in this study, which is still in progress,
indicated that despite the wide difference in the amount of thermo-
luminescence produced by different soils, the general shape of the decay
curves was quite similar. The most rapid decay occurred within the
first 24 hours after irradiation. For the soils examined, the percentage
decay during the 0.5 -24 hour period ranged from 10 to 26 percent.
The percentage decay during the 0.5 --2016 hour period ranged from 22
to 75 percent.

Interim Report

Persistence of Radionuclides in Soil, Plants, and Small Mammals in Areas
Contaminated with Radioactive Fallout

E. M. Romney, W. A. Rhoads, A. Wallace, and R. A. Wood

The persistence of radionuclides in soil, plants, and small mammals
was investigated periodically in areas contaminated with fallout from
above-ground nuclear detonations at the Nevada Test Site. Study sites
were established at various locations out to about 225 km from ground
zero. Emphasis was placed upon the movement of $^{90}\text{Sr}$ and $^{137}\text{Cs}$ from
abiotic to biotic components. Several neutron activation products also
were studied in fallout areas located within 5 km of nuclear excavation
tests. Radionuclides continued to be taken up through plant roots in
small amounts, as time progressed, and some continued to be deposited
on foliage as resuspended dust particles. The inhalation route of entry
became less important with passing time, whereas ingestion continued to
be the most important route through which radionuclides entered small
mammals living in old fallout areas. Long-lived $^{90}\text{Sr}$ accumulated
primarily in bone tissue, while $^{137}\text{Cs}$ accumulated in muscle and soft
tissue. Most of the neutron activation products are short-lived, but among those found in animal tissues were isotopes of Co, Mn, and W. Findings indicate that $^{90}$Sr and $^{137}$Cs will continue to move in small amounts from abiotic to biotic components in fallout-contaminated areas with passing time.


Ecological Attributes of Perennial Plants in the Northern Mojave Desert
E. M. Romney, A. Wallace, H. W. Kaaz, and J. D. Childress

Density, dispersion, species association and biomass of perennial plants native to the northern Mojave Desert have been analyzed. Predominant shrubs near Mercury, Nye County, Nevada, are Larrea divaricata, Franseria dumosa, Lycium andersonii, and Grayia spinosa. These shrubs, and 24 less common perennials, form discrete clumps of vegetation separated by bare areas of desert. The size and spacing of clumps is irregular, and as many as 10 different species may congregate with interlocking foliage. Non-destructive measurements, including heights and diameters, were made of all perennial plants in 25 plots each 30.5 in diameter. Shrubs were collected from adjacent areas, measured, dismembered, oven-dried, and weighed. Regressions of above-ground biomass on volume indices were calculated for 12 species. These regressions have been used to estimate total standing crops of shrubs in the study plots near Mercury.

Reference: Southwest and Rocky Mountain Division of the AAAS, Tempe, Arizona, April 1971. Abstract
Revegetation problems Following Nuclear Testing Activities at the Nevada Test Site

E. M. Romney, A. Wallace and J. D. Childress

Whenever vegetation has been destroyed at the Nevada Test Site as the result of nuclear activity, the Salsola species and native annual species and grasses have grown abundantly in subsequent years on those areas. Experience indicates, however, that decades of time are necessary for the perennial shrub vegetation on a disturbed site to return to its original state. In disturbed areas on Pahute Mesa, the germination and survival of native shrub seedlings has been abundant in recent years and sufficient to return that portion of the southern Great Basin Desert to its original condition. After severe drought periods many new seedlings have disappeared, not directly because of drought but because of browsing animal activity. In the Mojave Desert portion of the Nevada Test Site, the germination and survival of shrub seedlings have been much slower on disturbed sites than at Pahute Mesa. Animals have destroyed virtually all shrubs which we have transplanted into disturbed areas. Nevertheless, transplanting has been successful when protected from browsing animals, and this appears to be a practical means of shortening the vegetational recovery time for these disturbed areas.

Fixation and Utilization of Nitrogen in Desert Vegetation

E. M. Romney, O. R. Lunt, A. Wallace, and P. A. T. Wieland

Some seasonal surveys were made to see if nitrogen fixation reactions could be detected in the root zone of desert vegetation by the acetylene reduction method. Corroborative field studies also were made to determine the response of desert vegetation to nitrogen fertilization with and without supplementary moisture.

Root-soil samples from several different plant species showed positive reactions which are presumed to primarily involve symbiotic microbial endophytes because of the sensitivity of the reaction to temperature and moisture and its dependence upon the presence of plant roots. The following species of nonleguminous plants gave positive reactions: Artemisia spinescens, Artemisia tridentata, Hymenoclea salsola, and Tetradyntia canescens of the Compositae family; Coleogyne ramosissima of the Rosaceae family; Bromus rubens of the Gramineae family; and Krameria parvifolia of the Krameriacae family. Three members of the Leguminosae family resulted in a positive test: Lupinus argenteus, Dalea fremontii, and Astragalus lentiginosus. Lichens which were not identified also resulted in a positive test.

The greatest response of desert vegetation to nitrogen fertilizer occurred in winter annual species with a two- to three-fold increase in standing crop biomass. Among the perennial shrubs studied, Eurotia lanata and Grayia spinosa showed the greatest growth response to added moisture. Plant growth on plots fertilized at levels of 100 and 200 kg of nitrogen per hectare indicated no beneficial influence from excess nitrogen input into desert soil. Added nitrogen increased the total nitrogen content in the foliage of nearly all plants examined.

Interim Report

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Radiation Doses to Vegetation from Close-in Fallout at Project Schooner

W. A. Rhoads, H. L. Ragsdale, R. B. Platt and E. M. Romney

Project Schooner was a nuclear cratering experiment in the Plowshare Program for peaceful application of nuclear explosives. On the basis of information from two earlier experiments, Palanquin and Cabriolet, special dosimeters for measuring both beta and gamma radiation were placed in the open environment and on shrubs in the downwind area where fallout was anticipated. In addition, polyethylene sheets were placed over shrubs to determine whether the shrubs could thus be protected against radiation damage. For shrubs not covered, it was found that the gamma radiation doses were essentially the same as the doses measured in the open and away from shrubs; but there was a 15% reduction in dose under the sheets. The beta doses to unsheltered vegetation were, however, reduced by almost 50% compared to doses at 25 cm in the open. This reduction was attributed to self-shielding. Beta doses to the shrubs were still further reduced to 31% of the 25-cm beta dose in the open by shielding the shrubs from direct fallout contamination. The estimated LD$_{50}$ for Artemisia was 4449 rads; but the reduction in dose by the shelters was nearly sufficient to prevent damage to the shrubs, even though all other Artemisia shrubs in the center of the fallout pattern were killed. It was concluded that beta doses must be considered in protecting growing food crops and livestock, and that even minimal shelter to prevent direct surface contamination would be of great importance.

Some Interactions of Ca, Sr, and Ba in Plants

A. Wallace and E. M. Romney

Short-term uptake tests (48 hr) disclosed that increasing levels of Ca in solution cultures decreased both Sr and Ba uptake by bush beans and decreased the proportions of them remaining in roots compared with that transported to leaves. Barium uptake was greater than Sr, and the concentration of both of these elements was highest in roots and lowest in leaves. Calcium accumulation was highest in stems and lowest in leaves. Uptake of Sr and Ba by roots showed little temperature dependency, but long-distance transport to shoots was temperature dependent as is known for Ca. Strontium and Ca and also Ba to a lesser extent served as stable-element carriers for transport of $^{85}\text{Sr}$ isotope into bush beans, but Mg did not. A level of $10^{-2}\text{M}$ Ba in nutrient solution was toxic to the plants; that which was transferred to shoots killed the leaves. Its effect on permeability resulted in large transfer of $^{85}\text{Sr}$ to shoots. Long-term uptake tests (90 days) with tobacco grown in solution culture disclosed that Ca accumulated more in leaves while Sr and Ba accumulated more in roots. Two desert-plant species, Lycium andersonii and Lycium pallidum, showed interesting differences in their accumulation of these elements. L. andersonii tended to concentrate Ca in leaves, but Ba was concentrated in roots with Sr equally divided between roots and leaves. L. pallidum concentrated all three of these cations more in roots than in leaves.

Effect of Boron Levels on Two Different Plant Species which often Grow in Soils High in Boron

A. Wallace and E. M. Romney

Two desert plant species were grown in solution culture with varying levels of boron. Both appeared to be very resistant to accumulation of large amounts of boron but *F. dumosa* accumulated moderately high amounts of it at levels of ten or more ppm in the nutrient solution. Growth reduction was indicated for this species at 50 and 100 ppm boron in the nutrient solution. The high levels of boron resulted in decreased potassium levels in roots for this species. Growth seemed to increase for *A. hymenelytra* up to a level of 20 ppm boron in the nutrient solution, but leaf contents at this level were only 156 ppm of dry weight. Higher levels did not further increase boron contents of leaves. The distribution of both of these species in the desert is probably related to their ability to accumulate small amounts of boron in the presence of large amounts of it.

Reference: Western Section Ecological Society of the AAAS, San Diego, California, June 21-25, 1971. Abstract

Leaf Forms in Desert Vegetation

A. Wallace and E. M. Romney

Survival of perennial plants in the Mojave Desert is determined by at least two very basic phenomena. First, the foliage is structured to conserve moisture by closely regulating seasonal productivity as a function of moisture supply. Second, the perennial species either drop their leaves or the leaves withstand extreme dehydration
without harm when seasonal soil moisture becomes exhausted. These plants have the unique capacity to remain inactive under severe moisture stress for several months' duration and continue to survive drought years wherein very little primary productivity occurs.

Interim Report

Leaf Temperatures of Desert Perennials in Summer Months
A. Wallace

Temperatures of leaves or blades of *Opuntia basilaris* and *Yucca schidigera* in the a.m. and without wind blowing tended to be 7 - 13°C above ambient. The temperature inside the blades was about 5°C cooler than that at the surface when the surface was higher than ambient. In the p.m. and especially when the wind was blowing, leaf temperatures of all species were 1 - 2°C above ambient. In the a.m. they tended to be 1 - 2°C or more below ambient for species other than *Yucca* and *Opuntia*. Irrigation tended to decrease leaf temperature by several degrees for some species. *Krameria parvifolia* which is active late in the summer seemed to be physiologically active at very high temperatures.

Interim Report

Cycling of Stable Cs in a Desert Ecosystem
A. Wallace, E. M. Romney and R. A. Wood

Contents of stable Cs in several compartments of desert ecosystems represented at the Nevada Test Site have been determined by neutron activation. Potassium in the same compartments has also been determined
and Cs:K discrimination under natural conditions has been evaluated. From compartment sizes some estimates have been made on the rates of Cs cycling through the systems. The rates were low, but the stable Cs was circulating. Compartments studied include soil, several different plant species, arthropods, reptiles, and mammals. The contents of Cs within compartments were in the nanogram per g of dry weight range. There appeared to be a progressive narrowing of the K/Cs ratio going from plants to reptiles and mammals. The addition of stable Cs as CsCl to a soil obtained from the Nevada Test Site resulted in leaf contents in Atriplex canescens of 0.0279, 0.580, 5.15, and 24.0 ug per g of dry weight for application rates of 0, 5, 20, and 50 ppm, respectively. The applied Cs was slightly more available than was the original soil Cs. One must conclude that stable Cs is freely circulated in ecosystems although at levels lower than that of K by a factor of over 100,000, and that the stable Cs will have an influence in the cycling of $^{137}\text{Cs}$.


Seed Germination of Some Northern Mojave, Southern Great Basin Plant Species

A. Wallace

Seeds of approximately 50 plant species from the Nevada Test Site have been successfully germinated. Populations of both have been prepared for experimental study. In general very little precaution is necessary. Greatest problems are variability in seed populations and low percentages of germination. In some years viability is low and in some of these cases
it is related to insect injury of seed. Special seed treatment is necessary in the cases of *Oryzopsis hymenoides* (sulfuric acid scarification) and *Coleogyne ramosissima*, *Cowonia mexicana*, *Purshia tridentata*, and *Cercocarpus ledifolius* (chilling needed).

We have successfully germinated seed from about 50 species collected from the Nevada Test Site. We found evidence of seed dormancy for four or five species, but some species failed to germinate and as yet we do not know the reasons or whether or not there is need for leaching of inhibitors from seeds before germination would occur. Our methods of irrigation, however, may automatically do this. Our studies have not been designed to test conditions for germination, but merely to successfully germinate seed for other studies.

**Interim Report**

**Estimation of Some Aspects of Nitrogen and Phosphorus Cycling in the Northern Mojave and Southern Great Basin Deserts**

A. Wallace and E. M. Romney

Analyses of soils and of the standing crop in a *Larrea-Franseria* community and in an *Artemisia* community at the Nevada Test Site indicate that nitrogen is not a limiting factor under usual conditions of shrub growth in those communities. It was for annuals growing between shrub clumps, however. The soil organic matter in the *Larrea-Franseria* community contained about 770 kg nitrogen per hectare while the standing crop contained about 45 kg per hectare and of this about 10 kg was needed annually for the new growth. In the *Artemisia* community 239 kg nitrogen per hectare was in the standing crop with an estimated 55 to 65 kg needed
annually. The soil organic matter contained over 5000 kg of nitrogen per hectare. It could be expected that some nitrogen would be available for leaching from the system each year if atmospheric nitrogen were fixed, and if the system were in steady state. The acetylene reduction test has been used to identify sources of nitrogen fixed into the systems. The amount of phosphorus in the standing crop of the *Artemisia* community was around 60 kg per hectare and an amount equal to twice that was contained in the litter beneath the plants. If the transfer coefficient between the various compartments is sufficiently large, the system could be maintained with no phosphorus stress.

Reference: Ecological Soc. of Amer. (Western Section), San Diego, California, June 1971. Abstract

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### The Home Ranges of Some Rodents in Rock Valley

B. G. Maza, N. R. French, A. P. Aschwanden

Home ranges of four heteromyid, two cricetid, and one sciurid rodents have been analyzed in terms of mean recapture radii over a period of seven years:

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of animals</th>
<th>Number of captures</th>
<th>Mean recapture radius (meters)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Perognathus formosus</em></td>
<td>1433</td>
<td>39204</td>
<td>15.38</td>
</tr>
<tr>
<td><em>P. longimembris</em></td>
<td>222</td>
<td>4480</td>
<td>18.96</td>
</tr>
<tr>
<td><em>Dipodomys merriami</em></td>
<td>117</td>
<td>2405</td>
<td>37.28</td>
</tr>
<tr>
<td><em>D. microps</em></td>
<td>283</td>
<td>7207</td>
<td>26.48</td>
</tr>
<tr>
<td><em>Onychomys torridus</em></td>
<td>48</td>
<td>427</td>
<td>61.57</td>
</tr>
<tr>
<td><em>Peromyscus crinitus</em></td>
<td>65</td>
<td>241</td>
<td>59.03</td>
</tr>
<tr>
<td><em>Ammospermophilus leucurus</em></td>
<td>31</td>
<td>72</td>
<td>71.91</td>
</tr>
</tbody>
</table>

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Note: Based on data collected in four study areas (A, B, C, D) and including both sexes, from August 1962 to November 1968. Long distance excursions omitted and multiple ranges segregated in heteromyids

More intensive analysis of the recapture radii of heteromyids was facilitated by the greater number of individual animals and the higher frequency of recapture. Three by two by three factorial analysis of variance involving three fenced plots, sex, and three different years indicated strong significant differences between males and females and between years, but only slight indications of differences between plots ($F = 3.33, F_{0.05} = 3.00$). In general, although individual home range sizes show considerable variation, there is no indication that the irradiation of Plot B has had a detectable effect on this parameter.

Interim Report

Comparison of Some IBP Population Estimation Methods for Small Mammals

During the summer of 1970 the fenced control plots in Rock Valley were used to test the efficiency of several trapping procedures. This work was undertaken in collaboration with personnel of the U. S. I. B. P. Grasslands biome (from Colorado State University) and the Savannah River Ecology Laboratory. The results of this study indicated that past procedures in Rock Valley (3 nights of consecutive trapping) successfully enumerate all of the individuals of most of the resident species. For example, following three nights of trapping in Plot C in mid-July, personnel of the Savannah River Ecology Laboratory trapped for another two weeks in the same area. Yet the extra two weeks of effort registered
only three individuals (two of these were diurnal ground squirrels) not trapped during the initial 3-day period:

<table>
<thead>
<tr>
<th>Species</th>
<th>Numbers of Mammals Trapped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>July 14</td>
</tr>
<tr>
<td>Perognathus formosus</td>
<td>105</td>
</tr>
<tr>
<td>P. longimembris</td>
<td>3</td>
</tr>
<tr>
<td>Dipodomys merriami</td>
<td>8</td>
</tr>
<tr>
<td>D. microps</td>
<td>2</td>
</tr>
<tr>
<td>Ammospermophilus leucurus</td>
<td>-</td>
</tr>
<tr>
<td>Onychomys torridus</td>
<td>10</td>
</tr>
<tr>
<td>Totals</td>
<td>128</td>
</tr>
</tbody>
</table>


Respiratory Metabolism and Body Water Turnover Rates of Perognathus formosus in its Natural Environment

R. K. Mullen

The respiratory and water metabolism of Perognathus formosus has been determined with the use of water labeled with the isotopes deuterium and oxygen-18. Laboratory determinations, made in conjunction with the gravimetric Haldane method, closely approximated results obtained by previous investigators studying the metabolism of other heteromyid species. Mean oxygen consumption and carbon dioxide excretion were 2.71 mM/g per day and 1.94 mM/g per day respectively for the isotope method and 2.62 mM/g per day and 1.90 mM/g per day respectively for the Haldane method. Mean body-water half-life was 15.6 days.
Determination of respiratory and water metabolism in the field over a 4-month period yielded mean values ranging from 3.71 to 7.13 mM/g per day for oxygen consumption and 3.04 to 5.83 mM/g per day for carbon dioxide excretion. Body-water half-life ranged from 3.8 to 6.5 days based on isotope turnover with results in an overestimate of the actual biological half-life of body water. Based upon these field results it has been proposed that laboratory determinations of these values are meaningful only insofar as they reflect metabolism in an artificial environment. Determinations so made bear no relationship to metabolism in a natural environment and may be misleading when used to buttress studies of the physiological ecology of heteromyid rodents.


Food Consumption by Heteromyid Rodents in Rock Valley

A. P. Aschwanden, H. O. Hill, B. G. Maza, N. R. French

Food consumption by heteromyid rodents in Rock Valley has been analyzed by identification of material in stomach contents. Ingested material was broken down in terms of leaves, fruits, seeds, flowers, insects, etc. Chi-square tests indicated no differences between four heteromyids in relative utilization of major food categories. However, these analyses did not attempt to discriminate between possible differences in species of plant materials consumed. All four rodent species appeared to rely heavily on the grass *Bromus rubens* and the cruciferous annual *Thelypodium lasiophyllum*. Various parts of these two species alone compose about 50% of the diets of the four major heteromyid rodents. Annual differences in relative abundance of certain
plant species reflect simple opportunism in the feeding of the rodents. For example, the presence of *Thelypodium* parts in stomachs (in 1966 and 1967) was roughly proportional to its availability as a food source.

In conjunction with these studies of food utilization by rodents, estimates were made of food production in Rock Valley during the years 1966, 1967, and 1968. Striking differences between years were revealed, and these differences are clearly related to varying success in reproductivity of the rodent populations.

Estimated production (kg/ha) of seed (1) produced by annual species in three Rock Valley study sites

<table>
<thead>
<tr>
<th>Year</th>
<th>A seeds</th>
<th>B seeds</th>
<th>C seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1966</td>
<td>58.8</td>
<td>93.8</td>
<td>61.2</td>
</tr>
<tr>
<td>1967</td>
<td>5.2</td>
<td>3.4</td>
<td>4.5</td>
</tr>
<tr>
<td>1968</td>
<td>77.1</td>
<td>52.9</td>
<td>64.2</td>
</tr>
</tbody>
</table>

Estimated production (kg/ha) of seed (1) and leaf produced by perennial species in three Rock Valley study sites

<table>
<thead>
<tr>
<th>Year</th>
<th>A seed</th>
<th>A leaf</th>
<th>B seed</th>
<th>B leaf</th>
<th>C seed</th>
<th>C leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1966</td>
<td>7.5</td>
<td>24.5</td>
<td>12.0</td>
<td>21.5</td>
<td>9.9</td>
<td>26.1</td>
</tr>
<tr>
<td>1967</td>
<td>.5</td>
<td>14.0</td>
<td>1.7</td>
<td>12.9</td>
<td>.8</td>
<td>15.0</td>
</tr>
<tr>
<td>1968</td>
<td>3.8</td>
<td>22.9</td>
<td>5.5</td>
<td>21.5</td>
<td>4.7</td>
<td>23.6</td>
</tr>
</tbody>
</table>

(1) Weights of seed presumed to be viable and/or edible e.g. not hollowed out by insects, etc. (or fruits where fruit weight was judged to be equal to seed weight)

*Interim Report*
Allelic frequencies in irradiated and nonirradiated populations of *Uta stansburiana*

F. B. Turner and C. O. McKinney

Analysis of allelic variation in continuously irradiated and nonirradiated populations of *Uta stansburiana* in Rock Valley, Nevada, revealed no significant differences in relative allele frequencies at 19 loci controlling selected proteins. The irradiated population did not differ significantly from control populations in three conventional measures of genetic variability.

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>Number of loci</th>
<th>Proportion of loci polymorphic</th>
<th>Average number of alleles per locus</th>
<th>Average individual heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plot A, Rock Valley</td>
<td>16</td>
<td>19</td>
<td>0.37</td>
<td>1.63</td>
<td>0.086</td>
</tr>
<tr>
<td>Plot B, Rock Valley</td>
<td>20</td>
<td>19</td>
<td>0.32</td>
<td>1.68</td>
<td>0.076</td>
</tr>
<tr>
<td>Plot D, Rock Valley</td>
<td>20</td>
<td>19</td>
<td>0.37</td>
<td>1.63</td>
<td>0.082</td>
</tr>
<tr>
<td>Rock Valley (3 areas)</td>
<td>56</td>
<td>19</td>
<td>0.47</td>
<td>2.11</td>
<td>0.081</td>
</tr>
<tr>
<td>16 others</td>
<td>22^a</td>
<td>19</td>
<td>0.27 ± 0.070</td>
<td>1.43 ± 0.012</td>
<td>0.054 ± 0.004</td>
</tr>
</tbody>
</table>

^a mean

*Interim Report*
Radiation-induced sterility in natural populations of lizards (Crotaphytus wislizenii and Cnemidophorus tigris)

F. B. Turner, P. Licht, J. D. Thrasher, P. A. Medica, and J. R. Lannom, Jr.

Leopard lizards (Crotaphytus wislizenii) and whiptail lizards (Cnemidophorus tigris) have been exposed to gamma radiation in a fenced 20-acre area since January 1964. Free-air exposure rates over most of the area were around 4-6 R/day. Average annual tissue doses have been estimated with implanted lithium fluoride microdosimeters at 400-500 rads/year for Crotaphytus and about half this for Cnemidophorus. Demographic data, and failure of mature female Crotaphytus in the irradiated area to assume typical reproductive coloration indicated absence of reproduction by this species in 1967 and 1968. Three female leopard lizards taken from the irradiated plot in May and June 1969 exhibited complete regression of ovaries, undeveloped oviducal walls, and hypertrophied fat bodies. One of three irradiated males collected at the same time was sterile. All of 20 control individuals taken in 1969 exhibited ovaries, oviducts, testes and epididymides normal for the season. The first sterile female Cnemidophorus was collected in June 1969. Of four more females taken from the irradiated area in June 1970, three lacked ovaries; one had ovaries and had recently laid eggs. Three males from the irradiated area in 1970 did not differ in sexual condition from three control males. Experimental administration of follicle-stimulating hormone to the three apparently sterile Cnemidophorus females collected in 1970 had no effect on oviducal growth.
The sterility observed in both species is attributed to long-term exposure to gamma radiation.


Ecological efficiency of consumer populations

F. B. Turner

It has been suggested that the ecological efficiencies (or gross efficiencies of yield to ingestion) of steady-state consumer populations may be constant 8-12%. It is now argued that, on the basis of available data, the ecological efficiency of populations of homeothermic animals cannot exceed 2-3%.

Reference: Ecology 51: 741-742

Demography of Uta stansburiana in southern Nevada


Between 1966 and 1967 populations of Uta stansburiana in southern Nevada increased about 40%. Over the next year they declined by about 50%. These changes have been explained in terms of annual differences in fecundity and survival. Most females laid five clutches of eggs in 1966, but only two or three clutches were laid in 1967. Adult survival between 1966 and 1967 was better than during the following year. The capacity for increase, $r_c$, was estimated as 0.0327/month and cohort generation time, $T_c$, as 15.32 months from the 1966-67 data. Data drawn from other natural populations of lizards were reviewed and the inferred
net reproductive rates \( (R_0) \) were compared with the history of the populations. Problems in the study of lizard populations were discussed, with particular emphasis on the difficulty in assessing egg production by species laying several clutches of eggs each season.


Densities and age-distributions of vertebrate populations in Rock Valley, Nevada

F. B. Turner, P. A. Medica and B. Maza

Densities and age-distributions of selected populations of rodents and lizards have been studied in three fenced 20-acre areas in Rock Valley, Nye County, Nevada, for the past 7 years. Species composition in study plots is almost identical, but relative abundances of species vary significantly. With few exceptions, fenced rodent populations may be successfully enumerated by three consecutive nights of trapping using a 15 m trap grid. Lizards are enumerated by direct counts. Densities of heteromyid rodents may vary by a factor of 3- to 4-fold; populations of lizards have shown up to 2-fold variations in density.

Biomass of spring populations of the pocket mouse, *Perognathus formosus*, has ranged from 74.7 g/ha in 1964 to 308.9 g/ha in 1966; that of the kangaroo rat *Dipodomys merriami* from as low as 9.7 g/ha in 1968 to as high as 65.6 g/ha in 1964. Spring biomass values for three populations of lizards have ranged as high as 178 g/ha for *Uta stansburiana*, 250 g/ha for *Cnemidophorus tigris*, and 102 g/ha for *Crotaphytus wislizenii*.

Lizards and tree frogs in an irradiated tropical forest

F. B. Turner and C. S. Gist

Populations of semiaboreal lizards (Anolis gundlachi and A. evermanni) and a tree frog, Eleutherodactylus portoricensis (the coqui), were studied in two 0.62-acre areas (the Radiation and South Control Centers) at El Verde, Puerto Rico, for a year before one of the areas was exposed to gamma irradiation and again for 84 days following the experiment. Small thermoluminescent dosimeters were implanted beneath the skin of 100 A. gundlachi just before irradiation.

In general preirradiation investigations showed that the behavior of individuals occupying the two study centers was identical and that the populations involved were of similar size and structure. The normal density of Anolis gundlachi was estimated at about 800 per acre, and the density of male Eleutherodactylus at around 400 per acre.

Animals were killed by irradiation, and the density of all species was obviously reduced within 15 to 20 m of the source. An estimated 200 to 250 Anolis gundlachi, mostly adults, were killed. The largest cumulative dose sustained by surviving A. gundlachi was about 8000 rads (based on seven recovered dosimeters). Young individuals apparently enjoyed better survival because of time spent below ground and because some individuals entered the population during the experiment. Two lizards of different species (A. cristatellus and A. krugi) were observed after the irradiation in the well-illuminated area that developed because of the fall of canopy leaves. Neither species was observed before the experiment.
Except for possible changes in the perch heights of male *Anolis gundlachi* and *Eleutherodactylus* and in the size distribution of surviving *A. gundlachi*, the postirradiation data revealed no differences that could be attributed to the irradiation. Comparisons involved weight-length regressions, growth rate of individuals, stratification, movements of individuals, and size distributions.

Although our data indicated that animals surviving the irradiation were essentially unaffected, we believe that a narrow zone existed in which various sublethal effects were at least temporarily manifested, but our methods did not demonstrate them. The radiation gradient design thus poses serious limitations in studies of irradiated vertebrate populations.


**Partition Behavior of Manganese(III) Between Bis(2-Ethylhexyl) Hydrogen Phosphate and Aqueous Perchloric, Formic and Acetic Acids**


In an extension of a previous study the liquid extraction behavior of Mn(III) as KMnO₄ has been studied radiometrically in systems between bis(2-ethylhexyl) hydrogen phosphate and aqueous BrO₃ solutions of HClO₄, HCOOH and CH₃COOH. The distribution ratios (K) were found to vary from 300 in HClO₄ media to greater than 10⁹ in CH₃COOH and HCOOH media. Furthermore the Mn-DEHP complexes have been studied spectrophotometrically and the tentative identification of the extracted species are
Mn(DEHP)$_3$ or the solvate Mn(DEHP)$_3$(DEHP)$_3$.


Radiochemical Support

Wet Radiochemistry. During the last six months approximately 300 samples of water were processed for Sr$^{90}$, Ce$^{144}$, Cs$^{137}$ and stable Ca.

Neutron Activation Analysis. During the same period 450 samples were analyzed for stable Cs and Rb using activation techniques.

Gamma Spectrometric Analysis. In excess of 300 samples of plants, soil and animal tissues, were analyzed for mixed fission products. This work extended over a 12 month period.

The Rapid and Carrier Free Isolation of Indium from Macroconcentration of Silver by Liquid-Liquid Extraction
R. A. Wood, S. T. Wakakuwa

A rapid and carrier free method for the isolation of indium from gram concentration of silver has been developed. The method is based upon the fact that indium in nitric acid aqueous media rapidly extracts into n-heptane solutions of bis(2-ethylhexyl) hydrogen phosphate with recoveries in the organic phase of greater than 99%. Silver, on the other hand, is extracted at less than 0.1% under identical conditions. The indium is quantitatively recovered from the organic phase with 12M HCl. This method can be readily adapted to mechanical techniques.
and thus will be useful in the isolation of In\(^{111}\) from Ag\(^{109}\) after a bombardment in the existing biomedical cyclotron.

**Interim Report**

**The Energy Metabolism of Four Species of Desert Rodents in Their Natural Environments**

R. K. Mullen

Study of the physiological ecology of animals in the strictest sense is the study of the physiological adaptations and responses of animals to their environments.

The double-labeled water, or D\(_2\)\(^{18}O\) method, was used to measure the CO\(_2\) production, oxygen consumption, and energy expenditures of four species of desert rodents (Perognathus formosus, Dipodomys merriami, D. microps, and Peromyscus crinitus) living in their natural habitats in the northern Mojave Desert.

Before the method was used with free-living rodents, validation studies were completed in the laboratory with *P. formosus*:

<table>
<thead>
<tr>
<th></th>
<th>Isotope</th>
<th>Haldane</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO(_2) production</td>
<td>1.82</td>
<td>1.75</td>
</tr>
<tr>
<td>(ml g(^{-1})hr(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(O_2) consumption</td>
<td>2.53</td>
<td>2.47</td>
</tr>
<tr>
<td>(ml g(^{-1})hr(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body water half-life</td>
<td></td>
<td>15.6</td>
</tr>
<tr>
<td>(T(_1/2) days)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The mean differences observed between the Isotope and Haldane methods compare favorably with the mean differences observed by other workers using similar techniques with laboratory rats and mice.

The isotope method was then applied to determinations of energy metabolism in the field for a one-year period. Over this period, CO₂ production varied in all species studies as indicated in the table below:

<table>
<thead>
<tr>
<th>Species</th>
<th>CO₂ Production (ml g⁻¹ hr⁻¹)</th>
<th>O₂ Consumption (Linear regression)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. formosus</td>
<td>2.62 to 5.80</td>
<td>Y = 7.80 - 0.13X</td>
</tr>
<tr>
<td>D. merriami</td>
<td>1.39 to 4.91</td>
<td>Y = 3.91 - 0.12X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Y = 7.78 - 0.20X</td>
</tr>
<tr>
<td>D. microps</td>
<td>0.36 to 4.71</td>
<td>Approx. same as for D. merriami</td>
</tr>
<tr>
<td>P. crinitus</td>
<td>4.42 to 7.51</td>
<td>Limited data</td>
</tr>
</tbody>
</table>

1) Where X is equal to temperature in °C.
2) "Winter Rate" of Oxygen consumption.
3) "Balance of year rate" of Oxygen consumption.

The data on O₂ consumption for both species of *Dipodomys* indicates that they experience diurnal torpor during the colder periods of the year, an observation without precedence.
Energy expenditure followed the pattern of $O_2$ consumption for each species. As would be expected, energy expenditure was inversely proportional to species body weight.


Persistence of Plutonium in Soil, Plants and Small Mammals

E. M. Romney, H. M. Mork and K. H. Larson

Periodic surveys during a 10 year period were made of the persistence of residual $^{239}$Pu in soil, plants and small mammals indigenous to fallout areas contaminated with $^{239}$Pu dispersed by high explosive detonations. Downward migration of fallout particles occurred in undisturbed soil profiles, and wind and water erosion accounted for some redistribution of the initial $^{239}$Pu contamination. Long-term cropping experiments showed a relatively low degree of $^{239}$Pu transfer from soil to plants, but there was a consistent increase in its accumulation in plant tissue during a 5 year cropping sequence. Plant uptake of $^{239}$Pu from soil was enhanced by DTPA chelating agent. Qualitative trends from these surveys indicate that the accumulation of residual $^{239}$Pu in kangaroo rats and jackrabbits was highest in bone tissue; considerable amounts also were found in lung tissue. Inhalation is known to be the major pathway for plutonium deposition in lung and bone, but the high levels found in the gastrointestinal tracts indicate that ingestion is also an important route through which these small mammals maintained contact with the residual $^{239}$Pu contamination in the environment.


Northern Ireland.
Effect of a Chronic Exposure to Gamma Radiation on the Shrub Ephedra Nevadensis in the Northern Mojave Desert

H. W. Kaaz, A. Wallace and E. M. Romney

A 33,600 Ci $^{137}$Cs source which was differentially shielded, to increase the uniformity of the distribution of radiation was set up on a 15-m tower in the center of a 9-ha plot in January 1964 in the Rock Valley area of the northern Mojave Desert. In the spring of 1969 the large majority of 5000 Ephedra nevadensis Wats. shrubs (Mormon Tea) within the plot failed to produce flowers and very little vegetative growth occurred on the species in contrast to a control plot to other nonirradiated areas. The 5-year cumulative radiation exposure throughout about 85 per cent of the plot ranged from 3.9 to 9.8 kR. Radiation effects on other species were non existent or doubtful. E. nevadensis has a higher interphase chromosome volume than do the other shrub species.


Ecology of the Nevada Test Site

J. C. Beatley, J. L. Reveal, C. W. Henderson, and K. MacKay

Following extremely heavy early spring rains in 1965, many thousands of seeds of Astragalus lentiginosus var. fremontii (Leguminosae) and Tridens pulchellus (Gramineae) germinated in areas of permanent study sites at the Test Site. The species are usually considered to be perennials. Year-round environmental measurements and plant data collections in the spring
of this and subsequent years enabled a history of the populations to be quantitatively documented in relation to rainfall on each of the sites.

In the *Astragalus* populations, which flowered and fruited in the spring of 1966, percentage of the population surviving until the spring of 1967 was directly correlated with rainfall, i.e., the greater the rainfall the greater the numbers of individuals surviving. Most plants, however, exhibited a biennial life cycle, and the occasional large spectacular populations of this species in southern Nevada vegetation are apparently biennials which germinated following extraordinary spring rains of the preceding year.

In *Tridens* populations, which germinate and reach maturity in the same spring season, the plants behave like annuals, and the perennial habit is expressed in only a small percentage of the populations.

The potential in higher elevation perennials for the biennial or annual life cycle at lower elevations may enable such species to belong to a greater diversity of plant communities in desert regions. Those individuals which successfully perennate give continuity to the presence of the species in the community, which is of potential significance to any dependent consumers in the community.


**Vascular Plant Inventory**

J. C. Beatley and J. L. Reveal

Among the 3000 plant collections of the 1970 season and spring of
1971, around 200 species were collected for the first time, and the total species now known from the 10,000 sq. mi. area of southern Nevada is more than 1100. Number of collections represented by specimens in the Test Site herbarium is 13,000. Around 4500 duplicates of the 1970 collections were distributed to various institutions, and descriptions of most of the new species are now in press.

Reference: Beatley, Janice C. Ecologic and geographic distributions of the vascular plants of southern Nye County, and adjacent parts of Clark, Lincoln, and Esmeralda Counties, Nevada (Supplement to UCLA 12-705). January, 1971


Chronic Low-level Gamma Irradiation of a Desert Ecosystem for Five Years

N. R. French

Populations of vertebrate animals, certain insects, and plants have been studied in three enclosed 8-hectare areas located in the Mojave Desert. They were enclosed by a fence to prevent rodents from entering or leaving the study areas. One area was irradiated almost continuously at a dose rate of 80 to 500 mr/hr. Animal populations were examined by capturing, marking and releasing individuals. Plants were examined for growth and for production of leaves, flowers, fruit, and seeds. The life span of the population of pocket mice, *Perognathus formosus*, in the irradiated area was shorter than in the other areas. No difference was detected in the number of a small lizard, *Uta stansburiana*, that survived from year to year. Females of a larger but less numerous species of lizard have become sterile in the irradiated area. All vertebrate animals
in the irradiated area have received exposures of 1 to 2 r/day. Certain species of plants have produced fewer flowers and fruits in the irradiated area. Plants have received exposures of 4 to 7 r/day. Although wild populations of small mammals are surprisingly sensitive to damage from chronic low-level radiation exposure, they are evidently able to persist under these conditions. There may be certain compensating mechanisms that become operative when the population is subjected to radiation stress.


Response of Desert Shrubs to Water Potential

O. R. Lunt, John Letey and S. B. Clark

Physiological responses of *Artemisia tridentata* to stresses induced by water potentials are being studied. Water potentials were allowed to fall to minus 60-70 bars using well established plants in containers. The confined root system made it possible to characterize water potentials throughout the rhizosphere. Transpirational loss differences between stressed and non-stressed plants became large after water potentials reached the range of about -30 bars in the stressed plants. The effects of limited water supply on net CO₂ assimilation were noticeable at water potentials of a minus few bars, but the compensation point was not reached until the soil was very dry, i.e., about -60 bars. It is interesting that transpiration dropped to about zero in the same range.
The data represent some of the first quantitative measurements on net CO₂ assimilation and transpirational losses in these moisture stress ranges. Additional work on this project is expected to give better insight into the function of water supply parameters on plant populations and distributions of ecosystems at the Nevada Test Site.

Interim Report
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A Comparison of Lung Scanning and Differential Bronchospirometry as Pulmonary Function Tests in Dogs

T. Isawa, J.R. Benfield, DeLores E. Johnson, and G.V. Taplin

The relative merits and accuracy of lung scanning and differential bronchospirometry in dogs for the estimation of differential ventilation and perfusion have been compared. Forty-three dogs, including 18 normal animals and 25 with unilaterally decreased pulmonary reserve, were studied by both bronchospirometry and radioisotope inhalation and perfusion lung scanning. Aerosols of Tc⁹⁹m or In¹¹³m tagged human serum albumin inhaled through an endotracheal tube were used for inhalation lung scanning, and suspensions of I¹³¹-MAA were employed for perfusion lung scanning.

There was good correlation (r = 0.968) between lung perfusion, judged from differential canine bronchospirometry, as compared with perfusion lung scans. The difference between the results by the 2 procedures was not statistically significant, with p value less than 0.01. However, similar comparison between values of ventilation percentage by bronchospirometry and inhalation lung scanning had a correlation coefficient of only 0.498. Three varieties of bronchospirometry tubes showed a tendency to reduce ventilation distribution in the left lung. A degree of left main-stem bronchial obstruction was considered to have caused the discrepancy in estimation of ventilation percentage between bronchospirometry with a tracheal divider and lung scanning with an endotracheal tube.

Lung scanning provides a reliable and reproducible estimation of not only differential but also regional canine lung function. It is a useful method to measure pulmonary function in a manner which supplements and enlarges upon differential bronchospirometry. The relative advantages of each of these 2 methods have been discussed, and illustrative data have been presented.

Immediate, Early, and Prolonged Lung Function After Autotransplantation
J.F. Benfield, T. Isawa, J.C. Nemetz, DeLores E. Johnson, and G.V. Taplin

Using a right bilobar pulmonary reimplant model, the postoperative recovery pattern of lung function was studied in dogs. Serial differential bronchospirometry, inhalation and perfusion scans, and blood gas determinations were made. Temporary intermittent total left lung atelectasis was created in order to test the response of the reimplanted lobes to respiratory stress resulting from acute right to left shunting. Function following bilobar reimplantation approached but never quite attained control levels. The reimplants functioned almost at maximum level immediately after operation. Within the first three days, there was regularly a significant fall in function which was best explained by inhalation scans. These suggested a transient functional obstruction at the bronchial suture line, consistent in timing and extent with postoperative edema. Recovery of function to near maximum levels was generally well underway by the end of the first postoperative week, and stability had regularly been reached by the end of the third postoperative week. This data suggests that the first week following human lung transplantation will be physiologically critical and that transient auxiliary respiratory support might well be required during that week.


Functional Assessment of Canine Lung Transplants by Radioisotope Lung Scanning Procedures

Thirty-four dogs with left lung allografts, 21 of which were fresh and 13 preserved, and 14 dogs with autografts were studied sequentially
by radioisotope inhalation and perfusion lung scanning procedures.

Allografts and autografts were found to function well immediately after operation. In autografts, both ventilation and perfusion were well maintained during the period of study. In allografts, ventilation deteriorated sooner and more markedly than perfusion. The ventilation-perfusion index decreased more rapidly and to a greater degree in the preserved transplants than in the fresh allografts. Partial bronchial obstruction at the bronchial anastomotic site was indicated on the aerosol inhalation scan as an area of excessive radioactive deposition. Such findings occurred transiently in allografts and autografts in the immediate postoperative period as a result of edema, at the time of rejection (7 to 30 days) in allografts, and many months postoperatively in autografts as a result of bronchostenosis by scar formation.

The ventilation and perfusion abnormalities that complicate lung transplantation can be identified in a practical, nontraumatic, and reproducible way with radioisotope inhalation and perfusion lung scintigraphy.


**Lung Scintigraphy and Pulmonary Function Studies in Obstructive Airways Disease**

T. Isawa, K. Wasserman, and G.V. Taplin

Eleven normal volunteers and 65 patients with respiratory disease were studied with a battery of lung function tests, chest roentgenograms, and three types of lung scintigraphy - radioaerosol inhalation, xenon 133 gas inhalation, and perfusion lung scan procedures.

Aerosol inhalation scans in normal volunteers showed uniform patterns of aerosol distribution nearly identical to their perfusion counterparts.
All patients with airway obstruction showed abnormal aerosol scans. There were two distinctly abnormal configurations in aerosol distribution patterns, central and peripheral, and combinations of each. The abnormal central and peripheral patterns corresponded respectively with the emphysematous and bronchitic categories described by Burrows and associates.

The aerosol inhalation scan as a sensitive indicator of airway obstruction is a useful counterpart to the perfusion scan and helps distinguish emphysematous, bronchitic, and mixed types of obstructive airway disease. It can disclose the location and magnitude of the bronchitic components and can be helpful in determining response to treatment. Performed sequentially, the aerosol scan can localize regional abnormalities of lung clearance in bronchopulmonary diseases.


Lung Imaging with $^{133}$Xe Gas versus Radioaerosol Inhalation (for Aiding the Interpretation of Perfusion Scans)

T. Isawa and G.V. Taplin

Lung-perfusion scanning has become an indispensable examination in patients with suspected pulmonary embolism. However, similar perfusion scan abnormalities are produced by obstructive airways disease even in the absence of radiologic abnormalities. Therefore, it is important to distinguish the scan abnormalities caused by vascular occlusion from those due to obstructive airways disease. $^{133}$Xe gas and radioaerosol inhalation methods are now available for determining airway patency and ventilatory abnormalities. This paper compares both types of inhalation procedures and describes their relative merits and limitations for increasing the accuracy of interpreting lung perfusion scans.
One hundred patients with various lung diseases, most of whom were initially suspected of pulmonary embolism, and 12 normal volunteers were studied. All patients had perfusion scan abnormalities but no roentgenographic abnormalities. Xenon gas was inhaled with a single-breath forced inspiratory vital capacity maneuver. Posterior views were obtained with a scintillation camera during breath holding and at 30-sec intervals during washout with air. An aerosol of either $^{99m}$Tc or $^{113m}$In-albumin solution was inhaled during normal tidal volume breathing. Four views of the chest were obtained with either a ten probe rectilinear scanner or a camera in all radioaerosol inhalation and perfusion lung scan examinations.

The xenon method gives an accurate measure of regional ventilation and dynamic airflow disturbances. Repeated studies can be done at short intervals. Disadvantages are that a scintillation camera is mandatory, breath holding is required, and only one projection is possible per examination. Also the resolution of scan abnormalities is relatively poor (4-5 cm) and the exact sites of partial airway obstruction are not visualized. The aerosol method requires no breath holding and is applicable in nearly all patients. Multiple views are obtained and direct comparison with perfusion scans is possible, giving accurate localization of ventilatory and arterial lung lesions with equally good resolution. Aerosol scans have characteristic patterns indicative of the main types of obstructive airways disease. Disadvantages are that airflow dynamics cannot be measured and the aerosol distribution patterns do not consistently provide a true measure of ventilation distribution in obstructive airways disease.
The xenon gas and the aerosol inhalation procedures just described each reveal regional ventilatory disturbances and thereby reduce the frequency of lung perfusion scan misinterpretation. They distinguish the normal or near normal ventilation of the ischemic lung of arterial occlusion from the non-ventilation of ischemic lung regions in obstructive airways disease and in bullous emphysema. For this purpose, the radio-aerosol method is preferred but for quantitating dynamic ventilatory abnormalities, radio xenon gas inhalation is superior.


Early and Delayed Changes in Regional Ventilation Following Experimental Pulmonary Artery Occlusion

T. Isawa, J.M. Criley, G.V. Taplin, and J. Beazell

It is believed that pulmonary embolism is accompanied by hypoventilation and bronchoconstriction. This concept is derived from findings obtained immediately after experimental embolization or pulmonary artery (PA) occlusion, but the duration of these effects is not known. Clinically, evidence of hypoventilation or bronchoconstriction is rarely demonstrable in embolized regions localized by lung perfusion scanning. The present study is presented to examine serially the effects of experimental pulmonary artery occlusion on regional ventilation in dogs and divergent clinical experience regarding pulmonary embolism and associated airway disturbances.

Fifteen dogs were studied in serial fashion for ventilation and perfusion changes following PA occlusion either with a balloon catheter or with a rubber balloon filled with contrast medium. To assess relative ventilation, either $^{133}$Xe gas, $^{99m}$Tc-albumin aerosol or both were inhaled.
spontaneously through an endotracheal tube. Perfusion changes were studied by using $^{133}$Xe gas in saline, $^{99m}$Tc- or $^{133}$I-MAA. A scintillation camera equipped with a video-tape recording system was used for lung imaging and quantification. Hemodynamic data, cineangiography, and chest radiograms were obtained. Autopsies were performed and macro- and microscopic examinations were made of lung specimens.

Relative hypoventilation with prolonged washout was found in the ischemic lung immediately and for 4-6 hr after PA occlusion. Breathing 8% CO$_2$ improved the unilateral hypoventilation slightly. After 6-8 hr ventilation returned to normal in 80% of the dogs and remained slightly decreased in the others. After 48 hr, unless complications such as pulmonary congestion, hemorrhage, or infarction developed, normal ventilation persisted in the ischemic lung for as long as two months. However, 70% of the dogs studied for several weeks or more developed some type of pulmonary complications. The main PA and right ventricular pressures became elevated promptly after PA occlusion and remained elevated after removal of the balloon 7 days later. Angiography confirmed the perfusion lung scan findings. Normal perfusion returned following PA occlusion of 48 hr or less duration but did not return when the occlusion lasted 7 days or longer.

In conclusion, hypoventilation and bronchoconstriction occur in the ischemic lung during the first few hours following PA occlusion. They do not persist unless parenchymal pulmonary complications occur.

Dynamic Studies of Liver Function with Radioisotopes

G.V. Taplin

The liver was the first major organ studied externally and dynamically by radioisotope techniques. Although the methods for estimating liver blood flow, hepatocellular function and biliary tract patency had the advantages of simplicity and complete safety, they never became widely accepted in hospital practice during the 1950s, and have fallen into disuse since 1960. However, with the advent of rapid imaging devices, dynamic or sequential liver scanning became practical and now promises to be a valuable procedure in distinguishing medical from surgical causes of jaundice. Other clinically useful procedures include the splenic injection of $^{131}$I macro-aggregated albumin (MAA) to determine intra and extra hepatic shunts by registering the rates of liver and lung accumulation. Most recently, dynamic measurements of the liver's dual circulation are now possible on a regional basis with cameras having areas of interest storage and replay capacity. By such techniques, it appears possible to distinguish malignant from benign liver lesions by differences in their blood supply.


Radioisotope Scanning: Current Status and Some Recent Advances

G.V. Taplin

Organ imaging with either rectilinear scanners or stationary scintillation cameras has achieved growing acceptance as a versatile tool in the diagnosis and management of disease involving most organs and organ
systems in the body. The upsurging interest in scanning has occurred mainly during the past five or six years with the development of scanners with convergent or focused collimators, larger or multiple crystal detectors, and with data display on x-ray film (photoscans) and/or color-coded dots (color scans). These improvements in instrumentation plus the introduction of new radiopharmaceutical agents, such as macroaggregated radioalbumin suspensions for lung perfusion scanning, technetium 99m pertechnetate for brain scanning, and other radiopharmaceutical agents labeled with this and other short-lived radionuclides, have extended the applications of scanning and provided organ images of higher resolution while reducing scanning time and radiation exposure to the patient.

The main reason for the popularity of scanning procedures is their capacity to reveal tumors and other space-occupying lesions as positive images in the case of brain and bone lesions and negative images in other organs of the body. Another feature of equal importance is the physiologic significance of the scan. For example, the lung perfusion scan is an objective test of regional lung function and a reliable measure of relative blood flow to all parts of the lung. Areas of reduced radioactivity indicate localized impairment of pulmonary arterial perfusion and function because ischemic lung tissue cannot carry on gas exchange normally. Scan images of the liver, spleen, kidneys, and thyroid are likewise tests of regional organ function. With recently available rapid scanners and camera type imaging devices, organ scans may be repeated in rapid sequence and thereby permit functional assessment on a dynamic basis.

This chapter is designed to acquaint the medical and surgical profession with the major clinical applications of radioisotope scanning.
as useful procedures that supplement radiography and other diagnostic modalities. Special attention is given to the detection of brain tumors, cerebral vascular lesions, pulmonary emboli, emphysema, lung cancer, primary and metastatic disease of the liver, biliary tract obstruction, unilateral kidney disease, and renal vascular hypertension.


Catabolic Pathway Differences Between $^{131}$I- and $^{99m}$Tc-Labeled Albumin Colloids and Microaggregates

K. Kitani and G.V. Taplin

This paper presents evidence from dog experiments that $^{99m}$Tc-labeled albumin aggregates are catabolized in the liver at different rates and by different mechanisms than the same suspensions labeled with $^{131}$I. With $^{131}$I albumin colloids (10-20 nm) externally monitored liver radioactivity levels decrease rapidly, while with the much larger size (1-5 microns) microaggregates of radioiodinated albumin the $^{131}$I release rates from the liver are much slower. On the other hand, both the colloidal and the micron-size albumin suspensions labeled with $^{99m}$Tc are removed from the liver at nearly the same rates as the $^{131}$I microaggregates regardless of the gross difference in their mean particle sizes. This reduction in liver turnover rates between the nm-size $^{131}$I colloids and the µm size $^{131}$I albumin microaggregate suspensions has been attributed to gross differences in the surface area between the two particles. However, surface area differences do not explain the slow and nearly identical $^{99m}$Tc-albumin colloid and albumin microaggregate liver release rates. During the period of liver release of radioactivity, blood levels show a secondary
rise with the $^{131}$I colloids and microaggregates; whereas, with the $^{99m}$Tc colloids and albumin microaggregate suspensions, only a gradual decrease in blood levels was observed.

The unique finding with the technetium-labeled albumin suspensions is that their $^{99m}$Tc-labeled dog radiation products are excreted in the bile, appearing and concentrating in the gallbladder during the first 2–3 hr. Furthermore, $^{99m}$Tc-albumin aggregate degradation products do not accumulate in the stomach as does "free" pertechnetate. A small fraction of the dose of injected technetium appears during the first hour or two in the urinary bladder.

In conclusion, $^{99m}$Tc-albumin colloidal and microaggregate suspensions are catabolized by different mechanisms within the liver than are radiiodinated albumin suspensions of the same sizes. Such suspensions give minimal radiation exposures when used for liver, spleen, and bone-marrow scanning. Also they may have a new and unique application in testing the proteolytic digestive capacity of the liver's Kupffer cells.


**Visualizing Biliary Excretion of $^{99m}$Tc-Albumin Degradation Products (A Test of Kupffer Cells Digestive Function?)**

K. Kitani, G.V. Taplin, M. Hayes, and DeLores E. Johnson

This work was undertaken to examine the potential usefulness of $^{99m}$Tc-albumin microaggregates for measuring the proteolytic digestive capacity of the reticuloendothelial system (RES) compared with radiiodinated albumin colloids and microaggregates used previously. The intended technique was to perform repeated scan examinations of the liver, spleen, and bone marrow to determine the turnover rates in each of the
three major organs of the RES. Early, during the course of this study with the short-lived $^{99m}\text{Tc}$-albumin preparations, biliary excretion, gallbladder concentration, and small bowel accumulation of radioactive material was observed. Therefore the technique was changed to use the biliary excretion of the albumin microaggregate degradation products as a potential measure of the liver's Kupffer cell digestive function.

Seventy-one camera studies were performed in 47 patients with various liver and RES disorders. Twelve patients including one volunteer without known hepatic or infectious disorders served as controls. The $^{99m}\text{Tc}$-albumin microaggregate suspensions (1–5 microns in diam) were prepared from 10–50-micron albumin macroaggregates by 8–10-min of ultrasonic agitation. Ten to 15 minutes after intravenous injection of 2.5 mCi and less than 1 mg of albumin as a test dose, abdominal scanning was performed and repeated 3 hr later. In selected cases, serial examinations were made to observe changes in the liver and abdominal distribution of activity versus time (1–5 hr).

In the 12 controls, radioactive material gradually appears in the gallbladder and the urinary bladder within 2 or 3 hr but little, if any, enters the small intestine by this time. However, by 5 hr or later, measurable activity reaches the small intestine via the biliary tract. As additional controls, patients who were being given $^{99m}\text{Tc}$-pertechnetate, albumin solutions, or albumin macroaggregates for other purposes were examined at intervals of 1–5 hr postinjection for evidence of biliary excretion of the pertechnetate ion or of labeled albumin radio degradation products. No filling of the gallbladder or accumulation of radioactivity in the small intestine was found although small quantities did appear in the urinary bladder during the first few hours.
The biliary excretion examinations made following $^{99m}$Tc-microaggregate injection showed an acceleration in the rate of excretion and an increase in the amounts of albumin degradation products entering the small intestine in patients with presumably overactive RES function, such as emphysema, Hodgkin's disease, and allergic dermatitis, whereas patients with hepatitis and cirrhosis usually had findings in the normal range or below.

In conclusion, the rate of biliary excretion of $^{99m}$Tc-albumin microaggregate degradation products appears to be a clinically applicable method for studying the proteolytic digestive capacity of the liver's Kupffer cells in health and disease.


Portal Streamlining After Mesenteric Vein Injection with $^{198}$Au Colloid

G.F. Gates and E.K. Dore

Amebiasis spreading to the liver via the portal vein tends to localize in the right lobe. The explanation offered is streamlining of blood from the superior mesenteric vein to the right lobe. However, splenic and portal venography in humans have produced contradictory results regarding streamlining. This study investigated streamline flow in the human portal system by injecting radiogold into various mesenteric veins. Additionally, since colon carcinoma can embolize to the liver, analysis of autopsy and liver scan data was done to uncover any preferential liver lobe metastases.

Twelve patients without liver disease who were undergoing laparotomy were selected. 200-300 μCi of $^{198}$Au colloid was injected into an identified mesenteric vein, and on the earliest possible postoperative day a scintiscan of the hepatic distribution of $^{198}$Au was done. Immediately
thereafter 2–5 mCi of $^{99m}$Tc-microaggregated albumin was injected in an arm vein, and a second liver scan was done. This provided a control for each patient's overall distribution of hepatic blood flow. Areas of interest of identical dimensions were set up over both lobes, and counts in the right area were expressed as a percentage of the total counts occurring in both areas. Therefore the distribution of blood flow from a selected mesenteric vein ($^{198}$Au) could be determined and compared to the control injection ($^{99m}$Tc).

Streamlining to the right lobe occurs but is dependent upon the mesenteric vein injected. Four out of five cecal vein injections streamlined to the right lobe along with one injection into a terminal ileal vein. A mid-jejunal vein injection showed a tendency to flow to the left. One out of four sigmoid vein injections also streamlined to the right.

Autopsies and/or liver scans of 61 patients with hepatic metastases secondary to colon carcinoma revealed that 1/3 had predominantly right lobe involvement. The remaining cases involved both lobes equally except one patient with predominantly left lobe involvement on scan.

Streamlining of blood in the human portal vein occurs and may account for the frequent occurrence of right lobe involvement in amebiasis. Streamlining may also account in part for the pattern of hepatic spread of colon carcinoma.

Comparison of $^{131}$I-Hippurate and $^{99m}$Tc-chelates for Monitoring Renal Homotransplant Function

M. Hayes and G.V. Taplin

Sequential kidney scanning has been reported to have distinct diagnostic value in a variety of renal problems. This study is presented to compare the usefulness of a short-lived radiopharmaceutical ($^{99m}$Tc-DTPA) with $^{131}$I-hippurate in the functional assessment of renal transplant recipients.

Twelve transplant patients were studied reportedly using a large crystal scintillation camera with dual-isotope capabilities. Serial 1 1/2-min kidney-bladder images were obtained for 20 min postinjection of a single intravenous bolus containing 300-μCi $^{131}$I-hippurate and 2 mCi $^{99m}$Tc-DTPA. Renograms and cystograms were obtained for both agents by area of interest quantification of magnetic tape data.

$^{99m}$Tc-DTPA gives better scan statistics and lower patient radiation exposures than $^{131}$I-hippurate. Renogram peak times are identical for both agents, however upslopes and downslopes for $^{99m}$Tc-DTPA (an agent which is cleared predominantly by glomerular filtration), are less steep than for $^{131}$I-hippurate which is cleared mainly by tubular excretion. Both agents are nearly equally sensitive to moderate changes in overall function; however, when function is severely depressed, $^{131}$I-hippurate is the more sensitive agent. In a few cases, both agents in combination give valuable information regarding selective depression of either glomerular filtration or tubular excretion; but in most instances, the functional abnormalities registered with each of the two agents is similar.

It is concluded that $^{99m}$Tc-chelates may be used as $^{131}$I-hippurate substitutes for evaluating the transplanted kidney when overall function
is fair to good. If a single radiopharmaceutical must be used, $^{131}$I-hippurate remains the agent of choice, especially when function is severely impaired.


Radiochemistry of Macroaggregated Albumin and Newer Lung Scanning Agents

G.V. Taplin and N.S. MacDonald

The original development of human serum $^{131}$I albumin macroaggregates for lung visualization by radioisotope scanning began at the UCLA Laboratory of Nuclear Medicine and Radiation Biology in January 1963 as an extension of previous work on shortening and simplifying Benacerraf's three-day protocol for preparing colloidal size $^{131}$I albumin to a one-hour clinical laboratory procedure. Solutions of human serum albumin can be converted to molecular aggregates of any desired range from 10 nm to 100 μm or larger by combinations and variations of albumin concentration, pH adjustment, heat treatment and simultaneous agitation.

The ideal particulate material for lung scanning in man is a suspension of particles made of a normal, rapidly metabolizable body constituent in the size range of 20-50 μm, which is non antigenic, reaction free, readily labeled in the clinic or hospital laboratory with short lived $^{99m}$Technetium or $^{113m}$Indium nuclides, and prepared with sterile, non-toxic, pyrogen free physiological materials. Its extraction efficiency by the pulmonary arteriolar capillary network should be 90 percent or higher and its removal rate from the lung must not exceed a few hours.

From a practical viewpoint, currently available MAA preparations labeled with $^{131}$I meet most of the requirements of the ideal agent except
for the $^{131}$I label, which gives more than ten times as much radiation exposure to the lungs as $^{99m}$Tc. However, its 2-3 week shelf life and commercial availability make it the agent of choice in most community hospital nuclear medicine facilities today.

The recent growth of "In house" preparations of MAA and of inorganic particles labeled with $^{99m}$Tc or $^{113}$In is likely to continue as the result of simplified procedures and materials (kits) made available from the radiopharmaceutical manufacturers. The most versatile preparation - albumin microspheres - in narrow size ranges, delivered unlabeled but in "kit" form for "In house" labeling with short lived nuclides shows promise for the future, provided that the early findings can be verified and the agent and kits meet the requirements of the Federal regulatory agencies. Much the same can be said for commercially prepared suspensions of unlabeled macroaggregates along with "kits" for "In house" labeling with $^{99m}$Tc.


Preliminary Imaging Studies with a Mosaic Crystal Image Intensifier Camera

M. Hayes

A new variety of image intensifier camera has been introduced which is designed to provide high resolution and large field size. This device employs divergent collimation, a mosaic of small crystals, and a pulse height analysis system. This study was undertaken to determine the imaging properties of this new camera. Some comparisons with another contemporary large field camera system will be made.
Method

Field size was demonstrated by obtaining images of a series of parallel radioactive lines on 1 inch (2.54 cm) centers.

Resolution was measured with a modified Kakehi phantom which consists of a series of radioactive lines with decreasing intervals.

Imaging Geometry was illustrated with scans of a three dimensional phantom containing three radioactive tubes which are placed at a 45° angle to the collimator face.

Imaging Speed was studied by determining the time to attain 200,000 counts (1,000 counts/sq cm) from a liver slice phantom containing 1 milli-cure of $^{99m}$Tc placed 4 inches (10 cm) from the collimator face.

Results and Conclusions

The mosaic crystal image intensifier camera achieved a 13 inch useable field size and was able to resolve 2 lines 1.0 cm apart at 4 inches on a Kakehi line source phantom. Moderate image distortion was produced. In an imaging speed determination, 200,000 counts were obtained in 1.7 minutes with 1 mCi of $^{99m}$Tc.

By comparison, an Anger scintillation camera with divergent collimation introduced slightly less image distortion and provided closely similar field size and imaging speed, but poorer resolution.

Interim Report
The Effects of Coronary Arterial Injection of Radioalbumin Macroaggregates on Coronary Hemodynamics and Myocardial Function

Norman D. Poe

The effects of intracoronary arterial injections of radioalbumin macroaggregates on coronary hemodynamics and myocardial function were studied in normal, anesthetized dogs to evaluate the possible use of this technique for determination of regional myocardial blood flow. Slow injections of macroaggregates containing less than 0.05 mg albumin with no particles greater than 60-70 microns in maximum diameter can usually be given directly into the left anterior descending coronary artery with no significant changes in coronary flow, myocardial contractility, arterial pressure or the electrocardiogram. As the mass of albumin and/or particle size are progressively increased, a sequence of functional changes evolves. Coronary flow is reduced followed immediately by transient hyperemia. Contractility falls. Only after marked flow and contractile changes have been produced do abnormalities in arterial pressure and the ECG develop. The course of events is similar to that resulting from progressive coronary arterial occlusion. It is concluded that carefully prepared radioactive particles, in amounts sufficient for external scintillation scanning, can be injected into the coronary arteries without detectable alterations in function, and that the technique is suitable for acute and chronic determinations of regional myocardial perfusion, both experimentally and clinically. The functional effects in the presence of coronary or myocardial disease are yet to be determined.

Regional Coronary Blood Flow Measurements: Radioactive Particles Versus K⁺ Analogs

Norman D. Poe

Preliminary reports estimating regional coronary blood flow with radioactive albumin particles are promising, but similar information might be obtained more safely with solutions of elements which concentrate in the myocardium. This study compares the results in 26 open-chest dogs after simultaneous injections of cesium-131 with technetium-99m labeled microspheres (10-100 µ), large albumin particles (10-100 µ) or small albumin particles (<40 µ). Scintiscans were performed in vivo and in vitro and multiple tissue samples counted to determine relative distribution quantitatively. Injections were made into the anterior descending coronary artery (I) or left atrium of normal dogs (II) or intra-atrially after ligation of the anterior descending coronary (III). Scan findings in each group were qualitatively similar except particles and spheres resulted in more limited and circumscribed flow patterns in I and more discrete ischemic defects in III. Quantitatively, the best correlation with cesium was found with the small particles (r = .845). Conclusion: Although uptake of K⁺ analogs may be altered in damaged cells, even without reduced blood flow, this method should be sufficiently reliable and safer for clinical application than the injection of radioactive particles.

Interim Report
Effects of Glucose-Insulin-Potassium Infusion on the Intramyocardial Distribution of Potassium/Cesium-131 in Experimental Infarction

Norman D. Poe

Injured and ischemic myocardium is known to lose large amounts of intracellular potassium. Restoration of the lost $K^+$ and repolarization of the cell membrane induced by a polarizing solution containing glucose, insulin, and $K^+$ (GIK) has been advocated as a means of treating acute myocardial infarction. However, the results have been equivocal and some authors report an increased incidence of arrhythmias in treated patients. To explore the changes in $K^+$ distribution induced by GIK which might cause altered conduction, serial scans using $131$-Cs as a $K^+$ substitute were performed in ten anesthetized open-chest dogs with experimental infarcts.

Localized myocardial damage, as evidenced by ECG, was produced by tying off the anterior descending coronary artery plus anastomoses if necessary. One mCi of $131$-Cs was given intravenously and a left lateral scan was obtained with the chest open to identify the ischemic area. A 10% glucose infusion containing 40 meq KCl and 20 units insulin/liter was begun at 2cc/min. Rescanning was performed after 30 minutes. A second scan was obtained following a similar infusion period, administered at the rate of 4-6 cc/min.

In approximately half the cases, primarily those with lesser degrees of ischemia, GIK did not alter the scan or $131$-Cs uptake by the myocardium. In the remainder there was an increase in the size of the infarct. This finding may be more apparent than real because it was accompanied by increases of up to 17% in the count rate of the normal myocardium.
without a corresponding detectable loss in the ischemic area. The more rapid infusion did not produce further change. The animals were then sacrificed. An in vitro scan was performed and 131-Cs distribution was verified by counts of multiple tissue sections.

Although no arrhythmias developed during the short duration of GIK administration in this study, it is possible that under the prolonged administration advocated clinically, the relative changes in distribution of intravascular \( k^+ \) may induce arrhythmias rather than protecting against them.


Fluorine-18 for Bone Scanning

L.R. Bennett, et al.

Studies involving F-18 for bone scanning have indicated that F-18 is satisfactory as a scanning agent for routine use. The agent is now being used clinically for bone scanning.

Final Report

Normal \(^{67}\)Gallium Scan

L.R. Bennett, R.C. Small, et al.

Gallium-67 has been shown to have a high uptake in a variety of soft tissue tumors. It also has a high uptake in liver, spleen, and bone, as well as excretion through the bowel which can present problems in interpretation. Based on 60 cases with total body scans, our findings in areas of the body free of cancer are presented.

In scans of the head, there is always some uptake in the region of the mouth; whether this is in the soft tissues or bone has not as yet been
determined. The amount of Gallium retained in this area after 24 hours is usually quite low; and since its distribution takes on a symmetrical pattern it has not interfered with tumor localization. A similarly low level symmetrically distributed uptake also occurs in the posterior portions of the skull, corresponding to the marrow-containing regions. The neck normally shows a small amount of \(^{60}\)radioactivity in the cervical vertebrae and occasionally in the soft tissues of the anterior neck.

In the thoracic area, there is a low but definite uptake in the ribs and scapulae on posterior scans. Again, this is not a problem since lesions in the ribs and vertebrae are usually much hotter than surrounding tissue. On anterior scans of the chest, the sternum often presents a more difficult problem in interpretation because it normally has a relatively high uptake; consequently, there is a very real chance of missing tumors in or just below the sternum.

The upper abdomen probably presents the greatest problems in interpretation. In the normal scan there is a high uptake in the liver and spleen as well as in the lumbar vertebrae and pelvic bones. Excretion by way of the bowel and urinary tract may also cause problems, particularly at 24 and 48 hours. It is obvious that laxatives and enemas will be necessary, probably beginning at 24 hours, and that no reliable information is gained by scanning before 48 hours.


The Use of Technetium Macroaggregates of Albumin for Lung Scanning

M.M. Webber, M. Cragin, et al.

A previously developed clinical method for tagging macroaggregates of albumin incorporating technetium sulfur suspension for lung scanning
has been used extensively over the period of approximately the past year and one-half. Initial animal toxicity studies failed to indicate any evidence of abnormal or adverse reaction to the macroaggregates of albumin prepared in this way. Inasmuch as the new preparation consists of macroaggregates of albumin, the safety of which has been previously established, and Technetium sulfur suspension, which is also being used on an almost nation-wide basis, there appeared to be little reason to suspect that there would be any clinical difficulties encountered with this substance. Nevertheless, it was considered important to determine whether any evidence of untoward human reaction involving the use of this material for lung scanning might be noted. The material has been in use for approximately one and one-half years in the Radioisotope Service of the hospital. A retrospective study was made of approximately one hundred patients who had received this pulmonary scanning agent. Review of their charts indicated no evidence of adverse reactions or other signs indicating difficulties attributable to the injected Technetium sulfur tagged macroaggregates of albumin. A number of patients exhibited nausea, fever, or other symptoms post injection, but these patients had a history of such recurrent symptoms prior to the injection. It was concluded that all evidence indicates macroaggregates of albumin tagged with Technetium sulfur suspension are a safe preparation for clinical use.

**Final Report**

**Demonstration of Vascularity of the Femoral Head**

M.M. Webber, M. Cragin, et al.

The question of whether or not the femoral head is vascular following fracture of the neck of the femur is often very difficult to answer.
Particles of Technetium sulfur colloid tagged with Radiotechnetium-99m accumulate in the bone marrow and demonstrate the phagocytes within the bone marrow of the pelvis and hip. In patients with diminished vascularity to the hip, it may be possible to show the absence of vascularity by use of a tracer outlining the bone marrow. A large series of patients was examined to determine the appearance of the bone marrow of the femoral heads in normal individuals. It was found that there is almost invariably symmetry in the amount of uptake in the femoral heads. Although the uptake may be very small on either side, it may also be quite large. This study involved patients who underwent the routine liver scanning procedure, which uses the same tracer. Inasmuch as the variants of the normal pattern have now been established, it is hoped that this method can be applied to patients who are suffering from fracture of the hip or other hip disease.

Interim Report

The Use of $^{131}$I Labelled Antibodies in the Detection of Coccidioidomycosis

J.D. Parker, M.M. Webber, et al.

A quick, painless diagnostic technique for differentiating the pulmonary form of chronic systemic fungal diseases from pulmonary tuberculosis is necessary. One possible technique is the administration of radioisotope labelled antibodies which might localize in areas of fungal infection and which could be detected in vivo with scintiscanning equipment. In this study, mice with localized subcutaneous Coccidioides immitis flank infection were given one of the three following $^{131}$I labelled rabbit globulin preparations: (1) globulin from a normal rabbit, (2) globulin from a
C. immitis infected rabbit containing precipitins to the fungal antigen, or (3) a non-IgG fraction of preparation #2 containing C. immitis precipitins. In each of the three experiments two mice were sacrificed at 3, 24, 48, and 96 hours after the injection of the globulin preparations and the amount of radioactivity was determined in the coccidioidal lesions and in several tissues. Also, at 96 hours scintiphotos were obtained of the lesions of two mice in each experiment using the Anger scintillation camera with a pinhole collimator. The mice given preparations #2 or #3 tended to have higher lesion-to-tissue radioactivity ratios than those given preparation #1, and their scintiphotos tended to show the lesions more clearly. The main problems encountered in the study were high body background due to prolonged circulation of the labelled globulin, and nonspecific protein localization in the coccidioidal lesion. Future work will require antibody purification and labelling with higher specific activity as well as reduction of body background.


Aluminum Ion Effect on Technetium Sulfur Colloid

M.M. Webber and M. Cragin

Because of several published reports indicating that the aluminum content of Technetium generator eluents has affected the stability of Technetium sulfur colloid labeled with Technetium-99m, a study was undertaken to determine whether there was significant aluminum in the eluents of various commercially available generators, and, if so, whether this aluminum affected the colloid as prepared by the method previously described at this institution. Data obtained indicated extensive
variability between various brands of commercial generators. However, there was no significant effect of even rather large quantities of aluminum on the preparation of Technetium sulfur suspension as made and previously reported by us and as stabilized by the addition of serum albumin.

Final Report

Variants of the Normal Lung Scan

M.M. Webber, L. Resnick, M. Cragin, and Winona Victery

Lung scanning is a commonly accepted nuclear medicine procedure, and a number of reports have dealt with the "normal" perfusion pattern, or the pattern of lung perfusion as seen in "normal" individuals. However, there has been no large-scale endeavor to determine whether there are variations of the theoretical normal which do not reflect the presence of a disease process. Because of this question, lung scans were performed on approximately 40 young, normal volunteers to determine whether any significant deviation from the theoretical normal could be detected. The study was designed in order to give us an idea of whether certain "abnormalities" might be expected even in the face of true clinical normal findings in the person undergoing the scan. Approximately 15% of these patients deviated to varying extents from the theoretical normal pattern. Approximately 5% had a substantial variation from the normal, which might have been interpreted as a perfusion deficit characteristic of pulmonary embolus or other pulmonary disease. This study indicates that even in the absence of known disease an occult disease or a variation of normal may be present which tends to mimic more serious disease states.

Interim Report

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Demonstration of Thrombophlebitis and Endothelial Damage by Scintiscanning

M.M. Webber, Winona Victery, and M.D. Cragin

In this study, we have evaluated a number of particulate $^{99m}$Tc-labeled tracers with respect to their ability to serve as an agent which would attach itself to fibrin deposits or clots as it circulated in their vicinity as a result of upstream injection. This was done in vivo on experimentally created lesions and in vitro, both microscopically and quantitatively. The materials which were evaluated were technetium-sulfur-labeled macroaggregates of albumin, technetium-sulfur-labeled macroaggregates of fibrinogen, technetium-labeled microspheres of albumin, and technetium-labeled aggregates of Sn (OH)$_2$.

Microscopically, macroaggregates of albumin demonstrated greatest uptake. The uptake on clots formed in vitro and assayed quantitatively indicated that three agents could be expected to have a high uptake in vivo. The uptake in injured vessels did not parallel these findings. In each of two animals studied, neither technetium-sulfur-labeled macroaggregates of fibrinogen nor the microspheres of albumin demonstrated high uptake. The presence of a lesion was confirmed with an injection of technetium-sulfur-labeled macroaggregates of albumin at the same area. Venographic studies before or immediately after morrhuate vein treatment had the same appearance, although uptake of radiotracer is very marked. Venographic irregularity is evident one day following morrhuate injection.

Comparison of several agents' ability to label clots indicates that macroaggregates of albumin and tin hydroxide are good agents for the evaluation of clot location in vivo. Macroaggregates of albumin are an accepted tracer for lung scanning, whereas tin hydroxide is not used in
the United States. Macroaggregates of albumin were therefore used for patient studies. Despite the numerous visual observations and variations of types of particles used in these studies, the actual mode of particle attraction by clots remains basically an enigma.


Cerebral Blood Flow Studies
M.M. Webber et al.

After six months' experience with flow studies on almost all patients who underwent brain scanning in the UCLA Clinic, only two cases were found in which additional information was acquired with respect to vascularity of lesions also seen on the normal brain scan. One case indicated decreased vascularity due to a vascular occlusion. Because of the low yield in cases seen by us, the flow study was felt not to be valuable as a routine procedure, but will be applied to selected cases as indicated.

Final Report

Measurement of the Depletion of Natural Body Potassium by Means of the Total Body Counter
N.S. MacDonald, I. Ban, and M. Hackendorf

Natural potassium is lightly radioactive due to the presence of about 0.01 per cent of the K-40 isotope. The gammas emitted by this radionuclide can be detected with the Total Body Counter, thereby making it possible to determine the amount of total potassium in the human body. An interesting application of this technique was recently carried out as part of an extensive program of the McDonnell-Douglas Corporation. Our contribution consisted in determinations of the amounts of potassium in
the bodies of six young, male volunteers before and after their confinement for three months in a full-scale, operating model of an experimental life-support module designed for extraterrestrial space exploration. Our significant finding was that each of the three men who did not exercise actively during their confinement lost about ten grams of body potassium—a depletion of about six percent of their body stores of this vital element. On the other hand, the three men who followed a schedule of regular, planned physical exercise maintained their normal body potassium level.

The counter facility can measure changes of as little as two grams in fifty of natural potassium, with a standard deviation of ±10 percent. This capability was utilized in another collaborative effort with the division of Thoracic Surgery of the School of Medicine. Repeated measurements of body potassium were made on forty dogs during the course of various experimental procedures. It was found that total body stores of potassium in dogs can be rapidly depleted by 20-25 percent by means of special diets.

Interim Report

Cesium-137 Levels in Human Adult Population in the Los Angeles Area
N.S. MacDonald, I. Ban, Anna Mae Flesher, and M. Hackendorf

One hundred eighty-nine measurements on adult residents of Southern California were performed with the Total Body Counter. These revealed that the mean quarterly values for Cesium-137 contamination in the bodies of males during 1970 was 26 picocuries per gram of potassium and 23 μCi/gm K for females. Thus, the rapid downward trend from the peak in 1965 which
flattened out during 1968-1969, has indeed been replaced by a flat trend during 1970. The levels of Cs-137 in these people are not decreasing, and there is some evidence that their body burdens may even be increasing very slightly. It must be emphasized that the accrued radiation dose to a person from these very small amounts of Cs-137 over the last decade was calculated to be less than 6 per cent of the absorbed radiation dose delivered by the radioactive natural potassium-40 inescapably present in his body.

Further measurements in this series will be sharply curtailed as a result of budgetary stringencies. A summary of results during the decade 1960-70 was published in "Nature" recently.


Turnover Rates of Immune Serum Globulins in Rheumatic Disorders - Measured with Radionuclide Labeled Proteins and the Total Body Counter

N.S. MacDonald, Anna Mae Flesher, I. Ban, and M. Hackendorf

The rates of turnover of immune globulin G and M were determined in 49 patients with rheumatic disorders. These studies performed in collaboration with Dr. Joshua Levy and others of the Department of Medicine, are aimed at elucidating the causes of rheumatic diseases and providing methods for objective evaluation of the efficacy of their treatment. Our contribution to the program consists of measuring the turnover rates of the two proteins (which have been tagged with radioactive Iodine-131 and Iodine-125, respectively), in blood and in the total body over a period of 3 weeks following their intravenous administration. These radioassay data from serial blood samples and total body counting, are then combined with other information obtained by the other collaborators.
(such as serum concentrations of the two immune globulins, plasma volume and body weight) to permit calculation of the daily rates of synthesis and of catabolism of these proteins.

By performing these determinations before and after chemotherapy, it was found, for example, that Azathioprene suppressed the daily synthetic rate of IgG by as much as 74 per cent in 7 of 9 patients. Production of IgM was reduced by like amounts in 8 of 9 patients. The concentrations of these proteins in the plasma were not greatly affected, probably because their rates of degradation also decreased during the 4-6 month treatment, thus compensating for the drop in synthesis. These changes were accompanied by clinical improvement in the patient's condition.

Interim Report

The Biomedical Cyclotron Facility; Progress Report

N.S. MacDonald and B. Cassen

Construction of the building to house the accelerator was begun on May 1, 1970 by a general contractor under an agreement with the University of California, Los Angeles, which provided the funds, and architectural and engineering supervision. The structure was accepted for occupancy on Feb. 7, 1971. The cyclotron itself was completed by the manufacturer (Cyclotron Corporation, Berkeley, California) and factory tests were observed and accepted on Sept. 18, 1971. The machine was shipped by truck with magnet pole pieces, yoke and dees all assembled. It arrived on Oct. 6, 1971, whereupon it was moved into the vault of the Facility for storage during completion of the building. A cyclotron engineer and radiation physicist were added to the staff of the Facility and, together
with the electronics engineer, received technical instruction and indoctrination during short stays at the Cyclotron Corporation factory. This basic operating staff, together with personnel of the Laboratory Shops Section undertook actual installation of the cyclotron, with assistance and guidance from the manufacturer's representative. The considerable amount of piping, wiring and mechanical work was essentially complete by the end of March, 1971. The run-in and performance tests were completed by mid-June and the accelerator was formally accepted from the manufacturer. The machine met or exceeded all performance requirements, including a full seven-hour continuous production of a beam of 22 MeV protons at a current of 50 microamperes at the external target. Construction of several items still required for target handling, and completion of hood exhaust systems, will postpone actual production runs of radionuclides for clinical usage until early July, 1971.

A large amount of effort was directed to the procurement, construction, assembly, and testing of ancillary equipment. These included a novel target transport system; a fairly elaborate, safety interlock system; a closed-circuit television monitor system with remotely controlled mirror periscope; gas target handling system; gas analysis equipment, gas chromatograph; solid-state, germanium detector system with 1600 channel analyser for gamma spectrometry; gamma area-monitoring system, continuous air-monitoring system for radioactive particulate and gaseous effluents; and special hoods, shields, etc. for the "hot lab".

A detailed safety manual for operations was composed, reviewed and approved by the appropriate campus, State and AEC regulating authorities.
Advantages of Kinetic Compared with Static Imaging of Intracranial Lesions

J.C. Kennady, Barbara J. Miller, and G.H. Wilson

Routine brain scanning of patients with $^{99m}$Tc- or $^{197}$Hg-labeled test agents is static imaging of any lesion that produces an alteration in the blood-brain barrier. In time the radiopharmaceutical will extravasate into the region of pathology and/or the compressed adjacent brain. The exact size of the lesion therefore cannot be accurately established nor is there any information regarding blood flow to the involved area or the degree of vascular compromise in the whole hemisphere.

Kinetic imaging (60 frames/sec) with the image intensifier videocamera shows the test agent ($^{99m}$Tco$_4^-$) bolus entering, distributing, and passing through the cerebral microvasculature. From videotape replay, integrated Polaroid pictures are obtained; a plastic grid with 1.5 cm$^2$ squares is placed over the TV monitor to facilitate quantification of regional cerebral blood flow (rCBF). Hence, the size and vascularity of the lesion can be seen and the blood flow in the involved region as well as global areas can be readily determined and compared with the control group.

Twenty-seven patients with no CNS abnormalities in the hemisphere were studied from the control group. Three parameters have been assessed in 29 1.5 cm$^2$ areas in each patient; the percent mean maximum concentration of $^{99m}$Tco$_4^-$, time from injection to beginning of downslope, and the inverse $T_{1/2}$ converted to rCBF. Total accumulative data indicating the highest mean percentages of the percent mean maximum $^{99m}$Tco$_4^-$ concentration are seen in the suprasylvian parietal areas (122.3 ± 10%); the mean injection to downslope time is 2.7 sec and the highest mean
rCBF values \((115 \pm 30 \text{ ml/100 gm/min})\) are in the superior and posterior parietal regions and the lowest \((60 \pm 22 \text{ ml/100 gm/min})\) are in the inferior frontal and temporal areas.

In patients with vascular tumors the percent mean maximum concentration of \(^{99m}\text{TcO}_4^-\) is higher and the regional blood flow rates are significantly slower within the tumor region. Similar pictorial results are obtained in patients with vascular tumors and chronic vascular disease; however, quantification of the latter group shows a higher percent test agent concentration in the cortex surrounding the vascular lesion with significantly slower blood flow rates in both regions.

A paramount advantage in kinetic brain imaging in patients operated upon is the high correlation found between postoperative return of function and the global regional CBF values. Patients with insignificant alterations in their rCBF rates showed rapid recovery, whereas, those with abnormal rCBF values in regions distant from the lesion had prolonged and incomplete functional return.


Comparison of Two Dynamic Gamma Imaging Camera Systems in Patients with Brain Tumors

J.C. Kennady

The image intensifier videocamera (Magnacamera) and the gamma scintillation camera (Dynacamera 2) have been used in the dual study of patients with brain tumors during the past six months. Following a rapid bolus internal carotid injection of \(^{99m}\text{Technetium pertechnetate}\), the passage through the lateral hemisphere is immediately seen on the iconoscope screen and recorded on videotape.

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Both systems have regional quantification capabilities from videotape replay. The image intensifier videocamera system has been previously described (Fourth International Symposium on Regulation of CBF, London, 1970). In addition, the gamma scintillation camera has a pulse height analyzer (set at 140 KEV) and ratio computer with a digital readout. Comparable hemisphere delineation and high count rates (excluding dural sinuses) requires a threefold increase in dosage of the test agent (2.5 vs 8 mCi) when using the gamma camera.

There is good correlation between the two cameras, the respective visual display with regional quantitation and these results with the location, size, and type of tumor at operation. The region of primary tumors show increased whereas secondary tumors have decreased $^{99m}$TeO$_4^-$ concentrations and slow relative blood flow indices compared with the other brain areas.

A significant number of patients have shown the radiopharmaceutical passing through the ophthalmic artery and over the supraorbital region producing slow relative blood flow indices in the subjacent anterior frontal regions. The respective arteriograms provide no clue for this observation. This further supports dynamically visualizing concomitant with quantifying intracranial hemodynamics.

Interim Report
S-17. **Comparison of Two Dynamic Camera Systems**

J.C. Kennady, Barbara Miller, E. Terao, and G. Wilson

S-21. **The Normal Lung Scan**


S-22. **Scintigraphic Localization of Blood Clots**

L. Resnick, Winona Victery, M. Cragin, J. Grollman, and M. Webber

S-28. **Effect of Aluminum on $^{99m}$Tc-Sulfur Colloid**

M. Cragin, Winona Victery, Margaret Bracken, M. Webber, J. Goodrich, W. Briner, H. Anderson, and C.C. Harris

S-32. **Portal Streamlining** (Honorable Mention Award)

G.F. Gates, E.K. Dore, and G.V. Taplin

S-41. **Method for Diagnosis of Intracardiac Shunts** (Gold Medal Award)

V.B. Bosnjakovic, L.R. Bennett, W. Vincent, Jeanne Larson, and R.J. Bennett
Decreased Growth Capacity of Passaged Gross Virus-Induced Mouse Lymphomas After Incubation with Antithymocytic Sera

Donna L. Vredevoe and Esther F. Hays

AKR or C3H/HeJ transplanted mouse lymphomas, originally induced by Gross virus, could be neutralized by in vitro incubation with an immuno-suppressive rabbit anti-mouse thymus cell serum. Neutralization was measured by significantly increased latent periods or decreased incidence (at a minimum of 90 days post-transfer) of lymphoma in syngeneic recipients of lymphoma cells incubated with antithymocytic serum when compared with syngeneic recipients of lymphoma cells incubated with normal rabbit serum.


Immune Response in Preleukemic Mice

Manuel Frey-Wettstein and Esther F. Hays

Humoral and cellular immunity was assessed serially in preleukemic AKR mice and Gross virus-injected C3H/HeJ mice over a period of 10 to 12 months. Quantitative gamma globulin, hemagglutinin and hemolysin titers after immunization with sheep red blood cells, and macrophage-migration inhibition were determined. Normal production of gamma globulins, as well as humoral antibodies was found in both of these experimental groups of mice, whereas macrophage-migration inhibition, which is believed to be a correlate of cellular immunity, appeared to be abnormal throughout the life of these animals, which were destined to develop a high incidence of lymphoma. Some aspects of this apparent dissociation between humoral and cellular immune response are discussed with regard to the
mouse virus lymphoma system, primarily involving the thymus, which was used in these studies.


Isolation and Transplantation of 10-20 Mouse Lymphoma Cells in a Semisolid Medium

Donna L. Vredevoe

Lymphoma was produced in 100% of C3H/HeJ recipients of 10-20 lymphoma cells embedded in gelatin droplets. Gelatin was optically clear and did not appear to affect the viability of transferred cells. The advantages of this technique over that of injecting cells into liquid medium are that (1) cell types can be selected for study and transfer, (2) cells can be quantitated exactly, and (3) the exact cells to be transferred can be photographed.


Increased Incidence of Lymphoma in C3H/HeJ Adult Mice Injected with Gross Virus and Antithymocytic Serum

Donna L. Vredevoe and Esther F. Hays

An increased incidence of lymphoma in adult C3H/HeJ mice injected with antithymocytic serum as compared with normal rabbit serum during a course of one to four weekly intraperitoneal injections of cell-free filtrates of Gross virus-induced lymphomas was noted. The latent period of lymphomas ranged from 119 to 298 days after initiation of treatment. The incidence tended to increase as the number of injections of cell-free filtrate was increased, with a maximum incidence reached at three injections.

To study their role in lymphomagenesis and the derivation of cells in developing tumors, GVH reactions were produced in Fl hybrid mice [(one parent with a predisposition to reticular neoplasms (SJL) and the other bearing a marker chromosome (T6)]. Mice were inoculated with SJL spleen cells. Controls were littermates given cell-free filtrates of SJL spleens and non-injected litters. Chromosome studies of spleen and lymph node cells were carried out in animals with acute GVH reactions and those with lymphoreticular neoplasms. More than half of the cell-inoculated animals had acute GVH reactions. Of the 53 mice surviving, 5 developed nonthymic lymphoma, and 18 had reticulum cell sarcomas. In 72 control animals, 3 nonthymic lymphomas and 12 reticulum cell sarcomas were found. Chromosome analysis of animals with acute GVH reactions revealed 34% of metaphases to be donor type. Three animals with nonthymic lymphoma also had significant numbers of donor metaphases. Donor cells were less prevalent in animals with reticulum cell sarcoma. Both cell-inoculated and control animals had a modal chromosome number of 40, however, aneuploidy was a feature of the reticulum cell sarcomas. In the spleen cell-inoculated animals the abnormal chromosome numbers were found principally in host cells. These studies show that lymphoma incidence is increased in mice with GVH reactions. Donor cells are present in these neoplasms and aneuploidy is a characteristic of the host tumor cell.

Morphology of Thymic Grafts Exposed to Lymphomagenic Virus

Esther F. Hays

Syngeneic thymic grafts in AKR mice were exposed in vitro to lymphomagenic virus, grafted subcutaneously or under the kidney capsule, and biopsied at intervals. No specific morphologic change was seen to precede the development of lymphoma in these grafts. Lymphoma was found to develop initially as foci of lymphoblasts in the cortex of a normal appearing thymus. Regeneration of syngeneic virus exposed thymic epithelial remnant grafts in AKR mice was compared to that of non-virus exposed grafts in the same strain and to those in a low incidence lymphoma strain. All of the grafts were found to regenerate to the morphology of normal thymus with a characteristic sequence of cellular events. These results imply that lymphomagenic virus acts by a direct transformation of cells in the thymic cortex which is not dependent on any preceding structural alteration of this organ and that regeneration of grafts composed of epithelial reticular cells was not impaired by the presence of the virus.
