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**LABORATORY OF NUCLEAR MEDICINE
AND**

RADIATION BIOLOGY MASTER

**UNIVERSITY OF CALIFORNIA
900 VETERAN AVENUE
LOS ANGELES, CALIFORNIA**

AEC CONTRACT AT(04-1) GEN-12

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UC-48 Biology and Medicine
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UNIVERSITY OF CALIFORNIA
LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY
ATOMIC ENERGY COMMISSION CONTRACT AT (04-1) GEN-12
900 Veteran Avenue
Los Angeles, California 90024

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8-7 ANNUAL PROGRESS REPORT *2227*

For the period ending

JUNE 30, 1972

UCLA #12-858

110
Printed in the United States of America. Available from Clearinghouse for Federal Scientific and Technical Information, National Bureau of Standards, U.S. Department of Commerce, Springfield, Va. 22151. Price: Printed Copy \$3.00; Microfiche \$0.65

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REPORT OF THE DIRECTOR

The following reports, organized on a divisional basis, succinctly comment on the major projects of the Laboratory staff. The listing of students and associated scientific personnel give further insight into the research involvement and teaching of our staff and associates.

My comments focus on changes in our programs and some of the notable events in fiscal year 1971-72.

During the year Dr. Eric B. Edney joined the Laboratory as Associate Director, and also as Division Chief for Environmental Biology (formerly Environmental Radiation Division). Under his leadership, several new programs are developing. Dr. Kenneth Nagy will be joining this division in the Fall, and other recruitment is anticipated. Laboratory staff from this division are participating in the IBP Desert Biome study. Rock Valley, at the Nevada Test Site, is one of the validation sites in the Desert Biome program.

The Division of Radiation Measurements was established during the latter part of the year with Mr. Gerald Huth as Division Chief. Dr. Jorge Llacer and Mr. John Ewins, along with Mr. Huth, form the nucleus of this group. Active recruitment is proceeding to assemble the staff. This division will develop a basic program in radiation detection and measurement, including supporting electronics, and seek applications in medical, environmental and other biological areas.

Greater emphasis will be placed on radiopharmacology in support of nuclear medicine, as a result of the addition of Dr. Gerald Robinson to the staff of our Nuclear Medicine Division during the past year. Currently, this division is seeking a physicist to meet needs in the Cyclotron area and for work on medical problems involving computer interfacing and instrumentation.

Staffing in other divisions has remained static during the past year. Training programs for undergraduates, graduate students and visiting scientists have been initiated with support coming from the Associated Western Universities.

We report, with sorrow, the deaths of two prominent members of the staff during the past year. Dr. Thomas Hennessy, who had been on the staff since 1954 and had served as Assistant Director from 1956 to 1969, died of a heart attack in July 1971. Dr. Benedict Cassen, a member of the Laboratory staff since 1947, and winner of the Distinguished Scientist Award from the Society of Nuclear Medicine for his invention of the scintillation scanner, died in March 1972 following a coronary attack. The scintillation scanner had a powerful effect in launching the field of Nuclear Medicine.

Dr. Stafford L. Warren, the founding Director of the Laboratory, shared the 1971 Enrico Fermi Award of the U. S. Atomic Energy Commission with Dr. Shields Warren. The award was for pioneering efforts in organizing and directing research on the hazards, biological effects and beneficial applications of ionizing radiation in biology and medicine.

Important progress on a number of important problems, of both fundamental and applied nature, are noted in this report. The work now in progress augers very well for the future. We express our appreciation to the Division of Biomedical and Environmental Research of the Atomic Energy Commission for support of this Laboratory.

LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY

University of California

Los Angeles

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ASSOCIATE DIRECTORS	Eric B. Edney, Ph.D. James F. Mead, Ph.D. George V. Taplin, M.D.
ASSISTANT DIRECTOR ADMINISTRATION	Clinton W. Longwill, M.A.

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DIVISION OF BIOCHEMISTRY

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Area of Interest

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Division Chief

Lipid Metabolism in Brain and Mem-
branes in Development and Aging

Dhopeshwarkar, Govind A., Ph.D.

Transport and Turnover of Lipids
in the Brain

Fulco, Armand J., Ph.D.

Control of Lipid Desaturation in
Bacilli

Howton, David R., Ph.D.

Radiation Chemistry of Lipids

Nevenzel, Judd C., Ph.D.

Lipid Composition, Function and
Metabolism in Marine Organisms

Wu, Guey-Shuang, Ph.D.

Radiation Chemistry of Lipids

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Leung, Sibyl

Quint, Joseph

Robinson, Stephen J.

SCIENTIFIC REPORTS

LIPID METABOLISM IN BRAIN AND MEMBRANES IN DEVELOPMENT AND AGING

The Alpha-Oxidation System of Brain Microsomes

James F. Mead and Roberta S. 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, O_2 , 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 ceribronic 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} 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, Fe^{+2} is oxidized to Fe^{+3} and the reaction ceases. It can be renewed by further ascorbate addition.

However, these experiments did not prove that the reaction studied in vitro is the same as that occurring in vivo and, indeed, the finding of Fe^{2+} and ascorbate as cofactors leads to the suspicion that a non-enzymatic reaction might be involved. In order to test this possibility, guinea pigs were raised on vitamin C-free supplemented diets. In 10 days, the deficient animals began to show definite signs of the disease. They were then killed and their brain microsomal preparations compared with those of the controls as sources of the alpha-oxidation enzymes. It was found that alpha-oxidation was about 80% reduced in the deficient brain but normal in the supplemented brain or in the preparation from the deficient brain plus 10^{-5} M ascorbic acid. Moreover, it was found that the site of the disability was not in the microsomal fraction itself but in the 10^5 xg supernatant, which contains the ascorbic acid. Thus it appears

that ascorbic acid is the true cofactor for alpha-oxidation both in vitro and in vivo. Present experiments are designed to elucidate its function and the mechanism of action of the enzyme system.

Interim Report

The Effect of Fatty Acid Structure on Membrane Properties

James F. Mead and Minerva G. Elepaño

It has been known for a long time that essential fatty acid deficiency is a disease related to membrane structure and transport, but the means to study the phenomenon in a simple system have not been available.

In this laboratory, a preparation of liver cells from normal rat liver has been found to exhibit some symptoms of EFA deficiency when deprived of EFA in the growth medium. Thus, growth is considerably slowed and the cells assume a different form, which can be reversed when linoleic acid (18:2) is added to the medium. Analysis of the cell membrane lipids indicates that all the so-called ω -6 series of polyunsaturated fatty acids (derived from 18:2) are low. They can be quickly restored if 18:2 is added to the medium. In an attempt to ascertain the sites of action of these polyunsaturated acids, control cells (no F.A. in the medium) and cells supplemented with 18:2 were maintained at various temperatures below 37°. The supplemented cells continued to divide even at 25° and some remained viable at 10°, whereas the control cells changed shape (rounded up), floated and ceased dividing even at 29°. Thus, one site of action of the ω -6 acids may be at the plasma membrane and efforts are in progress to determine its nature.

Interim Report

TRANSPORT AND TURNOVER OF LIPIDS IN THE BRAIN

Studies on the Blood-Brain Barrier

G. A. Dhopeswarkar, Carole Subramanian and James F. Mead

It has been shown, in a series of experiments using radioactively labeled fatty acids, that for fatty acids, there seems to be no blood-brain barrier in the original sense of the term. Thus, fatty acids injected intracarotidly, are taken up and incorporated into the brain lipids unaltered in less than 15 secs.

In a continuation of this study, an attempt was made to ascertain first, the form in which fatty acids are transferred to the brain and second, the reasons for the generally low extent of uptake from the diet in the adult. In several different experiments, the following information was obtained:

1) When the fatty acid was administered by intracarotid injection and the rat killed within 15 secs. the label was almost entirely in the form of FFA and the uptake by brain considerably exceeded that of liver.

2) With an intravenous injection, the label appeared largely in triglyceride and phospholipid and the uptake by liver far exceeded that by brain.

3) If the intravenous injection was used with an animal with the liver functionally removed, the label remained in FFA and the brain activity was far higher than that of the liver.

4) When phosphatidyl choline, labeled in the acyl side chains was injected, almost no activity appeared in the brain, but high activity was in the liver phospholipids.

The conclusions reached from these results are:

1) That the liver (and other organs) compete with the brain for circulating fatty acid.

2) That the liver (etc.) usually retains most of the fatty acid and converts it to triglyceride and phospholipid which are released into the blood.

3) That the brain can take up fatty acids very readily but cannot easily take up triglycerides or phospholipids and thus cannot utilize the products formed by the liver.

4) That the liver and other organs can store lipids, whereas the brain has no storage mechanism and can retain only those fatty acids for which there is a "vacant site" in a membrane phospholipid (e.g. a lysophospholipid). Otherwise, the fatty acid is released, possibly via cerebrospinal fluid.

Interim Report

CONTROL OF LIPID DESATURATION IN BACILLI

Biocynthesis of Unsaturated Fatty Acids by Bacilli

A. J. Fulco and Joseph Quint

In the past year, we have investigated the factors that determine substrate specificity for desaturation in Bacilli. An experimental procedure has been devised based on the simultaneous determination, in vivo, of temperature-dependent desaturase half-life and desaturation rate using various ^{14}C -labeled substrates in the presence of chloramphenicol. This technique allows one to determine whether observed substrate specificities arise from one desaturase acting differentially on several substrates or whether 2 or more desaturases are involved, each with its own narrow specificities. Using such a procedure, the in vivo substrate specificities and desaturase half-lives for a number of Bacilli desaturating systems have been determined. Desaturases of the Δ^5 -type have been found which show decreasing specificity in the order $\text{C}_{16} > \text{C}_{17} > \text{C}_{18}$ (i.e. most B. megaterium strains). The Δ^5 -desaturase of B. licheniformis, however, shows a specificity that is exactly the opposite - that is $\text{C}_{18} > \text{C}_{17} > \text{C}_{16}$. The Δ^5 -desaturase of B. pumilis shows little differential specificity towards these substrates. None of these systems acts on chain lengths of C_{14} or lower and in every case, only one Δ^5 -desaturase was present which acted on all 3 substrates. In B. licheniformis, Δ^{10} -desaturation is also observed with the specificity being opposite (i.e. $\text{C}_{16} > \text{C}_{17} > \text{C}_{18}$) to Δ^5 -desaturation in the same organism. Our most recent evidence strongly suggests that, in B. licheniformis, Δ^{10} - and Δ^5 -desaturation is carried out by the same enzyme and that specificity is changed by temperature of incubation.

Interim Report

LIPID COMPOSITION, FUNCTION AND METABOLISM IN MARINE ORGANISMS

The Lipids of Edible Marine Crustaceans

Nirmala K. Menon and J. C. Nevenzel

The muscle tissue of the large crustaceans (prawns, shrimps, lobsters and crabs) are widely eaten by gourmets and peasants alike. This is lean meat, containing 2 - 5% total fat (dry wt basis), of which only 6% is wax esters and triglycerides while 20-35% is sterols and 35 - 58% is polar material, including phospholipids and glycolipids. Lipid energy reserves are apparently not stored in the muscle of these animals, and the bulk of the fat probably represents membrane lipids.

Our original interest was in the sterols, specifically in detecting desmosterol in crustaceans generally. The rationale behind this interest is that, like all other members of the phylum Arthropoda, the crustaceans are thought to be unable to synthesize cholesterol de novo from acetate, yet they contain normal amounts of this sterol, and presumably it is an essential constituent of crustacean membrane lipids. Current knowledge suggests, however, that a major source of cholesterol in crustaceans is from the modification of dietary phytosterols via desmosterol to cholesterol. The amount of desmosterol present in crustacean tissues, then, is more likely to reflect the dietary intake of phytosterols than to be characteristic of the species. By gas chromatography we have tentatively identified desmosterol in penaeid prawns (local supermarket shrimp) and in the blue crab, Callinectes sapidus, constituting about 1% of the total free sterols in each animal; cholesterol was 98+% in all samples.

The polar lipids of these crustaceans differed from those of marine fishes in containing little or no sphingomyelin and by the presence of major amounts of glycolipids. Preliminary values for some components are given in Table I; those for the major lipid classes were determined

TABLE I. Lipid Classes in Marine Crustaceans (Wt % of total lipid)

Constituent	Penaeid prawn	<u>Callinectes sapidus</u>
Wax esters	1.9	
Sterol esters	0.3	
Triglycerides	4.4	
Sterols	35.9	20 (min.)
"Phospholipids"*	50.3	34.9
Phosphatidyl ethanolamine	4.6	5.0
Phosphatidyl choline	10.2	1.3
Phosphatidyl serine)	7.5	4.3
+)		
Phosphatidyl inositol)	3	2.6

* Total fraction includes free fatty acids

gravimetrically after separation by column chromatography using a particular silicic acid from which free fatty acids are eluted only with methanol. The values for individual phospholipid classes are from quantitative thin-layer chromatography (TLC), and that for glycolipid by gravimetric determination after elution with acetone from a second silicic acid column. The free fatty acid and the phospholipid fractions whose fatty acid compositions are given in Table II were separated on a DEAE-cellulose column,

TABLE II. Fatty Acid Compositions of Lipid Fractions (Wt. %)

Animal	Penaeid prawn						Crab
Lipid type	WE	TG	PC	PE	PS	PI	Glycolipid
Homolog							
14:0	tr	7.3	2.6	3.0	0.6	1.1	2.4
16:0	7.0	26.9	4.6	20.3	11.0	15.2	11.4
16:1	2.4	13.8	2.7	12.9	3.3	6.9	5.0
16:2	-	2.7	0.2	10.9	-	-	1.1
18:0	1.1	6.0	10.6	9.7	38.2	29.2	10.1
18:1	78.0	22.0	17.2	12.2	9.9	20.0	20.7
20:1	1.3	5.2	1.9	0.6	4.2	1.6	0.7
20:4	-	0.5	6.0	3.1	4.4	-	10.6
20:5	-	0.8	13.2	11.0	5.1	-	19.4
22:6	-	-	4.6	2.6	4.7	-	12.5
Total: :0	8.1	44.4	30.5	36.3	55.0	50.6	24.4
:1	90.8	47.0	25.5	25.7	21.2	34.2	28.3
:2+	1.0	7.5	43.3	38.0	21.4	6.9	47.3

WE = wax esters; TG = triglycerides; PC = phosphatidyl choline; PE = phosphatidyl ethanolamine; PS = phosphatidyl serine; PI = phosphatidyl inositol; :0 = saturated homologs; :1 = monenes; and :2 = polyunsaturated homologs.

thus resolving PS and PI completely; in both the prawn and the crab these two phospholipids were present in about equal amounts.

We believe the glycolipid present is most likely a monoglycosyl ceramide from its characteristics in TLC, a positive reaction with anthrone (on the TLC plate), and the presence of fatty acids. As yet we have not isolated or identified the expected sphingosine base or the sugar moiety.

Interim Report

RADIATION CHEMISTRY OF LIPIDS

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

David R. Howton and Guey-Shuang Wu

Since carboxylic acids are, in condensed states (liquid or solid), essentially completely "dimerized" via double hydrogen-bonding of their carboxy groups, it seemed to us quite probable that exposure of such substances to ionizing radiation might result in "cross-decarboxylation" by way of a mechanism (details of which have been outlined by us in J. Am. Chem. Soc. 89, 516 [1967]) having the interesting feature that ionization of one molecule would result in decarboxylation of the other to which the first is hydrogen-bonded. It seemed possible, too, to test this hypothesis by determination of the ratio of hydrocarbons (penta- and heptadecane) produced by irradiation of equimolar mixtures of crystalline palmitic and stearic acids which were, on the one hand, self-, and on the other, cross-paired with respect to their H-bonded "dimeric" states. We were therefore gratified to find that Co^{60} γ -irradiation (1 Mrad) of a sample of the self-paired substances (prepared by thorough stirring together of equimolar amounts of the two finely-divided crystalline acids) gave a $\text{C}_{15}/\text{C}_{17}$ hydrocarbon ratio of 0.955, while identical treatment of a sample of the cross-paired palmitic-stearic acid "compound" (prepared by slow cooling of a molten mixture: see Degerman and von Sydow, Acta Chem. Scand. 12, 1176 [1958]) yielded a 1.036 ratio of hydrocarbons.

It appeared to be desirable, however, to investigate possible effects of one troublesome (and unavoidable) feature of these experiments, namely, that while the cross-paired material is a solidified melt (and thus essentially voidless), the other (self-paired), consisting of closely packed crystals, is only about half as dense - i.e., contains about 45% void space. To provide some assessment of whether this difference in condition might have appreciably influenced the outcome, samples of palmitic and of stearic acids were irradiated (dose: 1 Mrad) individually both as firmly settled small crystals (sample about half void) and as solidified melts (voidless); the following (G) yields of hydrocarbons (i.e., decarboxylation products) were obtained:

<u>acid</u>	<u>with void</u>	<u>voidless</u>
palmitic	4.212	4.431
stearic	3.379	3.552

These data are of interest from two standpoints: first, they reveal that the presence of voids results in an apparent reduction (by about 5%) in hydrocarbon yield; and second, they permit calculation of the $\text{C}_{15}/\text{C}_{17}$ hydrocarbon ratio expected from an equimolar mixture of the two acids and show that this ratio ($G_p Z_p / G_s Z_s = 1.122$, the Z's representing the number of electrons per molecule of each acid) is considerably greater than that observed (0.955) on irradiation of the voidy mixture.

These observations seem most easily and acceptably understandable in terms of the possibility that diffusion of hydrocarbons out of the fatty acid crystals (and loss, when the sealed ampules, under pressure of

accumulated fixed gases - hydrogen, carbon dioxide, etc., are opened) may have significantly effected past results and their interpretation. Since diffusion out of the finely-divided solids would be more rapid and extensive (than out of solidified melts) in a given period of time, the lower yields obtained from material in this condition is explicable. Moreover, since pentadecane would diffuse out of crystals containing it more rapidly and extensively than heptadecane, the discrepancy between the observed and calculated C_{15}/C_{17} ratios is understandable.

Most seriously, of course: the fact that irradiation of the voidless cross-paired palmitic-stearic-acid "compounds" gives a higher C_{15}/C_{17} ratio than the voidy self-paired mixture would appear to be explainable in other terms and is thus of uncertain significance with respect to the involvement of the cross-decarboxylation reaction mechanism, proof of the existence of which awaits further study.

Interim Report

DIVISION OF DEVELOPMENTAL BIOLOGY

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Herschman, Harvey R., Ph. D.

Area of Interest

Development of Heart and
Control of Myosin Synthesis

Developmental Radiobiology

Mammalian Cell Biology

Macromolecular Specificity
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Stern, Andrew

SCIENTIFIC REPORTS

ENZYME INDUCTION AND CONTROL BY HORMONAL AND SERUM FACTOR

Hormonal Induction of Enzymes in Cultured Glial Cells: Differential Inhibition by β -Amanitin, Cordycepin, and Actinomycin D
J. de Vellis and D. English

The rat brain astrocytoma cell line, RGC₆ is characterized by several specialized functions of glial cells, including the cortisol induction of glycerol phosphate dehydrogenase (EC 1.1.1.8) (GDPH) and the induction of lactate dehydrogenase (LDH) by norepinephrine (NE). Cyclic AMP appears to be the mediator for the action of NE but not for cortisol (J. Neurochem 15:1061, 1968; Cellular Aspects of Neural Growth and Differentiation, D. Pease, Ed., pp 23-32 Univ. Calif. Press 1971; de Vellis and Brooker, Fed. Proc., in press). In the present study, the effect of inhibitors of RNA synthesis on GDPH and LDH inductions was investigated. Cells were grown for 9 days to a density-inhibited confluent monolayer, using Ham's F10 medium supplemented with 10% fetal calf serum. Alpha-Amanitin (10^{-5} M), a specific inhibitor of RNA polymerase (RP'ase) II (Science, 170:447, 1970), blocked the induction of GDPH but not LDH. Actinomycin D (AMD) which at 0.05 μ g per ml inhibits only rRNA synthesis, blocked the induction of GDPH but not LDH. AMD which at 1 μ g per ml. inhibits all RNA synthesis, and cordycepin, an inhibitor of mRNA (PNAS 67:1878, 1970), blocked GDPH and LDH inductions. The data suggest that both enzyme inductions require de novo synthesis of mRNA but that their mRNAs are transcribed by different nucleoplasmic RP'ases. The GDPH mRNA is synthesized by RPase II and therefore the LDH mRNA is probably produced by RPase III. The effect of low level of AMD suggests that the synthesis of rRNA is also required for GDPH induction unless the synthesis of GDPH mRNA is as sensitive as rRNA to AMD.

Interim Report

Effects of Cortisol (C) and Norepinephrine (NE) on the Uptake of Analogs of Amino Acids and Glucose into Cultured Glial Cells.
M. A. Shinwari and J. de Vellis

The rat brain astrocytoma cell line, RGC₆, is characterized by several specialized functions of glial cells including the cortisol induction of glycerolphosphate dehydrogenase (EC 1.1.1.8) (GPDH) and the induction of lactate dehydrogenase (LDH) by catecholamines. Cyclic AMP is the intracellular mediator for the action of NE but not for C (J. Neurochem. 15 15:1061, 1968; Cellular Aspects of Neural Growth and Differentiation, D. Pease Ed., pp 23-32, Univ. Calif. Press, 1971; de Vellis and Brooker, Fed. Proc., in press).

The incorporation of appropriate labelled precursors into lipids, proteins, RNA and DNA was altered by treatment with C or NE. In order to assess the metabolic significance of these changes it was deemed necessary to measure the effects of C and NE on membrane permeability. C₆ cells were grown for 8 days to a density-inhibited confluent monolayer, using Ham's F₁₀ medium supplemented with 10% fetal calf serum. Time course of uptake and concentration curves were carried out for each compound tested. Incubation with radioisotopes lasted for 20 min. in Krebs Ringer Phosphate buffer or culture medium. Treatment with C for 5 days progressively decreased the uptake of ¹⁴C-aminoisobutyric acid (AIB) and ¹⁴C-2-deoxyglucose (dG) to a final level of 60% of control. The time course was a mirror image of GPDH induction which after an 8-fold rise reaches a new steady state in 5 days. One hour following the addition of NE to cell cultures the uptake of AIB and cycloleucine was increased to 130% of control level whereas dG was decreased to 65% of control. Dibutyryl cyclic AMP had similar effects. At 24 h the stimulatory effect of NE on AIB and cycloleucine uptake had disappeared. These effects of NE correlated well with the rise and fall in the intracellular level of cyclic AMP. The data suggest that cyclic AMP is the mediator of the NE effect on membrane permeability. The data also show that the effect of C and NE on membrane permeability of glial cells is differential and time-dependent.

Interim Report

Effect of Catecholamines on Cultured Glial Cells: Correlation Between Cyclic AMP Levels and Lactic Dehydrogenase Induction J. de Vellis and Gary Brooker

A rat astrocytoma cell line, RGC₆ has several specialized functions of glial cells, including the cortisol induction of glycerol phosphate dehydrogenase (GPDH) and the norepinephrine (NE) induction of lactic dehydrogenase (LDH). Dibutyryl cyclic AMP (0.5 mM) induced LDH but not GPDH. Cortisol did not increase cyclic AMP or LDH. Following the addition of catecholamines (10⁻⁹ to 10⁻⁵) there was a rapid rise in cyclic AMP (up to 30 fold) which preceded LDH induction. However, only a 6 fold increase in cyclic AMP was necessary for maximal LDH induction. Submaximal concentrations of catecholamines had a correlative effect on cyclic AMP and LDH levels. Isoproterenol, NE, epinephrine, epinine, octopamine and phenylephrine were active and blocked by propranolol. Tyramine, histamine and dopamine were inactive. The first 3 hours after NE represented a cyclic AMP dependent RNA phase, whereas the subsequent LDH synthesis (after 6 hours) did not require elevated cyclic AMP levels. The effect of catecholamines on glial

cells appears mediated by a beta receptor and the rise in LDH is initially triggered by a rise in intracellular cyclic AMP.

Interim Report

Regulation of the Tyrosine α -Ketoglutarate Transaminase of RLC cells by Dexamethasone and Insulin

L. E. Gerschenson and M. Allen

The enzyme tyrosine α -ketoglutarate transaminase of RLC cells, cultured in chemically defined medium was found to be regulated by the synthetic corticoid dexamethasone and insulin. On the basis of induction kinetics and the use of RNA and/or protein synthesis inhibitors it was concluded that dexamethasone elicits its effect(s) at the transcriptional level while insulin does it at the post-transcriptional level. Insulin only affects the transaminase activity in cells pretreated with dexamethasone. Concentration and time course studies suggests as an explanation for this phenomenon the possibility that dexamethasone might induce the synthesis of a different substance (no tyrosine transaminase mRNA) necessary for the insulin effect with a different half life than the transaminase or its messenger.

Insulin was found to increase the rate of protein synthesis (^{14}C -leucine incorporation) in cells not pretreated with dexamethasone which suggest something peculiar about the transaminase regulation, probably at the translational level.

Dexamethasone was found to induce an increase in the rate of RNA synthesis (^3H -Uridine incorporation), and an inhibition in the rate of protein synthesis.

Interim Report

Regulation of the Tyrosine α -Ketoglutarate Transaminase of RLC cells by Cyclic AMP

L. E. Gerschenson and M. Allen

Cyclic AMP has been described to be an inducer of tyrosine α -ketoglutarate transaminase in the rat liver of the whole animal and insulin has been described to elicit its effects in liver by lowering the level of the cyclic nucleotides. Since insulin was found to be an inducer of the transaminase on cells pretreated with dexamethasone, attempts were made to study the effect of the addition of Dibutyryl Cyclic AMP (DBCAMP) on the levels of the transaminase.

10^{-3}M DBCAMP was found to induce an increase on the transaminase

activity only in cells pretreated with dexamethasone. This effect is additive with insulin and partially inhibited by actinomycin D.

From these results it was concluded that CAMP appears not to be involved in the mechanism of action of insulin and that CAMP appears to regulate the transaminase at the transcriptional level.

Interim Report

Inducible Chemical Porphyrin in RLC Cells

M. Kussell and L. E. Gerschenson

The Chemical allilisopropylacetamide and ethiocolanolone have been found to induce fluorescence in RLC cells. These chemicals are known to induce experimental porphyria in animals and liver-organ cultures. This condition is due to an increased synthesis of the enzyme δ -Aminolevulinate synthetase and the chemicals act by inducing such enzyme. We are studying the effect of the above mentioned chemicals in the regulation of that enzyme.

Interim Report

The Mechanism of the Action of the Heat Stable Serum Factor

I. Harary and Delia Casanella

We are now investigating the mechanism of the effect of the heat stable factor by examining its affect on the turnover of myosin. 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.

The effect of the heat stable factor is inhibited immediately by cycloheximide, a protein synthesis inhibitor. It is inhibited by actinomycin D but only after 8 hours of incubation. It appears that the messenger for the synthesis of myosin is longer lived than the average heart cell protein and that the heat stable factor main effect is on the synthesis of messenger RNA.

Interim Report

Separation of a Heat Stable Fraction for Myosin Synthesis

I. Harary, F. Hoover, Barbara Farley, E. Sato and Kasturi Datta

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 F₁₀ and serum [complete growth medium (CGM)] there occurs growth, and a five-fold increase in total myosin. 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.

Attempts to fractionate the serum factor has led to the preparation of a heat stable fraction from the serum which supports myosin in the heart cells without cell division or growth. The myosin serum factor is capable of withstanding boiling but not ashing. It is dialyzable and appears not to be protein in nature since it can withstand papain treatment. Preliminary attempts to assess its specificity indicate that its action is mainly on heart cell myosin. It has no effect on lactic dehydrogenase or creatine phosphokinase nor do skeletal muscle cells require serum for maintaining their myosin level.

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 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.

However, since its size and specificity pointed to its hormonal nature we cut across our search for the actual hormone by testing cyclic AMP, the secondary agent common to a group of hormones and tissues. Butyryl cyclic AMP penetrates cells and was as active in supporting the myosin level as the heat stable factor. Consequently this narrowed the search to those hormones which act through cyclic AMP. We have subsequently discovered that epinephrine is as active as cyclic AMP and the heat stable serum factor. We are now in the process of determining whether the heat stable factor is epinephrine.

Interim Report

ORGAN SPECIFIC PROTEIN SYNTHESIS

S-100 Protein in Clonal Cell Cultures

H. R. Herschman and B. Grauling

We have previously isolated two clonal cell lines which synthesize this protein [Lightbody, Pfeiffer, Kornblith, and Herschman, J. Neurobiol. 1, 411 (1970); Pfeiffer, Herschman, Lightbody, and Sato, J. Cell Physiol. 75, 329 (1970)]. The former, accumulates the protein during all phases of growth. The latter cell line, derived from a chemically-induced rat astrocytoma, 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 radio-labelled precursors specifically into the S-100 protein.

There are contradictory reports in the literature concerning both (a) the types of neural cells which contain this protein, and (b) its synthesis and degradation in brain. One of our objectives in isolating cell lines that continue to synthesize this protein was to establish an experimental system in which these questions could be examined without the presence of complicating issues, including heterogeneous cell populations and the existence of a blood-brain barrier. In order to devise a specific and quantitative assay for incorporation of radio-active amino acids into S100, I chose to work with the CHB cell line, since it accumulates this protein at all times. A technique for specifically and quantitatively isolating radioactively labeled S100, a brain-specific protein, from cultured human glial cells (CHB) has been developed. The necessary specificity is achieved by precipitation labeled S100 in antibody excess with carrier S100 and anti-S100 antibody, followed by acrylamide gel electrophoresis of the solubilized precipitate in the presence of sodium dodecyl sulfate. The specificity of the method is shown by applying the technique to cell lines which do not synthesize S100. Quantitative recovery is demonstrated by a second precipitation from a labeled glial cell extract. The differential rate of S100 protein synthesis in confluent CHB₄ cell is 0.06%. The S100 protein in confluent cultures of CHB₄ appears to be quite stable when compared to total protein, and is not rapidly degraded.

Recent experiments with the rat astrocytoma cell cultures suggest that the S100 protein is not synthesized while these cells are in exponential phase. These results suggest the exciting possibility that the initiation of S100 protein synthesis in this cell line is a density-dependent contact mediated induction of the synthesis of an organ-specific protein.

Interim Report

Synthesis of 14-3-2 in Cultured Neuroblastoma Cells

H. R. Herschman

Moore and his colleagues have isolated a protein, termed 14-3-2, which has been shown by serological techniques to be specific for neural tissues, and to be present primarily in neurons. We have, during the past year, made antisera to this protein, and confirmed its neural specificity. We have analyzed the major clonal isolates of the C1300 mouse neuroblastoma (Clone NB41 of Sato, Clone C1a of Schubert, Clones N18 and N4 of Nirenberg) and found them all to be negative. Recently, however, we have received a human neuroblastoma (IMR32) which appears to accumulate this antigen in culture. This protein is made constitutively by IMR cells; i.e., the relative amount of 14-3-2 (μg 14-3-2/mg protein) is constant throughout both exponential and stationary phase. We are currently beginning the analysis of synthesis and degradation of 14-3-2 in these cells, in analogy to our work on S100 in glial cultures.

Interim Report

Biochemical Basis for Genetically Determined Brain Weight Phenotypes

A. Dravid, R. Weimer, H. R. Herschman

Recent studies have demonstrated a striking amount of variability among genetically distinct stocks of house mice in both absolute and relative amounts of brain tissue and in several aspects of brain chemistry. At present, brains of mice of different stocks (inbred strains or selected lines) are known to vary in their size and weight. It is not known whether these genetically determined variations in brain-weight (and size) originate in variations in cell size or cell number, nor is it known whether neurones and glial cells are equally involved. To ascertain the involvement of neurones and glial cells, we have measured two nervous system specific proteins 14-3-2 and S-100 as potential markers for neurones and glial cells. Studies on DNA measurements on these brains indicate that variation in brain weight originate primarily in cell number. Existence of a secondary variation is indicated by the finding that DNA per unit weight in high brain-weight mice was significantly lower as compared with controls. No significant difference was observed in DNA per unit weight between controls and low brain-weight mice although the values were somewhat higher in the low brain-weight mice. Studies on the cell specific proteins 14-3-2 and S-100 indicate that the neuronal protein, 14-3-2 remains constant per unit weight in all three groups of mice whereas the glial cell (astrocytes) specific protein, S-100 is significantly decreased as compared with the controls. The decrease was much more pronounced in the low brain weight mice. These studies suggest that genetically determined variation in brain weight is a consequence of variation in cell number and the cells involved in the variation in number may be largely glial cells.

Interim Report

Distribution of Polysomes in Mouse Brain Tissue

M. P. Lerner, F.O. Wettstein, H.R. Herschman J.G. Stevens and
B. R. Fridlender

Polysomes were isolated from several different fractions of mouse brain tissue. After homogenization, the extract was centrifuged to yield a post-mitochondrial supernatant fraction and a pellet fraction. Sucrose gradient analysis of the material in the post-mitochondrial supernatant fraction indicated that 80 per cent of the ribosomes were present in polysomes and that little, if any, of the pellet fractions was present. Sucrose gradient analysis of the solution obtained after washing the pellet showed that very little polysomal material was present. The remaining pellet fraction was re-suspended in a detergent mixture of deoxycholate-Tween 40. Sucrose gradient analysis of the resulting detergent-soluble indicated that large amount of ribosomal material, in which 60-70 per cent of the ribosomes were associated in polysomes, were present.

In brain tissue from young animals, 20 per cent of the polysomes were found in the post-mitochondrial supernatant fraction whereas 80 per cent of the polysomes were released from the pellet fraction by detergent treatment. In contrast, in brain tissue from adult animals, 40 per cent of the polysomes were found in the post-mitochondrial supernatant fraction, whereas 60 per cent of the polysomes were released from the pellet fraction by detergent treatment.

Final Report

MEMBRANE FUNCTION AND SPECIFIC RECEPTORS

Estradiol and Progesterone Receptors and Carcinogenesis in Cultured Rabbit Endometrial Cells

L. E. Gerschenson, M. Allen and J. J. Yang

A method to culture endometrial cells from rabbit uterus have been developed.

A microtechnique to quantitative estradiol and progesterone receptors is being developed. These receptors are going to be used as specific markers for the cells. Preliminary work with chemical carcinogens, showed that the cells are easily transformed "in vitro" by them. The effect of the hormones on the rate of transformation and the effect of transformation on the hormonal dependency of the cells are under study.

Interim Report

Sialic Acid Masked Membrane Antigens of Clonal Functional Glial Cells

H. R. Herschman, J. Breeding and J. Nedrud

Antisera have been prepared to clonal cultured rat astrocytic cells (C₆ cells, CHB₄ cells). When cultured glial cells are grown in the presence of neuraminidase the serological activity of the cell membrane with homologous antiserum is increased extensively. A similar activation of serological activity can be effected by incubating washed harvested cells with this enzyme. Membrane bound N-acetylneuraminic acid is extensively reduced by either incubation. The activity responsible for promoting the enhanced serological activity co-chromatographs and co-electrophoreses with neuraminidase. Proteolytic digestion, unlike neuraminidase treatment, was unable to enhance the serological activity of cultured glial cells. This result suggests that growth in the presence of neuraminidase can be used to investigate the relationship of cell surface sialic acid to contact inhibition, hormonal induction, serum growth factors and a variety of other cellular phenomena.

Final Report

Antisera to Nerve Endings

H.R. Herschman and C. Cotman

In collaboration with Dr. Carl Cotman of U.C. Irvine, 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 organelles 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 preparation have been quantitated.

This antisera has been used in electrophysiological experiments designed to test its effect in EEG, flash evoked potential, and impedance (in collaboration with Drs. Anatole Costin and Dennis Havemann). These results while somewhat complicated and difficult to summarize suggest that antisera are able to promote specific physiological effects.

Recently we have isolated the 7S gamma globulin of this antisera and labelled a portion with I¹²⁵. Using a direct binding assay we have demonstrated the organ-specificity of this antiserum and determined that about 60% of the antibody activity is directed to the synaptic complex, or pre-post synaptic junction.

Interim Report

DIVISION OF ENVIRONMENTAL BIOLOGY

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Area of Interest

Animal Ecology

Spectroscopy

Nutrient and Radionuclide Cycling

Plant Ecology

Nutrient and Radionuclide Cycling

Nutrient and Radionuclide Cycling

Plant Ecology

Soil Factors

Nutrient and Radionuclide Cycling

Animal Ecology

Nutrient and Radionuclide Cycling

Chemical Problems

Visiting Scientist

Timothy Prout, Ph.D., Biology Department, University of California,
Riverside

SCIENTIFIC REPORTS

ANIMAL ECOLOGY

Responses of Desert Sand Cockroaches to Humidity and Temperature

E. B. Edney and R. A. Wood

The ability to respond appropriately to temperature and humidity is essential for any desert animal, but it has so far been difficult to study locomotory behavior of sub-soil animals without disturbing their environment. Arenivaga is a genus of cockroaches highly specialized for life below the surface of desert sand dunes, and we have begun a study of the movements of these insects in sand in response to gradients of temperature and humidity by tagging them with Technitium 99m (an isotope with a half life of about 6 hours). Tagged insects are allowed to move through sand in glass containers where temperature and humidity gradients may be established and monitored, and their position determined periodically by means of a scanning camera.

Interim Report

Rates of Water Turnover in Desert Insects

E. B. Edney and K. A. Nagy

The long term aim of this program is to identify the various avenues of water loss and gain, and to measure the rates at which these processes occur, in desert arthropods in near natural conditions. Techniques using tritiated water are at present being worked out, using the domestic cockroach, Periplaneta australiasiae. The experiments have not yet proceeded far enough to warrant any conclusions.

Reference: J. exper. Biol. 55: 253-272, 1971

Energy Flow in Southern Nevada Populations of Uta stansburiana

F. B. Turner, P. A. Medica, and B. W. Kowalewsky

Energy flow of Uta stansburiana in southern Nevada was estimated for a three-year period (1965-8) using a computer model calculating hourly changes in body temperatures of individuals and daily changes in the numbers and body weights of individuals making up the population. Annual energy flow ranged from 0.18 Kcal/m² in 1965-6 to 0.27 Kcal/m² in 1966-7. Annual production ranged from 0.033 Kcal/m² in 1965-6 to 0.056 Kcal/m² in 1967-8.

Ratios of production to energy flow varied from 18% (1965-6 and 1966-7) to 27% in 1967-8 (cf. 1-2% in birds and mammals), so Uta is

similar to ectothermic invertebrates in this respect. Uta annual energy flow (ca. 0.2 Kcal/m²) was only about 3.5% of Dipodomys merriami and 9% that of Lepus californicus in southern Arizona, but exceeded the energy flow of three of the eight mammals studied by the Chews. Annual production by Uta (ca. 0.046 Kcal/m²) was about the same as that reported for Dipodomys merriami in Arizona (0.053 Kcal/m²) and exceeded that of the other southern Arizona mammals.

The energy flow analysis was predicated on the lowest existing measurements of Uta respiratory metabolism (Roberts), and estimates of total metabolism using measurements by Dawson and Bartholomew were 3-4 times higher. The use of these latter respiratory rates would increase energy flow estimates roughly 3-fold and lower the production/energy flow ratios to around 6.5 - 8%. There is no clear-cut rationale for choosing between the two physiological models.

Reference: Program, 14th Annual Meeting, Society for the Study of Amphibians and Reptiles, Albuquerque, New Mexico. August 1971

Radiation-induced Sterility in Natural Populations of Lizards
F. B. Turner, P. A. Medica, and D. Smith

Between 1964 and 1966 minimum spring densities of horned lizards ranged from 1.0-2.5/ha in three 8-ha enclosures in Rock Valley, Nevada. One of these plots was subjected to continuous gamma irradiation from a centrally located source of ¹³⁷Cs. During this time numbers of horned lizards declined in all three plots. Between 1967 and 1970 numbers of horned lizards increased in the two control areas, but continued to decline in the irradiated plot. Female sterility owing to regression of ovaries is judged to be the cause of the population decline. Similar radiation effects have previously been observed among leopard lizards (Crotaphytus wislizenii), whiptail lizards (Cnemidophorus tigris), and side-blotched lizards (Uta stansburiana). Minimal survival of hatchling horned lizards to the age of 8 months was about 26-38%. Annual minimal survival of older lizards was 50-60%. Female horned lizards usually laid one clutch of eggs per year, but multiple clutches were observed in 1969. Conversely, no reproduction was observed in 1970. The maximum life span of horned lizards is at least 94 months.

Interim Report

Analysis and Modeling of a Desert Ecosystem

F. B. Turner, S. A. Bamberg, E. M. Romney, A. Wallace, T. Ackerman, J. Childress, H. O. Hill, H. W. Kaaz, B. G. Maza, P. A. Medica, and D. Smith

Ecosystems studies in the desert biome are organized into two areas:

i) analysis of specific ecological processes and the development of computable functions expressing rates of change of various compartments in terms of initial states and forcing functions, and the development of a mathematical model of a generalized desert ecosystem; and ii) periodic measurements of the states of various species populations and abiotic components of desert communities, so that the predictions of the model may be compared with changes observed in actual ecosystems. These 'validation' measurements are being made at four sites in western North America. Curlew Valley in northern Utah; near Tucson, Arizona; near Las Cruces, New Mexico; and in Rock Valley at the Nevada Test Site.

Abiotic and biological measurements were made on a 46.1 ha site in Rock Valley during 1971. Rainfall during the winter of 1970-1 was below normal so soil moistures during the first months of 1971 were never above 8-9%. Soil moisture declined steadily through August, but following a late August rain increased to 4-8% in September. By early December soil moisture had fallen again to 2-3%; after December rains they increased to 8-10% by the end of the year. Air temperatures were unusually high during mid-January of 1971, but followed normal patterns for the rest of the year.

Soil profiles in the vicinity of shrubs (as opposed to bare areas) exhibit aeolian deposits as an A₁ horizon, which also contain higher levels of organic matter and nutrients. An important generalization is that local geographic differences in soils are less pronounced than differences between soils beneath shrub clumps and that of bare areas. Soils of the validation site are underlain by a massive and strongly cemented caliche layer (at depths of 30 to 70 cm).

The validation site was divided into six reasonably homogeneous zones of vegetation, ranging in extent from 1.41 to 21.21 ha. Estimates of density and biomass of plants were developed for each zone and the site-wide estimates were obtained by summing zone data. The standing stock of perennial plants on the validation site as of February 1 was estimated as 1049 kg/ha. This estimate was based on live stem tissue only. During the spring maximum production of winter annuals was estimated to range from 2.06 to 7.88 kg/ha, depending on the zone in question. The overall site mean was 4.2 kg/ha, 76% of which could be attributed to but seven species. Production by winter annuals was only about 2% of that by perennials.

Production of new leaves and new shoots by seven major perennial species in the six zones was estimated to range from 160 to 230 kg/ha, and the overall site mean was about 207 kg/ha. This new growth ranged from 17 to 22% of the estimated February standing stock, and about 90% of the new growth was composed of leaves. Flower and fruit production was erratic and sparse, constituting only 3-4% of the production of new leaves and shoots.

Arthropods were sampled regularly in pitfalls and by vacuuming

shrubs. Various spiders, solpugids, and tenebrionid beetles were commonly captured in pitfalls. The local distributions of some of the tenebrionids were highly correlated with soil texture. Over 800 individual shrubs were vacuumed during the course of the season. More than 260 different kinds of arthropods were taken, of which all but a few mites were determined to family. Groups which, at one time or another, exhibited estimated densities exceeding 1000/ha were: chrysomelids, melyrids, tenebrionids, anthomyids, bombyliids, cecidomyiids, muscids, mirid bugs, membracids, braconids, eulophids, formicids, pteromalids, torymids, trichogrammatids, coleophorids, geometrids, gracilariids, phalaenids, pterophorids, pyralids, acridids, tanaocerids, and tettigoniids. A few groups exhibited estimated densities exceeding 5000/ha (cicadellids, cosmopterygid moths, mealy bugs, phloeothripids) and 10,000/ha (sminthurid collembolans, yponomeutid moth larvae, and thrips).

The most abundant mammal on the validation site is Perognathus longimembris. Spring densities of this species in the two trapping grids were around 10/ha (ca. 70-80 g/ha). Towards the northern edge of the validation site the relative abundance of P. formosus increased strikingly, owing to the more sandy soil. Other rodents exhibited spring and summer densities of less than one per hectare and the estimated spring biomass of all rodents was almost certainly less than 250 g/ha. Summer trapping, following the reproductive season, indicated very poor recruitment and observed densities were invariably lower in August than in April and May.

The most abundant reptile is the lizard, Uta stansburiana, and in the spring of 1971 observed densities ranged from 35 to 45/ha, with biomass estimates of 133 to 171 g/ha. The estimated biomass of four other species of lizards ranged from around 30-100 g/ha, with a total for the four species of around 400 g/ha.

Two species of birds bred on the validation site in the spring of 1971: Amphispiza bilineata and Toxostoma lecontei. Estimated breeding densities were about 0.46 and 0.04 pairs/ha (12.4 and 4.8 g/ha), respectively. Fringillids were the dominant birds during the spring--probably making up from 60 to 90% of the avian biomass.

Reference: Annual Report to the National Science Foundation, April 1972

CHEMICAL PROBLEMS

Partition Behavior of Mn(III) from Aqueous Bromate Solutions of Perchloric, Formic, and Acetic Acids into Bis(2-ethyl hexyl) Hydrogen Phosphate (HDEHP)
R. A. Wood, S. T. Wakakuwa, A. Beck, and T. H. Rutherford

Tracer level Mn(VII) as $303d\ ^{54}\text{MnO}_4$ is reduced in acid media of HClO_4 , HCOOH and CH_3COOH to weak divalent complexes. Upon the addition of sodium bromate Mn(II) is oxidized to HDEHP extractable Mn(III) species. The distribution ratios varied from 300 in HClO_4 media to greater than 10^3 in HCOOH and CH_3COOH systems. Stoichiometric and spectrophotometric studies have shown the extracting Mn species to be Mn_2O_3 and the solvent species to be $\text{Mn}[\text{H}(\text{DEHP})]_3$.

Final Report

Reference: J. inorg. nucl. chem. 33: 4291, 1971

The Partition Behavior of Ru(VII) and Tc(VII) in Systems between Bis (2-ethyl hexyl) Hydrogen Phosphate (HDEHP) and Aqueous Sodium Hypochlorite Solutions
R. A. Wood

Ruthinium and technitium are oxidized in alkaline solutions of NaOCl to perruthenate $[\text{M}_1(\text{RuO}_4)]$ and pertechnetate $[\text{M}_1\text{TcO}_4]$. Upon contact with n-heptane solutions of HDEHP the Ru(VII) and Tc(VII) ions partition in the organic phase with distribution ratios greater than 100. The relative stabilities of Ru and Tc-DEHP complexes are such that Tc can be quantitatively stripped from the solvent phase with distilled water. Alcoholic solutions of 6MNaOH are required to strip the Ru from the solvent phase. It is interesting to note also that the Ru-DEHP complex has been isolated. Spectrophotometric studies of this complex in the U.V. region show an absorption maximum at 330 m μ suggesting the $\text{M}_1(\text{RuO}_4)$ species. After aging periods of 4 hours, the absorption maxima shifts to 475 m μ suggesting the M_2RuO_4 species. Stability constants are presently being determined for these complexes.

Interim Report

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

An analytical method was developed for the rapid and carrier-free separation of $^{28}\text{ld}\ ^{111}\text{In}$ produced by the reactions $^{109}\text{Ag}(\text{He}^4, 2n)^{111}\text{In}$, $^{111}\text{Cd}(\text{p}, n)^{111}\text{In}$ and $^{110}\text{Cd}(\text{d}, n)^{111}\text{In}$. The method is based upon the

fact that indium in the presence of macro-concentrations of Ag(1) and Cd(11) preferentially extracts into n-heptane solutions of bis(2-ethyl hexyl) hydrogen phosphate (HDEHP) from aqueous 0.5M HNO₃ media. The Ag(1) and Cd(11) remain quantitatively in the aqueous phase. The distribution ratios (K) vary from greater than 10³ for indium to less than 10⁻² for Ag(1) and Cd(11). The indium is quantitatively stripped from the solvent phase with HCl. The total separation time is less than 20 min. This method is presently in use by the biomedical cyclotron facility at the UCLA Medical Center.

Final Report

NUTRIENT AND RADIONUCLIDE CYCLING

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 ppm of dry weight for application rates of 0, 5, 20, and 50 ppm.

Interim Report

Revegetation Problems Following Nuclear Testing Activities at the Nevada Test Site

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

Wherever vegetation has been destroyed at the Nevada Test Site as the result of nuclear activities, 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.

Interim Report

Larrea Ecotone Lines in the Frenchman Flat Area of the Nevada Test Site V. Q. Hale, E. M. Romney, and A. Wallace

Studies have been made of two different Larrea tridentata ecotone lines. Soil characteristics have been determined on both sides of each demarcation line and include soluble cations and anions, exchangeable cations, soil organic matter, soil pH and CaCO_3 , specific conductance and mechanical analyses all by horizons. Transects 500 meters long were established across each site and changes in vegetation were noted for each 50-meter increment. Mineral composition of the plants was obtained. Most relevant of the data were high specific conductance values for C horizons, high concentrations of soluble nitrates in the C horizons, and a very high percentage of exchangeable K (80% or more) in the A horizons of soil where L. tridentata failed to grow.

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}Sr and ^{137}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}Sr accumulated primarily in bone tissue while ^{137}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.

Interim Report

Increased Uptake of ^{241}Am by Plants Caused by the Chelating Agent DTPA A. Wallace

The chelating agent, diethylenetriaminepentaacetic acid (DTPA) has the ability to increase uptake by plants of several metals and it is widely used as a practical means of correcting iron chlorosis in plants. DTPA has been shown to greatly increase the uptake from soils of ^{241}Am by plants. In contrast, the chelating agent ethylenediamine di o-hydroxyphenylacetic acid (EDDHA) increased the uptake of ^{241}Am only slightly. The effect of EDDHA did not persist in the soil as much as did that of DTPA. PI54619-5-1 soybeans which are susceptible to lime-induced chlorosis were grown in calcareous Hacienda loam soil with micronutrient and chelate additions. ^{241}Am which had been mixed with the soil was greatly increased in plants to which iron DTPA had been added to the soil. Application of high levels of zinc or manganese salts decreased ^{241}Am content of plants only slightly indicating little, if any, competing effect. Valencia orange scions were grafted to rough lemon or trifoliate orange rootstocks and grown for 119 days with and without chelating agents in calcareous soil with which ^{241}Am had been mixed. Analyses of ^{241}Am were made after 85 days in leaves and stems of suckers growing from the rootstocks and also in new and old leaves and stems of the scions after 119 days. DTPA increased the contents of ^{241}Am in the shoots of both rootstock-scion combinations. The rootstock which is very subject to micronutrient deficiencies accumulated about as much ^{241}Am as did the other rootstock. Most accumulated ^{241}Am was transported to leaves of all the species studied. During a subsequent growth period after applications of ^{241}Am to bush beans, some of the ^{241}Am was transported from the old leaves to the new leaves and to new roots. The chelating agent DTPA had no effect on the retranslocation, however. Extraction studies with soil indicated that DTPA, but not EDDHA, could quantitatively extract ^{241}Am from soil.

Interim Report

Ecological Aspects of Plutonium Dissemination in Terrestrial Environments
E. M. Romney and J. J. Davis

The potential benefits to be derived for mankind from continued development of peaceful applications for plutonium cannot be denied. The technology of plutonium production and processing is already established, but the realization of its peaceful applications depends largely upon the development of methods for preventing its distribution in the environment. Because of safeguards and effective control measures, no accidental plutonium contamination of the public domain has imposed serious risks to a population group. Trace amounts of plutonium from above-ground nuclear detonations are contained in worldwide fallout; however, the levels of plutonium found in foodstuffs and other components of the environment are insignificant compared to the amounts known to be hazardous. There has thus been very little interest in the study of ecological aspects of plutonium contamination. The result is a paucity of information on the behavior of plutonium in ecosystems and its radiological effects on natural fauna and flora. The Nevada Applied Ecology Group is embarked upon a program at the Nevada Test Site to investigate the long-range effects of plutonium disseminated into the desert ecosystem. Emphasis has been placed upon standardization of analytical methods, delineation of contaminated areas, problems of resuspension and redistribution, food chain transport, and ecological effects.

Interim Report

Root, Stem, and Leaf Relationships among Eight Species of Mojave Desert Plants

A. Wallace, J. W. Cha, S. Bamberg, and J. Kinnear

Root growth of eight perennial desert plants grown in the glasshouse was followed as plants increased in size. Increasing root weight was statistically compared with increasing weights of stems and leaves. Generally linear regression adequately expressed the relationship between root weight and stem weight. The mean difference between observed and root weights calculated from linear regression for all eight species was 21.8%. The data indicate that it is possibly feasible to use linear regression in the estimation of root weights from observed (stem or shoot) weights or to estimate the below-ground component of the primary production from estimates of that distributed above ground. Estimates from leaf weights would give data in most cases as good as from stems. The mean per cent of whole plant that was root for the eight species was 17.7%. The mean proportion of the increase in plant weights that went below ground for the eight species was 19.5%. Shoots of *Franseria dumosa* plants were exposed to $^{14}\text{CO}_2$ and the distribution of ^{14}C in roots, stems, and leaves was measured at 1 week, 2 months, and 5 months. Only about 15% of the photosynthate was stored in the root. Much of that stored in stems was available for new leaf growth. The major storage organs were first the leaves and then the stems. Root

and stem weights were obtained for some field samples for the various species. The ratios were more variable than for glasshouse plants, but it was concluded that root biomass can be estimated for a population of at least, for some species, within a possible error of $\pm 10\%$ to 20% . It was also concluded that for *F. dumosa* the component of annual productivity going below ground was somewhere between the 15% shown by ^{14}C and the 26% of increased growth below ground shown by the glasshouse studies when plants increased in size.

Interim Report

Regulation of Heavy Metal Uptake and Responses in Plants

A. Wallace, S. M. Sufi, and E. M. Romney

Bush beans were grown in nutrient solutions with varying levels of calcium and chelating agents. Increasing calcium decreased Cu^{64} and Zn^{65} uptake by bush bean plants and the effect seemed to be non-competitive. EDDHA levels equal to or greater than that of ZnSO_4 increased uptake of zinc by plants when the concentration of EDDHA was 10^{-4}M or less; at 10^{-3}M levels of EDDHA, zinc uptake was greatly depressed. Nickel, cobalt, and, to a lesser extent, chromium were toxic to bush bean plants when 10^{-4}M of each was added to nutrient solution; EDTA overcame toxicity of nickel and cobalt more effectively than did calcium. Calcium overcame chromium toxicity more effectively than did EDTA. Calcium at 10^{-2}M resulted in far less toxicity than when plants were grown with lower calcium levels. Calcium and EDTA decreased plant uptake of zinc, copper, manganese, iron and cobalt.

Results with desert plant species illustrate that pH and soil temperature are important regulators of heavy metal contents of plants. Phosphorus level also regulates heavy metal contents of plants. Phosphorus-induced zinc deficiency is well known but phosphorus deficiency can result in excess uptake of zinc and other heavy metals as shown in studies with bush beans and desert plants. Interactions of these four types of regulators on heavy metal contents of plants and of related growth responses will be discussed.

Reference: Proc., 6th Internat. Colloq. on Plant Analysis and Fertilizer Problems, Israel, pp. 547-558, 1971

Effects of TIBA (2,3,5-triiodobenzoic acid) on Seed Production and Vegetative Growth of Alfalfa (*Medicago sativa* L.)

V. Q. Hale

TIBA (2,3,5-triiodobenzoic acid) was applied at low rates to seed alfalfa (*Medicago sativa* L.) in the field. Rates of 70 and 140 g of TIBA/ha resulted in seed yield increases of 60% over controls. Glasshouse tests showed that TIBA stimulated an increase in the number of stems per plant, and the greater number of stems probably accounted for the

increased seed weight. TIBA retarded vegetative regrowth in glasshouse experiments.

Reference: Crop Sci. 11: 678-679, 1971

Vegetative Propagation of Woody Shrub Species from the Northern Mojave and Southern Great Basin Deserts

P. A. T. Wieland, E. F. Frolich, and A. Wallace

Stem cuttings of 14 woody desert perennial shrub species from the Nevada Test Site were successfully rooted. At least one of these, L. divaricata, is a species for which earlier attempts to root had been unsuccessful (Chase and Strain, 1966).

Conditions for successful rooting varied with species. L. divaricata, A. hymenelytra, A. confertifolia, and C. ramosissima rooted more readily in soil than in vermiculite. IBA was not needed for Atriplex species or K. americana, L. divaricata, L. andersonii, F. dumosa; A. tridentata, and G. spinosa required mist or responded satisfactorily to mist rooting while other species disintegrated rapidly under the same circumstances. Other species rooted equally or better under dry conditions or even in a lathhouse where temperatures are lower. These include G. spinosa, E. lanata, E. viridis, and T. montana; E. viridis responded to bottom heat.

The species studied for which stem cuttings have failed to root include Krameria parvifolia and Juniperus osteosperma.

Reference: Madrona 21: 149-152, 1971

Carboxydismutase and Phosphoenolpyruvate Carboxylase Activities from Leaves of Some Plant Species from the Northern Mojave and Southern Great Basin Deserts

A. Wallace, V. Q. Hale, G. E. Kleinkopf, and R. C. Huffaker

Of 14 xerophytic plant species studied, only two (members of the Chenopodiaceae) exhibited high phosphoenolpyruvate (PEP) carboxylase and low carboxydismutase activities. Three other members of the Chenopodiaceae exhibited carboxylase activities characteristic of both the C_4 -dicarboxylic acid pathway and the Calvin cycle in the same species. Activities range from high PEP carboxylase and moderately high carboxydismutase to, conversely, high carboxydismutase and moderately high PEP carboxylase activity, including moderately high levels of nearly equal activities of each enzyme. Hence, gradations between extremes of the two photosynthetic systems can occur in the same species. Members of three other families (Ephedraceae, Zygophyllaceae, and Liliaceae) had carboxylase activities, characteristic of both photosynthetic systems

in the same species. The enzymatic activity of plants will continue to grow during hot summers and was characteristic of either the C_4 -dicarboxylic acid pathway or of both photosynthetic pathways.

Reference: Ecology 52(6): 1093-1095, Autumn 1971

Effect of Calcium Levels on Redistribution of Sr^{85} in Bush Bean Plants
A. Wallace

When bush bean plants previously exposed to Sr^{85} were transferred to solutions containing different levels of calcium without further addition of Sr^{85} , the calcium had relatively little, but some, effect on the redistribution of the Sr^{85} in the plants. Calcium did tend to restrict movement of strontium. Sr^{85} tended to concentrate in stems or old leaves, usually more so than calcium. New leaves particularly had lower Sr/Ca ratios than other plant parts indicating some discrimination in favor of calcium.

Reference: Plant and Soil 35: 415-420, 1971

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 some shrubs to determine whether the shrubs could thus be protected against radiation damage. The gamma radiation doses for shrubs not covered were found to be 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 with doses at 25 cm in the open. This reduction was attributed to self-shielding. Beta doses to the shrubs were reduced still further, to 31% of the 25-cm beta dose in the open, by shielding the shrubs from direct fallout contamination. The estimated LD₅₀ 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.

Reference: Proceedings, Symposium on Survival of Food Crops and Livestock in the Event of Nuclear War, Brookhaven Natl. Lab., September 15-18, 1970

Field Studies of Diet-Milk Transfer Coefficients at St. George, Utah
H. A. Hawthorne, S. D. Zellmer, and T. L. Hartsock

Feeding trials were made at a commercial dairy that utilized crops grown on the farm from 30 acres of land irrigated with water from the Virgin River. Twenty-two different alfalfa crops, and three ensilage crops (maize-sorghum plantings), were fed to dairy cows between June 1962 and September 1967. In this period 88 different cows entered into the milking herd, 22 of which were present at the last feeding trial. After an extended interval of "flushing" with the new crop of alfalfa, or other change in the diet, all the feeds consumed by the milking herd, and the milk produced in 10 days, were weighed (drinking water specimens were analyzed and their contributions to intake were calculated).

The mean daily per cow statistics, based on 32 feeding trials, were: milk production of 13.8 Kg, feed consumption (oven-dried weight) of 15.8 Kg, and a milking herd size of 26.2 cows. Mineral and radionuclide intake are given in the following table with the percent transfers and transfer coefficients. Each numerical value in the table is the mean for 32 10-day feeding trials.

Element	Daily Intake (per cow)	Transfer Diet to Milk (maximum)	Milk Transfer Coefficient
		%	%/Kg
K	380 g	5.5	0.40
Na	32.9 g	22.1	1.6
Rb	0.32 g	6.5	0.47
Cs	0.0042 g	5.0	0.36
¹³⁷ Cs	9.44 nCi	6.3	0.45
⁹⁰ Sr	9.71 nCi	0.88	0.064
Sr	2.92 g	0.62	0.045
Mg	43.3 g	3.4	0.25
Ca	195 g	7.4	0.55
P	45.1	27.4	2.0

We found Observed Ratios that were in the range reported elsewhere: these were 0.12 for Sr⁹⁰ and 1.1 for Cs¹³⁷. The ratios of the specific activities (pCi/g) for milk/diet were 1.5 for strontium (Sr⁹⁰/Sr) and 1.4 for cesium (Cs¹³⁷/Cs). Both ratios of specific activities indicated an enrichment of the milk with radionuclide relative to the stable element in the diet. Our preliminary interpretation is that the radionuclides were drawn from "pools" different from the stable element "pools."

Final Report

Gaseous Exchange of Mojave Desert Shrubs

S. A. Bamberg, A. Wallace, G. E. Kleinkopf, and H. Mork

Rates of gaseous exchange of common Mojave Desert shrubs have been determined under natural and manipulated conditions. Photosynthesis and transpiration rates were determined at several times during the season using a compensating null-point chamber. Performance of the plants is tested under ambient field conditions followed by manipulated conditions in the chamber of temperature and humidity. The plants' reaction to stress conditions of high temperature, low humidity, and high soil moisture tensions is compared throughout the growing season. Using the rates of gaseous exchange as an indicator of plant performance under natural conditions, additional stress conditions such as low-level radiation can be assessed. This work is being expanded to correlate the rates of gaseous exchange under various conditions with enzyme systems responsible for these processes.

Reference: IBP Progress Reports, June 1972

Density and Biomass of Plants in the Rock Valley Site, Nevada Test Site

S. A. Bamberg, T. A. Ackerman, H. W. Kaaz, and E. M. Romney

A vegetation analysis on a plot of 46.1 hectares in Rock Valley was conducted in connection with the IBP Desert Biome study. Plant cover, density, frequency and biomass were determined for each species of perennial and annual plant. Biomass was determined by dimensional analysis and broken down into component plant parts by ratios determined from whole shrub and partial destructive samples. Yearly increment growth and the contribution of biomass to litter were also determined for major shrub species. Results are calculated in numbers or weight per hectare. Overall density of the plot was 8,900 shrubs per hectare with six species accounting for 95% of both density and biomass. Above ground biomass values were 2,100 kilograms per hectare with 700 kilograms being standing dead material and root biomass 40% of above-ground parts.

Reference: IBP Progress Reports, June 1972

PLANT ECOLOGY

Predictive Model for Mojave Desert Ecosystems

J. C. Beatley

Biological/environmental relationships, as understood from ten years of field observations and data collections on 68 study sites at the Nevada Test Site, are incorporated into a word model for Mojave Desert ecosystems. The model, which deals primarily with the predictive behavior of the plant components in relation to environmental variables, may be summarized as follows:

Within the solar season the timing and amount of individual rainfalls is overall the variable most critical to the operation of southern Nevada ecosystems from year to year. Growth phenomena of the primary producers (vascular plants) in the systems are synchronized and are the direct results of the addition of water to the soils at particular points in time. Differences in rainfall over the land surface may have large consequences to the subsequent local success of the biological season.

Whether seasonal success is to be regional or local, and the extent to which the producer-consumer relationships will proceed, is not predictable except as the season progresses and the pattern of expression of the controls unfolds during the fall and/or winter months. Prediction follows the course of alternatives, and the alternatives are sufficiently diverse, and in themselves predictable, that in the multiplicity of desert environments it is virtually certain there will be a degree of success of biological populations which will ensure survival of the plant and animal species. Before a system could become truly disordered or non-functional a new season, with its predictable patterns for success, appears to restore the interrelationships to the level necessary for the continuing stability of the system components and the system itself.

Working versions of the word model are available in Modelling Report Series, No. 18, US/IBP Desert Biome, Logan, Utah.

Interim Report

Effects of Rainfall and Temperature on the Behavior and Distribution of *Larrea tridentata* (Creosote-bush) in the Mojave Desert of Nevada

J. C. Beatley

The effects of rainfall and temperature on the distribution and certain behavioral characteristics of *Larrea tridentata* (Ses. & Moc. ex DC.) Cov. (Creosote-bush) at and near its northern limits in the northern Mojave desert of southern Nevada, were investigated by way of data collected on 39 sites with *Larrea* and 20 sites without *Larrea*. The sites were located in eight drainage basins at elevations of 3000-5800 ft. over a 1,000 sq. mi. area of the Nevada Test Site. Data used were: (1) The rainfall records for 9 calendar years (1963-71) for each site; (2) maximum and minimum air temperature records for each site (1962-71); (3) percentage cover by all shrub species and by *Larrea*; (4) height and density data for *Larrea* on each site of its occurrence; and (5) percentage of germinable seeds from 29 of the *Larrea* populations for three seasons (1963-65) in relation to the seasonal rainfall for each site. The principal conclusions are:

- (a) Total percentage cover by shrub species is highly correlated with elevation ($r = 0.850$) and with rainfall ($r = 0.818$); shrub cover and elevation are less well correlated ($r = 0.689$). Percentage cover by *Larrea* follows two patterns of relationship

with rainfall: (1) Where average rainfall is low to intermediate, the same pattern as total shrub cover in relation to rainfall, and (2) on sites of high average rainfall, consistently low cover, a function of low density of Larrea on these sites. In general, in undisturbed communities, the taller the Larrea plants, the fewer there are of them; the height/number relationship is defined by the equation $\bar{Y} = a + b/X$. Numbers of Larrea plants are highly correlated with total plant volume as indicated by the sum of the plant heights ($r = 0.929$). Average height is not strongly correlated with average rainfall ($r = 0.572$), pointing to the importance also of other variables to this parameter; in the populations of tall plants (average 1 meter), however, the plants occur in low-density and on sites with high rainfall (average 6-7 inches).

- (b) The prevailing low minimum air temperatures and their extremes in the lowlands of enclosed drainage basins are inferred to be the primary cause of the absence of Larrea in three discrete vegetation zones in Frenchman Flat (Atriplex confertifolia, Lycium pallidum, and L. shockleyi), and over most of the basin floor of Yucca Flat where the communities are Atriplex and Grayia-Lycium andersonii. The low minimums in the lowlands of these basins result from cold air drainage phenomena year-round and the formation of a cold air lake of variable depth. Average extreme minimums on these sites were mostly below 0°F during winters of the 10-year record, and the extreme minimum for the network of sites was -18°F in one of the Atriplex communities of Frenchman Flat.
- (c) Larrea occurs over the bajadas of Frenchman Flat on sites above the lower layers of the cold air lake, and in Yucca Flat, where the species is at its northern limits; it is restricted to certain upper bajada sites and notably one site on the basin floor. Average extreme minimum air temperatures on all of these Larrea sites were above 1°F, and the absolute extreme minimum was -8°F. Upper altitudinal limits of Larrea apparently are not determined by extreme minimum temperatures since minimums in Coleogyne vegetation, which replaces Larrea altitudinally on the slopes, are well within the range of those on Larrea sites.
- (d) There is no pattern of relationship between extreme maximum temperatures in the region and the distribution of Larrea, although the highest maximums usually occur on non-Larrea sites (especially in the lowlands of Frenchman Flat).
- (e) The fidelity with which maximum and minimum temperature regimes obtain on given topographic sites, the influence of slight differences in elevation upon these regimes in the vicinity of playas, and certain inferred effects on vegetation, are illustrated (in a manuscript near completion) by data from nearby sites with a 5-ft. difference in elevation in southeastern Frenchman Flat; Larrea and

some of its common associates are present on the upper site and absent on the lower site.

- (f) Average rainfall on the Larrea sites ranged from 4.72 to 7.18 inches. Upper altitudinal limits of Larrea coincide with an average of around 7 inches of rain. Average rainfall of 6-7 inches, as rainfall was measured in this study, appears to be critical to the behavior of Larrea, and the low-density populations (and tall plants) occur in this rainfall range. Results of the germination trials support the inference of a deleterious effect of high rainfall on Larrea populations through time: There are high correlation coefficients (negative or positive, depending on the year) between the rainfall of the effective rainfall season and the percentage of germinable seeds; highest mean germination percentages (20-60%) occur with 3.5 to 5.5 inches of seasonal rain, and either lower or higher seasonal rainfall results in lower percentages of germinable seeds (0-20%).

The distribution and behavior of Larrea in the northern Mojave Desert, and the northern boundary of the Mojave Desert itself, are inferred to be under the major control of increases in rainfall with altitude and latitude. Minimum temperatures and their extremes appear to determine the sharp boundaries between Larrea and non-Larrea communities in the lowlands of enclosed drainage basins.

Interim Report

Vascular Plants of Ash Meadows, Nevada J. C. Beatley

The occurrence and regional distributions of the more than 200 vascular plant species (including several new species now described for the literature) collected since 1968 in Ash Meadows, the large Mojave Desert oasis in southern Nevada, were prepared for the information of plant and animal geographers, ecologists, and conservationists of the Country. Included are the kinds of vegetation to which each species belongs, floristic analyses, and the distributions in relation to the physiographic features of Ash Meadows and southern Nye County, Nevada. The area lies due south of the Test Site, and its many springs are fed in part by water moving underground from the Test Site.

Reference: UCLA Report #12-845, 59 p., December 1971.

A New Penstemon (Scrophulariaceae) and (Grindelia Asteraceae) from southern Nye County, Nevada J. L. Reveal and J. C. Beatley

A new Penstemon, P. pudicus, has been described from the Kawich Range, Nye County, Nevada. A member of section Saccanthera, the entity may be distinguished by its long flowers, bearded staminode, glabrous leaves and

anthers, and spreading habit. A new Grindelia, G. fraxino-pratensis, has been described from the Ash Meadows area of Nye County, Nevada (extending into adjacent Inyo County, California). It may be distinguished by erect or curved phyllaries, mostly entire to slightly dentate leaves (only at the apex), short ray lamina and resinous-punctate herbage.

Reference: Bull. Torr. Bot. Club, 98:332-335, (1971)

Russian-Thistle (Salsola) species in western United States
J. C. Beatley

Russian-Thistle populations in western United States consist of either of two species, or both, and are distinguishable at all stages in the field. Salsola pestifer is now replaced by an earlier name, S. iberica. The second species, S. paulsenii, which is the one not recognized in any of the plant manuals of the country, is the prevailing one in many areas of especially the Southwest. Where growing together they appear to hybridize freely, resulting in populations exhibiting varying degrees of genetic introgression.

Reference: Jour. Range Manage. Vol. 25 (1972)

The Physiological Behavior of Desert Shrubs
O. R. Lunt, A. Wallace, and H. M. Mork

Studies are continuing of plant parameters which control assimilation, photorespiration and water loss in desert shrubs. A null-point compensating system with an improved feedback mechanism is being used to make continuous, essentially automatic measurements of gaseous exchange under constant atmospheric conditions.

Two studies are continuing and a third one has been added:

1. The effect of soil moisture stress on the rate of photosynthesis and transpiration. S. B. Clark and John Letey.

Data obtained with Larrea tridentata are in the process of evaluation. The charts are being studied, and calculations are being made of the rates of photosynthesis and transpiration as they relate to soil moisture stress.

2. Fundamental characteristics of selected species of desert shrubs. H. M. Mork.

Data have been obtained on twelve more species of desert shrubs at three different locations and at three different times for some of them. The data are compared in the following table.

TABLE I

Comparative Data for Twelve Species Relative to Photosynthesis, Transpiration, and Compensation Point

Species	Measurement data Month-year-loc.	Photosynthesis (mg CO ₂ /g/hr) ²	Transpiration (g H ₂ O/g/hr)	C.P. (ppm CO ₂)
<u>Atriplex</u>	July '70 NMRB	22.2	2.10	5
<u>hymenelytra</u>	Feb. '72 Orchard	26.1*	1.14*	-
<u>Atriplex</u>	July '70 NMRB	16.3	1.95	11
<u>lentiformis</u>				
<u>Ephedra</u>	Mar. '72 RV	4.1	0.12	-
<u>nevadensis</u>				
<u>Eurotia lanata</u>	Mar. '72 Orchard	7.4	1.23	-
<u>Franseria dumosa</u>	June '70 NMRB	35.5	4.2	39
	May '71 RV	26.3(9)	1.72(9)	128
	Aug. '71 Glasshouse	20.3(10)	1.38(10)	-
<u>Krameria parvi- folia</u>	June '71 RV	42.1 (18)	1.69(18)	173 105
<u>Larrea tridentata</u>	June '70 NMRB	2.1	0.14	66
	May '71 RV	16.7	1.02	-
	Mar. '72 RV	9.3	0.95	-
	Mar. '72 Orchard	5.7	0.70	-
<u>Lycium andersonii</u>	July '70 NMRB	40.1	6.08	49
	June '71 RV	7.2	0.94	-
	Sept. '71 Glasshouse	19.4	3.50	-
<u>Lycium pallidum</u>	July '70 NMRB	37.9	4.26	39
	July '71 Glasshouse	24.6*	2.32*	-
<u>Lycium shockleyi</u>	July '70 NMRB	31.8	4.98	44
<u>Salsola pestifer</u>	July '70 NMRB	47.9	2.84	4
<u>Selinocarpus</u>	July '70 NMRB	25.3	2.55	51
<u>diffusus</u>				

*Values are mg/CO₂/sample/hour and g H₂O/sample/hour. Glasshouse refers to facility at Mercury. Figures in ()² are number of observations.

3. The effect of varying chamber temperature, CO₂ concentration, relative humidity and root temperature of the plant on the rate of CO₂ uptake and water loss. H. M. Mork.

Increasing the chamber temperature above 25° C tends to increase

the rates of photosynthesis and water loss up to about 40° C for Eurotia lanata at normal soil moisture. Other species under these or other conditions may be affected differently.

The CO₂ concentration in the chamber appeared to have a very significant effect on the plant process rates. Increasing the CO₂ concentration (range 90-375 ppm) increased the CO₂ exchange for Lycium pallidum almost tenfold while reducing the water loss by about ten percent. About the same range of increases in CO₂ concentration increased the CO₂ exchange about fourfold for Larrea tridentata with a reduction in water loss of about ten percent. This effect may be related to the moisture content of the soil in the field.

Relative humidity, also, influenced the rate of photosynthesis with much less effect on water loss. Decreasing the relative humidity (for Larrea tridentata) tended to reduce the CO₂ uptake and increase the water loss rate. In contrast, the net result on Eurotia lanata was an increase in photosynthesis and a decrease in water loss. For Lycium p. there was essentially no effect on either rate.

Results of the experiment with root temperatures indicate that the range needs to be extended up to perhaps 40° C or higher. The data (for the range 10 to 29° C) showed increases in both photosynthesis and transpiration for both Larrea tridentata and Atriplex hymenelytra in the Mercury glasshouse.

Interim Report

Improved Feedback Control for the Null-point Compensating System H. M. Mork, R. W. Farmer, and K. A. Flygare

A proportional feedback control has been designed to replace the original servo control unit in the Null-point Compensating System developed by Koller (1). It consists of two almost identical channels, one controlling CO₂ concentration and the other controlling relative humidity.

The improved feedback mechanism increased the stability of the system significantly more than the original servo control unit and enabled it to maintain constant atmospheric composition in the assimilation chamber under normal conditions. With this improved control, the Null-point Compensating System has the capability to make continuous, essentially automatic measurements of gaseous exchange under controlled conditions.

(1) Koller, Dov: Determination of Fundamental Plant Parameters Controlling Carbon Assimilation and Transpiration by the Null-point Compensating System (Improved Version). USAEC Report UCLA 12-797, 28 pp., Nov. 1970

Interim Report

SOIL FACTORS

Influence of Clinoptilolite on Sr90 and Cs137 Uptakes by Plants

H. Nishita and R. M. Haug

An experiment was conducted to determine the effect of clinoptilolite on the Sr90 and Cs137 contents of bean and barley plants grown in soils contaminated at the surface (experiment I). An experiment was also conducted to determine the release of Sr90 and Cs137 to clover plants upon continuous and prolonged cropping of a contaminated soil with and without clinoptilolite treatment (experiment II).

In experiment I, clinoptilolite was found to decrease the average Sr90 contents of plants. The percentage reduction of Sr90 contents of the different parts of the bean plants ranged from 48 to 70, 54 to 77, and 44 to 77 per cent in the leaves, stems and fruits, respectively, depending on the amount of clinoptilolite application and the kind of soil. The percentage reduction of Sr90 in barley plants under various treatments ranged from 57 to 77 and 57 to 79 for the leaves and stems and the heads, respectively.

In experiment II, eight harvests of clover were obtained. The application of Ca-treated clinoptilolite was effective in reducing Sr90 contents of the plants grown in the mineral soil used, but it had no effect in the organic soil. Depending on the harvest time, the average Sr90 contents of the plants grown in the treated mineral soil were 59 to 69 per cent less than that of the control plants. Clinoptilolite was effective in maintaining the reduced Sr90 contents of the plants throughout the experimental period. The Cs137 contents of the plants were not changed appreciably by the application of clinoptilolite.

Interim Report

Some Physical and Chemical Characteristics of Heated Soils

H. Nishita and R. M. Haug

It is known that the soil in the immediate vicinity of a nuclear detonation is exposed to heat ranging in temperature well above that causing fusion of geologic materials down to the ambient level. Our interest in heated soils stems from the fact that aside from the effect of ionizing radiation, one of the factors that needs to be known in assessing the growth response of plants in the immediate vicinity of a nuclear detonation is the effect of soil heating on the availability of the essential nutrient and nonessential elements to plants. Our previous paper (Soil Sci. 110: 61-70, 1970) has discussed the growth and the elemental composition of bean plants grown in a soil that was exposed to different temperatures up to 1,000° C.

In this study, the physical and chemical characteristics of five different soils that were heated at various temperatures to 1,000° C were examined. The properties examined include particle size distribution, pH, cation exchange capacity, electrical conductivity, water and ammonium acetate extractable Na, K, Mg, Ca and Sr, and total N, lime and organic C contents. Some of the results obtained are given below.

In general, the particle size distribution remained unchanged until the heating temperature was in the 300° to 400° C range. Above this temperature range, the sand fraction in all soils (except one) increased progressively with increasing temperature, while the silt and the clay fractions decreased. The cation exchange capacity generally decreased progressively with increasing temperature above 100° C. The pH of each soil decreased to a minimum at 200° C and then increased to higher values at higher temperatures. The organic-C content of the soils generally changed only to a small extent for heating up to 200° C and then decreased rapidly at temperatures above 200° C. The electrical conductivity of the saturation extracts of the heated soils indicated that the greatest extractability of ions occurred in the 200-300° C temperature range. The extractability of ions depended on the ionic species and the soil heating temperature.

Interim Report

Soil Thermoluminescence in Relation to Radiation Exposure under Field Conditions

H. Nishita and M. Hamilton

Experiments were conducted to determine the relationship of soil thermoluminescence to ionizing radiation exposure under the conditions of natural environment using the Cs137 radiation field at the Nevada Test Site. In experiment I, soil columns were buried in the ground and irradiated at a fixed distance from the Cs137 source. In experiment II, they were exposed on the ground surface at 12 different distances from the source. Soil thermoluminescence was determined in three ways, i.e., glow curve area below 200° C, glow curve height at 200° C, and "total area."

Both experiments showed that soil thermoluminescence is highly correlated with radiation exposure level. The first two methods of measuring thermoluminescence indicated above gave correlation coefficient $r > 0.915$. The "total area" method gave high correlation coefficient $r > 0.951$ for the noncalcareous soil, but low coefficient $r < 0.769$ with the highly calcareous soil studied, indicating that this method cannot be applied to the latter soil type. Under the experimental conditions that prevailed, the minimum radiation exposure level required to produce

measurable soil thermoluminescence was in the 20-85 R range.

Reference: Soil Sci. 111: 393-398, 1971

Distribution of Different Forms of Nitrogen in Some Desert Soils

H. Nishita and R. M. Haug

The distribution of different forms of N in the profiles of desert soils collected in different areas of the Nevada Test Site was examined. Among the soil profiles examined, the order of the amount of the different forms of N in the surface layer (0.0 - 7.6 cm) was always NO_2^- (not detectable) < extractable NH_4^+ -N < NO_3^- -N < fixed NH_4^+ -N < organic-N. This order may be changed in the sublayers. The extractable NH_4^+ -N may be greater than NO_3^- -N with the presence of trace amount of NO_2^- -N and/or the fixed NH_4^+ -N may be greater than organic-N. The amount of total-N was the greatest in the surface layer and usually decreased very sharply in the second layer (7.6 - 15.2 cm). Below the second layer, the total-N contents generally varied slightly. In the surface layers, the total-N was always accounted for primarily by organic-N. The amount of fixed NH_4^+ -N varied somewhat with different soils, but within any given soil profile, it generally varied slightly. The average absolute amount of it in the profiles collected in different areas and under different plant species ranged from 0.0031 to 0.0068 per cent by weight of oven dry soil. Expressed as percentage of total-N, the range was 3.4 to 63.3. The extractable inorganic-N ranged from 0.33 to 7.89 per cent of total-N depending on the soil and the profile layer. Exchangeable K concentration under the plant was 41 to 240 per cent greater than in the bare soil. This plus the higher organic matter content decreased the relative NH_4^+ -N fixation under the plant compared to its fixation in the bare soil.

Interim Report

An Integrator for Low Absorbance Samples in Atomic Absorption Spectroscopy

H. Nishita, R. Farmer, and S. Peterson

Since its establishment as a viable method by Walsh in 1955, atomic absorption spectroscopy has become a very important technique for chemical analyses. More than 60 elements are determined routinely by this technique. The sensitivity and the detection limits of these elements under specified analytical conditions have been reported in many books and reviews. From an analytical point of view, the important quantity is the minimum concentration of metal that can be measured with useful precision. It is common knowledge that the precision and accuracy of sample absorption measurements become increasingly unsatisfactory as the detection limit is approached. A large percentage of the analytical work done in our laboratory entails analyses of low absorbance samples.

Consequently, in order to improve the precision and accuracy of measurements, an integration readout system was incorporated into the spectrophotometer system. The circuit diagram of the integrator and its performance characteristics have been published.

Reference: Anal. Chim. Acta 58: 1-6, 1972

SPECTROSCOPY

Optical Emission Analysis of Biological Tissues

G. V. Alexander and L. T. McAnulty

A complete revision of the optical emission spectrometer and its computer programs has occupied most of our time during this past year. The integrator capacity of the spectrometer has been increased from 35 to 50 channels with 44 channels presently in use. These additional channels are being used to 1) improve the estimation of signal backgrounds, 2) extend the concentration range for the determination of manganese, and 3) add the elements Cd, Be, As, Sb, and Hg to the fixed program of the instrument. The control system for the spectrometer has been improved so that it is only necessary to position a sample in the arc chamber and then push a start button. The remainder of the operation including punching of the data is handled automatically.

After a modification of the computer program the new system was first used for analysis in January on a limited basis (2000 samples) and began full-scale operation at the end of April. A new computer program has been written which permits the evaluation of a three-dimensional background equation which in most cases is superior to the two-dimensional equation previously used. This program has been incorporated into a new program for determining concentrations. This new program permits analysis with a continual and automatic update of background data which greatly reduces the tedious hand calculation previously required to maintain the system.

Interim Report

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DIVISION OF NUCLEAR MEDICINE

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Areas of Interest

Development of new radiopharmaceutical agents and diagnostic procedures in nuclear medicine

Cardiac and tumor studies

Clinical applications of nuclear technology

Mechanisms of viral induction of mouse leukemia

Development and production of short-lived (cyclotron) radio-nuclides and their metabolic characteristics

Experimental physiologic studies of the cardiopulmonary system & myocardial imaging with cyclotron radionuclides

Radiopharmaceutical Development

Clinical applications in short-lived nuclides

Pulmonary studies in man and lung transplants. Investigations in dogs.

Radioaerosol inhalation studies

SCIENTIFIC REPORTS

LEUKEMIA BIOLOGY

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 or altered by the presence of the virus.

Reference: J. Surg. Oncol. 3:517-523 (1971).

Graft-versus-Host Reactions and the Viral Induction of Mouse Lymphoma

Esther F. Hays

The role of graft-versus-host reactions (GVH)² in the development of lymphoma was studied in mice carrying murine leukemia virus (MuLV) and a genetic susceptibility to lymphoma development. GVH reactions were produced by inoculating parental spleen cells into AKR x CBA/H-T₆T₆ and AKR x SJL/J F₁ hybrids. The spleen cells were obtained from animals with and without previous exposure to MuLV. Thymic lymphomas were found to develop in high incidence in mice receiving spleen cells from virus inoculated parental donors. The lymphomas were of host-cell type. Manipulations designed to increase the intensity of the GVH reaction were shown to have no relationship to the development of lymphoma. It was also demonstrated that AKR cells had an impaired ability to produce GVH reactions and yet were highly lymphomagenic when they came from virus inoculated donors. It was concluded that lymphomagenesis was directly related to the presence of virus in the spleen cells and not to the GVH reactions resulting from their inoculation.

Reference: Cancer Res. 32:270-275 (1972).

Development of Neoplasia and Karyotype Analysis in Mice with Graft-versus-Host Reaction

Esther F. Hays

When F₁ hybrid mice of a cross between SJL/J and CBA/H-T₆T₆ were given (1) parental (SJL/J) spleen cells, or (2) cell-free preparations of parental spleen cells, RCS developed with increased incidence in both groups when they were compared to non-injected controls. The presence of GVH reactions in the cell-inoculated animals did not influence tumor development. When C3H x CBA/H-T₆T₆ F₁ hybrids were given C3H spleen cells, visible GVH reactions did not develop. The incidence of mammary tumors in the cell-inoculated females of this hybrid cross was significantly greater than that in controls not receiving cell inoculations.

Non-thymic lymphomas occurred in the SJL/J x CBA/H-T₆T₆ hybrids and hepatomas developed in the male C3H x CBA/H-T₆T₆ mice with equal incidence in all experimental groups.

Karyotype analysis of the reticulum cell neoplasms showed small numbers of donor cells and aneuploid (malignant) cells of host origin.

No evidence was found in the mouse strains tested in this study, to support the hypothesis that the immunologic disorder associated with a GVH reaction could eventuate in lymphoma.

Reference: Cancer Res. 32:276-279 (1972).

The Effect of Actinomycin D on Lymphomagenesis of Thymic Grafts Exposed to Murine Leukemia Virus (MuLV)

Esther F. Hays

This study was designed to look at the role of an inhibitor of DNA dependent RNA synthesis, actinomycin D (ACT), in the establishment of infection with MuLV as measured by the development of lymphoma in thymic grafts. Thymus from AKR mice was exposed in vitro to ACT (1.0 µg/ml) for 3 hr and MuLV for 20 min, then grafted to thymectomized syngeneic hosts. Lymphoma developed in all of 15 control thymuses incubated in tissue culture medium and then MuLV, but it was found in only 1 of 11 thymuses exposed to MuLV after incubation with ACT. Thymus removed from suckling mice given MuLV 24 hr previously developed lymphoma in controls, but not when incubated with ACT prior to grafting. However, when the recipients were given MuLV as neonates and subsequently used in grafting experiments, lymphoma developed in both ACT and control incubated grafts. Grafts exposed to ACT were also found to regenerate and to restore the immune response of thymectomized hosts. These experiments show that ACT inhibited lymphoma development in thymic grafts when such grafts were exposed to this compound just prior to MuLV, as well as after viral infection had been established in vivo for 24 hr. ACT incubation did not inhibit lymphoma development in thymic grafts

placed on hosts previously infected with MuLV. ACT also did not affect graft regeneration or the ability of thymic grafts to function in producing normal immune responses in thymectomized animals. The inhibition of lymphoma in these experiments is a result of actinomycin D preventing the establishment of viral infection in thymic cells and not to a functional alteration of the thymus produced by a direct cytotoxic action of the drug.

Reference: Amer. Soc. Hematol. Program Book p. 65 (1971) (Abstract).

Granulocyte-Monocyte Colony Forming Capacity of Human Bone Marrow
C.G. Craddock and Esther F. Hays

An in vitro culture technique of marrow cells in soft agar has been developed and applied to studies with human marrow. The source of colony stimulating factor (CSF) has been serum from C57BL mice removed 4 hours after administration of endotoxin. Thirty-six patients with no obvious disturbance in granulopoiesis have been studied with two concentrations of the serum containing CSF. With this system reproducible increment in colony formation was observed. Marrow from patients with myeloproliferative disorders showed impairment of this response of ratio. Patients with acute myeloblastic leukemia showed poor colony growth, and when in remission with normal appearing marrows, showed an impairment of the response ratio. An increase in the response ratio was found in 1 patient with pH¹ positive, chronic granulocytic leukemia, and 2 patients recovering from drug-induced neutropenia. It has also been found that the presence of minor percentages of sarcoma cells, carcinoma cells, and leukemic lymphoblasts do not impede in vitro colony formation or impair the response ratio.

Interim Report

Granulocyte-Monocyte Colony Forming Capacity of Mouse Bone Marrow
Esther F. Hays and C.G. Craddock

Studies with mouse bone marrow have been carried out using the same system of soft agar and serum from mice previously given endotoxin, as in studies of human marrow. Again, two concentrations of serum have been used and a constant response ratio has been found for normal marrow. Preliminary studies have shown that AKR mice given Gross murine leukemia virus (GMuLV) have increased colony forming capacity. Such mice characteristically develop lymphoma between the second and third month of life. When their marrow becomes infiltrated with lymphoma cells, the number of colony forming cells decrease but the ratio of response remains the same. Studies of the marrow of C3H mice after neonatal thymectomy have shown no difference in colony forming capacity when compared with normal marrow. The administration of hydrocortisone to animals for 2 days prior to removal of marrow for study of colony forming capacity produces a striking decrease in the

number of colony forming cells without remarkably changing the differential count of the bone marrow.

Interim Report

Characterization of Various Transplanted Mouse Lymphoma Cell Populations Donna L. Vredevoe and Esther F. Hays

Single Gross virus-induced mouse lymphoma cells or low doses of such cells have been isolated and transplanted to syngeneic recipients. Three mouse strains have been studied: AKR, C3H/HeJ, and CBA/H (CBA/T₆T₆ donor lymphoma). Single cells were isolated and transplanted in a semisolid gelatin medium which minimized trauma to the cell and chances that the cell might adhere to glassware or instruments used for isolation and transfer.

Cells of the following types are being analyzed: 1) AKR: spontaneous, virus-accelerated, long-transplanted and short-transplanted; 2) C3H/HeJ: virus-induced, long-transplanted and short-transplanted; 3) CBA/H: virus-induced, long-transplanted and short-transplanted. In addition lines derived from the isolation and transplantation of single cells from any of these sources are being characterized. Work this year has shown that single mouse lymphoma cells can transplant lymphoma in syngeneic recipients, but that success of such transplantation is dependent upon the cell source. In the C3H/HeJ system long-transplanted cell lines transfer lymphoma with single cells whereas in C3H/HeJ virus-induced lymphomas the LD 100 dose is as high as 1×10^8 cells.

Lymphoma donor cell populations are being sorted as to 1) virus content (XC cell and newborn assays), 2) LD 100, 3) incidence and latent period of lymphoma upon syngeneic transfer of standard cell doses, 4) histology of lymphomas, and 5) theta antigen content.

It is the purpose of this project to eventually compare success of immunotherapy, applied with transplantation of syngeneic lymphoma cells, of mouse lymphomas of these characterized types. The objective will be to determine those tumor cell characteristics which are correlated with successful immunotherapy.

Interim Report

CARDIAC STUDIES

Comparative Uptake of Potassium and Cesium After Intravenous and Direct Coronary Arterial Injection N.D. Poe

Following intravenous administration of K-42 and Cs-131 approximately 5% of the injected dose concentrates in the myocardium, but there are rate

differences. Potassium reaches a peak between 5-20 minutes post injection and clears with a $T_{1/2}$ of about 6 hours; Cesium peaks between 1-2 hours and clears with a half-time in excess of 16 hours. These findings are in agreement with published data. With intracoronary injection approximately 70% of potassium is extracted on the first passage versus only 22% for cesium. Half-times are approximately 1 and 6 hours respectively. Were it not for the higher radiation dose and less optimal gamma emissions, K-43 would appear to be preferable to Cs-129 for myocardial scanning, particularly following intracoronary injection.

Final Report

Choice of Particle Preparations

N.D. Poe

Four classes of particles were investigated: albumin macroaggregates (MAA) 10-100 μ diameter and under 35 μ diameter; anion resin microspheres 10-100 μ and ultrafine microspheres under 35 μ . All were labeled with technetium-99m. Batches of each of these preparations were injected simultaneously with Cs-131 in three experimental models: left atrial injections in normal animals, left atrial injections in animals with ligation of the anterior descending coronary artery, and arterial injections directly into the anterior descending coronary artery. Twenty-eight dogs in all were studied. The hearts were removed and multiple full-depth sections taken for tissue counting. The cesium was distributed equally (within 10% range) throughout the normal left ventricle. The best particle/cesium correlation was found with the ultrafine microspheres. These, likewise, had the best internal correlation. After ligation of the anterior descending coronary artery, the perfusion defects obtained by scanning were qualitatively similar but quantitatively significantly different. With the particles, the defects were larger. Presumably, the cesium entered the ischemic area by anastomoses and recirculation. These observations were confirmed by *in vitro* tissue analyses. An analogous situation was found by direct intracoronary injection. The particles were restricted to a clearly defined region corresponding to the injected vascular bed. Although a substantial portion of the cesium was similarly localized, this material was found throughout the myocardium. The local distribution of the particles often was more erratic than the solution of cesium probably because of streaming and other mechanical effects of blood flow on the particles. It was concluded from this study that the ultrafine microspheres provide the most accurate and reproducible estimate of regional flow providing adequate mixing takes place prior to entering the coronary circulation.

Final Report

Cesium Kinetics in Acutely Ischemic Myocardium

N.D. Poe

To determine cesium turnover in acutely ischemic myocardium ischemic lesions were produced by ligation of the anterior descending coronary artery in open-chest animals. One hour before ligation 1 mCi of Cs-131 was given I.V. This would then be normally distributed in the heart prior to injury and/or infarction. The artery was then tied in a series of 15 dogs at 3, 6 and 24 hours. After ligation, Cs-129 and Tc-99m labeled microspheres were injected into the left atrium. Scans of the hearts were made followed by sacrifice. Sections of the myocardium were analyzed spectrometrically for relative radionuclide content: the Tc-99m gives relative flow; the Cs-131 indicates how rapidly cesium is lost from tissue of known degree and duration of ischemia; and similarly the Cs-129 shows cesium uptake in this tissue. Preliminary analysis of the data shows that very little (approximately 20% or less) cesium is lost from ischemic or infarcted myocardium in the first 3-6 hours but by 24 hours a definite loss is evident, the degree of which approximates the attained decrease in flow. Surprisingly, the Cs-129 values at all times correlated well with the Tc-99m microspheres. Differences were anticipated because cesium uptake is a function of both flow and cellular function while the spheres depend solely on flow. This question is being further investigated in acute infarction with restored flow. A series of chronically infarcted animals is being studied at intervals up to 6 months.

Interim Report

Clinical Myocardial Investigations with Cs-129 and K-43

N.D. Poe and L.M. Eber

The administration of Cs-129 to human volunteers commenced 9/24/71. To date 16 patients have received Cs-129 by the intravenous or intracoronary route or both. Of these, 5 patients had intracoronary and intravenous Cs-129 injection on separate days. Nine patients have had intravenous Cs-129 administration only and 4 patients have had the Cs injected directly into a coronary artery at the time of cardiac catheterization. Five patients had angiographically-normal coronary arteries. Most of the intracoronary injections were made into the left coronary system as this was most commonly involved in aneurysm production.

Heart scans obtained 1-2 hours following intracoronary Cs-129 administration were clinically useful in localizing underperfused areas of myocardium which corresponded to regions of abnormal ventricular contraction visualized by ventriculography. In the absence of computer image-enhancing systems, only grossly akinetic or paradoxing areas even correlated with filling defects on the scans. Areas of minor motion disturbances, such as localized hypokinesis, were not identifiable on the scans.

The images obtained after intravenous administration of Cs-129 were generally not of sufficient quality as to be clinically useful. Because of the poor target to background ratio, areas of myocardium known to be aneurysmic could not be identified on the scans. As our present equipment has no capacity for elimination of background, we plan to limit further intravenous studies with Cs-129 until we obtain an on-line computer modification (probably around Sept. 1, 1972).

Preliminary observations with K-43 suffer similar limitations to those noted above with Cs-129. However, as anticipated from the animal investigations, intracoronary injections of K-43 give superior results to those obtained from Cs-129.

Interim Report

Carbon-11 Labeled Carboxylic Acids

N.D. Poe and N.S. MacDonald

In an attempt to evaluate fatty acids as potential myocardial scan agents, several compounds have been prepared and tested in animals.

Relative myocardial uptake of acetate was evaluated in 2 dogs. Significant myocardial retention was not demonstrated. Distribution of octanoate was studied in 3 dogs and 2 rabbits. This material cleared more slowly from the blood than did the acetate but showed no definite myocardial concentration distinguishable from blood content.

Palmitate likewise failed to demonstrate substantial myocardial uptake as would have been anticipated from reports using C-14, but the precise composition of the material used was not determined. Further work in the area is in progress.

Interim Report

TOTAL BODY COUNTER ACTIVITIES

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

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

A total of 74 studies of the metabolic turnover rates of serum immune proteins (immune globulins, G and M) were made in patients suffering from rheumatic disorders. This work was conducted in collaboration with Dr. Joshua Levy and others of the Department of Medicine. The program is aimed at elucidating some of the factors in rheumatic diseases and providing methods for objective evaluation of the efficacy of therapeutic measures. Our contribution consisted in measuring the turnover rates

of the two proteins (which have been labeled 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 from total body counting were combined with other information regarding serum concentrations, plasma volume, and body weight to calculate the daily rate of catabolism and synthesis of these vital proteins. In a series of 18 patients with active rheumatoid arthritis it was found that 4 months of treatment with the immunosuppressive drug, "Azathioprine", reduced the rate of synthesis of immune globulin-M by 41% ($\pm 8\%$ S.D.) of their pre-treatment synthetic rate (in mg. per kg. per day). After an interim period during which a placebo was administered regularly, the synthetic rate was found in general to be somewhat higher than the pre-Azathioprine base line level. Six of these patients were then placed on Cyclophosphamide therapy, after which the IgM synthetic rate was found to have been reduced dramatically ($61\% \pm 11$ reduction)!. Immune globulin-G synthetic rates were also depressed by the drugs, but not to as great an extent. Cyclophosphamide decreased the synthesis of IgM more than Azathioprine, however. In another series consisting of untreated patients and normal subjects, it appeared that the synthetic rate for Immune globulin-M in 8 patients with psoriatic arthritis was similar to that in 7 normals (9.1 ± 4 mg/kg/day compared to the normal 6.0 ± 1), whereas 26 rheumatoid arthritic patients were making IgM (and catabolizing it) at a mean rate of 21.7 ± 6 mg/kg/day!

Interim Report

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

The Total Body Counter was used to determine the amounts of gamma radioactivity in a number of adult residents of Southern California. Body burdens of Cesium-137 (which enters human food supplies as "fall-out" of the debris from atmospheric nuclear detonations) have been monitored continuously at UCLA since 1959. During calendar 1971 the mean quarterly values for Cs-137 contamination in males was 23 picocuries per gm of body potassium, and 20 pCi/gm K for females. A total of 135 measurements were made. These levels are about the same as for calendar 1970 and suggest that human body contamination with Cs-137 has leveled off following the rapid decline of 1965-1968. Since atmospheric fall-out is declining, this may possibly imply that some Cs-137 is re-cycling and thus helping to establish a steady state condition. An alternative explanation might be that the observed level of Cs-137 represents a quantity of the nuclide which is more permanently fixed in the body than amounts which exceed this level. (Cs-137 in amounts above the nanocurie/gm K range appears to be metabolized in the body and excreted with the most important experimental "half-times" being between 1-3 months).

Interim Report

Biomedical Cyclotron Operations - Progress Report
N.S. MacDonald

The final acceptance tests of the accelerator were completed satisfactorily and the facility began productive operation on 12 July 1971. Mechanical performance has been excellent and "down-time" due to malfunctions or breakdown has been low. For example, during the period from 12 July 1971 to 23 June 1972, a total of 296 individual bombardments were performed during the period of 240 calendar working days. The accompanying table summarizes the production of radionuclides during this time. For each of these nuclides special techniques were devised and tested, not only for holding and bombarding the target, but also for chemically isolating the desired radionuclide after the bombardment. A number of novel "targetry" devices and appliances were designed and constructed and several new liquid-liquid extraction procedures were developed for separations of the radionuclide mixtures.

The radionuclide which was made most frequently was Cesium-129. We found that this could be obtained in moderate yields by alpha bombardment of purified sodium iodide powder pressed into a 1" diam. depression in an aluminum target plate. When necessary, isolation of pure Cs-129 completely free of iodine and Na-24,22 could be achieved by extraction of the aqueous solution with tetra-phenyl boron in benzene, and recovery from the organic phase by reextraction with HCL and neutralization. Identification was made by gamma spectroscopy with a Ge-Li detector and 1600 channel analyser, together with half-life measurements. After demonstration of the sterility and apyrogenicity of Cs-129 prepared in this way, the nuclide was approved for human use.

A simple technique for preparing Potassium-43 was devised. Argon gas was pumped into a gold-plated, hollow brass cylinder 8" long to 8-15 psig. This was bombarded end-on through an AL foil with alphas. The carrier-free K-43 was then washed out of the tube with physiologic saline solution and sterilized. A new circulating system is under development in which Argon gas is pumped through the target chamber, through a coil in a cooling bath and back to the target chamber, impinging on the aluminum foil window; thus cooling it and permitting bombardment with a higher beam intensity. Potassium-43 preparations were tested for pyrogens and sterility and approved for human use. Following preliminary animal studies the value of Cs-129 and K-43 for myocardial imaging in patients was taken under active investigation by Dr. N.D. Poe at the UCLA Medical Center. Fifty-six batches of Cs-129 and 22 of K-43 were furnished for this work.

Indium-111 has been reported to offer advantages for cisternal scanning and for localization of certain tumors. It is being evaluated by Dr. L.R. Bennett and his group in the UCLA Medical Center. We studied three different routes to this nuclide; bombardment of natural Cadmium (solid block, electroplated, and foil) with deuterons and with protons;

and bombardment of natural Silver with alphas. In-111 from an Ag foil target was less contaminated with other short-lived radionuclides of In and was free of the trace of long-lived In-114m, all of which appear in the product from Cd. However, protons on Cd foil proved to be the method for greatest yield of In-111. A liquid-liquid extraction method, using a heptane solution of diethyl-hexyl-phosphate was developed for the rapid, simple isolation of the radio-indium from the dissolved target metal. The In-111 is prepared routinely and 31 individual batches have been made available for the animal and human studies.

Considerable effort was devoted to the preparation of several aliphatic monocarboxylic acids, labeled with Carbon-11. These fatty acids are being investigated by Dr. Poe as radiopharmaceutical agents for myocardial imaging. The radionuclide itself was readily obtained by proton bombardment of a flowing stream of nitrogen gas containing a trace of oxygen. Carrier-free carbon-11 monoxide and carbon-11 dioxide were thereby produced. The CO was converted to CO₂ by passing the gases over hot copper oxide. The radioactive CO₂ was then bubbled into a stirred ethereal solution of the appropriate Grignard reagent. The reaction mixture was then hydrolyzed with HCl and the resulting carboxylic acid was extracted with NaOH or (NH₄)₂CO₃. Thus, for example, C-11-acetate was made from methyl magnesium bromide; C-11-octanoate from n-heptyl magnesium bromide; and C-11-palmitate from pentadecyl magnesium bromide. These acids were not isolated, but were used as aqueous solutions of the sodium salts for preliminary tissue distribution and organ imaging studies in animals. Although C-11 has only a 20 minute half-life, these chemical manipulations were rapid enough to make activities in the range of 5-20 millicuries potentially available for injection. However, the actual composition of these products had not been rigorously determined, so attempts were made to prove that the C-11 radioactivity was indeed incorporated into the desired carboxylic acids. Techniques of thin-layer chromatography and gas chromatography of the methyl esters are being applied to the problem. There is clear evidence that carrier-free, C-11 octanoate is the major reaction product, but the identity of a second, contaminating organic compound in the product has not yet been established. Work with C-11 labeled palmitic acid has been hampered by difficulties in inducing the reaction between magnesium metal and n-pentadecyl bromide to occur.

Nitrogen-13, in the form of carrier-free, N-13-ammonia was prepared by deuteron bombardment of either a flowing stream of methane gas, or of a layer of aluminum carbide powder. This radioactive ammonia was then used by Drs. Cohen and Spolter of the Sepulveda Veterans' Administration Hospital to prepare N-13 labeled glutamine and glutamic acid by enzymatic reactions. The yields of these radioactive compounds were quite low due to the 10 minute half-life of the N-13. However, the experiments were significant because of the practical knowledge gained in a promising field - the rapid and specific labeling of compounds with radionuclides by the use of enzymes fixed on a solid medium.

A number of other radionuclides were also prepared for exploratory purposes. Twenty-one batches of Fluorine-18 were provided for experiments in labeling acetate by exchanging F-18 for bromine atoms in ethyl bromoacetate (Dr. G.D. Robinson, Jr. has succeeded in preparing F-18 fluoroacetate in 3-5 mCi quantities). Other experiments included attempts at direct labeling of oleic acid with energetic F-18 atoms produced by deuteron bombardment of neon gas (unsuccessful, so far); Ruthenium-97 by alpha bombardment of natural molybdenum (for evaluation as a general labeling nuclide); Barium-135m from the bombardment of cesium chloride with helium-3 (for evaluation as a bone-scanning agent); Chlorine-34 from the (He-3, alpha) reaction on KCl (for studies of electrolyte movements in extravascular fluid spaces); and Tellurium-121 for tissue distribution studies.

Other activities included: the bombardment of soil samples with deuterons and alpha particles to study the dose dependence of production of thermoluminescent centers (Dr. Nishita); the production of radionuclides for labeling insects to permit tracking studies (Dr. Edney); and preliminary experiments on the analysis of N-15 by charged particle activation.

Interim Report

RADIOPHARMACEUTICAL DEVELOPMENT

Post-Preparative Processing of Micro-Particulate Agents

G.D. Robinson, Jr.

Particulate agents (5-50 μ), although initially developed nearly a decade ago, continue to be used in ever expanding variety of applications. Of special interest with some of the newer hemodynamic and/or perfusion scanning techniques are particulate radiopharmaceuticals with relatively narrow size ranges. A system which employs sedimentation from homogeneous suspension to fractionate particles into narrow size ranges has been developed. The particulate sample is brought into suspension and then is allowed to settle for an appropriate time interval. A given fraction of the uppermost portion of the settling suspension is removed, the removed volume is replaced with pure solvent and the process is repeated. Following several cycles at the initial settling interval essentially all of the particles smaller than some precise size limit have been removed from the system. The remaining particles are removed fractionally by repeated cycling at progressively reduced settling intervals. This is a universally applicable system which can be adapted to any type of particle/solvent system, with nearly any degree of precision in the size of individual particle fractions.

This system has been used to size microfine ion exchange resins (1-50 μ) in aqueous suspension. Fifteen cycles (25 percent removal) at each of 15, 10, 5 and 2 minute settling intervals gave particles in the ranges 1-10, 10-15, 15-20, 20-30 and 30-50 μ , respectively (~95% within the specific range). This technique is being applied to the sizing of aqueous suspensions of human serum albumin (HSA) macroaggregates and HSA microspheres.

Interim Report

Analogues of Biologically Interesting Compounds

G.D. Robinson, Jr.

The participation of small ions or molecules in specific metabolic processes is potentially a powerful mechanism for improvements in the localization of radiopharmaceuticals in target organs. A program has been initiated to develop gamma-emitting analogs of some biologically active molecules by utilizing some of the short-lived isotopes produced with the Biomedical Cyclotron.

Potassium fluoride in a polar, aprotic solvent is commonly used to prepare a variety of fluoroorganic compounds from the corresponding, bromo, chloro or iodoorganics. This interhalogen exchange reaction has been adapted to prepare a series of F-18 fluorocarboxylic acids. Initial work (with F-18 fluoroacetate) involved a 2-4 hr. reaction at 200°C between dry KF-18 and ethyl chloroacetate in acetanide or diethylene glycol solvent.

The product was isolated by gas chromatography. Recently the technique has been modified by utilizing F-18 fluoride absorbed on anion exchange resins as a dry reagent for exchange (150°C; 1 hr.) with ethyl bromocarboxylates (no solvent required). As before, the product is isolated by gas chromatography. The biological behaviors of the F-18 fluorocarboxylic acids are being evaluated with particular attention to their abilities to follow the metabolic routes of normal carboxylic acids.

Interim Report

Radio-Rose Bengal-HSA Microspheres: A Versatile Radiodiagnostic Agent G.V. Taplin, G.D. Robinson, Jr., and M. Hackendorf

Micro-particulate radiopharmaceuticals (1-5 μ) are rapidly removed from the circulation by the reticuloendothelial system (RES). 1-5 μ size I-131-rose bengal-human serum albumin (HSA) microspheres have been prepared by heating a stirred homogenized suspension of I-131-rose bengal-HSA complex in cottonseed oil at 125°C for 1 hr. The particles are separated from the oil by washing with diethyl ether and centrifuging. "Surface bound" I-131-rose bengal is removed from the dried particles by washing with a 6% HSA solution.

Studies in dogs have demonstrated the rapid initial deposition of the microspheres in the RE cells of the liver and spleen, with subsequent excretion of the I-131-rose bengal label via the biliary tract beginning 40-60 minutes post injection. This is in marked contrast with the slower uptake of I-131-rose bengal alone, which is extracted exclusively by the polygonal cells of the liver with excretion via the biliary tract beginning 10-20 minutes post injection.

Potentially this agent should permit more accurate and quantitative assessment of Kupffer cell digestive function clinically because the rose bengal label is excreted exclusively by the hepatic cells. Also it provides a means for demonstrating at the cellular level, by autoradiography, that the I-125-rose bengal in albumin microspheres initially engulfed by the RE cells is subsequently transferred to the hepatic cells and excreted in the bile. Clinically, the new agent may have special value as a dual-purpose liver-spleen scanning agent for the detection of space occupying lesions and for subsequent imaging of the biliary system in jaundiced patients as an aid in distinguishing intra- from extrahepatic causes of biliary obstruction.

Reference: J. Nucl. Med., 13, 470 (1972).

Inorganic Complex Anions: Potential Bone Scanning Agents
G.D. Robinson, Jr., N.S. MacDonald, and M. Hackendorf

Attempts to prepare and evaluate the complexes of Tc-99m (IV) and In-113m (III) with disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP) as potential bone seeking radiodiagnostic agents met with minimal success. Analysis by thin-layer chromatography (TLC) indicated that less than 25% of reduced $^{99m}\text{TcO}_4$ (ascorbic acid alone and with FeCl_3) was bound to EHDP. Direct complexation of In-113m (III) with EHDP has been somewhat more promising (in some case > 90% binding), but a reliable preparative routine has not yet been developed.

Interim Report

CLINICAL APPLICATIONS

Demonstration of Vascularity of the Femoral Head Using Technetium Sulfur Colloid

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

The purpose of this study is to determine the feasibility of demonstrating the vascularity of the femoral head using technetium sulfur colloid. Hip disease including fractures of the femoral neck raises questions relating to the blood supply of the femoral head. An index of such vascularity may prove valuable in demonstrating the prognosis of the healing of a fracture of the femoral neck and could be very helpful as a guide to the orthopedic surgeon in determining if immobilization, pinning or prosthetic replacement of the femoral head is necessary.

The method used in this study depends upon the ability of phagocytic cells located within the bone marrow to concentrate particles of ^{99m}Tc -sulfur colloid. These particles are normally used for liver and spleen scans. However, sufficient accumulation within the bone marrow is commonly noted to outline the bony structure of the pelvis. The method used in this study consists of intravenous administration of such radiocolloid in doses commonly used for liver scans, studying the area of the pelvis including the femoral heads by photoscan technique, and correlating the scans obtained with roentgenograms taken in the same position by an overhead x-ray tube. The procedure has been performed over a period of two years in patients who were scanned primarily for question of liver disease. These patients were examined to obtain information on the normal appearance of distribution of phagocytic activity within the bone marrow of the pelvis. In essentially all instances, symmetry of uptake was noted. Some patients demonstrated very excellent uptake of tracer within the femoral head, but symmetry was characteristic of normal patients. Patients with known hip disease were also studied, asymmetry was noted in such patients. In cases where the femoral head was surgically absent and replaced with a prothesis, no uptake in the area could be seen. In cases of degenerative

joint disease, asymmetry was noted with increased uptake in the involved joints. Studies on patients with acute fractures of the femoral neck are currently in progress.

Concentration of technetium sulfur colloid in bone marrow can be elevated by momentarily increasing marrow circulation during the injection of the tracer colloid. This can be accomplished by treating the extremity with microwave diathermy before injection of the tracer. Studies in rabbits have shown a 40% increased concentration of tracer in treated tibiae over nontreated. A similar effect can be accomplished with epinephrine which apparently gives bone marrow circulatory preference, possibly as a result of vasoconstriction of the splanchnic circulation. Although epinephrine has shown an average of 63% increase in concentration of the tracer over the control group, its side effects may be undesirable.

In conclusion, there is value in using colloidal material as an index of blood flow to the femoral head. Uptake is symmetrical in patients with no known hip disease. Asymmetry of uptake in the region of the femoral head correlates with a disease process of the femoral head.

Interim Report

Detection of Subclinical Arthritis with Technetium Pertechnetate or RISA M.M. Webber, W.C. Dick, and M. Cragin

Early detection of arthritis is important in the prevention of irreversible joint damage. Increased vascularity of joints, an antecedent of clinical arthritis, can be detected with pertechnetate-99m or RISA. Preliminary studies on rats with induced rheumatoid arthritis have shown that the extent of experimental arthritis can be estimated by the concentration of tracer in the joint. This information may be clinically useful in delineating a program of preventive drug therapy.

Interim Report

Radiocolloid Lymphangiography

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

Radiocolloidal gold-198 is the popular tracer for lymphatic system visualization; however, the long half-life and high energy beta ray emission of gold-198 gives undesired tissue irradiation. Two radioisotopes, indium-111 and technetium-99m sulfur colloid, were evaluated as suitable replacements due to their relatively short half-lives and low beta emissions. Lymphatic system visualization in rabbits and dogs using these two radionuclides was comparable to scintiscans done with gold-198. In addition, there is the advantage of reducing irradiation to the surrounding tissue. Because of large particle size leading to

slow movement and its short half-life, Tc-S appears less desirable than In-111 colloid in these preliminary studies.

Interim Report

The Use Of Iodine-131 Labeled Antibodies in the Detection of Coccidioidomycosis

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

A diagnostic technique for differentiating the pulmonary form of chronic system fungal diseases from pulmonary tuberculosis would be useful. One possible technique is the administration of radioisotope labeled 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 infection were given one of the three following I-131 labeled 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 3, 24, 48, and 96 hours after injection 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 labeled globulin, and nonspecific protein localization in the coccidioidal lesion. Future work will require antibody purification and labeling with higher specific activity as well as reduction of body background.

Final Report

Radioisotope Angiography

V. Bosnjakovic, L.R. Bennett, et al.

A radioisotope angiographic method for the diagnosis of intracardiac shunts has been developed and is under investigation. Visualization of flow through the heart is made with a scintillation camera placed in a modified left anterior oblique position with information stored in a tape system for later replay and analysis. Two radioactive tracers are injected intravenously in rapid sequence: xenon-133 in saline to outline all intracardiac venous blood flow exclusive of the pulmonary venous return, and technetium-99m as the sulfur colloid to visualize all chambers. Time-activity curves obtained from regions-of-interest selected over the heart chambers are obtained during the replay of the tape. Comparison of curves

from the two isotopes makes it possible to identify chambers and the approximate magnitude and location of shunts. The method also appears to be capable of detecting the existence of shunts at the atrial or ventricular level.

Interim Report

Indium-111 EDTA Cisternography

R. Morton, L.R. Bennett, et al.

Indium-111 has been termed the ideal isotope for cisternography by many researchers. This is due to its high photon yield, low energy gammas, lack of beta radiation, and suitable half-life. The use of In-111 labeled transferrin for cisternography has been reported by others. The present study was designed to show that In-111 EDTA would also give high quality scans. It was expected that the whole body dose would be less than either the popular I-131 HSA or In-111 Transferrin since EDTA would be rapidly excreted by the kidney once the complex was absorbed from the cerebrospinal canal. A second part of the study involved quantitative measurements over the head and trunk to estimate a biological half-life. Last, the study was to show that In-111 EDTA in the cerebrospinal canal would be safe for human use.

Indium-111 chloride was complexed with Calcium Disodium Versenate. The pH was adjusted to 7.4 with NaOH and the preparation was autoclaved. A dose of 250 μ Ci was injected into the cisterna magna of 5 dogs, the spinal arachnoid space being too small for easy injection. Scintiphotos were obtained with a gamma camera at 2, 6, 24, 48, and 72 hours, while quantitative measurements were made with a 2-inch scintillation crystal at the same time, with the addition of a count at injection time and at one hour. The crystal was placed 12 inches from the head and 12 inches from the trunk with a lead shield at the neck separating the two areas. The urine was collected and counted to determine the percent dose excreted. Also, cerebrospinal fluid (CSF) samples taken prior to injection and seven days later were analyzed for cells and protein.

The Indium-111 EDTA gave a particularly high ratio of CSF activity to body background, yielding high quality scintiphotos. The scintillation probe counting yielded a biological half-life of about 12 hours and the urine data were in general agreement. For animals in which the Indium-111 EDTA was suspected to have leaked from the subarachnoid space into the circulatory system (extensive bleeding through the spinal needle) the biological half-life was appropriately shorter. The urine data, corrected for decay, showed that 80-90% of the Indium-111 was recovered in the urine by six days post injection. This further substantiates the claim of whole body absorbed dose reduction. The laboratory analysis of CSF showed no evidence of an inflammatory reaction to the Indium-111 EDTA.

It is concluded that Indium-111 EDTA gives high quality scintiphotos and is safe for human use. Quantitative studies using a scintillation probe are useful in estimating turnover rates of the isotope, and are expected to be of diagnostic value when used in humans with interthecal injection. Likewise, urine counting will add some quantitation to cisternography.

Reference: Master's Thesis, "Investigation in the Use of Indium-111-EDTA for Radioisotope Cisternography," University of California, Los Angeles, 1972.

Interim Report

Thrombosis Scanning: A Reliable and Useful Test for Diagnosis of Intravascular Clots

M. Webber, Winona Victery, and L. Resnick

A simple scintigraphic method for diagnosis of deep venous thrombosis in the extremities using Technetium-99m macroaggregates of albumin has been developed (1). The accumulation of the radiopharmaceutical technetium sulfur colloid macroaggregates of albumin (used in lung scanning) on fibrin when injected intravascularly is the basis of the procedure. The clinical usefulness and reliability of the procedure were evaluated in patients suspected of thrombophlebitis and in normal patients. 1) The results of the thrombosis scan correlated with those of lower extremity venography in 10 of 13 patients. Of the three patients with no correlation, all had positive thrombosis scans and negative venography. 2) Sixty patients with clinical evidence of thrombophlebitis or pulmonary emboli, or both, were studied with thrombosis scans followed by lung scans to determine the correlation between the studies. Eighty percent of patients with pulmonary emboli had positive thrombosis scans. Twenty percent of the patients studied had a positive blood clot localization and negative lung scans, which could indicate pre-embolic diagnosis of venous thrombosis. Only 12% of the 60 patients had positive pulmonary emboli and negative thrombosis scans. Numerous patients evaluated by this test demonstrate marked uptake particularly in femoral and pelvic areas. 3) Radionuclide accumulation in 80% of the ninety normal subjects was minimal. A limited amount of uptake (usually in the calf area) occurred in 20% of the normals. Diagnosis of deep vein thrombosis with the scintigraphic procedure described here is a reliable test, correlating well with venography and the presence of pulmonary emboli. Thrombosis scanning may be very useful in bedridden patients in determining the presence of intravascular disease, before clinical symptoms develop. A few positive scans may occur in ostensibly normal individuals; the cause is a matter for speculation.

References: 1) Radiology 100:93-97, 1971
2) J. Nucl. Med. 13:476, June 1972.

Interim Report

Lung Function Studies

H. Morinari and G.V. Taplin

Lung scintigraphy studies in chronic obstructive airway disease, bronchogenic carcinoma and pulmonary embolism are continuing under Dr. Morinari at Harbor General Hospital who has replaced Dr. Isawa as of July 1971. Another continuing project is a study of lung reimplantation and transplantation in dogs - to determine whether or not lung denervation impairs ciliary activity and other lung clearance mechanisms. Preliminary results of two types of lung clearance studies indicate that the denervated lung removes 10-50 μ m size albumin macroaggregates deposited (via bronchial catheter) in the major bronchi in amounts less and at rates slower than from the opposite normal lung. The same statements are true for inhaled radioalbumin aerosol particles in respect to those deposited in the trachea and main airways. However, those reaching the lung periphery are retained for many hours at least.

Interim Report

Inhalation Studies with Submicron Sized Radioalbumin Aerosols

M. Hayes and G.V. Taplin

Two other studies were initiated, each having to do with particle size of radioalbumin aerosols. The first was to devise means for the delivery of submicron sized radioalbumin aerosols. This project was initiated because submicron size aerosols should behave more like inert gases as indicators of air flow and penetrate to certain emphysematous lesions into which larger (1-5 μ) aerosols fail to penetrate although they can be shown to have airway communication following radio xenon inhalation. Progress in this direction has been obtained by use of a heating chamber and/or by alterations in the length and caliber of the tubing and/or by placing additional baffles in the aerosol delivery line to the patient. These maneuvers have also improved the lung images following radioaerosol inhalation in specific respects. First, aerosol deposition in the trachea, major airways and stomach has been nearly eliminated in normal cooperative subjects and is far less of a problem in patients with airway disease. These improvements have not been accompanied by loss of capacity to detect airway abnormalities (as "hot" spots) which is an advantageous feature of aerosols for lung imaging.

Interim Report

Radioaerosol Bronchography Development

G.V. Taplin and K. Lyons

Another related study is the attempted development of Radioaerosol Bronchography (G.V. Taplin and K. Lyons): Such a procedure using relatively minute quantities of test material (1.0 mg albumin aggregates

labeled with 0.5 to 1.0 mCi Tc-99m) could be employed without hazard to many patients in whom standard bronchography using grams of radiopaque materials is contraindicated. By the time of this writing (June 1972) numerous attempts to deliver 5-10 μ microaggregates of albumin by inhalation as an aerosol have failed. Ultrasonic nebulizers reduce the size of the particles to 2.0 μ or less during the 5-10 minutes of the inhalation procedure. Mechanical nebulizers (Bennett and Bird types) have the same effect and while giving excellent lung parenchyma images they do not increase large airway deposition as hoped and expected. Albumin microspheres (5-10 μ) are not disrupted by high pressure or ultrasonic nebulization but these large particles fail to deposit in the trachea. They are captured in the delivery system by impaction and only the small particles reach the lungs - and these fail to deposit in sufficient quantities in large airways to permit visualization. Other methods and agents are under study.

Interim Report

Appearance of the Lung Scan in Normal Individuals

M.M. Webber, Winona Victory, L. Resnick, and F. Ben-Issac

This report describes the lung scans obtained in a population with no evidence of pulmonary or other disease. A fraction of these subjects demonstrated perfusion defects. In order to determine if such defects correlate with pulmonary function tests, an equal number of subjects who had normal or abnormal scans were followed up with pulmonary function tests, including arterial blood gas determinations and spirometric tests.

The study group consisted of 46 volunteers aged 21 to 34 years (32 males, 14 females). All were nonsmokers. Each when interviewed denied any history of asthma, emphysema, or other lung disease. Lung images in four views were obtained using Technetium-99m macroaggregates of albumin, on an Ohio Nuclear dual probe rectilinear scanner. The films were evaluated independently by three physicians. The scans were considered to be abnormal in 10 of the 46 subjects (22%). Thirteen percent exhibited various perfusion defects, 4% of which would have been difficult to distinguish from a pulmonary embolus. These abnormalities included two patients with crescent-shaped defects, two with apical perfusion defects, one with a wedge-shaped defect, and one with a basilar defect. Nine percent demonstrated other abnormalities; e.g., loss of the normal perfusion gradient, hyperexpanded lungs. Six subjects from both the normal and abnormal scan groups were referred for physical examination, arterial blood gases, and spirometric tests, by a pulmonary function specialist who was unaware of the scan results.

In conclusion, the pulmonary function test results do not explain the variation from the "normal" lung scan in the majority of these volunteers. Many of these scan abnormalities may be entirely unrelated to any pathologic

process. Interpreters of lung scans should consider the possibility of variants in their concept of the normal. Whether or not the variant scans may predict future pulmonary disease can only be investigated with long-term followup.

Reference: J. Nucl. Med. 13:476, June 1972.

Interim Report

Liver and RES Studies

K. Kitani, G.V. Taplin, and H. Morinari

Rapid Liver Accumulation of Human Serum Albumin Sensitized Dogs. This investigation was undertaken to determine the fate of heterologous human serum albumin (HSA) solutions, when injected into dogs previously sensitized to this commonly used radiopharmaceutical agent. However, either as colloidal size (10-20m μ) or 1-5 μ microaggregate suspensions, HSA is rapidly removed from the blood in dogs and man ($T_{1/2}$ = 2-3 min.) while it accumulates in the phagocytic cells of the liver-spleen and bone marrow (RES organs). In previous (1965) toxicity studies in dogs to 10-80 μ albumin macroaggregate suspensions, tracer doses (<0.1 mg/kg) were not sensitizing, but single large doses (20 mg/kg or more) nearly always produced immediate, severe anaphylactoid reactions when administered 7-10 days or longer after the sensitizing dose.

Following radio-HSA injections, blood samples were taken at frequent intervals for 3 hours and were assayed for radioactivity content. The heart and liver areas were monitored during the first 15 minutes. The distribution of radioactivity in the heart, liver, and spleen, was visualized sequentially by γ camera imaging.

In normal dogs, HSA remains in the circulation during the initial 3-hour period and the heart and liver areas are visible as blood pools only. Repeated 1.0 mg doses of HSA and single doses of 10 mg/kg failed to alter the blood disappearance rates of HSA or to produce observable reactions. However, in dogs given single 20 mg/kg or thrice repeated 10 mg/kg doses, both the I-131 and Tc-99m HSA solutions were rapidly removed from the blood and found in the liver at 15 minutes by camera imaging. At 3 hours, Tc-99m had entered the gallbladder as previously found and reported with Tc-99m albumin microaggregate suspensions. For control purposes, in similarly (HSA) sensitized dogs, the fate of canine serum albumin I-131 was not altered and no reactions were observed, in contrast to the anaphylactoid type reactions encountered in sensitized dogs.

In conclusion, the rapid accumulation of labeled HSA solutions in the liver of dogs previously sensitized to heterologous HSA is most probably caused by their immediate conversion in the blood to particulate antigen-antibody (albumin-globulin) complexes which are removed by the RES organs

by phagocytosis, as are other particulates, such as carbon particles, bacteria, and viruses. The catabolic pathway of this albumin-globulin complex following initial Kupffer cell trapping, appears to be quite similar to that of Tc-99m albumin microaggregates, which are also initially engulfed by the RE cells degraded to smaller molecular size, transferred to the hepatic cells and then excreted in the bile. Finally, the grossly altered behavior and metabolic fate of HSA solutions when injected into sensitized dogs is of both practical and scientific interest. It could give rise to serious pitfalls in animal studies with this agent, but more importantly, these findings warrant further investigation and confirmation at the cellular level through immunologic and autoradiography studies now in the planning stage.

Reference: J. Nucl Med. 13:442-443 (June 1972) (Abstract).

Metastatic Liver Disease - Correlative Studies

G.V. Taplin, K. Lyons, R. Gray, and E. Terao

A collaborative project has been initiated at the UCLA Center for the Health Sciences - to study various liver lesions by three separate procedures namely; I.V. liver scans with Tc-99m sulphur colloids, hepatic arteriography followed by liver scanning after injection of Tc-99m albumin macroaggregates (MAA) in the catheterized hepatic artery.

These three procedures complement each other and when used in selected patients wherein such information is vital to proper management, they are hoped and expected to improve diagnostic accuracy and to aid in subsequent treatment. Once the patient has been catheterized and studied angiographically, the added scan following arterial MAA injection adds no significant risk while providing extremely useful information regarding the distribution of the liver's arterial blood supply on a regional basis. Such information is of particular value in locating small areas of increased arterial flow not detectable by either routine scanning or hepatic angiography. The triple technique should definitely aid the diagnosis of both primary and metastatic liver tumors because they are almost exclusively supplied by the hepatic artery. Such tumors should give findings distinctly different from cysts and abscesses which are generally devoid of blood supply, either arterial or portal venous. Initial clinical results appear most promising.

Interim Report

Kidney Function Studies

M. Hayes, T.C. Moore, and G.V. Taplin

The kidney transplant studies at Harbor General Hospital during fiscal year 1972 have been presented at national meetings and described

in two publications and in another paper in the J. Radiology (June 1972). The latter is entitled "Radionuclide Procedure in Predicting Early Renal Transplant Rejection".

Radionuclide procedures generally are more sensitive and responsive indicators of sudden changes in kidney function and blood flow than are serum chemistry levels, which require time to reach new equilibrium states. Furthermore, the radionuclide tests, particularly the sequential kidney-bladder imaging procedure, provide evidence for or against intra and post renal obstruction, information which is not given by standard tests of kidney function.

Finally, in the management of kidney transplant recipients, radionuclide procedures, especially the B/K ratio, derived from the sequential kidney-bladder camera imaging procedure, are considered to be more sensitive and reliable than other methods for obtaining objective evidence of transplant dysfunction in the pre-rejection period.

Reference: Radiology 103:627-631, June 1972.

DIVISION OF RADIOBIOLOGY

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Staff

Area of Interest

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Radiation Effects on DNA and
Their Modification

Bennett, Leslie R., M.D.

Nuclear Medicine

Byfield, John E., M.D.

Radiation Biology
Clinical Oncology

Kay, Ernest, Ph.D.

Gamma Radiation Effect on
Protein

Lee, Y. C., Ph.D.

Radiation Biology
Nuclear Metabolism

Strickland, E. Hardin, Ph.D.

Biological Applications of
Circular Dichroism

Ward, John F., Ph.D.

Radiation Biochemistry of DNA

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Lewis, Hazel, Ph.D.

Weintraub, Isaac, M.D.

Rubin, Herbert, M.D.

Graduate Students

Fretto, Larry

Lucas, Joseph Nathan

Loksen, Salmen

Mc Creary, Nancy

RADIATION CHEMISTRY OF NUCLEIC ACID CONSTITUENTS

Nanosecond Pulse Radiolysis Studies of Aqueous Thymine Solutions

Leslie M. Theard, Frank C. Peterson, and L. S. Myers, Jr.

Nanosecond pulse radiolysis of aqueous thymine solutions has been studied by producing optically detectable concentrations of transients with 10-nsec electron pulses from a Linac and employing an optical system with a signal rise time of 5 nsec. In deaerated 10^{-2} M thymine solutions containing 1 M ethanol at pH 6.0, thymine radical anion, T^- ($\lambda_{\max}$ 325 nm), is present at the end of the pulse and its decay over the subsequent 10^{-6} sec is only slight. At pH 4.0, T^- decays exponentially with a half-life of ca. 10^{-7} sec, and upon decreasing the pH to 3.5 the decay half-life decreases in proportion to the increase of acid concentration, suggesting that T^- decays via $T^- + H_3O_{aq}^+ \rightarrow TH + H_2O$ with a reaction rate constant of $(6.4 \pm 0.3) \times 10^{10} \text{ M}^{-1}\text{sec}^{-1}$. The slow decay of T^- at pH 6.0 suggests that for the reaction $T^- + H_2O \rightarrow TH + OH^-$, $k \leq 10^4 \text{ M}^{-1}\text{sec}^{-1}$. An absorption signal presumed attributable to TH is present following decay of the T^- signal, with $\lambda_{\max}$ also at 325 nm and an intensity about half that of T^- . Formation of the H-atom adduct of thymine, TH' , produced via the reaction of $H + T \rightarrow TH'$ was observed to occur during the period 10^{-8} to 10^{-6} sec following the pulse for deaerated 10^{-2} M thymine at pH 0.65. The rate constant for TH' formation is $(6.8 \pm 0.2) \times 10^8 \text{ M}^{-1}\text{sec}^{-1}$, and the absorption spectrum ($\lambda_{\max}$ 400 nm) of TH' is different from that of TH. The data suggest that H-atom addition to thymine occurs at the 5,6 double bond while protonation of T^- occurs elsewhere. The submicrosecond decay of T^- and TOH resultant from their reactions with oxygen was observed; rate constants for the reactions are 4.3×10^9 and $1.9 \times 10^9 \text{ M}^{-1}\text{sec}^{-1}$, respectively.

Reference: J. Phys. Chem. 75, 3815-3821 (1971).

Transfer of Electrons from Thymine Anion Radical to 5-Bromouracil

L. S. Myers, Jr., Aida Warnick, Margie Gruca, and L. M. Theard

It has been suggested that one mechanism by which substitution of 5-bromouracil (BU) for thymine (T) in DNA causes sensitization to radiation involves transfer of electrons from other bases to BU (Zimbrick, Ward, and Myers, Int. J. Rad. Biol. 16, 505 (1969)). We have observed this reaction in a model system by pulse radiolysis. Radiolysis of thymine in dilute aqueous argon saturated solutions containing 1 M t-butanol produces hydrated electrons, e_{aq}^- , which

react with thymine to give thymine anion radical ($T^{\cdot-}$). This substance has an optical absorption maximum at ~ 325 nm and with doses of 1000 rads, a half-life of ~ 60 μ sec. Radiolysis of BU under similar conditions produces uracil which does not absorb at 325 nm. (Hydrated electrons react by dissociative electron attachment with BU to give uracilyl radical which immediately abstracts hydrogen from the butanol to give uracil.) Radiolysis of solutions containing mixtures of T and BU in proportions such that e_{aq}^- reacts mainly with T, gives an absorption with λ_{max} 325 whose intensity is consistent with competition for e_{aq}^- by T and BU, and whose half-life decreases with increasing BU concentration. The decrease in half-life is believed to be due to transfer of electrons from $T^{\cdot-}$ to BU. Similar experiments under conditions such that $\cdot OH$ instead of e_{aq}^- attacks T and BU do not show a similar dependence of decay rate on BU concentration.

Reference: Abstracts, Biophysical Society Annual Meeting, Toronto, Canada, (Feb. 1972), p. 229a.

Electron Spin Resonance Studies of Free Radicals in Irradiated Single Crystals of 5-Methylcytosine

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

Exposure of single crystals of 5-methylcytosine to ionizing radiation gives rise to electron spin resonance (E.S.R.) spectra which at room temperature can be explained as resulting predominantly from two chemically distinct stable free radicals produced in the crystals. One is formed by addition of a hydrogen atom to the 6-position of the pyrimidine ring. Its essentially isotropic hyperfine spectrum is generated by interaction of the unpaired electron in a 2p-orbital of carbon atom C(5) with three equivalent protons of the 5-methyl group and two non-equivalent protons attached to carbon C(6), all protons being in the β -position. The second radical results from removal of a hydrogen atom from the 5-methyl group. The unpaired electron of this radical is localized mainly in 2p-orbitals of the methyl carbon and of ring carbon C(6) giving rise to a hyperfine spectrum from interaction with the two remaining α -protons of the methyl group and the α -proton attached to C(6). All couplings are anisotropic with principal values of $A_x = 24.9$, $A_y = 16.3$ and $A_z = 8.6$ gauss for the methyl protons and $B_u = 12.5$, $B_v = 8.6$ and $B_w = 2.1$ gauss for the C(6)-proton. Measurements at low temperatures reveal the presence of a third type of radical featuring a doublet ESR resonance which is tentatively assigned to the radical anion of 5-methylcytosine. These findings are discussed in connection with results reported for related compounds.

Reference: Int. J. Radiat. Phys. Chem. 3, 117-129 (1971).

RADIATION BIOCHEMISTRY OF NUCLEIC ACIDS AND THEIR CONSTITUENTS

A Molecular Mechanism for 5-Bromouracil Radiosensitization

J. F. Ward and J. D. Zimbrick

We have previously postulated a mechanism for 5-bromouracil (BU) radiosensitization (Zimbrick et al., Int. J. Rad. Biol. 16, 505, 1969). The hydrated electron, produced by irradiation of water, reacts with BU forming a bromide ion and a uracilyl radical. The latter can react further by abstracting a hydrogen from a C—H bond. BU sensitizes because, in contrast to the other DNA bases, it reacts irreversibly with electrons. Two aspects of this mechanism have been further investigated. A. Other radiation induced radicals have been examined for their ability to donate an electron to BU. Reaction with BU was monitored by measuring bromide yields with an ion specific electrode (Orion Model 94-35). The hydrated electron adducts of cytosine and thymine can donate electrons to BU, the reaction is rapid occurring at a diffusion controlled rate. The radical ions CO_2^- and O_2^- also react with BU. B. The subsequent reactions of uracilyl radicals have been examined: Uracilyl radicals can abstract hydrogen atoms from the deoxyribose moiety of deoxynucleotides. This hydrogen abstraction leads to breakage of the sugar phosphate bond and is therefore equivalent to a DNA strand break. (The uracilyl radical formed by photolysis (257 nm) of BU reacts in a similar way.) These findings suggest that BU is a focus for reducing species damage in DNA and that the damage is in the form of a strand break.

Reference: Abstracts, Biophysical Society Annual Meeting, Toronto, Canada, (Feb. 1972), p. 230a.

γ -Radiation of Aqueous Solutions of Thymidine 3'5' Diphosphate (pTp): A Model System for Strand Breaks in DNA.

J. F. Ward and I. Kuo

In this model system radiation induced release of inorganic phosphate (iP) is considered equivalent to strand break production. On irradiation of an oxygenated solution of pTp (0.5 mM) $G(\text{iP})$ measured immediately is 0.20. $G(\text{iP})$ increases to 0.4 in 8 hrs and 0.55 in 45 hrs. If the solution is cooled to 0°C or adjusted to pH 4, there is no post-irradiation increase in iP but in 1 NaOH $G(\text{iP})$ increases to 1.5 in 8 hrs and 2.2 in 45 hrs. Low yields ($G < 0.1$) of labile phosphate esters (LPE) were obtained if pTp was irradiated in N_2 or if thymidine 5' monophosphate (TMP) was irradiated. The immediate yield of phosphate corresponds to the release of two phosphate ions

from one molecule of pTp. The resulting product is thymine substituted in the 1N position. Breakdown of the LPE does not lead to an increase in any product containing the 2 ^{14}C label of pTp. A volatile product containing ^{14}C was formed on irradiation of 2 ^{14}C pTp or TMP in oxygenated solution but not in nitrogen. Thus the LPE are formed as a consequence of base damage and release the 3' phosphate (low yield of LPE from TMP). This model predicts: (1) Immediate strand breaks in DNA release a base moiety leaving both a 3' and a 5' phosphate end group; (2) Base damage produced LPE which decay, breaking only the 3' sugar phosphate bond.

Reference: Abstracts, Radiation Research Society Annual Meeting, Portland, Oregon (May, 1972) p. 30.

Radiation Induced Damage to DNA - Model Systems Used to Investigate Molecular Mechanisms

John F. Ward

Dilute aqueous solutions of 5' deoxynucleotides and thymidine 3'5' diphosphate (pTp) have been used as model systems for the investigation of the mechanism of strand break production in DNA. Radiation induced reactions which lead to breakage of the sugar-phosphate bond are equated to strand break producing events. The yields of inorganic phosphate were correlated to the various products of radiation which were separable on an anion exchange column. Typical results indicate the formation of an immediate yield of inorganic phosphate $G(\text{iP}) = 0.2$ caused by reaction of hydroxyl radicals with the sugar moiety of the solute pTp. If oxygen is present during irradiation labile phosphate esters (LPE) are formed. Some LPEs are unstable at neutral pH ($G = 0.35$) while others decay only in alkali ($G = 1.9$). These LPE appear to be formed by radiation induced damage to the base moieties of solutes. The implications of these data to measurements of strand breaks produced in DNA will be discussed.

Reference: Abstracts, American Chemical Society Symposium on "Biochemical Effects of Radiation, Fort Collins, Colorado (June, 1972), p. 8.

Damage to DNA at Low Radiation Doses

John F. Ward and I. Kuo

In previous work (J. F. Ward and I. Kuo, Int. J. Rad. Biol. 18, 381-390 (1970)) we examined the effect of chloride ions on the radiation induced destruction of the base moieties of DNA. The DNA was

irradiated in aqueous oxygenated solution at pH 7. Base damage was measured as the decrease in ultraviolet absorption spectrum after heat and formaldehyde denaturation. For native DNA there is always a lag in the destruction of ultraviolet absorption, no appreciable base damage occurs until ~ 5 krad (0.015% DNA). This leads us to the hypothesis that some preliminary damage to DNA is necessary before OH $\cdot$ free radicals (produced from irradiation of the water) are capable of attacking the base moieties. This hypothesis is supported by pulse radiolysis work (L. S. Myers, Jr., Personal Communication) in which it was shown that the absorption of the DNA-OH $\cdot$ transient radical increases markedly with repeated radiation pulses.

In order to test the above hypothesis we have treated DNA with DNase (0.4 μ gm/ml) for various lengths of time, thus introducing "nicks" into the DNA backbone. These "nicks" should simulate the radiation induced strand breaks and permit the free ends of the DNA in the neighborhood of the "nicks" to open out allowing OH $\cdot$ radicals to react with the base moieties. After DNase treatment the lag in base damage was reduced, and eventually removed completely (5 hours of enzyme treatment at room temperature). The number of "nicks" produced in this time approximates the number of strand breaks produced by radiation during the lag. The significance of this result "in vivo" is far reaching. Enzymatic processes may be capable of repairing the strand breaks produced at low doses, but other repair systems will be called on in order to repair base damage produced at higher levels of radiation.

Interim Report

RADIATION BIOCHEMISTRY OF PROTEINS AND AMINO ACIDS

Absorption and Circular Dichroism Spectra of Ribonuclease-S at 77 $^{\circ}$ K Joseph Horwitz and E. Hardin Strickland

The absorption and circular dichroism spectra of RNase-S and RNase-A were compared in the near ultraviolet at 77 $^{\circ}$ K. The vibronic structure which emerges at 77 $^{\circ}$ K is different in the two enzymes between 280 and 293 nm. Analysis of the vibronic structure shows that a single tyrosine residue (O-O transition at 288.5 nm in RNase-A) has its bands shifted by approximately 2.5 nm to shorter wave lengths in RNase-S, indicating a change in the environment of this tyrosine residue in RNase-S. The x-ray data available on RNase-S strongly suggest that this residue is tyrosine 25.

Reference: J. Biol. Chem. 246 (11), 3749-3752 (1971).

Effects of Hydrogen Bonding and Temperature Upon the Near Ultraviolet Circular Dichroism and Absorption Spectra of Tyrosine and O-Methyl Tyrosine Derivatives

E. Hardin Strickland, Meir Wilchek, Joseph Horwitz and Carolyn Billups

To gain information about the properties of tyrosyl residues buried within proteins, the circular dichroism (CD) and absorption spectra of tyrosine derivatives have been investigated in nonpolar solvents. N-Stearyl-L-tyrosine n-hexyl ester dissolved in methylcyclohexane (O-O band at 283 nm) was used to measure the effects of hydrogen-bonding agents. Adding low concentrations of dioxane, N,N-dimethylacetamide, 1-butanol, or methanol causes a 1- to 4-nm red shift in the absorption spectrum and a 10 to 25% increase in the dipole strength. The results with N,N-dimethylacetamide suggest that a hydrogen bond between a tyrosyl hydroxy group and a carbonyl oxygen of the peptide backbone may be one mechanism for producing a large red shift in proteins.

CD spectra were recorded after the hydroxy group of N-stearyl-L-tyrosine n-hexyl ester had been hydrogen bonded. Dioxane and N,N-dimethylacetamide cause the CD spectra to red shift and intensify to the same extent as do the absorption spectra. Evidently hydrogen bonding to these compounds does not alter the conformation of this tyrosine derivative. In contrast, hydrogen bonding of N-stearyl-L-tyrosine n-hexyl ester to butanol or methanol causes a 50% loss of rotatory strength, suggesting an altered conformation. The dependence upon alcohol concentration is the same for both the CD and absorption alterations (half-maximal effect at 30 mM alcohol). Evidence is presented that a polymeric form of the alcohol may simultaneously hydrogen bond to both the hydroxy group and the amide oxygen atom of N-stearyl-L-tyrosine n-hexyl ester.

At concentrations greater than 15 μ M, N-stearyl-L-tyrosine n-hexyl ester is partially aggregated in methylcyclohexane, causing changes in the CD and absorption spectra. The aggregate is proposed to be mainly a dimer in which the hydroxy group of each tyrosine residue is hydrogen bonded to the amide oxygen of the other residue ($K \approx 1000 \text{ M}^{-1}$).

To minimize aggregation, N-acetyl-O-methyl-L-tyrosine ethyl ester (N-Ac-O-Me-L-Tyr ethyl ester) and N-stearyl-O-methyl-L-tyrosine n-hexyl ester were studied. O-Methyl tyrosine derivatives have the same vibronic structure as does tyrosine, but the wave length position is not much shifted by polar organic solvents (O-O band at $283.5 \pm 0.5 \text{ nm}$). The rotatory strengths of O-methyl-L-tyrosine derivatives are nearly identical with those of L-tyrosine derivatives in corresponding

solvents. For N-Ac-O-Me-L-Tyr ethyl ester dissolved in methylcyclohexane at 297 K, the rotatory strength is especially large (1.3×10^{-40} c.g.s. with $\Delta\epsilon = 0.75 \text{ M}^{-1}\text{cm}^{-1}$ at 277 nm).

Upon cooling N-Ac-O-Me-L-Tyr ethyl ester (dissolved in ether-isopentane-ethanol solvent) from 297 to 140 K, the negative rotational strength is intensified 10-fold. These results are interpreted in terms of temperature shifting the equilibrium distribution of the various conformers of N-Ac-O-Me-L-Tyr ethyl ester. The variable temperature CD technique may provide a way to detect motility of the tyrosyl side chains in proteins.

Reference: J. Biol. Chem. 247 (2), 572-580 (1972).

Radiation Effect on Lysozyme

Ernest Kay

The biological function of proteins depends on their conformation. As changes in circular dichroism (CD) and absorption spectra reflect an alteration in protein conformation, the CD and absorption spectra provide means for analyzing conformational changes induced by ionizing radiation. The reaction of the radical anion $(\text{CNS})_2^-$ is highly selective towards tryptophan. When a dilute lysozyme solution, saturated with N_2O and O_2 , is exposed to Co^{60} gamma-irradiation in the presence of KCNS , the enzymatic activity of the lysozyme decreases. The radiation damaged lysozyme has an appreciably different near ultraviolet absorption spectrum and diminished tryptophanyl CD bands. We are presently attempting to purify the individual products of radiation damage so that specific lesions may be characterized in detail. The affinity chromatography is an ideal tool for such purpose because the damaged products have different affinity for substrates and inhibitors from the native lysozyme. Thus far 3 fractions have been isolated from irradiated lysozyme. Two of these are minor products at low doses of γ -rays and lacking enzymatic activity. They have different electrophoretic mobilities and gel-filtration elution volumes from native lysozyme. The major fraction has approximately the same elution volume and electrophoretic mobility as native lysozyme. The enzymatic activity of the major product, however, is only half that of the native enzyme. The degree of heterogeneity of the major product in the irradiated lysozyme has not been determined.

Interim Report

CELLULAR AND MAMMALIAN STUDIES IN RADIATION BIOLOGY

Neuroblastoma Differentiation in Vitro

J. E. Byfield and Ivana Klisak

Additional experiments have been performed on the Murine C-1300 neuroblastoma cell line which can be induced to differentiate in vitro by number of manipulations. We have primarily compared the effects of various inhibitors of DNA replication as analyzed by tritiated thymidine incorporation. A direct correlation exists between the ability of each agent to induce differentiation versus their ability to inhibit DNA synthesis. These parameters include cell poisons such as cytosine arabinoside, Bleomycin, Mitomycin-C, methotrexate, etc., as well as physical manipulations such as x-rays. It is the conclusion of the study thus far that the C-1300 cells are phenotypically differentiated and the inducing agents only block cell cycling, thereby allowing morphogenesis to be more completely expressed. The cell line appears, however, to have considerable usefulness in the analysis of repair of single strand breaks in non-cycling cells. We are currently studying this aspect using single strand rejoining assays and unscheduled DNA synthesis using radiographic techniques. If successful, it will be possible to study repair in cells as they pass from the cycling stages into noncycling stages. Since the cells can be exposed to cytosine arabinoside and will remain differentiated and viable for substantial periods of time, it should be possible to determine whether there is a decline in radiation repair ability in the absence of repeated cycles of DNA synthesis. This would have obvious relevance to normal cells in vivo such as neurons, myogenic cells etc. In addition, the C-1300 system has been analyzed for the presence of tumor-specific transplantation antigens. It has been determined that these cells can elicit a weak syngeneic antitumor response in A/J mice and a stronger response in F-1 hybrids of related strains. Using in vitro cytotoxicity assays it is possible to show that the differentiated cells continue to express the tumor-specific transplantation antigens. This shows that morphologic and functional differentiation does not preclude the expression of some parts of a malignant genome. In addition, visual estimates of the A-type RNA virus present in these cells suggests that during differentiation there is a suppression of viral multiplication since the numbers do not appear to increase despite persistence of the virus in the differentiated cells. These studies are being continued because of the possible relevance of this information to the analysis of the human neuroblastoma.

Interim Report

In Vitro Determination of Radiosensitization

Y. C. Lee, J. E. Byfield

Efforts to develop a new screening procedure for the identification and elaboration of radiosensitizing drugs have been continued. Utilizing the L-1210 murine leukemia strain, it has been possible to identify the inhibitory effects of several useful chemotherapeutic agents on repair replication. Different assays of radiation repair have been measured including non-conservative replication, single strand rejoining, inhibition of net thymine incorporation, inhibition of net thymine incorporation in cells exposed simultaneously to cytosine arabinoside, and inhibition of normal semi-conservative replication identified on alkaline CsCl gradients. The evidence to date indicates that the various chemotherapeutic agents differ widely in their ability to inhibit repair as measured by these different assays. In general, those drugs which have indicated some usefulness clinically appear to be the best inhibitors, regardless of the type of assay used to study repair. Extensive efforts were made to reduce the radiation dose required to elicit repair of single strand breaks. Using a newly modified alkaline sucrose gradient technique it has been possible to demonstrate single strand breaks and their subsequent repair following doses as low as 200 rads. Several of the useful chemotherapeutic agents can be shown at high concentrations to inhibit this type of single strand repair, but only actinomycin D is useful at meaningful concentrations. These results indicate that the national screening efforts aimed at elaborating chemotherapeutic agents are probably not developing the most useful agents in terms of radiosynergism. The screening assay now developed can probably be used to improve upon this situation. In addition, we have developed several malignant human cell lines which should be useful in comparing the inhibition of single strand repair to radiation repair measured by the Elkind-Sutton method of split dose repair analysis. During the forthcoming year we plan to evaluate additional drugs, comparing inhibition of single strand repair to inhibition of colony formation.

Interim Report

Thermal Bone Marrow Expansion

J. E. Byfield, I. Klisak, L. R. Bennett

Experiments have been conducted studying the effect of elevated ambient temperature on the induction of new bone marrow in the peripheral limbs and tails of rodents. Elevation of the temperature from 25°C to 34°C causes the transformation of fatty bone marrow to erythropoietic red marrow. This process begins within one day and

reaches a peak at approximately one month. Techniques for studying this have been worked out and include quantitative ^{59}Fe uptake, ^{59}Fe radioautography, and stem cell analysis using clone formation in syngeneic mouse spleens. The results indicate that the stem cells responsible for thermal marrow expansion are located in the tail itself and are present from birth. The neonatal mouse contains functional red marrow in the tail which regresses to the fatty state immediately after birth. By one month it has reached an active state, but can be reactivated by temperature elevation. Local origin of the stem cells has been confirmed by split dose experiments and by the use of rat/mouse chimeras. Using an alkaline phosphatase stain specific for rat cells it has been shown that the induction of thermal marrow expansion in the mouse/rat chimeras involves host mouse cells rather than rat donor cells. To arrange a satisfactory split dose technique using the mouse tail, it was found that the tails could be rendered hypoxic and therefore radioresistant by placing a ligature at the base. Such ligated mice undergo an increase in radiation resistance; all of the non-hypoxic marrow could be deleted by total body radiation. Only the hypoxic tail marrow remains viable. Using this resistance test, it was found that a split dose had no effect on a resistance conferred by tail marrow even if during the period between the split the ligature was removed in order to wash out potentially circulating stem cells. This type of experiment therefore confirms the results of the histologic examinations. Thermal marrow expansion offers some obvious applications in Radiation Therapy in humans where extensive radiation to the bone marrow is required. In addition, it may be useful in extending the range of chemotherapeutic agents which are marrow toxic. These potentialities will be studied further in the next year.

Interim Report

REVIEWS ON RADIATION INDUCED CHEMICAL CHANGES IN NUCLEIC ACIDS LEADING TO BIOLOGICAL DAMAGE

Radiation Damage in Living Organisms: Initial Chemical Changes and Their Modification

Lawrence S. Myers, Jr.

Several convincing lines of evidence suggest that damage of DNA is a major factor in the responses of living organisms to ionizing radiation. The initial damage can occur when energy from radiation is deposited directly in DNA, or when reactive species produced by energy deposition in water diffuse to DNA and react with it. The

result is formation of DNA free radicals. These radicals can be conveniently studied in vitro with the aid of model compounds: purine and pyrimidine bases, nucleosides and nucleotides. (DNA can be regarded as a linear phosphodiester polymer in which the carbon atoms of the main chain are parts of a deoxyribose unit, and a purine or pyrimidine base is attached to each deoxyribose to form a short side chain.) Hydrogen atoms and hydroxyl free radicals, produced from water, add to the bases and abstract hydrogen from the deoxyribose units to give free radicals on the portion attached. The yields of base radicals are approximately proportional to the relative amounts of each base in the nucleic acid. These radicals are stable with respect to rearrangement, and in pure aqueous solutions decay much more slowly than do radicals on small molecules. The principal results of this kind of damage are formation of altered bases and breaks in the main chain. Either result may have serious consequences for living organisms. Free radicals formed by deposition of energy directly in DNA have not been characterized with certainty; temperature, base composition, moisture content, and possibly secondary structure appear to be important parameters, but their role has not been defined precisely. Proposed chemical mechanisms for the sensitization of nucleic acids to radiation by 5-bromouracil and N-ethylmaleimide and for protection by sulfhydryl compounds will be described. In addition, the relevance of this work to the overall biological response to radiation and attempts to protect mammalian organisms from harmful effects of radiation will be discussed.

Reference: Transactions, American Nuclear Society, Las Vegas, Nevada, 15 (1), 449 (June, 1972) (Abstract).

The Radiation Chemistry of Nucleic Acids
Lawrence S. Myers, Jr.

This was a refresher course which covered effects of ionizing radiation on nucleic acids and nucleic acid constituents (purine and pyrimidine bases, nucleosides, and nucleotides), and reactions by which radiation effects may be modified. Recent contributions of pulse radiolysis and electron paramagnetic resonance spectroscopy were emphasized.

Reference: Abstracts, Annual Meeting of Radiation Research Society, Portland, Oregon (May, 1972) p. 66, Fa-1.

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105. Wolfgram, F., Mona E. Fewster, and James F. Mead: Lipids and Amino Acids of Multiple Sclerosis Myelin. In: Symposium on Selected Topics in Human Chemical Neuropathology, W. Tourtellotte & L. Amaducci (ed.), Organo Societa Italiana Di Neurologia, Firenze, Italy (1972).
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SCIENTIFIC EXHIBITS

1. Gates, G. F., H. W. Orme, E. K. Dore, and G. V. Taplin. S-28 Measurement of Cardiac Shunting in Cyanotic Children. SNM 19th Annual Meeting, July 11-14, 1972, Sheraton-Boston Hotel, Boston, Mass. (Scientific Exhibit).
2. Webber, M. M., and J. Goodrich: The Effect of Aluminum on Technetium Sulfur Colloid. Am. J. Roent., Rad. Ther. and Nucl. Med. 113:4:791-792 (December 1971) (Abstract of Exhibit).

HONORS AND AWARDS

James F. Mead, Ph.D.

1971 recipient of the Alton E. Bailey Award in recognition of his exceptional contribution in the elucidation of the metabolism of polyunsaturated fatty acids. Awarded by the North Central Section of American Oil Chemists' Society.

Stafford L. Warren, M.D.

Dr. Stafford L. Warren and Dr. Shields Warren were selected jointly for the Enrico Fermi Award for 1971. The award consisted of \$25,000 and a gold medal and citation for each. The citation read as follows: "For the imaginative, prescient and vigorous efforts of Dr. Shields Warren and Dr. Stafford Warren, independent yet coordinated, which made possible the early development of atomic energy so as to assure the protection of man and the environment, and for the establishment of a biomedical research program, which has resulted in many substantial applications of ionizing radiation to diagnosis and treatment of disease and to the general welfare." Awarded by the Atomic Energy Commission in Washington, D.C., May 18, 1972.

Special Recognition

J. C. Beatley, Ph.D.

Member of the Editorial Board, Ecology (1969-71).

Leslie R. Bennett, M.D.

Visiting Professor, University of Cincinnati, November 1971.

Speaker, Oak Ridge Symposium, November 1971.

Guest Speaker, Argentine Society of Nuclear Medicine, October 1971.

J. de Vellis, Ph.D.

Elected: President, Neurochemistry Club, Los Angeles.

Elected to Membership in International Society for Neurochemistry and American Society for Neurochemistry.

Review Committee, Committee for Advanced Science Training (CAST), California Museum of Science and Industry.

Govind Dhopeswarkar, Ph.D.

Invited Speaker, Patel Chest Institute, University of Delhi, India, and at the Radiation Medicine Centre, Parel, Bombay, June 1972. Topic: Transport and Turnover of Lipids in the Brain.

E. B. Edney, Ph.D.

Invited Speaker, Plenary Session of the Entomological Society of America, Los Angeles, December 1971.

L. E. Gerschenson, M.D.

Invited Speaker, American Cancer Society, 1st Workshop in Liver Cell Culture, McArdle Institute for Cancer Research, University of Wisconsin, Madison, Wisconsin, June 1972.

Outside Reviewer of grants for the American Cancer Society, California Division, California Cancer Institute, UCLA and National Institute of Health.

Isaac Harary, Ph.D.

Member of Editorial Board, Journal of Molecular and Cellular Cardiology.

Invited Speaker - University of Miami, April 1972.

Esther F. Hays, M.D.

Invited Speaker, Pennsylvania State Conference on Virus Tumorigenesis and Immunogenesis in April 1972. Review article to be published with Proceedings of the Conference, by Academic Press.

H. R. Herschman, Ph.D.

Invited Speaker, Department of Psychiatry, Washington University School of Medicine, St. Louis, Mo.

Invited Speaker, Department of Microbiology, University of Connecticut Medical School, Farmington, Connecticut.

O. R. Lunt, Ph.D.

Teaching Participant for the Regional Training Course on the Application of Nuclear Techniques in Plant Biochemistry with Reference to Protein, organized by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations, in cooperation with The Government of Colombia, at the Instituto de Asuntos Nucleares, Bogota, Colombia, November 2 to December 3, 1971.

James F. Mead, Ph.D.

Member, Editorial Board, Journal of Neurochemistry.

Invited Participant at Ciba Conference on "Nutrition and Brain Development" London, England, October 1971.

University Lecturer, University of London, October 1971.

Chairman, Sessions on Biochemistry at Joint Meeting of Japan Oil Chem. Soc. and American Oil Chem. Soc., Los Angeles, California, April 25, 1972.

Lawrence S. Myers, Jr., Ph.D.

Appointed Guest Scientist, Chemistry Department, Brookhaven National Laboratory, 1971 -

Invited Paper: Radiation Damage in Living Organisms: Initial Changes and Their Modification, meeting of the American Nuclear Society, Las Vegas, Nevada, June 1972.

Invited to give Refresher Course on "The Radiation Chemistry of Nucleic Acids", Meeting of the Radiation Research Society, Portland Oregon, May 1972.

Invited to serve as Biophysicist on the Staff of the Biology Branch of the Division of Biology and Medicine of the U. S. Atomic Energy Commission.

E. M. Romney, Ph.D.

Invited Speaker, Eleventh Hanford Biology Symposium on the Biological Implications of the Transuranium Elements, Richland, Washington, September 1971.

George V. Taplin, M.D.

Was selected Corresponding Member (Honorary), at the Ninth Annual Meeting, Gesellschaft für Nuclearmedizin, Zurich, Switzerland, December 1971.

F. B. Turner, Ph.D.

Invited Speaker, National Science Foundation review and site visit, U.S.I.B.P. Desert Biome Program, Utah State University, Logan, Utah, June 1971.

A. Wallace, Ph.D.

Invited Speaker, Symposium on Uses of Radiation and Radionuclides in Soil-Plant Studies of Trees, Vienna, Austria, December 1971.

John F. Ward, Ph.D.

Invited Participant, Symposium on Molecular Basis of Radiation Biology, Hebrew University of Jerusalem (January 3-5, 1972). Topic: Mechanisms of Radiation Induced Strand Breakage in DNA.

Invited Participant, Symposium on Biochemical Effects of Radiation, Rocky Mountain Regional Meeting of the American Chemical Society, Ft. Collins, Colorado (June 30-July 1, 1972). Topic: Radiation Induced Damage to DNA.

Invited Speaker, Radiology Department, Harvard Medical School, March 9, 1972. Topic: Molecular Mechanisms of Radiation Damage to DNA.

Invited Speaker, Radiology Department, Stanford Medical School, April 19, 1972. Topic: Mechanisms of Strand Break Production in DNA.

Invited Review, Advances in Radiation Biology 1973 - Molecular Mechanisms of Radiation Induced Damage to Nucleic Acids.

Scientific Exhibits, Society of Nuclear Medicine's 18th Annual Convention
Biltmore Hotel, Los Angeles, June 28-July 1, 1971

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