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DEPARTMENT AND LABORATORIES OF
NUCLEAR MEDICINE AND RADIATION BIOLOGY

CONTRACT NO. AT(04-1)GEN-12

1068109

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UCLA 444
Progress Reports

THE UNIVERSITY OF CALIFORNIA
Los Angeles Campus
SCHOOL OF MEDICINE
Department and Laboratories of
Nuclear Medicine and Radiation Biology
10875 Le Conte Avenue
Los Angeles 24, California
Contract No. AT(04-1)-GEN-12

SEMIANNUAL PROGRESS REPORT

FOR THE PERIOD ENDING JUNE 30, 1959

1068110

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12.

γ -irradiation of water (G_H 2.78, G_{OH} 2.28), indicates that the two types of free radical react very differently with olefinic substances: $H^\cdot$ adds to the olefinic center and does not abstract hydrogen from the methylene group adjacent to the double bond; $HO^\cdot$ is apparently the agent responsible for formation of the intermediary allylic radicals (by abstraction of H from the oleate molecule), but does not add to the center of unsaturation. On the other hand, $H^\cdot$ reacts readily with aliphatic radicals, but not with allylic radicals, while $HO^\cdot$ does the reverse preferentially.

Interim Report.

The Spontaneous Change in Ferriprotoporphylin in Alkaline Solution.

Mary-Louise Rothschild and Lawrence S. Myers, Jr.

It has been reported in the literature that FPP (ferri-protoporphyrin) undergoes spontaneous changes as it stands in dilute alkaline solution and that these changes are reflected in the visible absorption spectrum. The nature of this reaction has been investigated by following the spectral changes of 10^{-5} M FPP in .1 N, .01 N, .001 N NaOH and in .1 NaOH plus 10^{-4} M KCN, sodium diethyldithiocarbonate, 8-hydroxy quinoline, or NaCl. In addition, the effect of irradiation on a 52 day old solution of FPP in 0.1 N NaOH was compared with that on fresh FPP and ferrideuteroporphyrin solutions, and the spectral properties of a butanol insoluble compound isolated from an 'aged' FPP solution were investigated.

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The results, taken together, show that in aerated alkaline solution FPP is converted to another iron porphyrin pigment and that this pigment probably differs from FPP in the nature of the side chains on the 2 and 4 positions.

Reference: Nature 182, 1671 (1958)

A Study of the Reaction between Ferriprotoporphylin and Hydrogen Peroxide in Alkaline Solution and Characterization of the Chief Reaction Product.

Mary-Louise Rothschild, Jane Leonard, and Jean Filbert

It has been reported previously that when ferriprotoporphylin, FPP, in 0.1 N NaOH is treated with dilute solutions of H_2O_2 another iron porphyrin pigment is formed, FPP', which can be separated from unreacted FPP by a difference in solubility in the two phase system of n-butanol and NaOH- CO_2 solution, pH 9.6. The reaction between FPP and H_2O_2 has now been studied in the pH range from 7 to 13. The results indicated that the formation of FPP' from H_2O_2 was most efficient at pH 9.6. FPP' was isolated and recrystallized, and extinction coefficients were determined for the pigment in 0.1 N NaOH and in alkaline pyridine and dithionite. The visible absorption spectrum of FPP' is very similar to that of FPP but it is shifted about 4 m μ toward shorter wave lengths in pyridine and dithionite and the

myelination while those of the aged animal may be due to various degenerative diseases. In any event, these changes appear to form a fairly good index of the aging process — one which can be followed readily and which, with more modern techniques presently available, may give additional valuable information.

The similarity of the end results of aging and chronic low-level irradiation has prompted many to consider that they have similar mechanisms. Be this as it may, it is possible that both processes will be reflected in the lipid composition of the brain. The low rate of turnover of these lipids render them particularly susceptible to irreversible alteration by various damaging influences. This should be particularly true of the polyunsaturated acids of the brain, which have important functions as constituents of the glycerophospholipids and which may be renewed very slowly if at all.

In order to investigate these possibilities, 80 weanling rats were divided into 2 groups, one of which was kept as the control group. The other was treated in the same manner but was x-irradiated 5 days a week with 15 r per day. At intervals, representative animals from both groups are killed and the brain lipids are extracted and separated on the silicic acid column into cholesterol esters, triglycerides, cholesterol, diglycerides, monoglycerides and phospholipids. The phospholipids will be further fractionated by silicic acid chromatography and the lipid esters will be saponified and the resulting fatty acids separated by gas chromatography.

Results to date have shown that in new born rats the brain lipids were 2.25 per cent. In weanling rats, the lipid content had increased to 8.2 per cent. At 49 days, the per cent lipid of the control brains was

still 8.2 per cent, but that of the irradiated animals was 9.2. At the same time, the per cent of cholesterol increased from 19 to 24 per cent of the lipid and the total phospholipid decreased from 78 to 72 per cent. The corresponding values for the 49-day irradiated animal were 26 per cent cholesterol and 53 per cent phospholipid.

A continuation of these studies should produce further important results.

Interim Report.

Effects of Essential-Fatty-Acid Deficiency and X-Irradiation
on the Rat Plasma Cholesteryl Ester Spectrum

David R. Howton and Sam Hashimoto

Arachidonic acid comprises more than half of the fatty acids involved in the plasma cholesteryl esters of the normal adult male rat, the remainder consisting of about equal amounts of linoleic, oleic, and palmitic acids. In the fat-deficient animal (raised to cessation of growth on a linoleic-acid-free diet), oleic is the major acid of these esters, accompanied by lesser amounts of eicosatrienoic and palmitoleic and a trace of eicosatetraenoic acid. An acid spectrum intermediate between these extremes is discernable in the adult animal after as few as 15 days following withdrawal of linoleate from the diet. Although whole-body x-irradiation produces little change in the plasma cholesteryl ester spectrum of other animals, that of the fat-deficient rat shows marked alteration 3 days following radiation, concentrations of polyunsaturated acids increasing at the expense of the more saturated components. Under none of the various conditions employed in the present experiments did the arachidonate-linoleate ratio of these esters depart greatly from 5:1.

Reference: UCLA-435. (A brief summary of the findings in this study was presented at the 1959 Deuel Conference on Lipids and is slated to be published in the American Journal of Clinical Nutrition.)

Effect of X-Irradiation on Estrogen-Induced Protein Synthesis in the Chick.

Ole A. Schjeide, Sue Simons and Nancy Ragan

Under none of the experimental conditions nor in any of the stages studied was whole body-X-irradiation (400-750 r) followed by significant changes in serum concentrations of estrogen-induced lighter lipoproteins, X_2 -lipoglycoproteins or X_1 - phosphoproteins. Small but statistically significant increases in total serum lipids and dense lipoproteins were observed, as were decreases in serum albumin. No indications of gross changes in turnover of serum proteins (labeled with methionine- or cysteine- S^{35}) were detected following whole body irradiation.

Interim Report

Effect of X-Irradiation on Cellular Inclusions in Livers.

Ole A. Schjeide, Sue Simons and Nancy Ragan

Wet Volumes of Cellular Inclusions in Livers
of Control and Irradiated Chicken Embryos.*

Age (Days)	<u>Nuclei</u>		<u>Mitochondria</u>		<u>Microsomes</u>	
	No X	X	No X	X	No X	X
13	.6 (.4-1.0) ⁴	.4 (.3-.6) ³	1.8 (1.2-2.3) ⁴	1.7 (1.2-2.2) ³	.6 (.5-.7) ⁴	.8 (.8) ³
18	.5 (.3-.7) ⁶	.5 (.3-.8) ⁴	1.7 (1.4-2.0) ⁶	1.3 (.9-1.6) ⁴	.7 (.4-.9) ⁶	1.0 (.7-1.4) ⁴
21	.2 (.1-.3) ⁵	.3 (.2-.5) ⁵	1.3 (.9-1.8) ⁵	1.5 (.9-2.0) ⁵	.7 (.5-1.0) ⁵	.9 (.6-1.6) ⁵
25	.5 (.4-.7) ³	.6 (.3-.8) ³	1.6 (1.4-1.8) ³	1.5 (1.3-1.7) ³	1.0 (.9-1.0) ³	.9 (.8-1.0) ³

*All embryos and chicks were X-irradiated (500-600 r) three days prior to sacrifice on day indicated. Ranges are shown in brackets. Superscripts give numbers of determinations.

Probability values comparing groups:

21 days No X and 500 r (Mitochondria) = .5
 18 days No X and 500 r (Mitochondria) = .05
 18 days No X and 21 days No X (Nuclei) = .01

Percent reduction by irradiation when calculated from paired experiments:

13 days (Mitochondria) - 20 per cent reduction
 18 days (Mitochondria) - 24 per cent reduction

Probability values calculated from percentage differences within paired groups. This eliminates differences between starting materials:

Probability of 13 day Mitochondria being reduced by irradiation
 = 0.1

Probability of 18 day Mitochondria being reduced by irradiation
 = 0.01

The Radiation Sensitivity of Cells and Colonies of S-3
He La Cultures

Stanley R. Person and Edward Sato

We have irradiated (Co^{60} - γ rays) populations of single cells and micro colonies of S-3 He La cultures, where the colonies contain approximately 2, 4 or 8 cells/colony. The survival curves (assay-macroscopic colony forming ability) for 2, 4 and 8 cell stages and for single cells are identical. This indicates that a cell interaction exists. That is, a lethal ionizing event in one cell of a colony eventually leads to the death of the entire colony, although it does not spread to neighboring colonies.

We have also irradiated mature colonies (500-1000 cells/colony) and split these into single cells for plating at various times after irradiation. These were also assayed for macroscopic colony forming ability. A maximum increase in the slope of the resulting survival curve (two to three fold) was noted when the colonies were split into single cells and plated at 30 hours or longer after irradiation.

We have also considered the possibility of the isolation of a toxic product. However, so far we have been unable to directly demonstrate the existence of such a substance.

Interim Report

Lethal and Sub-Lethal Damage to E. coli Caused by Tritium
Decay in Thymidine

Stanley R. Person and Hazel Leah Lewis

When a thymineless strain of E. coli (15_{T-}) is grown in a mineral medium containing tritiated thymidine (specific activity of 1 atom of tritium per 10 molecules of thymidine) and stored at -78°C there are approximately 50 decays/day/bacterium. Therefore, effects caused by the decay of this isotope may be studied in a reasonable time period. This label was chosen because there is a possibility that observed effects may be due to the removal of a single H-atom from a known position in a molecule of thymidine. The high specific ionization from the β -particle accompanying decay would seem to make this assumption implausible. However, we have found that E. coli B and its radiation resistant mutant show equal killing efficiencies (ability to form colonies) when exposed to tritiated thymidine. This is in sharp contrast to the very different killing efficiencies observed when the same two strains are irradiated with 50 or 250 KVP X-rays.

The killing efficiency per transmutation, for 15_{T-} is 1% at -78°C and increases to 50-100% as the temperature of storage is increased to $+45^{\circ}\text{C}$. A lactose to non-lactose

fermentation mutation, induced by the decay of tritium in tagged thymidine, was detected by the EMB agar method. The number of mutants detected was too small to determine a mutation frequency accurately, but it is about 4×10^{-4} .

Interim Report

The Ineffectiveness of Radiation on the Apparent Viability of Lymphocytes in Freshly Drawn Blood.

Benedict Cassen and Willi Gutfreund

The technique developed by this laboratory (J. Lab. Clin. Med. 52, 778, 1958) for separating lymphocytes from freshly drawn whole blood was found to work well on rabbit's blood obtained by cardiac puncture. The separated lymphocytes showed typical ameboid motions 8 hr. after separation when observed under a phase microscope. Samples of blood irradiated in vitro and receiving a dose of about 1000 roentgens showed after 6 hr. the same viability as the controls. In each sample 25 lymphocytes were observed, almost all showing ameboid motions. Cell counts showed that lymphocytes were not lost by rapid lysis or by being phagocytosed during irradiation. Although there are some rather remote possibilities that prevent an absolutely rigorous logical conclusion, these observations are offered against any hypothesis to explain the rapid fall off of lymphocyte count after total body radiation on the basis of a direct lethal effect of the radiation on mature lymphocytes.

It is not ruled out that they are sublethally affected. If the lymphocytes are long-lived cells, as appears necessary from the results of several investigators, it appears that new hypotheses are required to explain their rapid disappearance from the circulation after total body radiation. This work was reported at the Pittsburg (1959) meeting of the Radiation Research Society, in which report some new hypotheses are considered.

Interim Report.

The Response of the Kangaroo Rat (*Dipodomys merriami*, Mearns) to Acute Whole Body Irradiation

T. J. Haley, R. G. Lindberg, A. M. Flesher, K. Raymond, W. McKibben, P. Hayden

Kangaroo rats (*Dipodomys merriami*, Mearns) received acute whole body irradiation at doses of 25 to 550 r. At 550 r the ST_{50} was 10.3 (9.7 - 10.9) days and total mortality was 93 per cent/14 days. These animals normally have a pronounced lymphocytosis (75 to 85 per cent of the total leukocytes) which was changed to a lymphocytopenia by irradiation. This effect was dose dependent being much less at the lower dose levels (25 to 50 r). A slight anemia developed on the ninth post-irradiation day at doses of 450 to 550 r. The hematocrit also decreased at this time and there were fluctuations in the total number of erythrocytes coinciding with these changes. There were only slight changes in body weight, adrenal weight and kidney weight by the ninth post-irradiation day; however, spleen weight showed an abrupt decrease. The initial spleen weight averaged 55 mgm while on

the ninth day it was 10 to 38.5 mgm depending upon the radiation dose. In most respects the responses of the Kangaroo rat to various doses of acute whole body x-irradiation closely resemble those obtained with the CF-1 mouse.

Reference: Report UCLA-440, May, 1959. Accepted for publication by Radiation Research.

Point Source Beta Irradiation of Bone

Lawrence E. Detrick, H. C. Upham and Thomas J. Haley

Stable Sr or Sr⁹⁰ beads were implanted within the femur of CFW rats to study bone changes due to localized radiation (330×10^3 to 638×10^3 rep/cm tissue, bead surface dosage for 6 months). Stable beads had no effect on the bone and Sr⁹⁰ beads did not affect the differential blood cell count or histology of 7 other organs. Although all Sr⁹⁰ femurs were fractured no cancerous tissue was found. Fractured segments healed together by a fusiform fibrotic encapsulation centered over a mass of necrotic tissue. This capsule consisted of highly active periosteum, perfuse laminations of fibroblasts actively differentiating into cartilagenous osteoid and newly formed bone. When the Sr⁹⁰ bead capsule separated from the femur, both femur segment canals were plugged with newly developing bone and bone marrow filled the marrow cavities, being somewhat hyperplastic distal to the fracture and less dense than normal at the fracture end. Osteocytes were present in all lacunae of all performed shaft bone. When the Sr⁹⁰ bead remained in contact with the femur a necrotic mass formed. Two to 3 mm away the shaft bone was dead, avascular, acellular

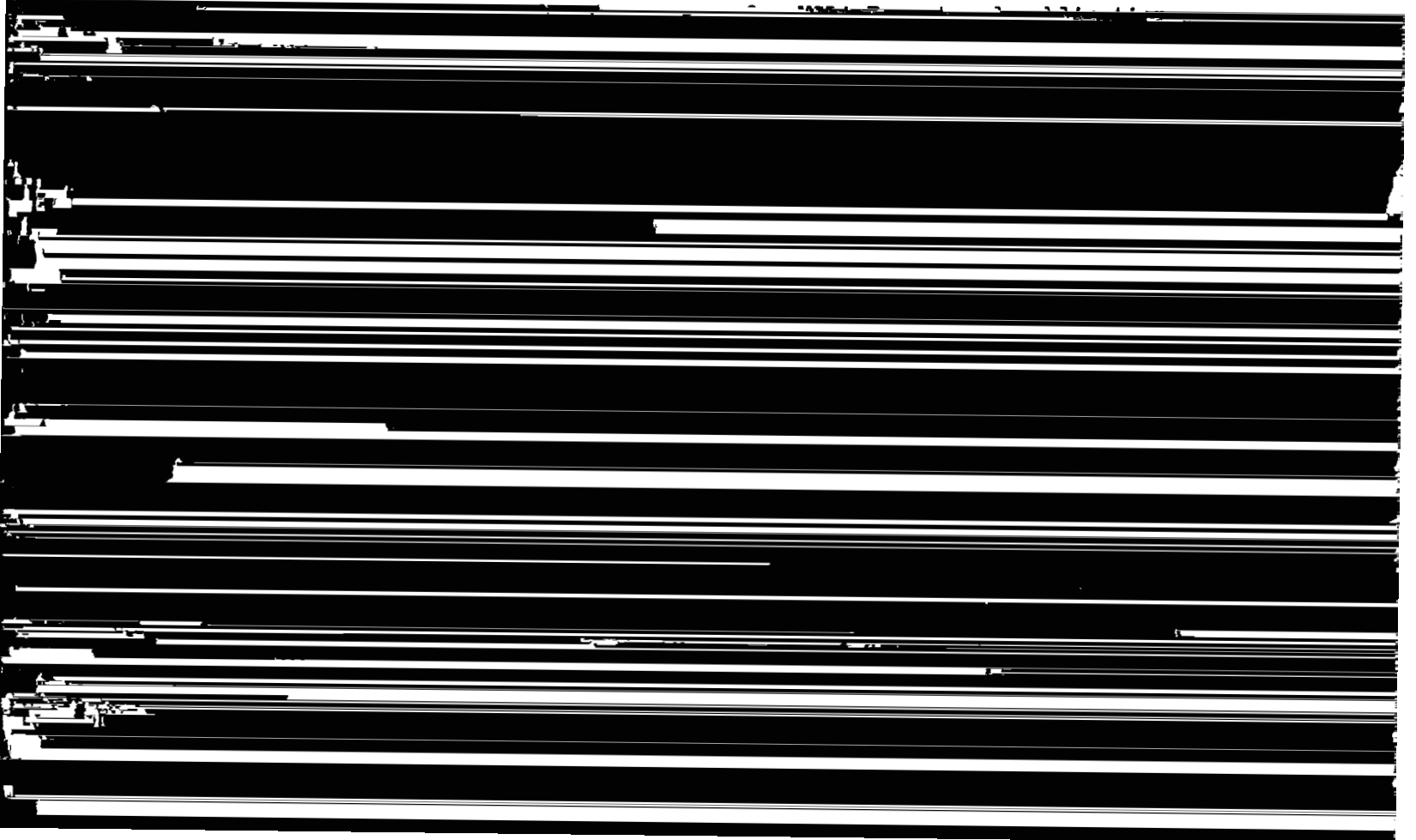
and eroded. Normal marrow appeared 6-8 mm down the shaft and small capillaries were seen below the necrotic capsule. The Sr^{90} beads will be reimplanted for weekly serial sacrifice (2-6 weeks) and determination of the rate of histological damage and the extent of attempted tissue repair at this early date.

Reference: Published in Fed. Proc., 18, 384 (1959).

Influence of X-Ray Irradiation on Thiamine Transport in Rat Intestine

Lawrence E. Detrick, C. P. Miles, H. C. Upham, A. K. Dunlap and T. J. Haley

Thiamine transport experimentation on the irradiated rat intestine has been completed. Experimental animals have been increased in number to 160: 80 each, irradiated and non-irradiated; 8 rats per group; 10 time intervals studied over a post-irradiation (PR) period of from 1



Central Nervous System Effects Following Whole Body X-Irradiation of Cats

Thomas J. Haley and H. Gangloff

Following 400 r total body x-irradiation behavior, spontaneous and evoked brain electrical activity were studied in unrestrained cats with chronic electrodes implanted in cortical and subcortical areas. All cats showed increased alertness immediately after irradiation and also during the second day. Anorexia, lack of drive and ataxia were seen after 4-6 days, with death after 5-11 days. Spontaneous brain electrical activity was practically unaltered in the cortex, thalamus, caudate nucleus and tegmental reticular formation. However, the hippocampus and amygdala consistently showed spontaneous spike discharges firing at a maximal rate 3-7 hours after irradiation and decreasing later. Reticular arousal thresholds decreased significantly immediately after irradiation, returned to control level later and then showed little change or a slight increase. The threshold of the recruiting response to thalamic stimulation remained unchanged immediately after irradiation but was slightly increased later. Hippocampal seizure thresholds remained unaltered except in one animal. Electrode positioning was verified by histological examination. General effects of irradiation included leukopenia, hemorrhage and general malaise but little or no diarrhea was apparent. Similar results have been obtained with 200 r acute whole body irradiation, although none of the animals died. Further work covering irradiation of the head or body alone will be completed during the next period.

Reference: Published in Fed. Proc., 18, 399 (1959), Also to be

presented at the 21st International Congress of Physiological Sciences, Buenos Aires, August, 1959. Also accepted for publication in *Experientia*.

The Biohydrogenation of Fatty Acids

James F. Mead

In 1937, Rittenberg and Schoenheimer carried out experiments designed to assess the amount of hydrogenation of unsaturated fatty acids occurring in mammalian tissues. The conclusion reached was that as much as 30 per cent of a fed unsaturated acid may be hydrogenated to the corresponding saturated acid. This conclusion has been accepted as fact for the past 20 years despite some evidence from this and other laboratories that it is not correct. An experiment was therefore designed to clarify this situation.

Palmitoleic acid was obtained from macadamia nuts and was labeled with C^{14} in the carboxy group as described elsewhere. Palmitic acid was separated from the organ and depot fat of rats to which this labeled acid had been fed and was decarboxylated to compare the activity of the carboxy group with that of the remainder of the chain. It was found that the activity of the carboxy group was about 3 times as great as that of the other odd carbon atoms (in which the activity resides in acids derived from carboxy-labeled acetate). Since in palmitic acid isolated in a similar manner from rats fed other carboxy-labeled acids, the carboxy group has the same activity as the other odd carbons, it is evident that some hydrogenation of the fed palmitoleic acid occurred.

However, although the fed palmitoleic acid had a total activity of

8×10^7 disintegrations per second per mg., the isolated palmitic acid had a specific activity of only 8.4 d.p.s. per mg. of which only 20 per cent was derived by hydrogenation (from excess activity in the carboxy group). A rough calculation from these values reveals that only about 0.26 per cent of the ingested palmitoleic acid was hydrogenated.

Therefore, it can be said with some confidence that hydrogenation of fatty acids by the mammalian organism is an almost negligible process.

Interim Report

Study of Abnormal Proteins and Polypeptides

N. S. Simmons and J. Kinnear

Preliminary investigation has shown that low molecular weight basic proteins and polypeptides such as protamine (P) and histone (H) react with normal human serum proteins to give modified electrophoretic patterns. These altered patterns show increased globulin fractions formed apparently by interaction between (P) and (H) and the serum albumin component. Since these changes are almost exact duplications of abnormal patterns seen in various disease processes, it is strongly suggestive that the presence of low molecular weight basic polypeptides in the circulating blood is to be suspected. However, since these low-molecular weight components may be dialysable and since standard electrophoretic techniques require dialysis of the plasma or serum prior to analysis, it was necessary to devise a technique for sample preparation and analysis avoiding dialysis. This has been done. Preliminary investigation has shown that .1 M tris(hydroxymethyl) aminomethane (TRIS) when titrated with HCl at 0° has the midpoint of its titration curve at pH 8.6. This is the pH at which the usual

veronal buffer system is used. When pooled samples of normal human sera were made 0.1 M TRIS and .05 M HCl, at a 3.6×10^{-3} mhos/cm. Suitable adjustment of similar buffer concentrations were made with NaCl to give a solution with the same specific conductance. When the serum was subjected to electrophoretic analysis it was found that the ascending and descending patterns were not enantiomorphic and a large boundary anomaly was present in the ascending pattern. Empirical adjustments of the buffer conductivity with NaCl showed that conditions could be found in which the patterns were enantiomorphic and the boundary anomaly absent. The following Table shows the ratio of ascending and descending albumin peak movement as a function of the NaCl concentration of the buffer and its specific conductance. It can be seen that when the serum, diluted 10 fold, is made 0.10 M TRIS and .05 M HCl and .01 M NaCl and run against the various buffers listed, the ascending and descending patterns show linear enantiomorphy with a buffer containing .10 M TRIS and .05 M HCl and .03 M NaCl.

TABLE I

Buffer				Specific Conduct.	Ratio Ascend./Descend. Pk. Movement
.10 M TRIS	.05 M HCl	.02 M NaCl	3.6×10^3	Mhos/cm	$1.21 \pm .03$
.10 M TRIS	.05 M HCl	.025 M NaCl	3.9×10^3	Mhos/cm	1.13
.10 M TRIS	.05 M HCl	.030 M NaCl	4.0×10^3	Mhos/cm	$1.04 \pm .01$
.10 M TRIS	.05 M HCl	.035 M NaCl	4.1×10^3	Mhos/cm	.92
.10 M TRIS	.05 M HCl	.040 M NaCl	4.3×10^3	Mhos/cm	Unstable Boundary

It has therefore been found possible to obtain excellent electrophoretic patterns of normal human sera, when the latter is diluted ten-fold and made .10 M TRIS — .05 M HCl — .01 M NaCl, and run against buffer

.10 M TRIS — .05 M HCl — .03 M NaCl. This system is now being used to investigate the serum proteins in various disease states and the interactions between normal serum proteins and low molecular weight basic polypeptides.

Thyroxin Action on Acyl Phosphatase

Isaac Marary and Phyllis Wright

The study of the interaction of acyl phosphatase with thyroxin analogues was continued. Further studies with other analogues have strengthened the conclusion that maximum inhibition is obtained with a diphenyl ether having a 4' hydroxyl group, 3,5,3',5' iodines, and a propionic acid in the 1 position.

The possibility that the inhibition was a result of the presence of the phenolic and carboxylic acid groups at a set distance from each other was explored. These two groups could be conceived as binding with two positive groups on the protein. The distance of these two groups from each other in thyroxin was calculated to be 11.5 Å. The pK of the phenolic group is 6.5 and that of the carboxyl was assumed by analogy with tyrosine to be about 3.0. The role of the 3',5' iodines would therefore be conceived as increasing the acid strength of the phenolic group. The 3,5 substituted iodines do not contribute to the acid strength and neither are they essential for inhibition.

However, it was found that this hypothesis was not supported. Dicarboxylic acids of varying lengths, between 10 and 13 Å, gave only small inhibitions. It was also found that thyroxin, with an acetylated phenolic group, maintained its ability to inhibit.

A report that N acetylated amino acids and a derivative isolated from the thyroid, thought to be acylated thyroxine, could stimulate enzymatic acetylation of amines led us to test the effect of such compounds on acyl phosphatase. N-acetyl tyroxine, phenylalamine, and glycine had no effect on the enzyme. Synthetic N-acetyl thyroxine gave 70% of the inhibitory activity of thyroxine at a test pH of 5.4. At a test pH of 7.4 the N-acetyl derivative failed completely to inhibit while thyroxine maintains the same level. These observations indicate that a positive charge in the thyroxine is necessary for inhibition of the enzyme at pH 7.4 and not at 5.4.

At pH 5.4 the enzyme should have a preponderance of positive charges as compared to pH 7.4. If we assume that both a negative and a positive charge on the protein are active in thyroxine binding, but that the positive charge is more important, then the loss of the amino group on the thyroxine should cut down the inhibition but not eliminate it. Thus, the negative group of the thyroxine (carboxyl group) could bind with the preponderant positive charge on the protein, and the positive group of the thyroxine (amino group) could bind with the weak negative charge on the protein. Loss of positive charge through acetylation would lessen the binding but not eliminate it.

At pH 7.4 the positive charge on the protein is considerably less if not lost. The negative charges are increased. Thus the positively charged amino group now becomes important for binding to the protein and the negatively charged carboxyl group is less important. Elimination of the charge on the amino group through acetylation, therefore, eliminates

the major binding site.

The loss of inhibitory activity of thyroxin physiological pH's by acetylation may result in an increase of the activity of acyl phosphatase. The possibility of enzymatic acetylation of thyroxin in the peripheral tissues leading to an increase in acyl phosphatase activity is being investigated.

Interim Report

Toxicological Studies on Polyphenyl Compounds Used as Atomic Reactor Moderator-Coolants

Thomas J. Haley, L. E. Detrick, N. Komesu, P. Williams,
H. C. Upham and L. Baurmash

A study has been made of certain aspects of the toxicology of organic polyphenyl compounds proposed for use as moderator-coolants in atomic reactors. Only monoisopropylbiphenyl, irradiated monoisopropylbiphenyl and terphenyl mixture (OMRE) were irritating to the conjunctiva in rabbits and only the unirradiated moderator-coolants caused skin irritation in rabbits. Both the moderators and their components were highly damaging to guinea pig skin following intracutaneous injection. All of the polyphenyl compounds except irradiated monoisopropylbiphenyl produced sensitization and chemical necrosis. The latter produced only necrosis. Monoisopropylbiphenyl, ortho- and meta-terphenyl were the only polyphenyls causing death after inhalation, although all of the compounds produced some of the following symptoms: nasal congestion with rhinitis, lachrymation, labored respiration, erythema of the ears and paws. The following histopathological changes were also observed: acute tracheal necrosis, acute tracheobronchitis, pulmonary edema, bronchopneumonia, atelectasis

and petechial hemorrhages. All such toxic effects can be prevented by protective clothing and respirators.

Reference: To be published in Toxicology and Applied Pharmacology
for September 1959. UCLA-441

A Pharmacological Study of Mellaril, 3-Methylmercapto-10-[2-(N-Methyl-2-Piperidyl)-Ethyl]-Phenothiazine Hydrochloride

Thomas J. Haley, A. M. Flesher, K. Raymond, N. Komesu and
P. Williams

The pharmacological properties of a new phenothiazine drug, Mellaril, 3-methylmercapto-10-[2-(N-methyl-2-piperidyl)-ethyl]-phenothiazine hydrochloride have been studied. It has been shown that the drug produced a dose dependent hypotension which was at least partially peripherally mediated. There was no significant effect on respiration until exitus. Electrocardiographic changes indicated that cardiac anoxia was produced. The compound has adrenergic blocking, slight antihistaminic, very weak atropine-like activity but no ganglionic blocking activity. The drug produced hypothermia, potentiated hexobarbital sleeping time and prevented epinephrine death in mice.

Reference: UCLA-439. Accepted for publication in Toxicology and Applied Pharmacology, to be published in July. Also published in Fed. Proc. 18, 399 (1959).

Antibacterial Activity Associated with Lactobacillus
Acidophilus

James G. Vincent, Robert C. Veomett, and Richard F. Riley

Lactocidin, an antibiotic-like agent, has been obtained from all tested strains of L. acidophilus from the rat, rabbit, hamster, and man, and from most of the L. acidophilus strains isolated from the mouse. The broad antimicrobial spectrum of crude lactocidin furnished a mechanism to explain the influence of L. acidophilus on the ecology of the mucosal areas.

Two methods of assay of lactocidin were devised to follow its production in agar cultures and its purification through silicic acid chromatography. However, the product of a 2500-fold purification of lactocidin was unstable and had lost its capacity to function bactericidally in the presence of serum.

The identification of lactocidin may furnish a means of selecting effective strains of L. acidophilus for oral administration. It is suggested that oral lactobacillus preparations might be useful in the treatment of intestinal conditions leading to post-irradiation bacteremias.

Reference: UCLA-434. Submitted for publication in the
Journal of Bacteriology.

Screening of Compounds for Prophylaxis Against Radiation Injury

Thomas J. Haley, A. M. Flesher and N. Komesu

In 1957, Haley et al found that quinoxaline 1,4-di-N-oxide reduced x-radiation mortality in mice by 50%. Two mechanisms were involved, reduction of bacteremia and interaction with x-ray produced oxidizing radicals. Comparisons have been made of other N-oxides using groups of 20 CF-1 mice and the same radiation conditions as before. The 250 mgm/kgm oral dose of drugs was given 24 hrs. prior to irradiation with 550 r. The two quinoxaline derivatives significantly increased the ST_{50} day but had less effect on total survival than quinoxaline 1,4-di-N-oxide. Erythromycin N-oxide significantly reduced the ST_{50} day and total survival while its anhydro derivative was equivalent to quinoxaline 1,4-di-N-oxide as a radiation prophylactic. All of the above compounds are readily absorbed, excreted slowly in the urine and exert antibiotic effects so the radiation bacteremia could be reduced. On the other hand, not all of them can interact with equal facility with the radiation produced oxidizing radicals. Examination of the chemical structures involved indicated that an amine oxide either in an unsaturated ring e.g. quinoxaline or within

one carbon atom of a double bond e.g. anhydroerythromycin is necessary if oxidizing radicals are to be prevented from exerting their deleterious effects. In the dimethyl substituted quinoxaline compounds difficulties in oxidizing the methyl groups are probably the reason for the decrease in protectant activity even though Francis et al showed that hydroxylation in the 2 position occurs in vivo. With erythromycin N-oxide, the double bond is lacking and the compound can be oxidized only with difficulty even in vitro. Thus it would appear that amine oxides with the above chemical structures can reduce mortality from ionizing radiation when administered orally 24 hrs. prior to exposure.

Reference: Accepted for publication in Nature.

Radiation Protection Studies

J. L. Leitch and J. Moore

In the last semi-annual report, UCLA No. 431, preliminary data were presented on the effects of AET (2-aminoethylisothiuronium bromide - hydrobromide) and of 5-HT (5-hydroxytryptamine creatinine sulfate) on the radiation syndrome. This work has been continued using x-irradiation comparable to that previously described.

However, a new parabolic filter 0.46 mm. Cu was built in

cooperation with Dr. Stanley Person of the Radiobiology Division. This unit was calibrated and the field was found to be flat within ± 1.2 per cent providing the field did not extend beyond the half-angle of 19.5° . This filtration system had a half-value of 1.9 mm. Cu.

Mice were treated 20-30 minutes prior to irradiation with a 0.25 ml. dose containing $10 \mu\text{M}$ of AET plus $1 \mu\text{M}$ of 5-HT. All data were calculated by the method of Litchfield (J. Pharm. Exptl. Therap., 97, 399-408, 1949) for time per cent effect curves. All calculations were carried out at a probability level of $p = 0.02$ since it was found previously that evaluation of compounds would obtain greater significance at these producible limits. The data were compared to experiments with a midline dose of 460 r x-irradiation without treatment and also to a second series at the same irradiation level but given 1×10^{-5} M AET per mouse 20-30 minutes before irradiation. These data are summarized in Table II.

TABLE II

Summary of Survival Time Data for Mice Treated
With AET Alone or a Combination of AET and 5-HT

Treatment in μ M per mouse	Radiation Dose		ST ₅₀ day	Slope	60-Day Mortality %
	Total r	Rate r/min.			
None	460	19.4	7.2	1.53	95.8
10 μ M AET	460	20.0	21.7	2.62	69.5
10 μ M AET	460	19.6	41.0	10.25	48.6
plus	900	20.0	12.2	1.74	63.6
1 μ M 5-HT	1200	19.3	6.4	1.71	100.0*
None	1200	19.3	3.95	1.193	100.0**

* All mice of this group dead after only 20 days.

** All mice of this untreated group dead after only 8 days.

Although final calculations have not been made for the treated mice receiving 1200 r x-irradiation, the available data indicate that the ST₅₀ and slope values are approaching those of untreated mice receiving only 460 r x-irradiation. Of particular interest is the fact that a slight modification of the survival time and slope values at the 1200 r level is suggested by the data from the mice treated with the combination of AET and 5-HT. Whether or not this modification is significant must wait for the completion of the observations on the treated 1200 r series. The ST₅₀ day shows an approximate linear relationship to the reciprocal of the x-ray dose within the ranges

studied.

Work is in progress to extend the above investigations to different rates of irradiation and also to determine whether or not other therapeutic compounds show the same relationship between the ST_{50} values and the reciprocal of the radiation dose.

Interim Report

The Effect of Radiation Exposure Rate Upon Acute Mortality in Mice

O. M. Meredith, Jr., G. V. Taplin, W. Coffman and J. Post

The following is an abstract of a paper presented at a national meeting of the Radiation Research Society in Pittsburgh, May 17-20, 1959. This paper will be submitted for publication as soon as the 35 curie Cobalt⁶⁰ source is available to round out experimental data.

"Dose rates of about 20 r per minute are customarily used in radiation protection studies, mainly because of convenience. Also, higher dose rates are known to cause little change in mouse mortality. However, during fallout from detonation of thermonuclear devices, the radiation exposure rates are likely to be much slower. Therefore, mouse mortality studies were made at various dose rates including those which simulate acute fallout conditions. Results demonstrate that mouse mortality is dose rate dependent with both 250 KVP x-ray and Co⁶⁰ gamma radiation exposures. For example, x-ray exposures

given at the usual rate of 20 r per minute yield a mean LD 50/30 value of 480 r, whereas when the rates are reduced to simulated fallout conditions (3.0-1.5 r per minute) the corresponding mean mortality value is 640 r. This intensity effect is equivalent to a dose reduction of about 160 r. The most likely explanation is that the longer exposures permit a relative dominance of tissue repair mechanisms over simultaneous injurious actions. These effects are analogous to reduced mouse mortality values which follow multiple fractionated versus single continuous acute exposures with the same total radiation dose. This information indicates the need for extending dose rate studies to much slower rates. Furthermore, protective agents of proved value should be reinvestigated under these conditions and used in the form of long acting or depot preparations."

Interim Report.

Studies on the Effects of Nucleic Acid Preparations from Leukemic Cells of AKR Mice Injected into Newborn C3Hf/Gs and (C3Hf x AKR) F_1 Hybrid Mice.

Esther F. Hays, Norman S. Simmons, Jeanne Carr, Ione Crawford, and Elinor Thorpe

Data from this laboratory has indicated that subcutaneous injections of nucleic acids prepared from leukemic and non-leukemic organs of a high leukemia (AKR) strain into (C3Hf x AKR) F_1 Hybrid Mice might be responsible for

increasing the incidence of leukemia observed in these animals. (Nature 180: 1419-1420, Dec. 21, 1957).

Data obtained in the past six months has indicated that in two groups of this same hybrid cross, injected both subcutaneously and intravenously with nucleic acids from leukemic cells when newborn, has not resulted in an increased incidence of leukemia. The numbered results of these experiments are shown in Table III.

Table III- Results of Injection of Leukemic Nucleic Acid Preparations into Newborn (C3Hf x AKR) _F ₁ Hybrid Mice				
	No. Animals Injected When Newborn	No. Devel. Leukemia	Non-Inj. Controls	No. Dev. Leukemia
Pub. Data (Nature 1957)	31	11	65	5
DNA-RNA S.C. Add'l Data	25	6	49	10
DNA-RNA I.V.	43	7	45	6

The second group of experiments involves injecting leukemic (AKR) nucleic acids intravenously into newborn C3Hf mice. (Previous subcutaneous injection of similar preparations in this same strain did not result in the

development of leukemia.) And, further, considering the possibility of genetic transformations being involved, these same animals received intraperitoneal injections of cell-free extracts of leukemia (AKR) tissues at 6 weeks of age. These extracts had been previously shown to be leukemogenic when given to newborn C3H mice, but not to C3H mice of 6 weeks of age.

A summary of this data is presented in Table IV and does not either confirm or deny the above hypothesis. These animals are now all over 1 year of age.

Table IV - Results of I.V. Injection of Nucleic Acids (DNA-RNA) I.V. into Newborn (N.B.) C3Hf/Gs Mice Followed by Cell-Free Extract at 6 wks. of Age			
Experimental		C o n t r o l s	
	DNA-RNA N.B. + Cell-Free Ex- tract 6 Wks of Age	DNA-RNA N.B. + Saline 6 Wks. of Age	No. Inj. Alternate Litters
No. of Ani- mals	43	32	94
No. Developing Leukemia	1	0	1

Interim Report

Sex Chromatin in Cultured Human Tissues

Charles P. Miles

Thirty-eight tissue culture lines or primary cultures were examined for sex chromatin. In origin, 29 were female, and 9 male. With the exception of 1 amnion culture, none of the male cells showed characteristic sex clumps. The one exception showed peripheral clumps with an incidence ranging to 12%. Such positive cells were presumed to be contaminants of maternal origin. This case with other amnion cultures will be discussed in greater detail in a subsequent publication.

Of the 29 tissue cultures of female origin 6 were classified as cancer and 23 as benign. The malignant cultures were so called only if they were derived from cancer and showed the appropriate cytologic characteristics in in vitro growth. This precaution in classification is necessary since many primary explants of cancer tissue give rise only to cytologically benign fibroblast-like cells presumably of stromal origin.

Of the benign cultures 11 were derived from lesions of the uterine cervix, 9 from amnions of female infants, 2 from thyroid lesions, and 1 from an endometrial carcinoma.

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Of the cultures exhibiting cancer characteristics in vitro, 4 stemmed from epidermoid carcinomas of the cervix, and 2 from ovarian carcinomas.

Twenty-six of the 29 cultures displayed characteristic peripheral sex chromatin clumps in at least some of the cells. In a number of cases the incidence appeared low, but this may probably be attributed in part to fading of the stain in the older preparations. The negative cases comprised the 2 arising from ovarian carcinomas and 1 arising from an amnion. These cultures were respectively approximately $2\frac{1}{2}$ months, $3\frac{1}{2}$ years, and $2\frac{1}{2}$ years of age. The youngest was in the 9th transfer. The amnion was that designated A185 by Zitcer and Dunnebacke. Samples of the 4 subline strains Nos. A1, A2, A3, and A4 were all negative. The sex chromatin positive cultures varied from 2 to 55 days in age. One was carried through 5 transfers, none of the rest through more than 3.

These cases with those previously reported justify the tentative inference that sex chromatin clumps appear invariably in the primary explants of human female tissues - benign or cancerous, but that the sex chromatin feature is eventually lost in later transfers. Further evidence

on this point is provided by recent primary explants of H.Ad.#1. H.Ad.#1 is a tumor of female origin which has been maintained in heterotransplant for almost 3 years and 70 generations. The recent explants in tissue culture do exhibit sex chromatin in contrast to the culture previously reported as negative. In this one case a change (loss of sex chromatin) which occurs in tissue culture does not occur in heterotransplant. As previously reported, the culture of H.Ad. #1 which did not show sex chromatin had been in vitro for 1½ months. If our assumption is correct, this would represent the earliest reported loss of sex chromatin. The longest reported survival of sex chromatin appears to be in the case described by Orsi & Ritter as 10 weeks, 9 transfers. Sex chromatin might eventually prove to be a convenient indication that cells cultured in vitro have not undergone "transformation". On the other hand, while preliminary cloning experiments do not favor such a possibility, it has not definitely been ruled out that "transformation" of female cells into established strains is actually a process of selecting out the sex chromatin negative cells in the original explant.

Whether or not the 2½ year old negative female amnion culture reported here had become malignant, it did show a very high mitotic rate (74 mitoses per thousand cells in one count), and moderate variation in nuclear size. It may be true that the hypothetical development of cancer in tissue cultures of benign origin is invariably accompanied by a loss of sex chromatin. Since primary explants of female cancer developing in vivo have invariably shown sex chromatin in vitro when appropriately stained (but not always in tissue section), we may speculate that at least in this one respect hypothetical malignant change in vitro is not identical with cancer development in vivo.

Reference: Submitted for Publication in Nature.

Morphology and Functional Relations of Sex Chromatin in Cultured Amnion Cells.

Charles P. Miles and Audrey Koons

About 25% of sex chromatin clumps in female amnion cells in tissue culture may be distinguished from compar-

able male clumps calculated relative to the characteristic



size comparable to sex chromatin occur in the nuclei of both sexes, but with a higher incidence in female cells. Sex clumps of characteristic form are seldom evident at any stage of mitosis; and the incidence of peripheral sex clumps varies inversely with the number of mitoses in the culture. Clear spaces in the nucleoplasm adjacent to the clumps or extending from clumps to nucleoli may sometimes represent invaginations of the nuclear membrane. Apparent chromatin strands extending from sex chromatin to nucleoli may sometimes represent the walls of such invaginations; in other cases the strands may represent actual threads. After treatment with hypotonic saline sex chromatin is no longer seen. Nucleoli are often seen close to or merged with sex clumps. Occasional appearances suggest excretion of material through the nuclear membrane in the vicinity of the sex chromatin.

Reference: Submitted for Publication in Experimental Cell Research

Use of Isotopes for Studying the Behavior of the Blood-Brain Barrier During Concussion

Benedict Cassen, Richard Neff, Marjorie Moody, Herbert Gass.

A technique was developed for concussing mice in a moderately reproducible fashion by giving them a blow on the head by a mass

Whether or not the 2½ year old negative female amnion culture reported here had become malignant, it did show a very high mitotic rate (74 mitoses per thousand cells in one count), and moderate variation in nuclear size. It may be true that the hypothetical development of cancer in tissue cultures of benign origin is invariably accompanied by a loss of sex chromatin. Since primary explants of female cancer developing in vivo have invariably shown sex chromatin in vitro when appropriately stained (but not always in tissue section), we may speculate that at least in this one respect hypothetical malignant change in vitro is not identical with cancer development in vivo.

Reference: Submitted for Publication in Nature.

Morphology and Functional Relations of Sex Chromatin in Cultured Amnion Cells.

Charles P. Miles and Audrey Koons

About 25% of sex chromatin clumps in female amnion cells in tissue culture may be distinguished from comparable male clumps solely by virtue of the characteristic morphology of the female clump. Peripheral chromatin clumps in male nuclei are significantly less than half the size of female clumps. Non-peripheral chromatin masses of

released by a cocking mechanism from a spring loaded modified hypodermic injector (Hypospray). The mice were stunned from twenty minutes to an hour and would usually act normally afterwards. P^{32} phosphate was injected intravenously via the tail vein one minute after concussion. Animals were sacrificed at various times after injection, the brains removed and brehmsstrahlung counted in a well counter. The longer the time during concussion that the P^{32} was in the blood, the higher the brain count. The counts were very significantly higher than non-concussed controls or animals that had recovered from concussion. We believe this to be the first definite evidence that the blood-brain barrier permeability to phosphate ions is reversibly increased during reversible concussion. Checks were made on variation of blood content of the excised brain. Evidence was obtained that when there was no visible hemorrhage the blood activity was negligible.

Interim Report.

Preparation of C^{14} -Labeled Unsaturated Fatty Acids

Judd C. Nevenzel and James F. Mead

Methyl palmitoleate-1- C^{14} (200 mg., 10^5 dps/mg.) has been prepared in 5% overall yield (32% from $BaC^{14}O_3$) from palmitoleic acid isolated from the oil of macadamia nuts. The synthesis involved application of the decarboxylation-reconstitution technique originally developed in this laboratory (UCLA-183, J. Am. Chem. Soc., 74, 1109 (1952)) and since applied to the preparation of 1- C^{14} -labeled oleic (UCLA-356, J. Org.

Chem., 22, 319 (1957)), linoleic (J. Am. Chem. Soc., 76, 4970 (1954)), and α - and γ -linolenic acids (UCLA-416, J. Org. Chem., 23, 933 (1958)). Details of the preparation, characterization of intermediates, and use of the product in investigation of fatty acid saturation in vivo will be reported shortly.

In connection with studies in progress on the possible intermediacy of homo- γ -linolenic acid in the linoleic-arachidonic conversion, the nature of the anticipated biosynthetic processes is such as to make the carboxy-labeled substance unsuitable. Methyl 8,11,14-eicosatrienoate-2,3- C^{14} (methyl homo- γ -linolenate) was therefore prepared by employing the Kolbe electrolytic coupling reaction between γ -linolenic acid and the half methyl ester of succinic-2,3- C^{14} . Yield from the commercially available labeled starting material was 29%. The ester obtained (240 mg.) has a specific activity of 0.55 mc/mM.

Interim Report

Investigations with Rose Bengal I^{131}

G. V. Taplin, D. E. Johnson, and M. Hirsh

Work is being continued on the use of Rose Bengal I^{131} in investigating the disturbed physiology of the liver in patients with hepatobiliary tract diseases. A paper entitled "Rose Bengal I^{131} Findings Versus Standard Liver Test Results in Patients with Diseases of the Liver and Biliary Tract" was presented on June 19, 1959 at the annual meeting of the Nuclear Medicine Society in Chicago, Illinois.

An abstract of this paper follows:

"The Rose Bengal Hepatogram has been described previously as a highly sensitive test for liver functions in jaundiced patients, which also provides valuable diagnostic information regarding liver vascular capacity and bile flow interference. The latter feature makes it useful in the differential diagnosis of medical versus surgical jaundice. Since all standard tests for liver function may fail to detect clinically apparent liver disease, the Rose Bengal BSP blood clearance stress test was developed.

The need for further clinical evaluation of these tracer tests has been recognized by the authors and other investigators. This paper is presented to compare the Rose Bengal findings (500 tests) with the results of seven standard liver tests in large groups of patients with medical and surgical jaundice in whom the diagnoses has been established by clinical and laboratory methods and confirmed by either liver biopsy and/or operative findings and autopsy.

Rose Bengal-BSP blood clearance stress tests have been performed in 23 control subjects in whom values for the 45 minute BSP blood retention and tracer dose Rose Bengal blood clearance half-times have also been determined. Tracer and stress test results have been compared with BSP retention in 39 nonjaundiced patients having probable liver disease and in 25 individuals having

proved liver disease of mild to moderate severity.

The comparative findings indicate that the tracer hepatogram is the best indicator of biliary tract obstruction. It is less sensitive than the BSP for detecting mild liver damage in nonjaundiced patients. The Rose Bengal stress test is more sensitive than the BSP in the same patients and also in individuals having probable liver disease."

Interim Report

The Miokon I¹³¹ Renogram

G. V. Taplin and D. Johnson

Work has continued on developing an improved technique for evaluating individual renal function by substituting Miokon I¹³¹ for Diodrast during performance of the renogram. A paper entitled "The Miokon I¹³¹ Renogram" was also presented at the meeting of the Nuclear Medicine Society in Chicago on June 18, 1959. An abstract of this paper follows:

"The Diodrast I¹³¹ Renogram has been described previously as a useful clinical tool in urology and as a rapid screening procedure for detecting unilateral renal disease in hypertensive patients. The procedure requires careful kidney localization by x-ray examination and an angular positioning of the renal detectors to avoid liver interference, since Diodrast is partly removed by the liver and excreted in the bile.

This study was undertaken to improve the radioisotope renogram in three respects: (1) to simplify the positioning technique by using collimated detectors having larger fields of view; (2) to eliminate liver interference by substituting Miokon or Hypaque, which have insignificant liver uptake, and, thereby, permit perpendicular placement of the renal probes over the flanks; (3) to augment the test by obtaining additional information regarding renal function by making simultaneous measurements of tracer blood clearance half-times and by determining the per cent excretion of the tracer and PSP in 30 minute urine samples.

The modified Miokon I¹³¹ Renogram procedure has been studied in 100 patients with a variety of renal diseases. Also Diodrast and Hypaque have been used as test agents in many of the same individuals and in 15 normal subjects for comparative purposes. The new Miokon-PSP-Renogram has been demonstrated to be superior to the original Diodrast procedure in that positioning of the renal probes is less critical, liver interference with the right renogram is eliminated, and the blood clearance half-time and urinary excretion values not only give important information on total renal tubular function, but also aid in interpreting the individual renograms."

Interim Report

Studies of Liver Function and Hepatic Blood
Flow Using Rose Bengal and Colloidal Gold

G. V. Taplin, D. Johnson, and O. M. Meredith

During the past six months a clinical investigation on this subject has been conducted at the Harbor General Hospital. Approximately 70 patients with a variety of liver disorders, including cirrhosis in various stages, acute infectious hepatitis, biliary obstruction, and hematochromatosis, and suitable control subjects have been studied. All patients were tested with the Rose Bengal Hepatogram to determine liver vascular capacity, polygonal cell function, and bile flow interference. They were also tested with colloidal gold to measure liver blood flow. Furthermore, the preceding tests were supplemented by seven standard biochemical liver tests including the 45 minute bromsulphalein retention, the albumen/globulin ratio, serum bilirubin concentration, alkaline phosphatase, cephalin flocculation, and thymol turbidity. Several patients were studied repeatedly during the course of their diseases. The colloidal gold blood clearance half-time values varied from $2\frac{1}{2}$ to 5 minutes in normal subjects. These results were in accord with the findings previously reported by Vetter, et. al., at the Second International Conference on the Peaceful Uses of Atomic Energy at Geneva, Switzerland. Patients with mild uncomplicated Laennec's cirrhosis showed only slightly increased gold half-time values,

whereas, cirrhotic patients with advanced liver disease and ascites had half-time values ranging from 9 to 26 minutes. Individuals having acute infectious hepatitis and serum hepatitis and severe obstructive jaundice all had gold blood clearance half-time values of less than 6 minutes. Thus, it appears that the colloidal gold test is a valuable indicator for detecting the vascular defects in cirrhosis. It aids in evaluating the abnormally slow Rose Bengal blood clearance in patients with advanced cirrhosis. Furthermore, it may have value in the differential diagnosis of jaundice.

A new project is underway using the same tests in conjunction with other standard biochemical liver tests to evaluate the effect of various anesthetics on liver function. In these studies liver biopsies are being obtained pre-operatively and immediately following operation. This pathological biochemical data should be of great assistance in evaluating the reliability and accuracy of the Rose Bengal test for detecting liver damage.

Interim Report

Radiostrontium Studies in Animals

N. S. MacDonald, M. Hepler, and E. Brooks

Comparisons of the behavior of tracer amounts of bromide ion and strontium ion were continued. A mixture of Br^{82} and Sr^{85} was injected into a femoral vein of rats, each of which was bearing a left tibial fracture. One day after the injection both tibiae were obtained from each rat and the burdens of both radioactive species determined by the use of gamma-ray scintillation counting with a single-channel spectrometer. In terms of the per cent of the dose retained per whole tibia, the values for Sr^{85} were all about ten times as great as for Br^{82} , in fractured as well as in intact bones. For each animal the per cent of the dose retained in the fractured tibia was greater than that in the contralateral, intact tibia, for Br^{82} as well as for Sr^{85} . These differences can be more easily compared when expressed as increments. For example:

$$\Delta \text{Sr} = \frac{(\% \text{ of dose in left tibia}) - (\% \text{ of dose in right tibia})}{\% \text{ of dose in right tibia}} \times 100$$

At four and five days post-fracture, ΔSr values ranged from 22-54, while the corresponding ΔBr values for an individual rat was 2.5-3 times greater than the ΔSr value for that animal. However, at eleven and twelve days, when the calcification of the fractures was most vigorous, the values were more nearly equal, ΔBr ranging 200-264. The values for control animals, bearing no fractures,

were 0 ± 10 . Reasonable interpretations of these findings are being sought. For example, the apparent avidity for Br^{82} of the tissue at the fracture site, at a time before extensive new bone has been formed (4-5 days) may be due to an enlarged "chloride space" or fixation in the hematoma at the site. The great increase in Sr^{85} retention in eleven to twelve day old fractures is attributable to the presence and continuing formation of large amounts of new calcium bone salts in the callus at this period in the normal course of healing.

The double tracer technique was used in another fashion in an attempt to learn something of the kinetics of calcium transfer between mother and fetus. Since two radioisotopes of calcium were not available, Sr^{85} (a pure gamma emitter) and Sr^{90} (a pure beta emitter) were used as substitutes. In collaboration with Dr. Hutchinson of the Department of Obstetrics, who initiated the study and performed the delicate surgical operation, $\text{Sr}^{90} - \text{Y}^{90}$ was injected into a vein of a pregnant rhesus monkey nearing the end of her term. At the same time Sr^{85} was injected into a vein of the fetus. Plastic cannulae had been inserted so that samples of maternal blood, fetal blood, and amniotic fluid could be taken at intervals. After $1\frac{1}{2}$ hours both were sacrificed and selected tissues, as well as both carcasses, were assayed for Sr^{90} and Sr^{85} content. These analyses were performed by conventional methods; gamma scintillation counting for Sr^{85} and

beta counting with a Geiger-Muller tube for Sr^{90} . As expected, the rate of disappearance of an isotope from the blood of the animal receiving the injection could not be described as a single exponential function. The Sr^{90} required approximately 10-15 minutes to reach a maximum concentration in fetal blood (at which time it was 60 per cent of the concentration in maternal blood.) The Sr^{85} required 15-20 minutes to reach a maximum concentration in maternal blood and this maximum was only 5 per cent of the Sr^{85} concentration in the fetal blood at that time. This disparity in peak concentrations may be due primarily to the much greater dilution of Sr^{85} entering the maternal blood pool, compared to Sr^{90} entering the fetal blood pool. However, the disparity in times, if confirmed in subsequent experiments, will require a more subtle explanation. Transport of strontium (and presumably, of calcium) in both directions across the placenta is rapid. The gross distribution of both radioactive tracers is given in Table V. The concentrations in selected tissues are presented in Table VI.

Fetal bone was more avid for both Sr^{90} and Sr^{85} than was maternal bone, spongiosa more avid than compact bone and the ratio of concentration in spongiosa to that in compact bone was about the same, whether the strontium came from the maternal or fetal blood. As an interesting side light, the mother monkey was found to have an

accidental phalange fracture of uncertain age. The concentration (c/s/gm of ash) of both Sr^{85} and Sr^{90} of the callus in this area were, respectively, 5.7 and 5.5 times the concentrations in the corresponding contralateral finger. Here again, as far as the process of calcification is concerned, it appears to make little difference whether the radiostrontium (and by inference, the calcium) is derived from the maternal or the fetal blood. These studies will continue as soon as another pregnant monkey is available.

Interim Report.

TABLE V

GROSS DISTRIBUTION OF THE RADIOACTIVITY RECOVERED
FROM ALL SAMPLES AND CARCASSES $1\frac{1}{2}$ HOURS AFTER
INJECTION OF RADIOISOTOPES

	<u>Fetus</u>	<u>Mother</u>	<u>Placenta</u>
Sr^{90}	5.7%	88.1%	6.2%
Sr^{85}	11.1%	73.0%	15.9%

30 μc of Sr^{90} - Y^{90} were injected intravenously into the maternal circulation. Simultaneously 19 μc of Sr^{85} were injected into a vein of the fetus by a surgical operation.

TABLE VI

CONCENTRATION OF RADIOACTIVITIES IN MONKEY
TISSUES 1½ HOURS AFTER INTRAVENOUS INJECTION
OF Sr⁹⁰ INTO THE MATERNAL CIRCULATION AND
Sr⁸⁵ INTO THE FETAL CIRCULATION

	c/s/gm. wet tissue			c/s/gm. of ash		
	Liver	Kidney	Muscle	Tibia shaft	Tibia end	Shaft/end ratio
Fetus Sr ⁹⁰	8	14	29	220	738	3.6
Sr ⁸⁵	3	5	4	97	373	3.9
Mother Sr ⁹⁰	37	69	15	72	304	4.2
Sr ⁸⁵	2	4	1	4	16	4.0

Radiostrontium Metabolism in Humans

N. S. MacDonald, M. Hepler, and E. Brooks

Two studies of the uptake and excretion of Sr⁸⁵ in human subjects suffering from osteoporosis (non-senile) were made. These patients were on metabolic balance regimes so that full data on daily calcium and nitrogen balance, as well as Sr⁸⁵ excreted, will be available. Both studies were conducted with equipment designed especially for obtaining radioisotope osteograms. This gear for bedside use was assembled with the aid of the Electronics Section of the Laboratory. It consists of a NaI crystal for gamma scintillation counting whose output is routed to two single-channel pulse height analysers (transistorized models obtained from HASL). The output

of each analyser may be recorded on a ratemeter-recorder combination or on a separate scaler. After proper adjustment of channel widths a mixture of iodinated human serum albumin (RISA) and Sr^{85} may be injected intravenously and the rate of accumulation of both materials at selected sites in the body may be determined simultaneously. The interpretation of these RISA- Sr^{85} Osteograms and correlation with balance data is incomplete, but it appears that in these patients the rate of transport of the Sr^{85} to the bone was normal. (With "senile osteoporosis" this transport appears to be reduced, possibly because of reduced capillary blood flow.) These studies are continuing with the collaboration of members of the staff of the Departments of Radiology, Medicine and Surgery.

Interim Report.

Studies of Liver Function During Chronic CCl_4 Poisoning with the I^{131} Rose Bengal Liver Function Test and the Au^{198} Minimum Liver Blood Flow Test

O. M. Meredith, Jr., G. V. Taplin, W. Coffman, and J. Post

The outstanding advantage of use of the I^{131} Rose Bengal and Au^{198} colloidal gold tests in study of the development of cirrhosis induced by chronic CCl_4 poisoning is the opportunity to identify those states in which polygonal cell secretion of I^{131} Rose Bengal has been impaired by alterations of the liver blood flow, due to disruption of the normal hepatic architecture, through estimation of the minimum

liver blood flow with the use of Au^{198} colloidal gold.

A second objective in the present investigation has been to establish experimentally, under controlled conditions, the basis for use of the bromsulfalein (BSP) I^{131} Rose Bengal stress test for detection of impairment of liver function. The use of BSP, administered simultaneously with a tracer dose of I^{131} Rose Bengal, has been intended to indicate depression of liver secretion of Rose Bengal when the radioactive tracer dose alone does not place adequate stress upon the physiological process to show up a deficiency in function. (It has been reported previously that simultaneous administration of 5 mg/kgm of BSP with the radioactive tracer dose did not significantly alter the physiological response, as indicated by measurement of blood retention of Rose Bengal, from that observed following administration of the tracer alone (UCLA No. 420).)

The accompanying tables contain results from recordings made with a gamma-ray scintillation detector placed over the head of animals following intravenous administration of either I^{131} Rose Bengal or Au^{198} colloidal gold. Evaluation of the responses has been based upon comparison of 95 per cent confidence range for individual control animal response and the means of the experimental groups for a particular perimeter since a significant loss of animals due to poisoning has reduced the number of observations available. Effort

in this preliminary investigation has been directed toward determination of a poisoning regime for future work which would induce the greatest amount of cirrhotic involvement with a minimum loss of animals in a reasonable time.

Use of Au¹⁹⁸ colloidal gold to gain an index of liver blood flow has shown a steady level in the high normal range in Table VII with a single marked exception of an animal in Group II which has marked prolongation of the blood clearance half-time at 210 days et seq. Such a change should be indicative of the beginning of a severe cirrhotic state, but conclusive evidence is not yet available. In general, there has been no significant alteration of the minimum liver blood flow over the period of study.

In Table VIII the tracer and stress tests for Groups I and II showed a significant difference in response following administration of 50 mg/kgm of CCl₄ twice weekly per os during the initial phases showing that the stress test can indicate abnormal response while the tracer test falls in the normal range. As the experiment proceeded, however, the difference in these responses became reduced until the 109th day or later with Groups I and II when tracer and stress dose became similar or reversed in relation to one another. The differences in these last cases may not be entirely significant since it is not possible to perform all of the test measurements on the same day due

to increasing biological background as the tests are performed. The routine procedure has been to test animals as late as possible following the most recent poisoning and prior to the next scheduled poisoning, and to complete the remaining tests in the next poisoning cycle. Hence, the reported results are gained within a week of the stipulated elapsed time and all tests are performed under similar conditions as possible.

Thus, the use of the BSP-I¹³¹ Rose Bengal stress test has been useful for indication of liver impairment under conditions of this investigation, but in later phases the response tends to become similar to that for the tracer test usually at an abnormal level of response. An understanding of this complication must be deferred for future investigation.

Biopsy specimens of the liver have been obtained at frequent intervals from all experimental animals. Grossly these specimens show the development of hobnailed livers and there has not been a development of hepatic decompensation and ascites. Microscopic evaluation is forthcoming.

Interim Report.

TABLE VII

RESULTS OF AU¹⁹⁸ MINIMUM LIVER
BLOOD FLOW TEST IN CHRONIC CCl₄
POISONING OF RABBITS¹

	Elapsed time (days)	Number of animals ²	Blood clearance half-time (sec) ³
Group I	18	4	36 (24 - 44)
	53	4	31 (24 - 40)
	120	4	43 (40 - 46)
	185	3	37 (32 - 48)
	225	3	45 (45 - 54)
	286	3	40 (28 - 52)
Group II	7	5	33 (24 - 52)
	31	5	36 (24 - 48)
	109	5	41 (32 - 46)
	179	4	42 (38 - 48)
	216	2	50 (36 - 64)
	279	2	80 (40 - 120)
Group III	41	3	39 (32 - 44)
	72	3	40 (28 - 52)
	107	3	53 (48 - 56)
	170	3	40 (40 - 40)

Control Values

Number of animals	Mean	Standard error	95% Confidence Range for individual response
39	39 sec.	1.6 sec.	16 - 55 sec.

1. Female Dutch rabbits, 1-1.5 kgm, fed on a balanced commercial rabbit diet were used.
2. The experimental groups contained 6 animals each at time of inception.
3. The half-time measurements were determined from semi-logarithmic graphs of the Au¹⁹⁸ blood clearance and liver uptake curves. The mean value and range of the observations is presented.

TABLE VIII

RESULTS OF I^{131} ROSE BENGAL LIVER TESTS IN CHRONIC CCl_4 POISONING OF RABBITS¹

Poisoning Period (days)	CCl_4 Dose ² (mg/kgm)	Number of animals ³ by group	Rose Bengal Tracer Test per cent	Blood Retention ⁴ Stress Test per cent
18	50	4	30 (22 - 34)	45 (36 - 57)
53	5-10	4	31 (28 - 36)	60 (50 - 82)
84	10	4	51 (48 - 53)	57 (47 - 68)
120	10-15	I 4	49 (30 - 71)	58 (35 - 78)
185	15-25	3	40 (35 - 48)	36 (34 - 38)
225	25-15	3	39 (25 - 54)	44 (37 - 51)
286	15	3	38 (37 - 38)	46 (34 - 54)
7	5	5	28 (21 - 37)	29 (23 - 36)
31	5	5	25 (16 - 32)	
73	5-10	5		
109	10-15	II 5	45 (28 - 53)	42 (26 - 57)
179	15-25	4	47 (40 - 56)	50 (40 - 73)
216	25	2	45 (37 - 51)	55 (48 - 61)
279	15	2	48 (36 - 59)	40 (35 - 44)
72	50	3	33 (21 - 42)	67 (64 - 70.5)
107	50	III 3	47 (46 - 48)	57 (51 - 65)
170	50	3	58 (36 - 72)	61 (38 - 82)
	Controls ⁵	43	26.9 $\pm$ 5.9	32.8 $\pm$ 4.2

- Notes:
1. Female Dutch rabbits, 1-1.5 kgm., fed on a balanced commercial rabbit diet were administered CCl_4 in olive oil orally twice weekly.
 2. Changes in CCl_4 dosage indicated in column 2.
 3. Six animals per group initially.
 4. Per cent Rose Bengal blood retention at 10 minutes as related to the two minute level measured over the head.
 5. Mean plus or minus the standard deviation. The 95 per cent confidence range for control response is 15-38 per cent retention.

persisted in Ladino clover over prolonged cropping periods. Neither form of applied Ca reduced Sr^{90} uptake from neutral or alkaline-calcareous soils. It appears that further additions of Ca will provide little protection against crop uptake of the radiostrontium fission products from Ca-rich soils.

The Sr/Ca atom ratios and distribution factors for beans were dependent upon the concentration of these cations in the nutrient substrate and the method of assessing plant available Sr and Ca. When adequate levels of Ca were present for plant needs, the Ca content of the plant reached a maximum concentration. Upon further additions of excess Ca, the absorption of Ca proceeded at a reduced rate relative to the rate of Sr absorption, and a discrimination occurred in favor of Sr uptake.

Publication: SOIL SCIENCE 87:160-165, March, 1959.

Effects of Bicarbonate and Some Other Anions on the Shoot Content of P^{32} , Ca^{45} , Fe^{59} , Rb^{86} , Sr^{90} , Ru^{106} , Cs^{137} , and Ce^{144} in Bean and Barley Plants

J. A. Goss and E. M. Romney

Bean and barley plants were cultured in nutrient solutions and subsequently treated to include a control and 10 me. of NaCl , NaNO_3 , Na_2SO_4 , or NaHCO_3 added per liter. The content of radioisotopes in the shoots was determined after an 8-hour period on treatment.

The presence of added bicarbonate very significantly reduced the shoot content of all radioisotopes studied except Cs^{137} . This reduction was more severe in bean plants, particularly for P^{32} and Fe^{59} . A slight reduction was observed in the content of P^{32} and Ca^{45} in beans cultured on NaNO_3 -treated solutions and of P^{32} , Ca^{45} , and Sr^{90} by beans cultured on Na_2SO_4

treatments. The shoot content of Ca^{45} was reduced in barley cultured on NaCl , NaNO_3 , and Na_2SO_4 treatments. Barley appeared to be more tolerant of the bicarbonate anion than was the bean plant.

Publication: PLANT AND SOIL X:233-241, March, 1959.

Lime-induced Chlorosis Studied

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

These studies have indicated that iron deficient plants fix carbon dioxide in the dark more rapidly than green plants. Lime soils are known to have abundant supplies of carbon dioxide as carbonate and bicarbonates. Evidence is thus accumulating that organic acids and amino acids may be directly related to the causal mechanism of lime-induced chlorosis.

Publication: CALIFORNIA AGRICULTURE 13:3.

A Granular Collector for Sampling Fallout Debris from Nuclear Detonations

E. M. Romney, J. W. Neel, G. M. Le Roy, A. J. Steen, and K. H. Larson

A granular collector for sampling fallout debris from nuclear detonations was developed which consists of a shallow metal tray and a removable mylar plastic liner filled with 1/8- to 3/16-inch diameter polyethylene plastic granules. Fallout debris impinging upon the exposed collector tray during fallout is physically entrapped within the granular matrix and later separated from the matrix by wet sieving in isopropyl alcohol. The essentially pure fallout debris then may be separated into different particle size ranges by dry sieving. The efficiency of the granular collector was tested under laboratory and field conditions. Its performance during fallout was compared with gummed paper and resin-coated

plate collectors and samples of the native soil surface.

Publication: Report UCLA-432, January, 1959.

The Influence of K and Cs on the Release of Cs¹³⁷ from Three Soils

H. Nishita, E. M. Romney, G. V. Alexander, and K. H. Larson

Experiments were conducted to study the influence of K and Cs amendments on the uptake of Cs¹³⁷ by Ladino clover upon prolonged cropping of contaminated soils.

The Cs¹³⁷ uptake by plants increased as K concentration in the soil was reduced by cropping. The addition of K to soils with relatively high levels of K was ineffective in reducing Cs¹³⁷ uptake by plants. However, after the soil K was decreased to a low level of cropping, the addition of K to soils reduced Cs¹³⁷ uptake by plants.

Unlike the addition of K, the addition of carrier Cs to the soil markedly increased Cs¹³⁷ uptake by plants and reduced the K uptake. The addition of Cs even at a level severely injurious to plants increased Cs¹³⁷ uptake rather than reduced it. The threshold level for producing injury to clover appeared to be around 4.5×10^{-4} me. Cs per g of soil in Vina loam. The toxic effect of Cs added in injurious amounts to the soil was alleviated by the addition of K. The amount of Cs and K uptake by plants depended on the soil type.

Publication: Report UCLA-437, May, 1959. Submitted for publication in SOIL SCIENCE.

Factors Influencing the Biological Consequences of Environmental Contamination by Nuclear Debris

R. G. Lindberg

Experience in conjunction with weapons testing activities in the continental United States has shown (1) that radioactive fallout is immediately available to animals, and at near distances is accumulated and/or metabolized in microcurie levels of concentration, (2) radioactive contamination is persistent in the environment over a period of years, (3) radioactive substances resulting from fallout contamination are persistent in metabolic systems for a period of years, apparently in equilibrium with the environment, and (4) the potential hazard from internal emitters cannot be assessed on the basis of mr/hr intensities at any given location since the problem posed by internal emitters is dependent upon the biological availability of radioactive materials rather than the total radioactivity present.

Factors influencing the biological fate and persistence of radioactive fallout have been shown to be (1) the physical and chemical characteristics of the matrix in which the fission products occur which are in turn dependent upon the conditions of detonation, (2) the predominant fallout particle size in any particular area, and (3) the kind of soil and land usage concerned. The ultimate objective of these studies is to define the ways in which radiation can reach man through the environment.

Publication: Report UCRL: In press.

Report in preparation - Sr^{90} Content in Bones from Mule Deer and Big Horn Sheep from Environs Adjacent to the Nevada Test Site

R. G. Lindberg and J. H. Olafson

Sr^{90} content was shown to be of the same order as in Jack rabbits and Kangaroo rats sampled from comparable locations. Sr^{90} in deer bones appeared to correlate better with degree of rainfall in the sampling area than with operations at NTS. Big Horn sheep sampled from the Desert Game Range in the Sheep Mountain area of Clark County, however, while generally lower in Sr^{90} content than mule deer showed a significant increase between November, 1956 and November, 1957. This increase cannot be attributed to Operation Plumbbob either on the basis of published fallout patterns or rainfall. The maximum strontium values observed were 24.4 strontium units in a deer collected at Beaver Dam, Nevada in 1957 and 19.5 S.U. for a Big Horn sheep collected from Sheep Canyon, Nevada in 1956. This was collaborative work with the veterinarian assigned to the USAEC Las Vegas Field Office, Las Vegas, Nevada.

Some Environmental Factors Influencing Radiostrontium Uptake by Plants

E. M. Romney, W. L. Ehrler, A. Lange, and K. H. Larson

The amounts of Sr^{90} taken up from Vina soil varied among several different crop plants by a factor as high as ten on the basis of dry weight. The relative decreasing order of Sr^{90} uptake by mature plant tops was turnip - millet > Swiss chard > Ladino clover > broccoli > soybeans > barley > oats > wheat > spinach. For each of the cereal crops, the concentration of Sr^{90} in the grain was only about one-fifth of the Sr^{90} in the forage. Lowering of root temperature from 17° to 7° C significantly reduced Sr^{90} uptake by barley and beans during a 24-hour absorption period.

The Q_{10} values indicated that strontium uptake was chemically controlled rather than the result of physical phenomena.

Reduction of light intensity from 1,000 to 450 foot-candles significantly reduced Sr^{90} uptake by barley and beans. The uptake of Sr^{90} also was reduced as the light exposure period was decreased. One-third to one-half of the total amount of Sr^{90} obtained during a 24-hour absorption period was taken up independent of exposure to light.

Publication: PLANT AND SOIL (Submitted for publication).

Radio-ecological Aspects of Nuclear Fallout

K. H. Larson, J. W. Neel, R. G. Lindberg, L. Baurmash, H. A. Hawthorne, and G. V. Alexander

The radiation levels of fallout patterns during Operation Plumbbob were determined by the aircraft and techniques utilized by the U. S. Geological Survey and the Raw Materials Branch, AEC. In addition, five to ten mobile ground teams were employed. A conversion factor for aerial monitoring to equivalent ground was established, i.e., 50,000 counts/sec at an elevation of 500 ft was the equivalent of 1 mr/hr at 3 ft above the ground. Using this relationship, aerial- and ground-derived values generally agreed within ± 15 per cent.

The fallout patterns of seven tower-supported and three balloon-supported detonations were delineated to distances ranging from 100 to 600 miles from Ground Zero and representing the time of fallout of at least 12 hrs after detonation. Based upon available comparisons, balloon-supported shots appeared to produce less than 10 per cent of the total fallout produced by tower-supported shots of equivalent yield and detonation height over fallout times up to 12 hrs. It was estimated that

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10 to 30 per cent of the total radioactivity produced by tower-supported shots was accounted for by these measurements and criteria.

Particle size analysis of fallout samples indicated that tower-supported shots produced much greater amounts of fallout in size ranges greater than 44 microns in diameter than did balloon-supported shots, and in many locations the majority of the radioactive particles from balloon-supported shots was less than 5 microns in diameter.

An index of biological availability was based on solubility of fallout material in 0.1 N HCl and water. The solubilities of fallout material from tower-supported shots were of the order of seven per cent and one per cent on 0.1 N HCl and water, respectively, and the solubilities of fallout material from balloon-supported shots were of the order of 70 per cent and 15 per cent.

Gamma decay measurements based upon both prelocated field monitoring instruments and laboratory analyses indicated a general deviation from the $(\text{time}_1/\text{time}_2)^{-1.2}$ relationship. Calculations of infinite gamma dosage, based upon the above relationship, were estimated to be approximately 10 per cent high at a fallout time of 1 hr, approximately true at 1.1 hrs, and 10 to 70 per cent low at fallout times from 1.2 to 20 hrs. The measured gamma radiation decay is not a simple relationship. Beta decay measurements more closely approximated the $(\text{time}_1/\text{time}_2)^{-1.2}$ relationship.

The accumulation of fission products by native rodents sampled from various locations within fallout patterns was documented for five detonations during Plumbbob. The studies were considered a 'tracer' study with a purpose of defining some of the factors influencing the biological accumulation of radioactive fallout.

A comparison of the data from the five tower detonations studied shows a marked similarity in the patterns of biological accumulation when the data were related to the time of arrival of fallout at the collection location. Maximum total fission product accumulation in the animal tended to occur at locations corresponding to time of arrival of fallout of $H + 2$ to $H + 3$ hrs. Total fission product concentration in all tissues decreased with times of arrival of fallout from $H + 3$ to $H + 12$ hrs. In the case of the several tower-supported detonations studied, the fission product concentrations remained more or less uniform over distances at which the time of fallout arrival occurred between $H + 5$ and $H + 14$ hrs. However, in contrast, in the case of the single balloon-supported detonation documented, the rate of decrease with respect to distance is approximately constant over the distances at which the time of fallout arrival occurred between $H + 2$ and $H + 12$ hrs.

Between 82 and 87 per cent of the radioactivity found in the thyroid tissue of the native rodents was accounted for at $H + 72$ hrs in terms of 17 - 20 per cent iodine¹³¹ and 65 - 67 per cent iodine¹³³. Samples taken at $D + 20$ days contained only iodine¹³¹. Between 12.5 and 40.0 per cent of the several fission products (Sr^{89-90} , Y^{91} , Ce^{144} , Cs^{137} , and Ba^{140}) accumulated in bone were accounted for in terms of radiobarium and radiostrontium.

During the first three weeks following radioactive fallout contamination of an environment, the decline in residual radioactivity is due to radioactive decay according to $t^{-1.04}$ during the period $D + 3$ days to $D + 21$ days. Maximum daily redistribution of residual fallout was measured at 1.68 $\mu\text{c}/\text{sq ft}$ during Priscilla at $D + 16$ days and at 4.03 $\mu\text{c}/\text{sq ft}$.

during Smoky at D + 17 days (values corrected to a common time of H + 12 hrs). Air concentrations were measured during Priscilla and Smoky with readings ranging between 837 to 1,971 $\mu\text{C}/\text{M}^3$. The air concentrations were similar for both shots even though the degree of contamination was two to five times greater in the case of Smoky fallout. A diurnal cycle was demonstrated with all stations showing maximum readings at mid-day and minimum readings shortly after midnight.

Pre- and postseries soil samples were collected from 25 agricultural areas at distances ranging from 50 to 230 miles from NTS. At ten of the locations feed and milk samples were collected. A special agricultural sampling in conjunction with aerial and ground monitoring was conducted in South Dakota following the Diablo and Stokes shots.

Publication: Plumbbob Weapons Testing Report, WT-1488.

IMPROVEMENTS IN TECHNIQUES AND INSTRUMENTS

Chemical Dosimeters for Civil Defense

G. V. Taplin, K. Malin, and M. L. Griswold

Studies have been continued on the development of aqueous phenol red-trichloroethylene gamma-ray dosimeters for civil defense and for use in radiology to measure tumor doses directly and to study depth-dose distribution in phantoms. A paper entitled "Chemical Dosimeters for Civil Defense" was presented at the annual meeting of the Radiation Research Society in Pittsburgh, Pennsylvania on May 19, 1959. The abstract of this paper follows:

"External gamma radiations from fallout have become a major factor in civil defense since the development of thermonuclear devices which can contaminate large areas and injure tremendous numbers of people. Even rough estimates of the doses received by individuals and/or at specific locations within large target areas could have considerable psychological and medical value during the early phases of a nuclear emergency. Two types of direct reading chemical dosimeters have been designed for these purposes. The personnel dosimeter registers gamma ray doses of either less or more than $125 \text{ r} \pm 25 \text{ r}$ and, thereby, informs the individual that he has or has not received an exposure requiring early medical attention. The area monitoring dosimeter provides civil defense officials with

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exposure data (0-1000 r) at specific locations such as schools, fire and police stations, factories, and other highly populated areas.

Dosimeters of these types have been evaluated under both laboratory and field conditions during the past five years. Data will be presented to demonstrate their practical applicability in the radiological aspects of civil defense. Emphasis is placed on the psychological value to the large proportion of individuals whose radiation exposures may be below the level causing disability or requiring medical assistance."

Interim Report.

Aqueous Chemical Dosimeters for
Thermal Neutron Measurements

G. V. Taplin, K. Malin, and M. L. Griswold

A second paper entitled "Aqueous Chemical Dosimeters for Thermal Neutron Measurements" was also presented at the same meeting along with a ten minute colored movie which demonstrated the technique for preparing aqueous chemical dosimeters. This paper included the most recent studies on the effects of impregnating aqueous chemical systems with lithium⁶ chloride to increase their sensitivity to thermal neutron exposure. This work was performed in collaboration with Doctors Victor Bond and Eugene Cronkite of the Brookhaven National Laboratory. An abstract of this paper follows:

"This study was performed for two main purposes: (1) to aid

in the evaluation of previous field test data on the response of film and chemical dosimeters to mixed N-gamma bomb radiations; (2) to devise chemical methods for estimating both components of the N-gamma field in slow neutron reactor ports.

The response of aqueous phenol red-trichloroethylene dosimeters can be sensitized to thermal neutron exposures in nearly direct proportion to the amount of lithium⁶ added on the basis of the lithium⁶ N-alpha reaction. The addition of lithium chloride in amounts up to 2 per cent reduces the gamma-ray response of the same system by less than 50 per cent. Thus, lithium-free and lithium⁶-loaded dosimeters may be made which have nearly equal responsiveness to gamma radiations but have thermal neutron sensitivity differing by 20-fold or greater.

Such instruments permit direct measurements of both components in mixed N-gamma fields, and therefore, are suitable for research purposes and may also be applicable as crash dosimeters for use by reactor personnel in the event of accidental exposure to large doses of mixed N-gamma radiations."

Interim Report.

Low Level Gamma Counting

N. S. MacDonald, M. Hepler, and E. Brooks

The major design features of the human total-body radiation counter were drawn up and procurement of most of its components was completed. The facility will utilize a steel room of the type originated at the Radiological Physics Division of Argonne National Laboratory. It will differ from the Argonne room in door design and will have the added feature of an emergency port for use in the event of door blockage. The dimensions will be 8' x 8' x 8' with all walls, floor, and ceiling made up to 6" thickness by laminating $\frac{1}{2}$ " thick steel plate. This plate is now cut and sandblasted and assembly will begin shortly. The counting equipment will consist of four NaI-Tl crystals, 5" diameter x 4" thick with 3" photomultiplier tubes and individual high voltage supplies. The output of each detector will be amplified and analysed separately by a 50-channel subgrouping of a 200-channel pulse-height analyser. The circuiting will also permit integration of the outputs from any combination of the four detectors and analysis of the combined counts by the full 200 channels.

Samples of all materials going into the steel room will be monitored for gamma contamination with the 3" x 3" NaI crystal installed in the cylindrical steel shield described in the previous semi-annual report. This counting equipment includes a single-

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channel pulse height analyser and is of adequate sensitivity to detect the Cs^{137} contamination in current milk. For example, a 2.4 lb. sample of de-fatted milk powder from a local market was assayed for K^{40} and Cs^{137} . The latter activity amounted to $125 \mu\mu\text{c}$ of Cs^{137} per gram of K -- a value in agreement with data from the Los Alamos program. Sweepings from a parking area, including dust and "fallout ash" from a local brush fire showed activities attributable to Cs^{137} , thorium decay products and possibly Zr^{95} - Nb^{95} . Welding rods showed significant radioactivity, which was found to reside entirely in the external jacket of flux. Samples of weldments, steel plate, steel and lead shot showed no detectable activity.

Interim Report.

Preparation of Two Materials for Examination in The Electron Microscope.

F. W. Bishop and Ruth G. McCandless

1. Technique for examining tissue culture cells:

The tissue cultures are received growing on cover slips in small petri dishes. The buffered saline which covers the culture is carefully pipetted off, and the dish filled with cold isotonic 1% OsO_4 , buffered to pH 7.4 with veronal acetate. Cells are fixed for five minutes, the short fixation time giving optimum demonstration of nuclear detail. The osmium tetroxide is poured off and replaced by tap water, which is followed in turn by graded alcohols and

methacrylate monomer. We use 95% butyl methacrylate and 5% methylmethacrylate, a combination which produces a slightly firmer block than butyl alone. Two changes of monomer are used (one half hour each); the second one contains 2% benzoyl peroxide as catalyst.

In embedding the cultures use is made of solid cylindrical blocks of plastic. These are prepared by polymerizing methacrylate monomer in #0 gelatin capsules, and then dissolving off the gelatin in water. One end of the cylinder is cut as flat as possible with a razor blade. A drop of molasses-thick prepolymerized plastic is applied to this flat end and the block is then set down on a selected area of the culture, with the fresh plastic in contact with the surface of the culture. The drop of methacrylate penetrates the cells, and when this is polymerized the cell layer adheres to the bottom of the plastic cylinder. Several such preparations may be made from a single dish of culture, on the cover slip or on the bottom of the dish itself.

The dish is allowed to remain at room temperature for an hour, to allow the prepolymerized plastic to penetrate the cells, and is then placed under an ultraviolet lamp for at least 48 hours to polymerize the methacrylate. The plastic cylinders are left in place for at least another 48 hours to permit continued hardening. The blocks are then removed by setting the dish on dry ice for about 2 minutes. They are immediately placed in a dessicator, to come to room temperature without collecting moisture. These preparations are more readily removed from the bottom of the dish but they have a somewhat more

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nearly flat surface on the cover slip.

Small blocks, about 1 mm. cube, are cut out and mounted on wooden pegs. The culture cells are usually cut in a plane parallel to their plane of growth. Sections have also been cut at right angles to the plane of growth, yielding information on growth characteristics and thickness of these cells. Sections are cut on a Porter Blum microtome at 1/40 micron thickness. After mounting on 200 mesh copper screens, coated with collodion and carbon, they are examined and photographed in the EMU2-RCA electron microscope. In some instances we cut thin sections for the electron microscope, alternating with thick sections (2 to 4 microns) to stain with selective stains for light microscopy. A study is now being made of special metallic stains, such as ferric chloride, to intensify the differentiation of the DNA of the sex chromatin in electron micrographs.

2. Technique for the study of liver mitochondria in chick embryos.

The preparation procedure is as follows: The living embryo is dissected to expose the liver. A slash is made in the surface, and the cut area immediately dripped with cold isotonic 1% OsO_4 , buffered to pH 7.4. Dripping continues for 10 minutes, while the blood still circulates through the organ. A thin sliver of surface tissue is then removed to a pool of osmium tetroxide under the dissecting scope, where it is cut into narrow strips (about 1/2 mm.

wide). Fixation continues at 4°C. for 2 hours, with two changes of fixative. The long fixation time has been found necessary to bring out the fine structure of liver mitochondria. The tissue is embedded and mounted on a suitable support and is then sectioned.

Electron micrographs have been taken at relatively low magnification (x5,400) for mitochondria counts, and at about x12,000 for details of the fine structure of the mitochondria. A thick section (2 to 4 microns) is cut from each block and stained for light microscopy to aid in the identification of the area photographed, since the location of the cells within the lobule is of significance in evaluating the mitochondria count.

Experimental work on this problem is being continued in a study of the characteristics of liver mitochondria in day old normal chicks.

Interim Report.

Developments in Scintillation Counting for Application to Nuclear Medicine.

Benedict Cassen, Fred Larmie and Herbert Gass.

It was found that a slight yellowing effect in sodium iodide crystal pieces used in the cracked crystal tunnel counter mentioned in the last Semi-Annual Report could be greatly retarded by adding a small amount of Ionol (an antioxydant) to the silicone fluid in which the crystals were immersed. The Ionol was found to be soluble in the silicone. On reassembly an appreciable gain in sensitivity was

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obtained. For I^{131} an absolute counting efficiency of over 30% can be obtained on a 20 ml. sample. However, no defined spectrometric peaks could be obtained. A report on these developments was given at the Chicago meeting of the Society of Nuclear Medicine (June 1959).

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