THE UNIVERSITY OF CALIFORNIA

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SCHOOL OF MEDICINE

ATOMIC ENERGY PROJECT

CONTRACT No. AT—(04—1)—GEN—12

REPORT No. UCLA 431

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UCLA 431
Progress Reports

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

SEMI-ANNUAL PROGRESS REPORT

FOR THE PERIOD ENDING DECEMBER 31, 1958

LEGAL NOTICE

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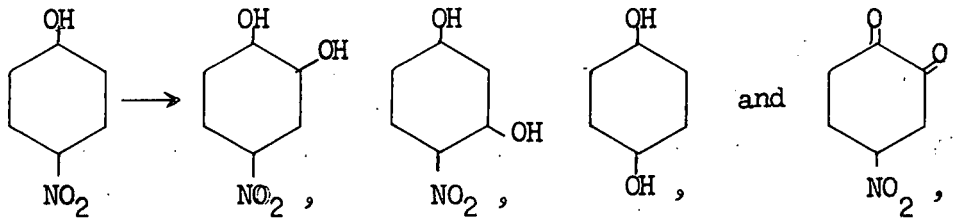
EFFECTS OF RADIATION ON CHEMICAL AND BIOCHEMICAL SYSTEMS

Irradiation of Aqueous Solutions of Nitrophenols

David R. Howton

The pioneering investigations of Stein and Weiss (cf. J. Chem. Soc. 2704 (1950); 3265 (1951) on the products of ionizing-irradiation of aromatic substances in aqueous solution lead to the expectation that irradiation of aqueous solutions of nitrophenols might involve attack at all possible positions, including those initially carrying substituents. Thus p-nitrophenol, for example, might give the following products,



I II III IV

the quinone (IV) arising via a process initiated by abstraction of a hydrogen atom from the original hydroxyl substituent.

Co^{60} γ-radiation of air-saturated solutions of p-nitrophenol in 0.1 N H_2SO_4 yields a series of ultraviolet and visible light absorption curves which are isosbestic until at least 25% of the original solute is destroyed, the isosbesticity implying that a single product (or a family of products in invariant ratio to one another and hence probably arising from a common precursor) is formed, and that this product (or products) is not attacked further at an appreciable rate by the substances produced by radiation of water.

4-Nitroresorcinol (II) has now been prepared (by nitration of resorcinol monobenzoate and saponification of the intermediate) and, together with 4-nitrocatechol (I, isolated chromatographically from commercially

available material), characterized spectroscopically. In contrast to the behavior of 3-nitrocatechol, both I and II are sufficiently stable in basic solution to permit studying these substances in such media. By taking advantage of the fact that the absorption maximum of I in 0.1 N NaOH (510 mμ) lies beyond the region of appreciable absorption of either II or p-nitrophenol, and that, in 0.1 N H_2SO_4, both I and II absorb light of wavelengths beyond the limit of appreciable absorption by p-nitrophenol, it has been possible to show that both I and II are indeed formed (G_I 0.67; G_{II} 2.2) but that these are not the sole products of the reaction; it has been possible to estimate I and II to comprise only about 26% of the total p-nitrophenol undergoing alteration.

From the data of Stein and Weiss (loc. cit.) on the radiation of aqueous solutions of nitrobenzene and of phenol, it is possible, assuming that the directive effects of the two substituents of p-nitrophenol act independently, to estimate G_I and G_{II} to be 0.27 and 0.20, respectively. It is thus clear that effects of the nitro and hydroxy substituents are interdependent.

These preliminary findings, together with the remoteness of the possibility that hydroquinone (III) comprises the bulk of the unidentified product, augurs well for substantial involvement of hydrogen atom abstraction from the original hydroxy substituent and hence, together with solution of the potentially more complex problem of the products of irradiation of aqueous o-nitrophenol solutions, for attainment of insight into certain features of the fundamentally interesting steric requirements of hydrogen atom abstraction by reactive free radicals.

Interim Report.

Effect of Gamma-Radiation on Ferriprotoporphyrin.

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

Exposure of 10^{-5} M ferriprotoporphyrin in .1 N NaOH to less than 15,000 r of γ-radiation results chiefly in a change in the molecule which leaves the porphyrin ring structure intact. This change was detected by a shift in the visible absorption peak at 612 mμ to 600 mμ and by about a 30% decrease in the Soret peak absorption. Dilute hydrogen peroxide and potassium permanganate will also cause this change. Evidence of the importance of the vinyl groups of FPP on the production of the new iron-porphyrin pigment was obtained by comparative studies with ferrideuteroporphyrin.

Reference: Nature 182, 316 (1958), UCLA-407

Radiation Induced Reactions of Ferriprotoporphyrin in Aqueous Alkaline Solution.

L. S. Myers, Jr., Mary-Louise Rothschild, Marianne Kersten, and Laura Cosi

Dilute air-saturated 0.1 N NaOH solutions of ferriprotoporphyrin (FPP) were exposed to cobalt-60 γ-irradiation and analyzed spectroscopically. The main path for the radiation induced decomposition is by a series of consecutive bimolecular reactions which give pseudo-first order kinetics. The first observed step appears to be an oxidation, possibly accompanied by polymerization, to a compound with an FPP-like spectrum (FPP') in which the resonating ring system of the porphyrin molecule remains intact. The second involves destruction of the resonating porphyrin ring system, possibly by a series of reactions initiated by radiation, and possibly involving more than one colored compound. Subsequent steps produce further colorless degradation products which partially protect the unreacted FPP and FPP'

and give rise to the apparent first order kinetics and inverse dependence of the rate constants on concentration. The yields for the first and second reactions are 7.1 and 0.3 molecules per 100 e.v. absorbed, respectively. While this scheme gives kinetic equations which approximate the data very well, there is evidence that other reactions are also occurring. Existence of FPP' was confirmed by removing FPP from irradiation solutions by extraction with n-butanol. FPP' remained in the aqueous phase. It is a stable compound which forms complexes with pyridine and CN^- ion.

Reference: UCLA-430

Effect of pH on the Radiation Induced Reaction of Ferriprotoporphyrin.

Mary-Louise Rothschild and Julianne Hitt

The rates of the two spectroscopically observable reactions that ferriprotoprophyrin undergoes when alkaline solutions are exposed to γ-radiation are affected in different ways by a change in pH. The rate of the initial reaction, the formation of FPP', varies directly with the sodium hydroxide concentration in the range 10^{-3} to 1N, whereas the second reaction, the rupture of the porphyrin ring structure, varies inversely with the sodium hydroxide concentration. This, together with previously obtained results on the effect of oxygen on the rupture of the porphyrin ring structure can be interpreted as evidence that the HO_2 radical, formed during the radiolysis of aerated water, reacts with the FPP', formed by low doses of γ-radiation, to cause the rupture of the porphyrin ring structure. If this interpretation is correct it indicates that the pK for the ionization of HO_2 is approximately 10 as some investigators believe, and not 2, as others believe.

Interim Report.

EFFECTS OF RADIATION ON BIOLOGICAL SYSTEMS

Lack of Glycoprotein Response in Irradiated Chicken Embryos.

O. A. Schjeide, Sue Simons, and Nancy Ragan

Polysaccharide associated with serum proteins has been found to be elevated in several clinical conditions including neoplastic disease, rheumatoid arthritis, tuberculosis, various infections and pregnancy. It has also been observed in the adult rat that increases in plasma polysaccharide take place by the 6th day following 700 r of whole body x-irradiation and remain elevated until about the 12th day post-irradiation. Shetlar et al. have intimated that increases in plasma polysaccharides under clinical conditions are related to tissue proliferation or repair.

It is of interest to test the serum polysaccharide response in various stages of chicken embryos and newly hatched chicks for 2 main reasons: (1) The abovementioned polysaccharide reaction to irradiation may be essentially lacking in the very young animal; (2) The normal course of increase of protein-bound polysaccharide (a doubling in concentration of both glycoprotein and seromucoid takes place at hatching) might, conversely, be inhibited by x-irradiation.

Results

No significant changes in serum glycoproteins or seromucoids were detected in younger animals as a result of irradiation. On the other hand, significant increases of both total serum polysaccharide and the seromucoid fraction were observed in irradiated 14-week-old capons.

As reported earlier, during the course of normal development, both serum glycoproteins and the seromucoid fraction are present in low concentration from the 10th to the 20th day of incubation. However,

between the 20th and 22nd day, during the period of hatching, a doubling in concentration of both of these moieties takes place in the serum. This increase is correlated with the fact that growth of both the liver and the whole body ceases abruptly at this time. It seems especially noteworthy that irradiation given prior to hatching had no detectable influence on the normal increase of serum glycoproteins and seromucoids. In contrast, dense lipoproteins (~S 0-10) which also normally appear in double concentration in the serum after hatching, are observed to be sharply reduced following the animal's exposure to 500 r of whole-body x-irradiation.

Previous studies in this laboratory have shown that serum albumin is significantly reduced in the developing chicken by 3 days post-irradiation. Utilizing the rat as an experimental animal, Shacter et al. (1952) have correlated this reduction of serum albumin with a decrease in total serum sulfhydryl. They have further related the fall in sulfhydryl with an increase in serum glycoproteins. It is of interest to note that these relationships do not exist in the embryo chicken. The drop in serum albumin (and hence presumably sulfhydryl) is not followed by an increase in glycoproteins or seromucoid.

It is possible that the embryo's failure to increase its serum glycoproteins following irradiation is due to its limited mechanisms with respect to the synthesis of these moieties. On the other hand, the embryo may lack homeostatic regulatory mechanisms present in the adult.

Conclusion

From the above results it would appear that embryo chickens and very young chicks are incapable of giving a strong serum polysaccharide

response when irradiation is employed as the stimulating agent. The cells and mechanisms for placing these moieties into the serum are, however, sufficiently well developed and radioresistant so that the normal increases in the serum are relatively slightly altered by 500 - 600 roentgens.

Interim Report.

The Effect of Water Content on the Sensitivity of T1 Bacteriophage to X-radiation.

S. R. Person and Hazel L. Lewis

When water is added to frozen dried preparations of T1 bacteriophage prior to irradiation (50 KVP X-rays from a berrylium window tube), their sensitivity to the radiation increases, as might be expected on a physico-chemical basis. While these data are contradictory to what has been observed at the cell level, we feel that our data reflect an effect more nearly due to water content because the viruses are metabolically inert when outside their host.

The lowest relative humidity at which the phage show an increase in radiation sensitivity is 13%. The sensitivity increases to its maximum value (about 2.5 times the minimum value) at a relative humidity of 27%. At relative humidities higher than this, the radiation sensitivity remains constant. The dry virus preparations are exposed to these relative humidities until an equilibrium has been established, as judged by experimental observation.

Since the virus suspensions need some protective material around them to survive drying, we do not know whether the water is absorbed by the viruses or the medium. We are investigating these two alternatives.

The radiation sensitivity also depends upon the nature of the medium in which the viruses are originally suspended and upon the method of drying these suspensions. We have some evidence indicating that these effects are also due to varying water contents and we plan to investigate this further using isotopically labelled water.

No oxygen effect was observed on dry virus samples prepared from different media and by different drying techniques.

Interim Report.

Phage Killing due to the Presence of High Specific Activity Tritiated Thymidine.

S. R. Person and Hazel Lewis

At present we are attempting to label T1 and T2 bacteriophage, or their appropriate mutants, with tritiated thymidine at sufficiently high concentrations so as to produce killing of the viruses in a reasonable time.

The recoil nucleus produced by the transmutation may not have sufficient energy to break chemical bonds and the effects due to ionizing events, caused by the emitted beta-particle can possibly be ignored due to the molecular dimensions of the viruses. Therefore, the cause of virus death may be due to the removal of a single H-atom from a DNA molecule and the resulting molecular rearrangements. It should be noted that this would be the first experiment of this nature where the label is in the "genetic part" of the virus DNA.

These experiments may offer unequivocal data concerning the fraction of the virus NA that is necessary for its duplication.

Interim Report.

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

L. E. Detrick, H. C. Upham, D. Highby, A. Dunlap and T. J. Haley

Detrick et al, (Radiation Research, 2, 483, 1955), reported that 600r whole body irradiation of rats, administered by 2 - 250 kvp Picker Industrial Units activated simultaneously, produced a survival time of 8.8 days in 50% of the animals. It also produced cellular damage, hydration and edema of the small bowel, engorged mesenteric lymph nodes and a depression of gastro-intestinal function (measured by glucose transport across an isolated intestinal loop in a Fisher-Parson perfusion apparatus) that persisted long after the histological appearance of the intestinal mucosa had returned to normal. Szony et al, (Nature, 179, 51, 1957), confirmed the latter observation for succinate oxidation in duodenal mucosa. The influence of irradiation on gastro-intestinal function has been further investigated employing thiamine hydrochloride in place of glucose, and irradiation was given by a single irradiation unit head. One hundred twelve rats were used (56 irradiated) and thiamine transport across the intestinal wall was studied over a 17 day post-irradiation period. All standard and biological samples were hydrolyzed with H_2SO_4 and incubated over night with clarase to reconvert cocarboxylase to thiamine. Quantitative thiamine determinations were made by the thiochrome method, the fluorescence of which was measured in a DU spectrophotometer. One irradiated rat died of pneumonia and 2 showed extensive petechial hemorrhages in the small bowel; otherwise the animals were in good physical condition throughout the experiment. On gross inspection and histological study the appearance, intensity and the regression of the hemorrhagic mesenteric lymph nodes were essentially the same in both the glucose and

thiamine study. The hydration and edematous syndrome of post-irradiation intestinal damage were present but less dramatic in the present experiment when compared to isolated perfused intestinal segment weight curves and histological sections of non-perfused bowel specimens. Degenerative changes were present in crypt cells 1 hour after irradiation. The greatest damage was observed at 24 hours and by the third day mitotic activity had resumed. The regular cell structure had returned and the epithelium appeared essentially normal, although some debris remained in the crypt lumen. Mucosal changes consisted of abnormally shaped, swollen, pale epithelial cells and nuclei. Dark staining, bizarre shaped masses of chromatin material were observed in the nuclei or cytoplasm between the cells and in the lumen. Capillaries of the villi were congested and the submucosal vessels were engorged with blood. Quantitative thiamine determinations of the control intestine perfusion and bathing solutions were more uniform in value and usually showed a greater thiamine transport across the intestinal wall than was found to occur after irradiation. The greater thiamine transport of the control animals resulted in a greater loss of thiamine from the perfusion solution and was reflected in the greater gain in the bathing solution. The trend in thiamine values of the perfusion and bathing solutions showed an approximate reciprocal relationship one to another.

TABLE I

Thiamine Transport from Perfusion Fluid Solution Across the Intestinal Wall of Irradiated and Control Rats (μY/cm. intestine/hr)

Solution	Experimental Rat Groups	Hours Post Irradiation 1	Days Post Irradiation 1	3	7	10	14	17
Bathing	Control	62	41	60	62	55	67	76
	Irradiated	18	37	45	25	29	33	25
Perfusion	Control	59	39	60	60	54	66	68
	Irradiated	17	31	31	24	23	33	36

Further determinations will be made on days 4,5 and 6, since this interval appeared to be a critical period in the study of the effect of irradiation on thiamine transport across the intestinal wall.

Interim Report

Point Source Beta-Irradiation of Bone

L. E. Detrick, H. C. Upham, A. Dunlap, D. Highby and T. J. Haley

Stable Sr^{88} or Sr^{90} beads were implanted within the femur of 43 CFW strain rats to study histological changes in bone, marrow, blood and body tissues due to localized β-irradiation of known intensity (330×10^3-638×10^3 rep/ccm tissue, bead surface dosage for 6 months). When sacrificed the differential blood cell count and histology of 7 organs studied showed no changes due to localized β-irradiation. Sr^{88} implanted control femurs were intact and the shaft site of implantation healed. The bone and marrow cytology were essentially negative except for fibrosis about the bead. At autopsy all Sr^{90} femurs had fractured. The fractured segments were healed together by a fusiform shaped fibrotic encapsulation

that centered over an intervening mass of necrotic tissue. Histologically the capsule consisted of the usual highly active periosteum, profuse laminations of fibroblasts actively differentiating into cartilagenous, osteoid and newly formed bone tissue. When Sr^{90} beads were found in the necrotic mass between the fractured femur tips, differentiating capsule-like tissue surrounded the area and faded into the capsule tissue. Both femur segment canals were plugged with newly developing bone and bone marrow filled the marrow cavities, being somewhat hyperplastic at the farther ends and a little less dense than normal at the fractured ends of the canal. Osteocytes were present in all lacunae of all preformed shaft bone. A comparable state of healing was observed in and around the femur segment that did not contain a Sr^{90} bead. When a Sr^{90} bead was found at the outer edge of a fractured femur segment necrotic material was observed around the bead and extended into the necrotic mass previously described. Only a few strands of acellular fibrotic tissue lay between the bead and shaft bone. For 2-3 mm from the fractured end down the femur the shaft bone was dead, avascular, acellular, of decreased density and eroded without benefit of osteoclasts. From the bead down the marrow cavity necrotic tissue faded into a cellular fibrotic plug, then into an area without marrow but in which small capillaries remained. Six to 8 mm down the shaft from the bead the marrow again appeared normal. Encapsulation also surrounded the greater portion of this segment. Osteoma, osteosarcoma and leukemic marrow changes were not observed under the conditions of the present experiment.

Interim Report

Effects of Whole Body Irradiation on the Nervous System

T. J. Haley and H. Gangloff

Serial electroencephalograms were taken from cats with implanted electrodes. The electrodes were placed in the cerebral cortex, reticular formation, amygdala, hippocampus, nucleus ventralis anterios, nucleus centralis lateralis and nucleus centrum medianium. The animals were studied chronically in the absence of anesthesia or drugs. When stable thresholds for arousal, recruiting and hippocampal seizure had been obtained after 6 to 12 weeks of study, the cats were subjected to 400r acute whole body x-ray irradiation. Irradiation was completed in 10 minutes, and electroencephalographic recording began 5 minutes later. Records were obtained every 3 hours on radiation day and twice daily thereafter for the first 5 days. Preliminary evaluation of the records showed that the spontaneous spike discharge rate of the hippocampus of one cat had increased from 12/hr pre-irradiation to 58/hr. 1 hour after irradiation, and it reached a peak of 212/hr within 6 hours. Thereafter the rate decreased to its pre-irradiation value. In two other cats, similar results of a lesser magnitude were obtained. Immediately post-irradiation, the spontaneous electroencephalogram of all animals showed a pronounced increase in a low voltage fast activity indicating a definite alerting phenomena had been produced. There are changes in the arousal and recruiting thresholds, but it is too early to draw any definite conclusions until more animals have been studied.

Interim Report

BIOCHEMICAL, PHARMACOLOGICAL AND TOXICOLOGICAL EFFECTS

The Biosynthesis of 5:8:11-Eicosatrienoic Acid

A. J. Fulco and J. F. Mead

It has been found by many investigators that one of the major changes accompanying the development of the fat deficiency disease in most animals is the appearance and increase of a trienoic acid. This acid was isolated from fat-deficient rats in This Laboratory and was found to be 5:8:11-eicosatrienoic acid. From this structure two origins could be logically proposed: hydrogenation of arachidonic acid or dehydrogenation and chain-lengthening of oleic acid. The former case would mean a further loss of the presumably vital arachidonic acid; the latter case would represent the formation by total synthesis of a polyunsaturated acid — an accomplishment not previously believed possible for the mammalian organism.

In order to decide between these possibilities, fat-deficient rats were given intraperitoneal injections of carboxy-labeled sodium acetate and the polyunsaturated fatty acids were isolated from their organ and depot fat by low-temperature crystallization. The C_{20} fatty acids were isolated by gas-liquid chromatography and this fraction was further separated into eicosa-trienoic and eicosatetraenoic acids by reversed phase chromatography. Oleic acid was also isolated from the mixed fatty acids by similar techniques. A sample of the active eicosatrienoic acid was oxidized with permanganate to give pelargonic and glutaric acids. Similarly oleic acid was also oxidized to give pelargonic and azelaic acids. A second sample of the eicosatrienoic acid was hydrogenated to arachidic acid, chromatographically separated from a trace of stearic acid and degraded stepwise. Table II gives the activities of these fractions. Since the activity of the pelargonic acid derived from the eicosatrienoic acid is of the same order as that derived from oleic acid,

it is evident that the former acid is not derived from arachidonic acid (which should have little or no activity beyond the carboxy group), but is synthesized from acetate. In order to test the possibility that the C_{20} trienoic acid is indeed formed from oleic acid, the arachidic acid prepared from it by hydrogenation was degraded stepwise and the activity of the fragments thus derived were compared with those derived similarly from oleic acid. These data are also presented in Table II. The distribution of activity in the oleic acid is as follows (in per cent of the total):

$$\overbrace{\underbrace{CH_3\text{-}(CH_2)_7\text{-}CH}_{39.3}=\underbrace{CH\text{-}(CH_2)_7\text{-}COOH}_{60.7}}^{77.1 \qquad 22.9}$$

The distribution of activity in the 5:8:11-eicosatrienoic acid is as follows:

$$\overbrace{CH_3\text{-}(CH_2)_7\text{-}CH}^{13.6}=\overbrace{CH\text{-}CH_2\text{-}CH=CH\text{-}CH_2\text{-}CH=CH\text{-}CH_2\text{-}CH_2}^{20.4}\text{-}CH_2\text{-}COOH$$

$$1.9 \quad 64.2$$

It can be seen that the ratio of the last 9 carbons of the eicosatrienoic acid to the third through the eleventh carbon atoms is the same as that for oleic acid thus strongly indicating that the former is derived from the latter.

From this study, it has become evident that the polyunsaturated fatty acids of the body are formed from any available fatty acids either taken in the diet or synthesized de novo. The process of formation has previously been described as dehydrogenation to form new double bonds in the divinyl methane relationship toward the carboxy group from the original double bonds. Chain-lengthening processes also occur to enable further dehydrogenation steps to take place.

TABLE II

Activities of Fatty Acids Derived from Acetate-1-C^{14}-Injected Rats

Component	Activity (disintegrations/sec/m. mole)
Oleic acid (contains palmitic acid)	16×10^3
Stearic acid — (II) (from hydrogenation of (I), free of palmitic)	11.8×10^3
Pelargonic acid (last 9 carbons of (I))	4.6×10^3
Azelaic acid (first 9 carbons of (I))	7.1×10^3
Benzoic acid (C_1 of (II))	2.7×10^3
Margaric acid (terminal 17 carbons of (II))	9.1×10^3
5,8,11-Eicosatrienoic acid (hydrogenated) — (III)	22.1×10^3
Pelargonic acid (last 9 carbons of (III))	3.0×10^3
Benzoic acid (C_1 of (III))	14.2×10^3
Benzoic acid (C_2 of (III))	0.41×10^3

A Study of Abnormal Human Plasma Proteins

Norman S. Simmons, Jean E. Kinnear and Morton Sigel

Lupus erythromastosis and certain other pathological conditions give rise to abnormal blood plasma proteins. In vitro production of the abnormalities should lead to better understanding of the mechanism of the disease. Dialyzable polypeptides such as protamine and histone might very well be expected to bind to albumin and globulins, producing higher molecular weight fractions.

Ultracentrifugation studies are too insensitive to adequately separate the different plasma proteins for molecular weight or concentration determinations.

Paper electrophoresis, while sensitive enough to separate the plasma fractions, suffers a disadvantage in that staining and elution techniques of analysis are relatively insensitive.

Moving boundary electrophoresis, using the schlieren technique for obtaining patterns, has proved to be entirely adequate.

Problems involving plasma preparation and instrumental operating procedure have been worked out. Preliminary studies with normal pooled plasma plus protamine (circa 10% of plasma) give electrophoretic patterns much like those reported in the literature for Lupus erythromastosis. The albumin fraction is greatly decreased, while the α_2 and γ globulin fractions are much increased. Increased amounts of protamine further decrease albumin and the α_1 globulin and greatly increase the γ globulin fraction.

The Specificity of Thyroxin Inhibition of Acyl Phosphatase

Isaac Harary and Phyllis Talmage

Previous reports have presented evidence for an inhibition of acyl phosphatase by low levels of thyroxine. Further investigation of this phenomenon has indicated that maximum inhibition is obtained with compounds having the diphenyl ether framework with a 4' hydroxy and 3,5,3',5', iodine ring substitution.

A comparison of the tetra-, tri- and diiodo derivatives of thyroxine indicates that 4 iodines are more effective than 3 and 3 more effective than 2. Thyronine itself has no inhibitory effect. The same conclusions can be drawn from examination of the iodine derivatives of thyropropionic, thyroacetic and throformic acids.

It can also be shown that the propionic side chain is more effective than the acetic which in turn is more effective than the formic. This is true for the tetra-, tri- and diiodo series of thyroderivatives containing these side chains.

A comparison of diiodothyranine and thyronine indicates that the presence of the 3,5 iodines has no effect in causing inhibition. From observation of the inhibition given by thyroxine as compared with diiodotyrosine and of that given by tetraiodothyroformic as compared with diiodohydroxybenzoic acid it can be shown that the presence of the phenyl ether group increases inhibition.

Comparison of tyrosine with phenylalanine, p-hydroxyphenylglycine with phenylglycine, p-hydroxybenzoic acid with benzoic and p-hydroxyphenylpyruvic acid with phenylpyruvic acid indicated that the para phenolic group also increases inhibition.

The higher inhibition given by tetraiodothyropropionic acid as compared to thyroxine indicates that an amino group in the side chain decreases inhibition.

It would seem therefore that the active center of acyl phosphatase fits specifically a tetraiodothyropropionic acid structure.

Interim Report.

The Organic and Amino Acids in Beans and Avocados Affected by Iron Chlorosis

W. A. Rhoads

Iron chlorosis, in particular, a kind of iron chlorosis called 'lime chlorosis', is a disease affecting cultivated plants around the world. The disease is manifest in yellowing of leaves, reduced yields, and at severity, death of the affected plants. The disease is usually remediable by the addition of iron into the plant, despite the fact that in some cases chlorotic plants may contain more iron than unaffected plants. It has been estimated that, in the United States alone, there are several million acres of crops on calcareous soils which are subject to at least minor or occassional chlorosis.

Many environmental factors are known to contribute to the onset of the disease; and, although the disease has been recognized for more than 100 years, a complete understanding of its cause has not been reached.

In an attempt to contribute to this understanding, the organic acids an amino acids in affected leaves of avocados, and roots and leaves of beans were measured with the thought that the content of these metabolites might give an insight into the metabolism of affected plants and thereby suggest causal mechanisms. To the same end, affected and normal plants were

allowed to incorporate $C^{14}O_2$, both in the light and in the dark, and the C^{14} distribution among the metabolites for time periods up to two hours was noted.

Results from beans showed that all measurable organic acids, except one, were increased in bean leaves suffering from lime chlorosis; and most free amino acids appeared to be increased also. It was previously known that malic acid (usually) and citric acid were increased. In this work it was shown that succinic and fumaric acids were increased. An acid tentatively identified as malonic acid occurred in either increased or decreased quantities in lime chlorotic leaves. It was, however, quantitatively, a major constituent of both green and chlorotic leaves.

Results from the $C^{14}O_2$ light fixation studies showed a large decrease in C^{14} incorporation in one of the organic acid components of lime chlorotic leaves compared to green leaves. Although this component was not certainly identified, there was evidence that it was alpha-ketoglutarate, which could provide a lead for future work into the immediate cause of chlorosis.

Since malic and aspartic acids were increased in chlorotic bean leaves, and since these have often been shown to be associated with dark fixation of CO_2, it was suggested that CO_2 fixation is involved in iron chlorosis. Subsequent experiments in dark fixation of $C^{14}O_2$ showed that both bean leaves and roots had the capacity to fix CO_2 in the dark, and that tissue from which iron had been withheld appeared to have a greater capacity to fix CO_2 than did tissue to which Fe had been available in the substrate.

An examination of the literature for possible relationships between CO_2 fixation and the symptoms of iron chlorosis, and environmental conditions leading to the onset of chlorosis yielded considerable evidence that CO_2 fixation may be responsible for some of the symptoms of chlorosis. Although a theory was presented by which CO_2 fixation might lead to Fe precipitation in the roots and to a consequent deficiency in the leaves, there was not sufficient evidence to make a conclusion of this nature.

Thesis presented and approved as partial requirement for Ph.D. degree. Candidate sponsored by the Environmental Radiation Division, Department and Laboratories of Nuclear Medicine and Radiation Biology, UCLA.

Some Pharmacological Properties of Mellaril, 2-methylmercapto-10-[2-(N-methyl-2-piperdyl) ethyl]-phenothiazine

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

Recently a new tranquilizer drug, Mellaril, became available, and it was of interest to determine its pharmacological properties. Comparison has been made with chlorpromazine to determine if the structural changes in the amine side chain had any influence on pharmacological activity. It was observed that Mellaril was twice as active against acetylcholine and ten times more active against serotonin spasm of the isolated guinea pig ileum than chlorpromazine (see Table III). Mellaril has a greater affinity for the acetylcholine receptors of the ileum and can be removed only after prolonged washing over a period of 150 minutes. Chlorpromazine does not have the same binding power on the tissue receptors. Both compounds showed an equivalent activity against barium and histamine spasms. Hexobarbital sleeping time in mice was increased to the same extent by both Mellaril and chlorpromazine over the dosage range of 1 to 5 mg/kg. Preliminary experiments on the hypothermic properties of Mellaril and

chlorpromazine in mice indicate that both compounds lower body temperature to the same extent, but Mellaril has a longer duration of action. This work should be completed prior to the next semi-annual report.

TABLE III

Antispasmodic Effect of Tranquilizers

Drug	Spasmogen	*ED_{50} & Range	*Slope & Range
Chlorpromazine Hydrochloride	Acetylcholine	5.80(2.41-13.92)	3.87(1.38-10.84)
	Barium Chloride	29.0(11.60-72.50)	2.80(0.97-8.13)
	Serotonin	9.0(4.00-20.25)	3.67(1.05-12.85)
	Histamine	0.48(0.26-0.87)	1.99(0.91-4.38)
Mellaril Hydrochloride TP - 21	Acetylcholine	2.50(1.40-4.45)	2.54(1.06-6.10)
	Barium Chloride	24.0(13.33-43.20)	2.56(1.01-6.58)
	Serotonin	0.99(0.58-1.68)	2.11(1.17-3.80)
	Histamine	0.65(0.34-1.24)	2.19(0.84-6.78)

*At P = 0.05

Interim Report

Local Toxicity from Organic Moderators

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

In the previous interim report, information was available on the sensitizing properties of the organic moderators MIPB, RMIPB and TPM. Other data showed that these materials and their component parts were extremely irritating to the trachea and pulmonary passages. Recently completed experiments have shown that none of the components of the TPM (terphenyl mixture)

are as irritating to the rabbit's skin as TPM itself. Furthermore, after reactor irradiation even TPM becomes non-irritating to the eye. Similar results were obtained when the compounds were applied to intact and abraded rabbit skin. However, such effects may be related to the fact that polyphenyl compounds have a low solubility and probably did not penetrate the skin. Intradermal injection of RTPM gave the maximum irritation index of 8. Furthermore, there was no diminution in edema, erythema or eschar formation observed during the injection series. At autopsy all animals receiving RTPM had adhesions at the injection sites and petechial hemorrhages on the underlying skin surfaces remote from the site of the test injection. RTPM also stained the area black and produced scars 10 mm in length. Necrosis and scarring were produced by 48% OTP, 30.5% MTP, 5.5% PTP and 16% biphenyls, but these effects were not as pronounced as with RTPM. Histological examination of the skin revealed that undiluted RTPM produced extensive acute cellulitis with focal abscess formation throughout the dermis and into the subcutaneous layer. There was some increase in eosinophils about these zones. A focal necrosis of the epidermis with subepidermal bleb formation was also observed. With 10% RTPM, there was moderate edema with moderate numbers of polymorphonuclear cells and lymphocytes in the dermis and subcutaneous layers. A similar reaction was produced by 1% RTPM and there were greater numbers of eosinophils in the dermis and subcutaneous fat. The reaction to 48% OTP was similar to undiluted RTPM. The histological examination of the skins from the animals receiving the other compounds has not as yet been completed, but in all probability the results will be similar to what has already been reported. Work on the inhalation of the low concentrations of all compounds is in progress, but it is too early to draw any definite conclusions.

Interim Report

RADIATION PROTECTION

Quinoxaline Compounds in Radiation Protection

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

Our previous observations on quinoxaline-1,4-di-N-oxide (Proc. Soc. Exp. Biol. Med. 96, 579, 1957) indicated that such compounds decreased mortality from radiation injury. It was established that part of this effect was related to an antibacterial action which decreased radiation-induced bacteremia. Upon this basis, 2,3-dimethyl-quinoxaline-1,4-di-N-oxide, 6-chloro-2,3-dimethylquinoxaline-1,4-di-N-oxide and 2,3-dithiol quinoxaline were evaluated at oral doses of 125 and 250 mg/kg and at intramuscular doses of 75, 125 and 250 mg/kg daily for three days or one day pre-irradiation with 550r acute whole body x-irradiation (LD_{100}). The results of the oral experiment are given in Table IV. Those groups marked with the asterisk indicate a significantly increased survival time. However, only 2,3-dithiolquinoxaline (Groups IV and X), 2,3-dimethyl-quinoxaline-1,4-di-N-oxide (Group XI) and 6-chloro-2,3-dimethyl-quinoxaline-1,4-di-N-oxide (Group XII) increased total survival. The dithiol compound produced diarrhea at all doses. When the three compounds were administered intramuscularly only the 6-chloro-2,3-dimethyl-quinoxaline-1,4-di-N-oxide was effective in increasing total survival (45% alive at 30 days). The results obtained with the 2,3-dimethyl compound indicated that doses of 75 and 125 mg/kg increased survival time but not total survival. The dithiol compound was toxic at all doses tested and decreased survival time significantly. Further work on the amines and amine oxides will be undertaken.

Interim Report - These data were presented at the American Chemical Society Division of Medicinal Chemistry, Chicago, Illinois, Sept. 7-12, 1958.

TABLE IV

Effect of Oral Quinoxaline on Radiation Mortality in Mice

Group	Medication	Day Pre-R	ST_{50} & Range Days	Slope & Range	Final Mortality %	Days
I	Saline Control	3,2,1	9.4(8.41-10.51)	1.29(1.19-1.40)	100	14
II	Di Me Q 125 mg/kg	"	*14.0(12.29-15.95)	1.35(1.21-1.49)	90	30
III	Cl di Me Q 125 mg/kg	"	9.4(8.03-11.0)	1.43(1.28-1.60)	90	30
IV	Di SHQ 125 mg/kg	"	*11.4(8.64-15.05)	1.85(1.47-2.34)	75	30
V	Di Me Q 250 mg/kg	"	9.6(8.57-10.75)	1.30(1.20-1.41)	95	30
VI	Cl Di Me Q 250 mg/kg	"	8.9(7.36-10.77)	1.56(1.36-1.78)	95	30
VII	Di SHQ 250 mg/kg	"	10.0(8.33-12.0)	1.52(1.33-1.73)	95	30
VIII	Di Me Q 125 mg/kg	Pre R-1	*12.2(11.49-12.95)	1.14(1.10-1.19)	90	30
IX	Cl Di Me Q 125 mg/kg	"	8.4(7.18-9.83)	1.44(1.28-1.61)	100	25
X	Di SHQ 125 mg/kg	"	*11.7(8.01-17.08)	2.22(1.62-3.04)	68.4	30
XI	Di Me Q 250 mg/kg	"	*12.4(10.12-15.19)	1.56(1.32-1.84)	78.9	30
XII	Cl di Me Q 250 mg/kg	"	*13.4(10.85-17.55)	1.60(1.35-1.90)	80	30
XIII	Di SHQ 250 mg/kg	"	*12.0(10.53-13.68)	1.33(1.21-1.46)	94	30

Di Me Q = 2,3-dimethyl-quinoxaline-1,4-di-N-oxide
Cl di Me Q = 6-chloro-2,3-dimethylquinoxaline-1,4-di-N-oxide
Di SHQ = 2,3-dithiol-quinoxaline

Radiation Protection Studies

J. L. Leitch, D. Maryn and J. Moore

All albino mice (DAL-Swiss) were exposed to total, whole-body, x-ray doses of 500 r, measured in air and carried out with a single Picker Industrial Unit operating at 250 KVP and 15 ma. The characteristics of this radiation were as follows: Filters: 0.21 mm. Cu inherent, 0.50 mm. Cu parabolic and 1.0 mm. Al; HVL = 1.7 mm. Cu; TSD = 70 cm; and dose rate = 21.3 r/min.

Each group of 60 mice were treated 20 to 30 minutes prior to irradiation with one of the following solutions:

a. $1x10^{-5}$ M AET/0.25 ml/mouse (AET = 2-aminoethylisothiouronium bromide-HBr)

b. $1x10^{-5}$ M AET and $1x10^{-6}$ 5HT/0.5 ml/mouse (5HT = 5-hydroxytryptamine creatinine sulfate)

c. Saline (0.9% NaCl) (0.25 ml. per mouse)

All data were calculated by the method of Litchfield (J. Pharm. Exptl. Therap., 97, 399-408 (1949)) for time per cent effect curves. Calculations are summarized below for the preliminary experiments:

Run Number	Treatment	ST_{50} day	Slope	Mortality 60 days %
4C	None	7.75	1.42	93.1
5C	None	5.9	1.49	94.9
4C and 5C	None	6.7	1.55	94.0
4A	$1x10^{-5}$ M AET/mouse	23.6	3.55	69.0
4B	$1x10^{-5}$ M AET/mouse plus $1x10^{-6}$ M 5HT/mouse	59.0	5.51	40.4

When the data for the two untreated groups, 4C and 5C, were analyzed at a level of $p = 0.05$, it was found that their respective ST_{50} values were significantly different. However, at $p = 0.02$, this was not the case. It was, therefore, concluded that in the evaluation of compounds of possible

therapeutic value the statistical analyses should be made at a p value of 0.02 instead of the usual level of 0.05. In this manner the evaluation of compounds will attain greater significance. The data from the two untreated groups were, therefore, combined so as to serve as a basis of comparison.

Both treatment procedures, 4A and 4B, very significantly increased the ST_{50} values and slope values, whereas the 60-day mortality was decreased. In addition it was found that the incorporation of 5HT into the treatment significantly modified the animal responses.

Interim Report

NEOPLASM INDUCTION

The Development of Leukemia and Other Neoplasms in Mice Receiving Cell-Free Extracts from a High-Leukemia (AKR) Strain

Esther F. Hays and William S. Beck

An 18.6 per cent incidence of lymphocytic leukemia was found in C3Hf/Gs mice given inoculations when newborn of cell-free extracts of leukemic tissues of spontaneously occurring leukemia of the AKR mouse. These leukemias occurred in mice under 14 months of age. No lymphocytic neoplasms were observed in control animals.

Similarly inoculated C57BR/cd mice showed an increase in the incidence of lymphocytic neoplasms when they were under 14 months of age when compared with noninjected controls.

Inoculated (C3Hf x AKR) F_1 hybrid mice likewise showed an increased incidence of lymphocytic leukemia in animals under 14 months of age. As in the C57BR/cd strain, the over-all incidence of lymphocytic neoplasms was not significantly different from that of the controls.

Parotid and mammary carcinomas and subcutaneous fibrosarcomas have been shown to develop in both C3Hf/Gs and (C3Hf x AKR) F_1 hybrid mice receiving AKR leukemic extracts in the newborn period.

Reference: Cancer Research, Vol. 18, No. 6, pp 676-81, July 1958

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, Eleanor Thorp

Preliminary work done in this laboratory has indicated an increased incidence of leukemia in (C3Hf x AKR) F_1 hybrid mice when injected subcutaneously in the newborn period with nucleic acids from tissues of leukemic and normal AKR mice (Nature 180: 1419-1420, Dec. 21, 1957). No increased incidence of leukemia was observed in similarly injected C3Hf/Gs or C57BR/cd mice.

This study has now been extended and altered in certain respects. In order to place the nucleic acids in more direct contact with the hematopoietic tissues and assure the retention of the full administered dose, newborn mice were injected intravenously (after Billingham & Brent, Proc. Royal Soc. 146:78-90). Intravenous injections of nucleic acids prepared from leukemic AKR spleens and lymph nodes have been made in over 50 (C3Hf x AKR) F_1 hybrid mice.

Further, considering the possibility of genetic transformations being involved in our previous findings, similar preparations of AKR nucleic acids were administered intravenously to newborn C3Hf/Gs mice. At six weeks of age these animals then have received intraperitoneal injections of cell-free extracts of AKR leukemic tissues. Sufficient time has not elapsed to record results of these two studies.

Interim Report

RADIOISOTOPIC STUDIES OF PHYSIOLOGY AND METABOLISM

Liver Function Studies with Rose Bengal I^{131}

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

Recent advances in the laboratory development and clinical application of radioactive Rose Bengal tracer tests for measuring liver blood supply, polygonal cell function, and bile flow interference are presented.

The use of a mixture of bromsulphalein (BSP) and Rose Bengal as a liver stress test is shown to be a means of increasing the sensitivity of the tracer test to the point where a negative stress test indicates the absence of significant liver pathology. Results of repeated stress and tracer test procedures in more than 200 rabbits with acute and chronic CCl_4 poisoning demonstrate the excellent correlation between histopathological changes and disturbed physiological functions.

In experimental cirrhosis produced by chronic CCl_4 poisoning in rabbits the rates of blood clearance of Rose Bengal measured over the abdomen (portal area) decreased much more than did those measured simultaneously over the head. These findings are indicative of a portal vascular functional defect in early cirrhosis. This dual blood clearance procedure appears to be readily adaptable for clinical use in investigating patients suspected of having portal hypertension.

Clinical Studies. Approximately 250 tracer dose Rose Bengal Hepatograms had been performed in 150 patients having a variety of disorders of the liver, including 50 surgical patients with obstructive jaundice. In most of the latter individuals, tests have been made repeatedly before and after operation. The blood clearance half-time and liver vascular capacity values provide reliable indices for evaluating polygonal cell function and disturbances of liver blood supply. Repeated radioactivity measurements over the liver and abdomen recorded during the initial 15-60 minute test period and subsequently after 2-4 hours and again in about 24 hours gave data from which the degree of bile flow interference

or biliary tract obstruction may be estimated on a purely mechanical basis. In nearly all jaundiced patients these measurements are sufficient to differentiate medical from surgical causes, especially when the test is repeated during critical periods. Serial hepatograms have reflected changes in biliary obstruction and polygonal cell function in most of the diagnostic problem cases more quickly and accurately than have indirect biochemical liver tests. However, in three patients having severe jaundice of several weeks duration liver function was so grossly depressed that an estimate of bile flow interference could not be made because of the associated impairment of bile formation and dye extraction capacity of the liver cells.

A 30-70 per cent reduction in liver vascular capacity and a 2-10 fold slowing of liver uptake and blood clearance rates are found in patients with portal cirrhosis. In such cases the rates of dye excretion from the liver and entry into the abdomen are proportional to the impairment of liver function, and thereby, demonstrate patency of the biliary tract. Patients having liver enlargement from chronic congestive heart failure show moderately prolonged blood clearance and liver uptake rates together with normal or increased liver vascular capacity values and no bile flow interference.

The features of the Hepatogram indicating biliary tract obstruction are: (1) a prolonged liver uptake curve, (2) delayed entry time and reduced rates of appearance of dye in the intestines, (3) abnormally high liver retention of dye at 24 hours together with low abdominal levels and incomplete blood clearance.

Interim Report Read at the annual meeting of the Radiological Society of North America, Chicago, Illinois, November 21, 1958.

Clinical Studies with the Miokon I^{131} Renogram

G. V. Taplin and D. Johnson

In the past six months 60 patients with a variety of renal disorders have been studied with Miokon I^{131} Renogram and new equipment. The latter consists of two sets of lead collimated scintillation detectors with two inch aperatures and with the one and one-half inch crystal withdrawn two inches into the shields. Otherwise, the equipment is similar to that originally used except that a third set is employed to register blood clearance by placing the detector over the side of the head. The new technique differs from the original in that the wide angle detectors are placed flush to the flanks and aimed directly over each kidney area as previously located by upright roentgenograms of the abdomen.

Miokon has been proved to be excreted almost completely by the kidneys and not partially by the liver, as is the case with Diodrast. Less than 0.5 per cent is excreted in the bile of patients recovering from cholecystectomies and uptake excretion curves over the liver area parallel to those of blood clearance measured over the side of the head. The configuration of the Miokon renograms obtained with the new detectors and technique is similar to Diodrast renograms except that the second segment rises to a peak value in 6-10 minutes rather than 3-6 minutes, as is the case with Diodrast. Also the blood clearnace rates are somewhat slower. The Miokon Renogram appears to be an improvement because Miokon is more kidney specific and the new technique with wide angle counters makes kidney localization less critical. During these studies eight hypertensive individuals have been demonstrated to have unilateral renal disease by this screening procedure and in all instances these findings have been confirmed by one or more urologic diagnostic methods. In addition eight patients have been tested with Diodrast Renograms as originally performed and also with the Miokon Renogram. In two instances where the Diodrast Renogram gave false information on the right side due to liver interference, the true state of renal malfunction

was demonstrated correctly by the Miokon Renogram technique.

Interim Report

Rose Bengal and Iodopyracet as Radiodiagnostic Agents in Liver and Kidney Diseases

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

The Rose Bengal Hepatogram is now established as a highly sensitive liver function test which also supplies diagnostic information on vascular abnormalities of the liver and on the degree of biliary tract obstruction. No other single procedure gives as much useful information for the differential diagnosis and management of patients with diseases of the liver and biliary tract.

The Rose Bengal-BSP blood clearance stress test is capable of detecting minimal liver damage in rabbits and asymptomatic liver disease in man. However, further investigation is needed for final assessment of its maximum sensitivity.

New tracer techniques using Colloidal Gold^{198} and Rose Bengal have been developed in animal studies for further evaluation of liver vascular disorders. Both procedures are clinically applicable.

The Diodrast Renogram is becoming recognized as a simple, rapid, nontraumatic office procedure for estimating individual kidney functions. It is especially valuable as a screening test for hypertensive patients suspected of having disease of one kidney. Most hypertensives having normal Renograms may be spared the discomfort, time and expense of hsopitalization and complicated urological diagnostic procedures.

Recent modifications in the instrumentation and the use of Miokon in place of Diodrast have increased the reliability of the Renogram while simplifying its performance.

These tracer tests are unique in providing data on multiple functions

simultaneously with complete freedom from drug reactions and the radiation exposures are many times less than from x-ray examinations of these organs.

Interim Report

Read at the New York Academy of Sciences Symposium on Radiodiagnostic Agents, October, 1958. To be published in the Transactions of the New York Academy of Sciences.

Studies with Colloidal Gold^{198} for Estimating Changes in Liver Blood Flow and Phagocytic Function

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

During the past six months external tracer techniques, identical with those used for liver function determination with Rose Bengal I ^{131}, have been employed to estimate liver blood flow in normal rabbits and changes induced by acute and chronic CCl_4 poisoning and by surgical interference with the liver blood supply. These studies were undertaken in conjunction with continuing investigations with Rose Bengal for two major purposes: (1) To improve present methods for evaluating liver blood flow and, (2) to develop experimental and clinically applicable techniques for estimating phagocytic function of the von Kupffer cells of the liver.

The experimental results for half-time values of blood clearance and liver uptake rates of Colloidal Gold^{198} have been analyzed by the method of Dobson and Jones (Acta Med. Scandinav, 144, Suppl. 273, 1952. Data presented in Figure 1 demonstrate that half-time values of liver uptake remain constant in relation to dose of carrier gold over the range of 10-100 μg/kg and then increase 6-8 fold as the carrier dose is raised from 100 to 5000 μg/kg. The reproducibility of the colloidal gold test when performed with carrier doses of gold below 100 μg/kg is presented in Table V. Findings shown in Table VI demonstrate that surgical ligation of the portal vein causes a 3-6 fold increase in liver uptake

half-time, whereas ligation of the hepatic artery produces no significant effect.

In acute, but mild, liver damage of rabbits from CCl_4 poisoning as detected by Rose Bengal testing, the half-time liver uptake values for colloidal gold show more variation than controls but no definite abnormalities (see Table VII).

The effects of chronic CCl_4 poisoning at varying dose levels are presented in Figures 2 and 3. These two groups of animals were tested repeatedly with colloidal gold using carrier doses below the critical level (100 μg/kg) and with tracer Rose Bengal as well as BSP Rose Bengal stress tests. Histologic examinations of liver biopsy specimens were made repeatedly in each group. Representative tissues showed typical evidence of cirrhosis including chronic inflammatory changes, fatty degeneration, and definite increase in perilobular fibrous tissue. In both groups during the acute phase of poisoning there was a greater effect on blood flow than on polygonal cell function, whereas during the chronic stages there was a progressively increasing impairment of both blood flow and polygonal cell function. In group I after the dose of CCl_4 had been reduced from initial levels of 50 mg/kg twice a week to 5 mg/kg twice a week, the colloidal gold test indicated a reversible reaction and only temporary impairment in liver blood flow.

Phagocytic Function of the RE System with Supercritical Doses of Colloidal Gold[198]

When colloidal gold is injected intravenously with carrier doses exceeding 100 μg/kg the half-time blood clearance becomes an index for measuring phagocytic capacity of the RE system. However, to use this test experimentally at daily intervals it is necessary to determine the dose in normal animals which does not interfere with phagocytic function or show an accumulative effect. Preliminary studies on the amount of carrier and the time interval between tests are shown in Table VIII. These findings indicate that the critical amount of

carrier for repeated testing must be kept below 150 μg/kg. Further studies are needed in this respect before using this test for evaluating radiation damage to the RE system or other purposes.

Interim Report

Table V

Colloidal Gold^{198} Liver Blood Flow Test in Rabbits Reproducibility of Blood Clearance and Liver Uptake Half-Time Measurements

Run	Number of Animals	Carrier Dose μgm/kgm	Average Half-Time (sec.) Blood	Liver
I	10	10	29.5	30.7
II	10	17	29.8	35.8
III	10	50	31.9	31.0
Overall half-time averages			31.0±0.9	34.5±1.2

Table VI

The Effects of Vascular Obstruction Upon Liver Uptake Rates of Colloidal Gold

Experiment	Number of Animals	Half-Times (sec.) After operation
Controls	30	34.5+1.2
Ligation of portal vein	5	140 (96-174)
Ligation of hepatic artery	3	34 (30-40)

Table VII

Effects of Acute CCl_4 Poisoning on the Rates of Colloidal Gold^{198} Liver Uptake in Rabbits(1)

Experiments	Number of Animals	Half-Time Values Liver Uptake (sec.)
Controls	30	34.5+1.2
24 hrs post poisoning	12	34 (30-42)
48 hrs post poisoning	12	39 (17-56)

(1) A dose of 75 mg/kgm of CCl_4 was given by gastric intubation.

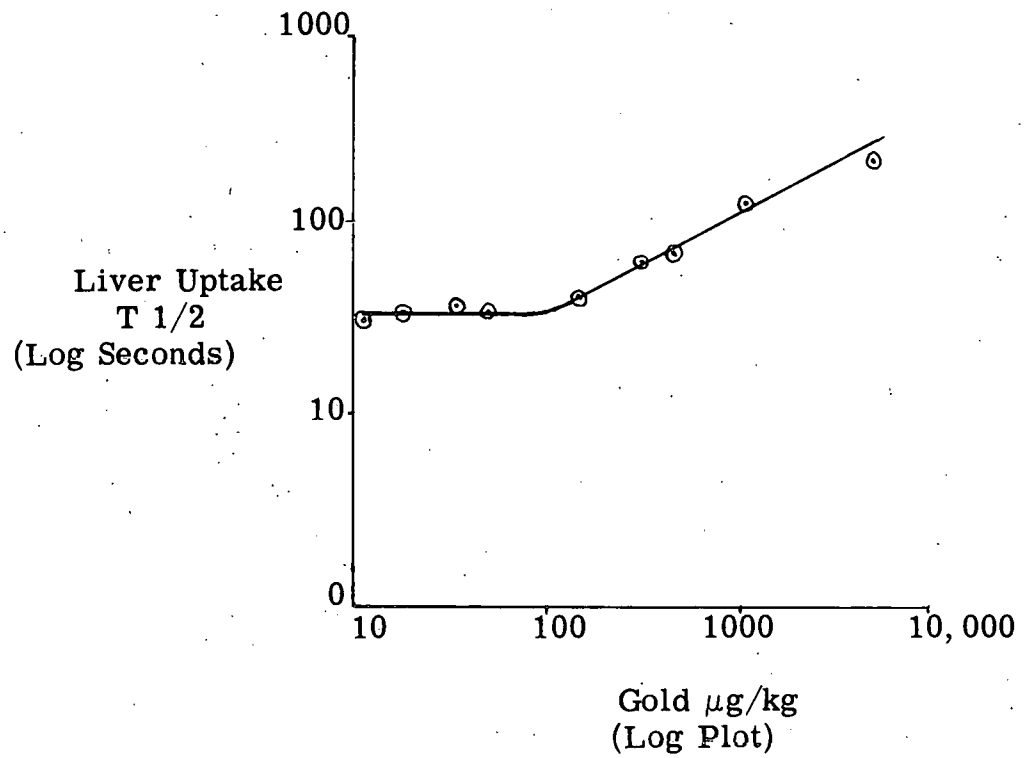
Table VIII

Effects of Amount of Carrier and Time Intervals on Liver Uptake Rates of Colloidal Gold^{198}

Date	Carrier Dose µgm/kgm	Number of Animals	Average Liver Uptake T 1/2 (seconds)
10-2-58	146	10	42.0
10-3-58	299	10	85.4
10-6-58	446	10	77.1

FIGURE I

Colloidal Gold^{198} Liver Blood Flow Test
Relation of Half Time of Liver Uptake and Dose of Carrier Gold[1]



1 Each point represents the mean value from 10 animals. The average standard error is 6 per cent of the mean for each group.

GROUP I

Effects of Chronic CCl_4 Poisoning on Blood Retention of Rose Bengal I^{131} and Liver Uptake of Colloidal Gold^{198}

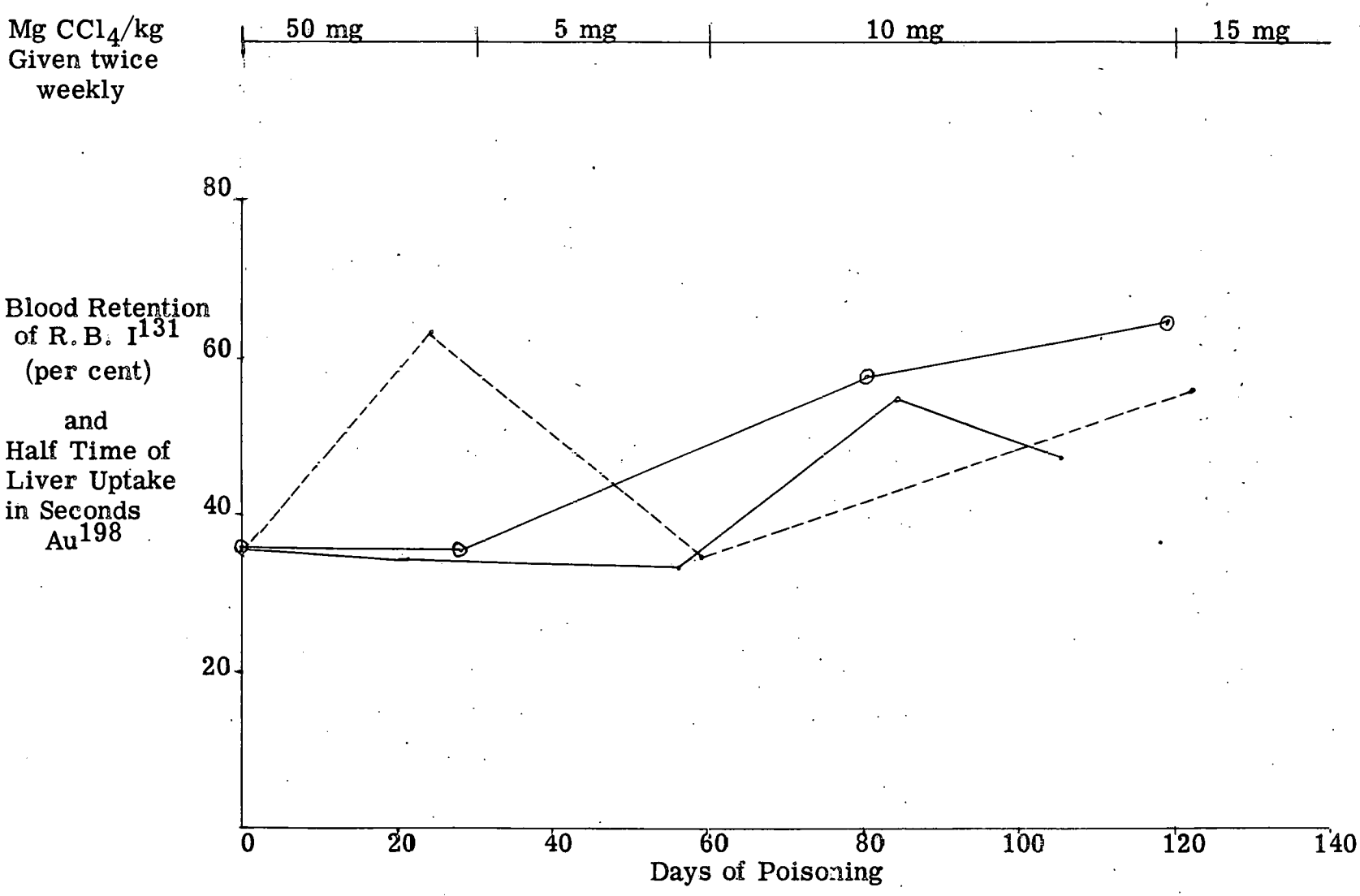
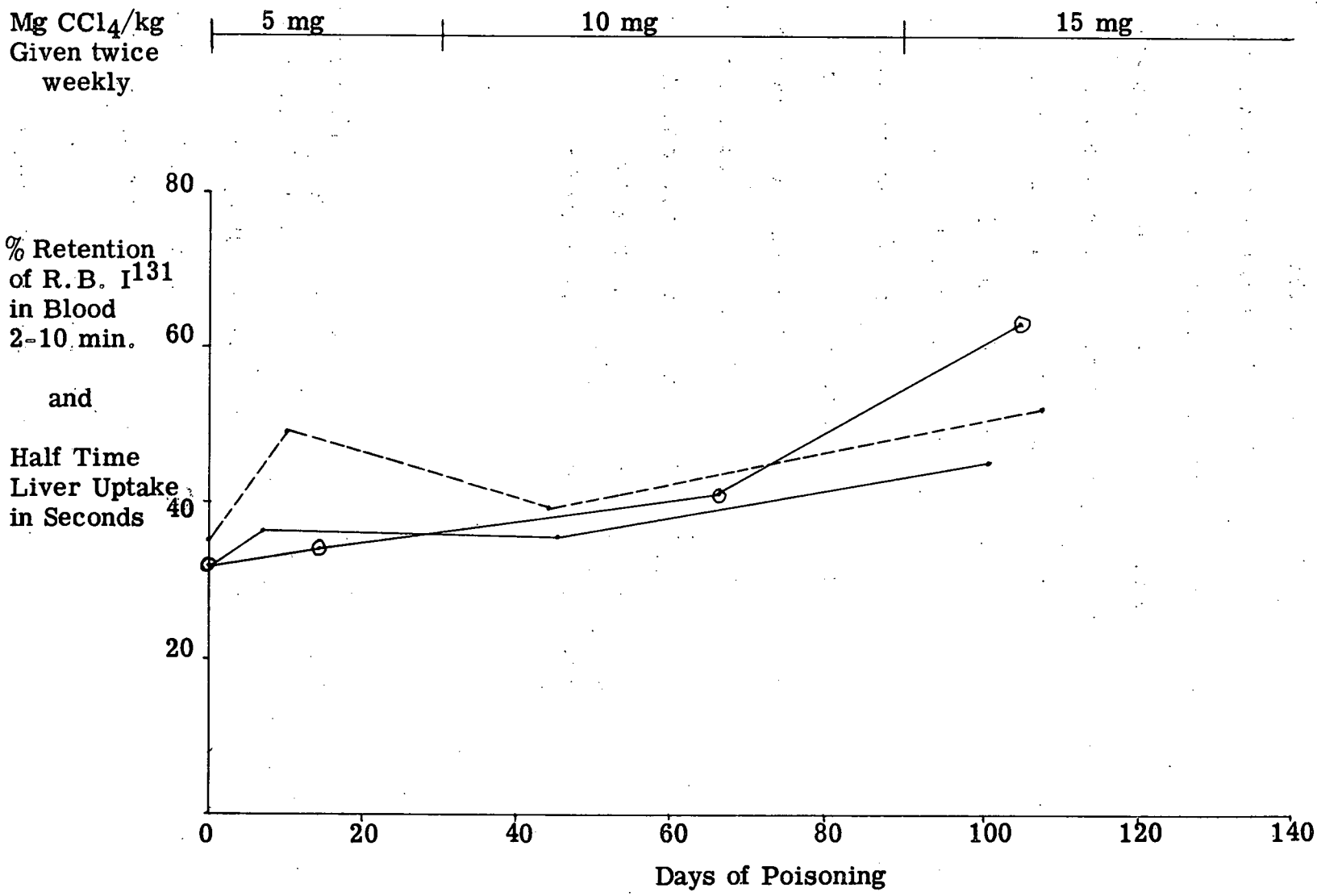


Figure 2

GROUP II

Effects of Chronic CCl_4 Poisoning on Blood Retention of Rose Bengal I^{131} and Liver Uptake of Colloidal Gold^{198}



R. B. I^{131} tracer dose . —— .
R. B. I^{131} stress dose ⊙ —— ⊙
Au^{198} tracer dose . - - - - .

Figure 3

Osteogram Studies

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

In order properly to interpret Sr-85 osteograms, one should attempt to evaluate the individual contributions to the observed shape of the osteogram due to several events, namely: intracapillary transport of the Sr-85 after injection into the blood; transport or diffusion out of the capillaries into the intercellular space; and deposition or fixation of the cation by the elements of bone tissue (see J. Lab. and Clin. Med. 52, 541 (1958)). Chloride ion would be an ideal tracer for the diffusion phase, but unfortunately no suitable, gamma-emitting isotope of chlorine is available. Bromine-82 was used as a substitute. This beta-gamma emitter has a half life of 36 hours. From preliminary experiments with normal rabbits and with rabbits bearing a healing tibial fracture, the following general statement can be made: The rates of appearance of bromide ion (Br^{82}) and strontium ion (Sr^{85}) in the tibia shaft or knee area following intravenous injection are about equivalent except in healing fractures and possibly in rapidly growing bone. These investigations are continuing.

Interim Report

Radiostrontium Metabolism Studies

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

When tracer amounts of radioactive isotopes of calcium and strontium are injected into the blood, there is a greater relative accumulation of the Ca in the bone tissue than of the Sr. Some evidence indicates that this is attributable to a greater "leakage" of Sr out of the kidney nephrons into the urine, rather than to an inherently greater avidity of bone tissue for Ca ions as compared to Sr ions. Double tracer experiments to elucidate this problem are underway, using Ca^{45} and Sr^{85}. A mixture of these isotopes is injected into the femoral veins of normal rats and rats which have undergone nephrectomy or

tibial fracture or both. The bones are removed for assay of their burden of Ca^{45} and Sr^{85}, one day after injection. These measurements are not yet complete.

The availability of two different carrier-free radioisotopes of strontium affords an excellent opportunity to investigate the dynamics of transport of strontium from the blood of a pregnant animal into the fetal circulation and return. Information of this sort may prove helpful in understanding the movement of calcium across the placenta as the fetus grows and its bones calcify. In collaboration with Dr. Hutchinson of the Department of Obstetrics and Gynecology of the Medical School, a study of this subject has been initiated. A pregnant Rhesus monkey, at term, was anesthetized and polyethylene cannulae were inserted into a maternal vein, and a fetal vein. A third cannula tapped the amniotic fluid. Sr^{85} was injected into the fetal circulation at the same time that Sr^{90} (in equilibrium with Y^{90}) was injected into the maternal circulation. Small samples were then withdrawn from the three cannulae at frequent intervals up to 70 minutes postinjection. Each sample is then assayed for Sr^{90} and Sr^{85} content. These measurements are in progress.

Interim Report

Developments in Scintillation Counting for Application to Nuclear Medicine.

Benedict Cassen, T. C. Crough, and F. Larmie

Efficiency tests were performed on the cracked crystal tunnel counter mentioned in the last Semi-annual Report. Efficiencies were found to be comparable with conventional well type, single crystal counters. The tunnel counter, however, had the advantage that much larger volumes of solution could be placed in the sensitive region. It was found that, as was suspected, the refractive index of the immersion liquid had little effect on efficiency

and that better results could be obtained with low refractive index silicones rather than high refractive index aroclors. Some drifts in efficiency with time were noted. The reason for these drifts, such as yellowing of the crystals, is being studied. Time varying absorption effects would be expected to show up as reduced resolution in the spectrometric pulse height distribution.

In connection with the effects of high fields on scintillations in NaI crystals, it was found to be difficult to obtain reproducible results and that crystals would suddenly break down dielectrically. Further exploratory experiments are in progress.

An improved and simplified photoscanner was put into operation at the Los Angeles Veterans Hospital Radioisotope Unit. New work on liver and brain tumor scanning of patients is now being pursued. A specially designed and constructed tungsten strip converging collimator was installed on this scanner. The design is novel in that strips of tungsten one inch wide are "egg crated" together to produce a cellular structure with septa converging to a five inch focus. It is used with a two inch sodium iodide crystal, and its over-all sensitivity for its degree of localization is extremely high, thus enabling very small doses to be used.

Interim Report

ENVIRONMENTAL RADIATION

Fate and Persistence of Strontium^{90} and Cesium^{137} in Various Locations Established Along Documented Fallout Patterns in Nevada and Adjacent Sites

K. H. Larson, J. H. Olafson, R. G. Lindberg, B. W. Kowalewsky, J. W. Neel, L. Baurmash, and H. A. Hawthorne

Soil, native rabbit, and, where available, milk samples were collected at 60 locations in Nevada, Utah and Wyoming along, and adjacent to, documented 'hot lines' from Plumbbob and previous Nevada test series detonations. The sampling program was initiated in June and concluded in September, 1958.

Strontium^{90} contamination levels, analyzed by soil-sodium carbonate fusion, have been determined at 11 areas in Nevada and Utah. The results of these analyses appear in Table I. The higher Sr^{90} contamination levels are associated with native areas which were selected as midline locations (by field monitoring) with respect to specific Nevada Test Site detonations. The agricultural areas, while subject to possible surface soil disturbance resulting from farm management practices, do not coincide with known midlines of fallout patterns.

The Sr^{90} analysis of native rabbit, milk, and additional soil samples is in progress.

Interium Report

TABLE IX. Sr^{90} Contamination Levels as of August, 1958 in Soils at Eleven Areas in Nevada and Utah

Area	Location	Field Description	Sr^{90} Activity (0-1" Depth)* mc/sq Mile	Sr^{90} Activity (0-1" Depth)* µµc/g Ca
Alamo, Nevada	1 mi south	Agricultural	21.3 ± 11.4	6.8 ± 3.8
Moapa, Nevada	8 mi north	Native veg.	142 ± 70.3	38.3 ± 20.7
	7.7 mi northwest	Agricultural	16.3 ± 8.07	2.5 ± 1.4
Riverside, Nevada	3.8 mi southwest	Agricultural	22.7 ± 4.76	9.6 ± 1.0
Elgin, Nevada	3.8 mi southwest	Native veg.	114 ± 63.6	140 ± 56.0
St. George, Utah	1 mi southeast	Agricultural	14.4 ± 6.29	4.5 ± 1.5
	5 mi north	Native veg.	45.6 ± 8.76	406 ± 84.5
Hurricane, Utah	1 mi southwest	Agricultural	12.4 ± 1.56	3.5 ± 0.9
Enterprise, Utah	0.7 mi north	Agricultural	7.46 ± 1.11	8.6 ± 1.3
	9 mi north	Native veg.	41.2 ± 5.95	51.2 ± 9.3
Enoch, Utah	2 mi southwest	Agricultural	16.7 ± 2.92	4.6 ± 0.50
Panguitch, Utah	Northwest city lt.	Native veg.	31.9 ± 12.2	14.9 ± 5.7
Columbia, Utah	3.1 mi south	Native veg.	67.2 ± 6.95	202 ± 148
Vernal, Utah	4 mi south	Agricultural	13.8 ± 4.95	8.7 ± 1.6

*Standard deviation values represent total variation, i.e., field sampling, laboratory sampling and counting.

Radiological Report on Southwestern South Dakota

H. A. Hawthorne, K. H. Larson, J. W. Neel, J. H. Olafson, and K. R. Price

Aerial survey and ground monitoring teams established that fallout was present and that gamma radiation intensities were less than 0.1 mr/hr in southwestern South Dakota, August 15 - 17, 1957. Decay rates of contaminated plant material indicated the fallout complex 'effective time of origin' was between the times of detonations of Shots Diablo and Stokes. 'Hot spots' were observed within the radiation field.

Soil Strontium^{90} levels from 14 to 40 mc/sq ft were found with an average of 21 mc/sq mi: soil beta radioactivity from mixed fission products ranged from 9 to 44 c/sq mi along the 28.3 mi-long arc sampled.

Fresh milk collected on August 17, 1957 had 21.8 μμc Sr90/g Ca and 29.9 μμc Cs^{137}/liter on August 17, 1957.

Reference: UCLA report in press.

Sr and Cs Content of Milk Collected During October, 1957

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

Milk was collected from seven farms during Operation Plumbbob. Five of these farms were on the midline of fallout from one or more detonations. Levels of Cs^{137} ranged from 18 to 88 μμc/liter: Sr^{90} varied from 4 to 37 μμc/g Ca in the milk from these farms. The ranges for the seven farms, sampled during the Operation, were from 13 to 88 μμc Cs^{137}/liter of milk and from 2.6 to 37 μμc Sr^{90}/g Ca. Sr90, as Sunshine Units, declined more than twofold over a 40-day period after Smoky at a farm on the midline at 260 miles from Ground Zero.

Interim Report

Further Evaluation of Fallout Patterns from Balloon-Supported and Low-Yield Atomic Test Devices

K. H. Larson, J. W. Neel, L. Baurmash, and R. Rowland

Investigations of fallout patterns originating from balloon-supported and low-yield devices were conducted during Operation Hardtack, Phase II, at the Nevada Test Site under the designation of Program 37, Civil Effects Tests Operations.

Fallout patterns from one tower- and two balloon-supported detonations were delineated, despite low radiation intensity levels, utilizing previously established aerial survey equipment and techniques used by the U.S. Geological Survey. The aerial surveys were performed by USGS personnel employing a Douglas DC-3 aircraft under contractual arrangement with Civil Effects Tests Operations, Division of Biology and Medicine, AEC.

The monitoring equipment used in the USGS DC-3 aircraft has been described in detail by the designers, Davis and Reinhardt (Health Physics Progress Reports, ORNL-877, October, 1950, ORNL-1174, p. 15, October, 1951, and ORNL-1684, p. 11, January, 1954). The detectors consisted of a battery of six NaI (Tl) crystals 4 in. in diameter by 1 in. thick and one crystal 1.5 in. in diameter by 1 in. thick. These crystals were used interchangeably, depending upon the counting rate desired. An Atomic Instrument Supply Company, Model 306, positive high-voltage supply was used to feed six No. 6364 photomultiplier tubes operating at approximately 1,500 volts. The signal from the photomultipliers was fed through a mixing preamplifier, discriminator, rate meter, and vacuum-tube volt meter, which recorded the counting rate on Esterline-Angus recorders.

The rate meter was calibrated periodically throughout each flight by a Frahm resonant-reed controlled oscillator giving a pulse frequency of 500.2 cycles/sec. Since the equipment had to operate over a wide temperature range, an instrument check procedure utilizing a Cs^{137} source was used. The discriminator was set at the energy level of the Cs^{137}, and, after the cesium was placed in position under the crystals, the amplifier was adjusted to a predetermined value above background.

Calibration of the nonlinearity of the equipment was obtained by using radium sources of various values placed at a distance of 124 cm from the bottom of the array of crystal-photomultiplier cans in the aircraft. The instrument lag which is dependent upon the time-constant of the rate meter was equivalent to a ground distance ranging from 400 to 750 feet. However, when radiation readings were transposed to maps having a scale of 1:500,000 or approximately 8 miles to the inch, the effect of instrument lag was of no consequence.

The internal accuracy of this equipment was estimated to be $\pm$ 5 per cent.

The USGS DC-3 aircraft was utilized for both general and detailed pattern delination and went into operation on the day after shot or later in order to minimize the possibility of the aircraft becoming contaminated by persistent airborne fallout. A serpentine pattern was flown along the bearing of the estimated hot line, or along predominate land marks such as roads, and continuous radiation-intensity readings were taken by the recording gamma-radiation monitoring equipment. The flight patterns were, in actuality, a series of various straight-line bearings navigated by predetermined visual location points over as level terrain as practicable.

An air speed of 140 ± 20 miles per hour was maintained at an altitude of 500 ± 25 ft above ground level. A position plot was maintained by an observer, utilizing a view finder, who marked the position on a map and also actuated a marking-system switch over recognized visual reference points, which, in turn, placed fiducial marks on all record tapes and camera film. All flight patterns were recorded by a 35-mm gyrostabilized continuous strip-film camera.

The construction of iso-intensity fallout pattern maps depended upon the conversion of radiation intensities recorded by the aircraft in terms of counts/second to mr/hr at three ft above ground surface. Conversion factors for several models of survey instruments with ion chamber, G-M tube, or scintillation crystal detectors were determined by coincident ground and aerial survey of contaminated areas. The response of the various ground survey instrument models varied as much as 35 per cent in fallout fields, and variation was observed with respect to different types of detector as well as different models having the same type of detector. Using the USGS equipment described above, a radiation intensity of 77,000 c/s at 500 ft elevation was determined to be equivalent to one mr/hr at three ft above ground surface as measured by the Tracerlab AN/PDR-TIB ion chamber survey instrument calibrated against a Co^{60} standard source.

Interim Report

The Influence of Stable K and Cs on the Release of Cs^{137} from Three Soils

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

In the previous project (SOIL SCIENCE 86: 195-201, 1958), it was found that as the available K concentration in the soil was depleted by cropping the uptake of Cs^{137} by clover plants increased. The present project was

designed to study the effects of adding stable K and Cs to the soil on the uptake of Cs^{137} upon prolonged cropping.

The addition of stable K to soils up to levels equivalent to 442 pounds per acre had very little, if any, enhancing or depressive effect on the uptake of Cs^{137} by plants. As found previously, irrespective of the initial K concentration in the soil, the uptake of Cs^{137} increased as the K concentration in the soil was reduced by prolonged cropping. The addition of stable Cs to the soil up to 752 pounds per acre enhanced the uptake of Cs^{137} by plants. The addition of stable Cs at the rate of 752 pounds per acre was toxic to plants and growth was greatly reduced. The threshold level of Cs toxicity in the soil studied appeared to be about 125 pounds per acre.

Interim Report

IMPROVEMENTS IN TECHNIQUES AND INSTRUMENTS

Development of Slow-Neutron Sensitive Chemical Dosimeters

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

The relative thermal neutron versus gamma ray sensitivity of single-phase, aqueous phenol red-trichloroethylene dosimeters has been increased as much as twenty-fold by adding lithium chloride to the system. The slow neutron response is proportional to the amount of $lithium^6$ added. Instruments containing 10 per cent USP lithium chloride and 0.5 per cent $lithium^6$ chloride or approximately equal amounts of $lithium^6$ have nearly the same sensitivity to thermal neutrons (see Table X). Addition of $lithium^6$ chloride in concentrations up to 0.5 per cent causes little reduction (20-30 per cent) in gamma ray sensitivity, while increasing the thermal neutron response by more than six-fold. Thus, it

appears entirely possible to prepare lithium^{6} impregnated single-phase phenol red-trichloroethylene dosimeters which have thermal neutron sensitivity values 10-20 times greater than similar instruments without added lithium.

The results of these preliminary studies indicate the possibility of using differential chemical dosimetry for measuring mixed slow neutron gamma radiations in biomedical research including depth dose measurements which are needed in neutron capture therapy of deep-seated lesions.

Interim Report

Table X

Thermal Neutron Response of Phenol Red-Trichloroethylene Dosimeters Containing Varying Amounts of Lithium^{6} and Lithium^{7}

Lithium Content	N Response minus gamma contamination (roentgens)(1)	RCE(2)	N(3)	Factor
None	181	3.04	12	1
0.1% Lithium^{7} Chloride	257	4.31	6	1.4
10.0% Lithium^{7} Chloride	1235	20.7	6	6.8
25.0% Lithium^{7} Chloride	3483	58.5	6	19.3
0.5% Lithium^{6} Chloride	1190	20.0	6	6.6

(1) Response of instruments exposed at three dose levels adjusted to NVT = 1.22×10^{12}
Gamma contamination in neutron beam measured with 30 similar chemical dosimeters and film, sheilded within one inch thick lithium metal container

(2) RCE = Relative Chemical Effect, i.e., response to thermal neutrons equivalent to 1.0 r Co^{60} gamma rays.

(3) N = Number instruments exposed in thermal neutron port of Brookhaven Reactor.

Further Developments in Special Uses of Chemical Dosimeters

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

(1) Monitoring of Radio-biological Experiments. Single-phase phenol red-trichloroethylene dosimeters have been used for monitoring the integrated exposures of mice to 250 KVP x-rays during radiation protection studies as a cooperative effort with the Radiation Therapy Section. During these studies efforts have been made to improve the accuracy and reproducibility of such exposures. Results of the last six months work demonstrate good correlation between chemical and physical methods of dosimetry and with the biological response of mice. However, further improvement is needed in both chemical and physical measurements because errors of 5 per cent or more still occur. Chemical dosimetry of these exposures may be improved by using more instruments per experiment and by making minor changes in the preparation of the dosimeters used for this purpose.

(2) Gamma Ray Dosimetry in the 0-10,000 r Dose Range. Because of recent interest in high range gamma ray dosimetry for agricultural purposes, efforts have been renewed in this field. Single-phase aqueous dye trichloroethylene dosimeters have been prepared to register exposures in the 0-10,000 r dose range. The system showing the best radiation characteristics and stability features is prepared with 2 per cent bromthymol blue saturated with trichloroethylene and adjusted at pH 9.0. Dosimeters prepared from this system may be read visually with an accuracy of $\pm$ 10 per cent and with greater precision by simple photoelectric colorimetry.

(3) Personnel Dosimeters for Civil Defense. Because a need still exists for estimating individual human gamma ray exposures, especially in the event of

fallout from detonation of thermonuclear weapons, work has continued on the development of direct reading chemical dosimeters. Special attention has been given to the development of a single step device which indicates an accumulative gamma ray exposure of either less or more than 100 ± 25 r.

The phenol red-trichloroethylene system prepared in neutra glass ampules five inches long fulfills these requirements. Such dosimeters can be produced about ten times more cheaply than standard ionization chamber dosimeters now being employed for this purpose. Their value would be two-fold: (1) psychological, that is, reassurance that the individual had not received a serious radiation exposure; (2) as an aid to medical emergency teams in the separation of physically uninjured groups into those who do or do not need special medical attention for radiation injury during the emergency period.

Interim Report

Low Level Gamma Ray Counting

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

Plans are being made to construct and operate a Total Body Counter capable of identifying and measuring very small amounts of gamma radioactivity in human beings. The installation will be similar to that developed by Marnelli, Rose and Miller at the Argonne National Laboratory, to the extent that low background counting rates will be obtained by use of a room with steel walls and γ-spectrometry will be based on NaI scintillation detectors. In building such a counting facility it is essential to examine representative samples of all materials going into the steel room and to use only those which show the absolute minimum gamma ray activity. For this purpose, as well as for general utility in low lead level counting of bulky biological samples such as urine, live laboratory animals, etc., an inexpensive but effective steel shield has

been designed and constructed. Table XI presents its physical description.

Table XI

Cylindrical Steel Shield for Low Level Counting

Shape	-	Semi-cylindrical, resting on a concrete pad, 5'x5'x2"
Height, overall	-	4-1/2'
Outside diameter	-	56"
Working space inside	-	36" high, 24" diameter
Total weight	-	7 1/2 tons of iron
Top and bottom	-	Stacked 1/4" plate totaling 5" thick
Wall	-	16" annular space filled with scrap steel punchings, each 1/2" or less in longest dimension
Access	-	A slot in the wall, 12" wide, 36" high
Door	-	A sliding slab, 40" high, 20" wide, 5" thick, from stacked 1/4" plate; rolling on 4 bearings, weight approximately 1200 lbs.
Cost	-	Total for all materials, just over $1000

The cost for the iron was about one-third of what it would have been had steel plate been used exclusively. This economy was achieved by using scrap in the form of small steel punchings obtainable from metal scrap vendors. This material pours easily and packs fairly well. Its effective density was about 270 lbs per cubic foot or approximately 56 per cent of the density of solid steel. The radiation attenuation power of this material was crudely evaluated after the shield was completed. A collimated pencil of radiation from a 10 mc. radium source was directed at the inside surface of the wall and counts at the exterior surface were taken with a NaI crystal scintillation detector mounted in a lead shield having a 1" collimation hole. Counting rates at the spot where the "undeflected beam" emerged showed that the 16" of scrap punchings attenuated the gamma radiation from Radium to the same degree as 7 7/8" of solid iron. For the present, a 3" x 3" NaI crystal detector, canned in copper

and having a quartz window, is installed in the shield and evaluation of the assembly is proceeding. The K40 gamma rays originating from 10 grams of KCl in a plastic vial placed atop the crystal give a counting rate (betas totally absorbed in an Al disc) of 45 c/m above a background of 303 c/m. This represents detection of about 6 per cent of the theoretical number of gamma photons emitted. Equipment for pulse height analysis will soon be procured so that a detailed analysis of the background and the overall counting sensitivity can be made.

Interim Report

Development of an Underwater Spark Pulser for Studying the in vivo Elastic Properties of Tissues as Affected by Natural or Radiation Aging.

Benedict Cassen and Melvin Martin

A USLA Report is now being prepared to describe a new instrument that has finally been completed and is in satisfactory operation. This device was developed for the purpose of measuring possible elastic changes in in vivo tissues on patients as they might be affected by natural aging or localized irradiation in radiotherapy. This is a preliminary attempt in a program to get more objective correlation of physically measurable quantities with the aging process. Although the developed instrument can be used to measure the velocity of sound in tissue, it has already been shown that it can more interestingly measure in one test the increase in sound absorption with frequency. The initial pulse produced by an underwater spark produces a wide continuous spectrum of high frequencies. In water this pulse is transmitted without change in shape showing that all component frequencies are transmitted with equal efficiency. In tissue the rapid rise of the initial pulse is made less rapid after transmission. It is

interpreted that the tissue absorbs and attenuates the high frequency components of the pulse more than the low frequencies. As the instrument now works very well, plans are being made to use it on a hospital patient survey to see whether or not age correlations can be obtained. The patient feels essentially nothing but a feeble tick at the point where the transmitting transducer is pressed against the skin.

Interim Report

An Improved Illuminating System for The Electron Microscope

Francis W. Bishop

The illuminating system is as important in the electron microscope as it is in the conventional optical microscope. For the first several years the source of electrons in the commercial electron microscope consisted of a V shaped filament heated by the passage of a local current, and the electrons emerged from a hole in a covering cap which was at the same potential as the filament. This complete source was referred to as the electron gun. The illumination from a gun of this type was very weak and the distribution of the electrons over the surface of the viewing screen was irregular.

The next great advance was the so-called self-biased gun in which the cap through which the electrons emerged was maintained at a negative potential with respect to the filament. This voltage was obtained automatically by allowing the beam current to pass through a resistance, and the voltage drop so generated was applied to the gun cap. (This element is variously known as the Wehnelt cylinder, the grid-cap, etc.) The negatively charged grid-cap acts as a lens and by symetrically repelling the electrons as they

approach the cap, squeezes the electron beam into a tight, concentrated pencil which brightly illuminates the specimen. This great concentration of electrons (smaller than one grid opening in the specimen support at crossover) may cause breakage of the specimen by thermal or electro-static effects or result in the production of artifacts. The rate of contamination of the specimen by bombarded materials in the microscope is great at the intensity of beam current commonly used with the self-biased gun. (150-200μ amps)

The most modern microscopes now use two condenser lenses; one of which adjusts the beam diameter so that the final screen is completely illuminated; the second condenser lens spreads the area of this beam over a fixed condenser aperture. The over-all result is that the size of the illuminated field and and the intensity are independently variable, and the bombardment of the specimen except in the wanted areas is avoided. This system is easy on the specimen, and the illumination is relatively great. It is possible to operate satisfactorily with a beam current of 60μ amps or less (higher bias voltage) and with this smaller diameter pencil electrons, to use a smaller condenser aperture than usual. (250μ or less) In the older electron microscopes the condenser aperture was approximately 625μ in diameter and the beam current 150-200μ amps.

In an effort to reduce some of the undesirable phenomena inherent in our older instrument, including effects discussed above, certain experiments have been performed. Since it is already possible, by operating controls from the outside, to interchange and center instantly the condenser apertures, and to raise and lower the filament in the gun a known distance, our instrument is peculiarly suited to the experiments which will be described. The standard bias resistor was found to be 1/2 megohm, and the standard condenser

aperture was 625μ in diameter. We had been able to use one of 250μ but the illumination was somewhat low. In trials of high values of bias resistors up to over 70 megohms, it was found possible to use a condenser aperture of only 50μ in diameter, or less than half that of the smallest found on the most modern instruments, and beam currents which ranged from 1 to 5μ amps. About 2.5μ amps seems to be adequate for illumination. These two factors would have reduced the illumination to less than 1/5000 of its original value if it were not for the fact that the increased bias voltage squeezes the smaller pencil of electrons through the fine aperture.

There are a number of advantages that occur at once. The contamination rate has dropped to a new low level. This produces a clearer micrograph of any material at any magnification. It is possible to search for useful fields without the rapid production of contamination artifacts. The second immediately useful effect is the great increase in depth of focus. This makes the focussing of the microscope much less critical and the yield of accurately focussed pictures greater than before. The filament life is greatly increased, probably due to a reduction in positive ion bombardment. If this high-biased system were combined with the double condenser lens, the ultimate in illuminating systems would probably be achieved. As it is, it has greatly improved the performance of our older instrument.

Interim Report

Technique for Electron Microscope Studies of Rat Tissue

F. W. Bishop and Ruth McCandless

The possibility of studying radiation damage to biological materials by means of the electron microscope has been under consideration for some time.

At present there is a project being carried on in collaboration with the Pharmacology and Toxicology Division to study the effect of radiation on the small intestines of rats. The technique involves embedding the tissue in plastic and preparing ultra-thin sections. A small flap is cut in the intestinal wall close to the level of interest, and cold, 1% osmium tetroxide is dripped into the lumen of the intestine, while the circulation stops. (circa 10 minutes) Then a piece or pieces of partly fixed tissue are removed to a pool of osmium tetroxide on a glass plate and fixation is completed. (About 1-2 hrs.) Following a rinse the tissue is dehydrated in ascending concentrations of alcohol, starting with 70%, for 10 minutes each. The tissue pieces are then cut to final size. Lastly they are placed in two changes of 100% monomer of N butyl methacrylate.

At any convenient time the tissue pieces are placed in #0 gelatin capsules containing partially polymerized N butyl methacrylate. If necessary the tissue section is pushed to the bottom of the capsule. Polymerization is completed under an ultra-violet lamp. When the plastic is hard, the embedded tissue is cut out of the capsule, remounted and properly oriented on a wooden peg which is made to fit a Porter-Blum microtome. Sections 1/4$0\mu$ thick are cut routinely and these are mounted on specimen screens. When these sections are placed in the microscope for examination and are subjected to the electron beam they need additional support. This is provided by a thin carbon film which is produced by evaporating carbon in a vacuum system. Two different procedures are in use; one is to evaporate the carbon film onto the screen before the section is placed on it, and the other is to evaporate the film on top of the section after it is placed on the screen. Each system has some advantages.

The results which are being obtained from these electron microscope studies will be reported later.

Interim Report

The Spectrographic Analysis of Plant Ash for Trace Levels of Alkali Metals

Geo. V. Alexander and Peter Taylor

An emission spectrographic method has been developed for the determination of concentrations of cesium, potassium, lithium, rubidium, and calcium in plant ash. The method was developed primarily for the determination of cesium and potassium in plants grown on soils spiked with various levels of stable cesium.

Since a method has been developed in this laboratory for the determination of strontium and calcium in plant ash and has been used quite successfully for several years, it was decided to modify this procedure for the determination of cesium and potassium.

Most of the sensitive lines of the alkali metal atoms are in the far visible or near infrared region of the spectrum and therefore an "infrared" photographic emulsion is required for their detection. In this particular case Eastman Type 1-L was used. Preliminary observations of plant ash samples indicated that adequate sensitivity could be obtained for all elements of interest in the spectral region from 6600A to 8600A.

The choice of an internal standard for spectrographic analysis with photographic detection involves two major considerations: (1) the excitation and volatilization characteristics associated with the analysis line and the internal standard line must be as similar as is practicable in order to attain maximum precision, and (2) a close proximity in wavelength between

the two lines is required in order to minimize effects resulting from variations in the photographic emulsion. It is generally impossible to fully satisfy both of these points and therefore a compromise must be sought. After considering all of the elements which produce lines in the 6600-8600A spectral region, barium was selected for the internal standard. The spectrographic buffer composition was adjusted to make the volatilization of barium coincide as nearly as possible with that of cesium.

The resulting procedure is as follows:

Sample: The plant ash is thermally ashed at 400° C for 4 hrs. (Higher temperatures or excessive ashing times resulted in loss of cesium.)

Buffer-internal standard solution: 7.5 mg NaCl, 22.5 mg $MgCO_3$ and 0.50 mg $BaCO_3$/ml 6 N HCl. (All materials are analytical reagent grade.)

1. The sample is dissolved in the buffer-internal standard solution at a concentration of 7.5 mg/ml.
2. One drop (approx. 75λ) of the sample solution is dried into the top of a titted wafer-top electrode (Fig. 4). The addition of the tit to the wafer-top electrode used for strontium and calcium analysis results in more uniform intensities for the alkali metals, however, it does not affect the intensity ratios.
3. The dried sample electrode is excited with a 5 ampere DC arc using a 2 meter 24,400 line per inch ARL grating spectrograph. An Ednalite, type O-2 filter is used in front of the slit to eliminate second order lines.
4. The spectrum is photographed on Eastman type 1-L film which is developed for 5 minutes in D-19 at 68° F.
5. The spectral lines are densitometered with an ARL comparitor-densitometer and converted to intensity ratios by means of a Seidel transformed

emulsion calibration curve.

Figure 4

Titted Wafer-top Electrode

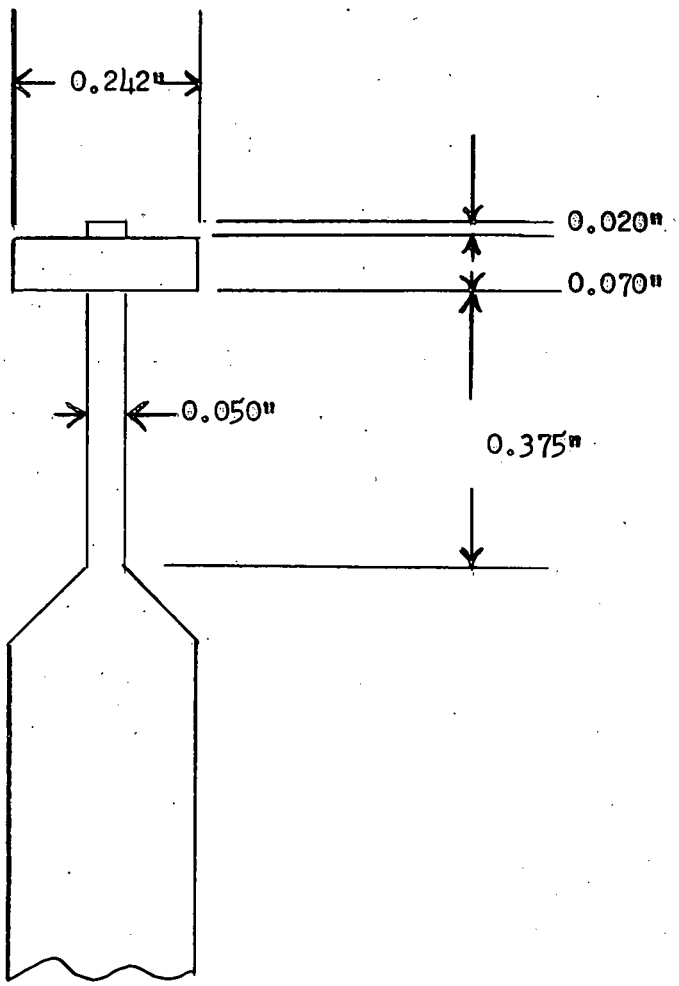


Table XII presents the various line combinations used for the analysis.

Table XII

Analysis Line	Standard Line	Concentration * Range in %	Variance %
Cs 8521.1A	Ba 8560.0A	0.01 - 0.3	10.5
Rb 7800.2	Ba 7780.4	0.001 - 0.03	8.5
Ca 7148.1	Ba 7060.0	5 - 40	4.1
K 6939.0	Ba 7060.0	5 - 40	8.0
Li 6707.8	Ba 6693.9	0.0001-0.003	8.2

* The concentration range for cesium has been extended to 10% by diluting the original sample solution 1:10 with buffer-internal standard solution.

The variance is somewhat higher than that observed for this method when used in the analysis for strontium and calcium, in which case chromium is used as the internal standard. This relatively high variance does not appear to be due to differences in excitation potential between members of the line pair, e.g., the excitation potentials for K 6939.0 and Ba 7060.0 are very similar while those for Rb 7800.2 and Ba 7780.4 are quite different. The relatively normal variance observed for calcium suggests that the photographic-densitometric variance is not unusually high. It appears that the higher variance must then be due to slight differences in volatilization rates or to effects resulting from the differences in line shape between the alkali metal and the barium lines. These two possibilities will be studied in some detail.

The method has been used to analyse 110 plant ash samples and is being used to analyse 80 soil leachates.

Interim Report