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UCRL-2111
Unclassified-Health and Biology

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

Contract No. W-7405-eng-48

MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

October, November, and December, 1952

February 18, 1953

Berkeley, California

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* Previous Quarterly Report UCRL-2001.

I. THE METABOLIC PROPERTIES OF VARIOUS MATERIALS

Joseph G. Hamilton, M.D.

TRACER STUDIES

Versene Studies*

Joseph G. Hamilton and Kenneth G. Scott

Introduction

Because of the potential hazard of the fission products and fissionable materials to individuals who may have been exposed to them, considerable interest has been devoted to the pharmacology of these radioactive substances as well as the development of methods for removing them from the body. Any procedure which results in a greater than normal elimination of these materials from the body has value from two points of view, for it can reduce the ultimate hazard to the contaminated individual, and second it allows a more accurate estimation of the degree of contamination which may be suspected in personnel exposed to such hazards.

One of the compounds which has received attention as a decontaminating agent has been the sodium and calcium salts of ehylene diamine tetra-acetic acid later referred to as "Versene". The pharmacology of this organic compound has been reviewed in detail by the Bersworth Chemical Company.[1] Popovici, et al[2] investigated the control of serum calcium levels using the sodium and calcium salts of Versene. They demonstrated that the sodium salt was many times more toxic than the calcium salt due to the fact that the former compound combined with the serum calcium and produced death by hypocalcemia. Cohn, et al[3] has recently investigated the effects of the calcium salt upon the deposition and retention of radio-yttrium using rats as the experimental animals. In their studies the Versene salt and radio-yttrium were administered intraperitoneally, either shortly before or after radio-yttrium was given. The animals were sacrificed at intervals up to 5 days and a very marked diminution of the skeletal uptake was observed in the treated animals. The greatest effect was noted in those animals which had been pretreated and these skeletal uptake of approximately 1/10 that of the control groups and the content in the liver was decreased by a factor of approximately 20. Earlier studies at this laboratory have demonstrated that oral administration of the calcium salt of Versene increased the urinary excretion of plutonium.[4]

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* The radioautography and histology of the monkeys and the Versene experiments have been in part reported previously, but it was felt desirable to include some of the new data in order that a more complete picture could be obtained even though there is a certain repetitive factor present.

Methods

The several separate experiments which comprise this investigation are summarized in Table I. Wherever administered, Versene was given as the Calcium salt at a pH of 7. Female rats weighing from 200 to 310 grams were used as test animals.

The treated animals and their control received plutonium 238. The dosage varied in the several experiments from 0.18 to 0.09 μc. The plutonium was administered as plutonyl nitrate in the presence of 0.01 bromate which kept it in +6 valance state and 0.1 M citrate which forms a complex with plutonium thereby preventing the formation of a plutonium colloid.

The 92-year plutonium 238 was used rather than the longer lived plutonium 239 in order to secure a higher degree of specific activity and thus approach a plutonium concentration as to the number of atoms that might be present in human beings who may have been exposed to this radioelement and who may have of the order of 0.1 mg of the longer lived isotope plutonium 239 stored in their body.

In all five experiments the treated and control animals were maintained in metabolism cages, each cage containing from 3 to 5 rats. The urine and feces were collected separately. The organs and tissues removed at the time of sacrifice were assayed for plutonium content and these included: spleen, blood, kidney, gastrointestinal tract, muscle, skin, injected leg of the fourth group as well as the "balance" of the rat. The balance represented the ashed remainder of the rat after it had been skinned, the skeleton removed from the ash and the calculated amount of plutonium in blood, muscle and skeleton subtracted from the total plutonium content of the balance. In the case of the animals which received plutonium by intramuscular injection an additional correction was made since the injected leg was removed at the proximal end of the femur. Thus the balance may be presumed to represent structures such as glandular, lymphoid, connective tissue and cartilage. Most of the tissues were assayed for their plutonium content by the technique which has been described in detail earlier by Scott, et al.[5] In other cases specimens containing relatively large amounts of calcium such as the skeleton, balance, liver, skin, urine and feces necessitated the use of a second assay technique in which the chelating agent Thienyl Trifloroacetone was used to separate the plutonium from the tissue ash. This chemical, commonly called TTA, will effect a quantitative removal of plutonium from animal residues when the following method is employed.

TTA Method for Separation of Plutonium from Tissue Ash

1. Add 50 cc of 2 N HNO_3 plus 0.1 M hydroxylamine hydrochloride solution to ashed sample and let stand for at least 2 hours.
2. Put 10 cc sample in centrifuge cone.
3. Add 3 cc of $La(NO_3)_3$ without stirring (20 mg $La(NO_3)_3$ per cc).
4. Add 10 cc of 12 M HF and stir.
5. Centrifuge for 5 minutes at 2000 rpm.

TABLE I

Dosage Schedule to Treated Animals

Exp. No.	No. of Animals	Time of Sacrifice Following Plutonium	Dosage in mg to Each Animal	Route of Plutonium Administered
1	3	1 day	2 mg Versene intravenously once at time of injection. 3.5% in diet starting 24 hrs. before plutonium injection.	intravenous
2	3	15 days	same as above	intravenous
3	10	15 days	186 mg Versene intravenously once at time of injection. 3.5% in diet starting 24 hrs. before plutonium injection.	intravenous
4	10	15 days	An average dosage of 140 mg each day for 5 days. None for following 3 days. 155 mg daily for next 7 days. Given either intraperitonealy or subcutaneously.	intramuscular
5	10	50 days	No Versene for first 25 days. Given 162 mg daily by intraperitoneal injection from 25 to 50 days.	intravenous

6. Pour off supernatant when precipitate is well packed down.

7. Add 100 cc of 1.5 M HF to cone and stir up precipitate.

8. Centrifuge again for 5 minutes at 2000 rpm and pour off supernatant when precipitate is well packed down.

9. Stir precipitate to break it up. Add 25 cc $Al(NO_3)_3$ and stir until precipitate dissolves.

10. Add 0.25 0.1 M $NaNO_2$.

11. Stir and let stand at least 15 minutes before pouring into a separatory funnel.

12. Add 10 cc of TTA solution containing 25 grams of TTA in 500 cc of benzene and shake for 10 minutes.

13. Let stand one hour and drain off bottom layer of liquid.

14. Wash twice with distilled water.

15. Add 10 cc of 8 M HNO_3 and shake for 15 minutes.

16. Let stand for 5 minutes.

17. Drain product into porcelain dish.

18. Rinse through funnel tube once with 8 M HNO_3.

19. Transfer and evaporate on gold or platinum dish, and count.

Results

The results obtained from the first two experiments, the methods of which are summarized in Table I, showed that the oral administration of Versene is not an effective way of removing plutonium from the rat. The simultaneous injection of Versene intravenously at a dosage of 8 mg per kilogram of body weight had little effect on altering the eventual distribution of plutonium in the tissues in animals which were also being fed 3.5 percent Versene in their diet, and sacrificed 1 day after the administration of plutonium.

Although group 1 in Table I had a higher urinary plutonium excretion no significant reduction was observed in the amount of plutonium found in the skeleton, and most soft tissues. The increased excretion which was observed probably represented a loss of plutonium from other soft tissues such as "balance". The amount of plutonium excreted in the urine and feces of the control group was 0.89 percent and 2.23 percent respectively. The group which received Versene excreted 3.37 percent and 2.36 percent of the plutonium administered in their urine and feces respectively. In the second experiment, in which the Versene was fed for a period of 15 days after plutonium administration, the muscle of the Versene-treated animals had a significant reduction of their plutonium content. The percent of plutonium per gram of muscle in the controls

was 0.36 percent and 0.14 percent in the Versene-treated rats. The plutonium found in skeleton and other tissues with the exception of the balance was not significantly different in the two groups. The fecal excretion was not significantly different in these two groups. The prolonged feeding of Versene did increase the urinary excretion of plutonium its being 10.5 percent in the second treated group as compared to 1.9 percent in the controls.

The parenteral administration of large amounts of Versene to rats following a dose of plutonium is capable of reducing the plutonium stored in most tissues of the body. When plutonium was administered intravenously and Versene given as shown in Table I, experiment 3, a significant decrease of plutonium in the tissues of rats sacrificed 15 days following plutonium administration was observed. These data are summarized in Table II. There was nearly an eightfold increase of urinary excretion with the administration of Versene. The amount of plutonium found in liver, muscle, skin and balance of these treated animals was significantly less than that in the controls. The amount of plutonium retained by the skeleton was nearly one-half that of the untreated control animals. A comparison of this data with that obtained in the first two experiments indicates that the greater effect was apparently due to the administration of large amounts of Versene parenterally as compared to the action of this compound when present in the diet, since the treated animals in groups two and three had the same diet.

The results obtained by the multiple injections of large amounts of Versene in animals on a normal diet are summarized in Table III. In general the results are very similar to those noted in the third experiment. An interesting observation in the fourth experiment was the effect of Versene upon the greater than normal absorption of plutonium from the site of the injection which was by the intramuscular route.

Figure 1 presents graphically the effect of Versene upon the urinary excretion of plutonium for the second, third and fourth experiments. There was apparently some increase of urinary excretion in all three groups and it seemed greatest in the fourth group.

Figure 2 indicates that the parenteral administration of Versene nearly a month after plutonium was given produced quite a prompt and definite increase of its excretion. The complete distribution of plutonium in the tissues in the fifth experiment is shown in Table IV. The fecal excretion of the Versene-treated and control animals was nearly the same. The depletion of plutonium in the tissues and organs was minimal. The greatest effects were seen with spleen, skin and balance. Here the plutonium content was reduced by nearly a factor of two. The skeletal content of plutonium was significantly lower in the Versene-treated animals but the reduction was not as great as that observed in the soft tissues.

Discussion

These studies indicate that prolonged oral administrations of Versene at levels that approached a significant degree of toxicity did not produce a pronounced depletion of plutonium in the soft tissues and had little or no effect upon the skeletal content of this element. The parenteral administration of small amounts of Versene likewise appears to be productive of little effect. The parenteral administration of large amounts of Versene at the same time plutonium

TABLE II

The Distribution of 0.09 μc of Pu^{238}, 15 Days After its Intravenous Administration in the Tissues of Control and Versene-Treated Rats. Values are Expressed as Percent of Dose per Organ and Gram Wet Weight Corrected to 100% Recovery. Actual Average Recoveries were 89.7% for the 10 Animals in the Control Group and 89.7/ for the 9 Animals in the Versene Group. Versene was Given in the Diet Primarily. See Table I for Versene Dosage.

	Control				Versene			
	% per organ	Mean Std. Error	% per gram	Mean Std. Error	% per organ	Mean Std. Error	% per gram	Mean Std. Error
Spleen	0.60	±0.06	0.67	±0.08	0.17	±0.05	0.20	±0.09
Blood	0.40	0.04	0.02	<0.01	0.28	0.05	0.02	<0.01
Liver	8.77	0.52	0.89	0.04	3.59	0.38	0.39	0.04
Kidney	1.37	0.13	0.76	0.07	0.50	0.08	0.26	0.05
G.I. Tract	0.88	0.07	-	-	0.32	0.04	-	-
Skeleton	55.4	0.92	2.62	0.12	30.6	0.20	1.35	0.09
Muscle	1.39	0.13	0.01	<0.01	0.71	0.10	<0.01	-
Balance	2.70	0.15	-	-	0.90	0.10	-	-
Skin	1.48	0.12	0.03	<0.01	0.51	0.06	0.01	<0.01
Urine	6.16	0.19	-	-	50.6	1.28	-	-
Feces	20.9	0.77	-	-	11.7	0.29	-	-

TABLE III

The Distribution of 0.11 μc of Pu^{238}, 15 Days After First Intramuscular Injection in the Tissues of Control and Versene-Treated Rats. Values are Expressed as Percent of Dose per Organ and Gram Wet Weight, Corrected to 100% Recovery and Representing only the Plutonium Absorbed from the Injection Site. The Actual Average Recoveries were 90.9% for the 9 Animals in the Control Group and 86.7% for the 10 Rats in the Treated Group. The Versene was Administered Parenterally. See Table I for Versene Dosage.

	Control				Versene			
	% per organ	Mean Std. Error	% per gram	Mean Std. Error	% per organ	Mean Std. Error	% per gram	Mean Std. Error
Spleen	0.44	±0.05	0.54	±0.03	0.20	±0.05	0.28	±0.08
Blood	0.47	0.04	<0.04	-	0.16	0.02	0.01	<0.01
Liver	6.50	0.52	0.97	0.09	2.12	0.16	0.27	0.01
Kidney	0.94	0.06	0.61	0.05	0.38	0.03	0.25	<0.01
G.I. Tract	0.80	0.12	-	-	0.31	0.03	-	-
Skeleton	63.6	0.70	4.39	0.17	34.4	1.77	2.04	0.12
Muscle	1.21	0.13	0.01	<0.01	0.66	0.06	<0.01	-
Balance	4.29	0.57	-	-	1.08	0.19	-	-
Skin	1.03	0.10	0.04	<0.01	0.36	0.03	0.01	<0.01
Urine	4.10	0.15	-	-	47.1	2.18	-	-
Feces	16.6	0.71	-	-	13.2	1.03	-	-
Left Leg	24.4				3.79			

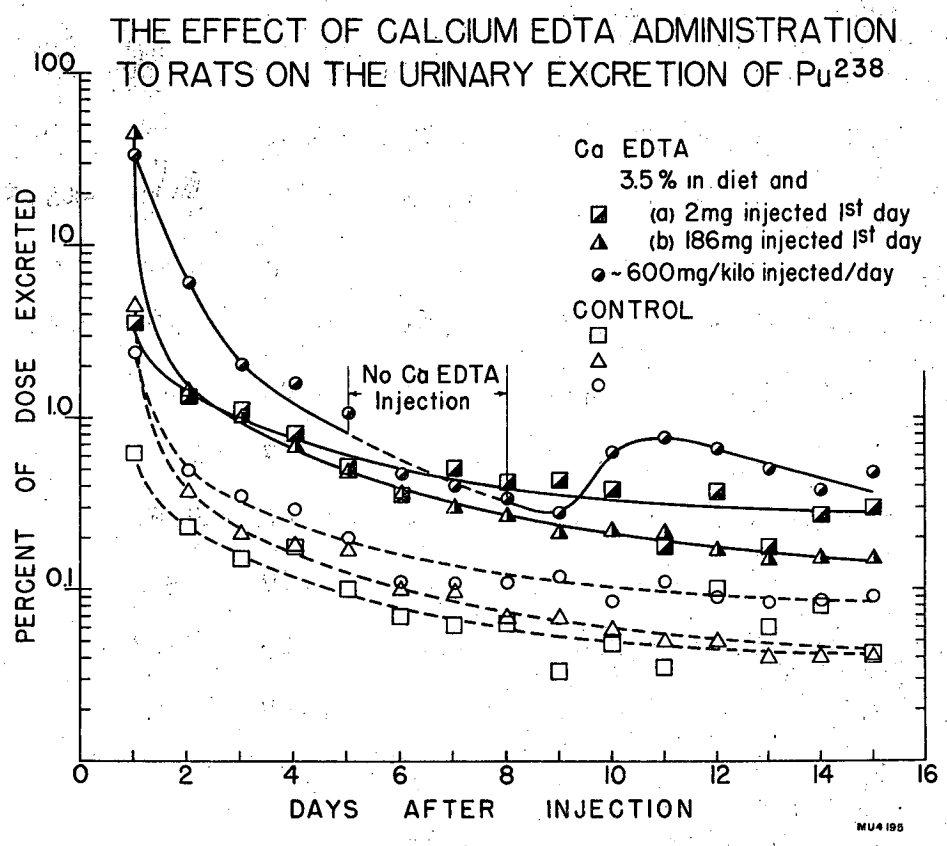
THE EFFECT OF CALCIUM EDTA ADMINISTRATION TO RATS ON THE URINARY EXCRETION OF Pu238
Ca EDTA
3.5% in diet and
(a) 2mg injected 1st day
(b) 186mg injected 1st day
~600mg/kilo injected/day
CONTROL
No Ca EDTA Injection
PERCENT OF DOSE EXCRETED
100
10
1.0
0.1
0
2
4
6
8
10
12
14
16
DAYS AFTER INJECTION
MU4195

Fig. 1

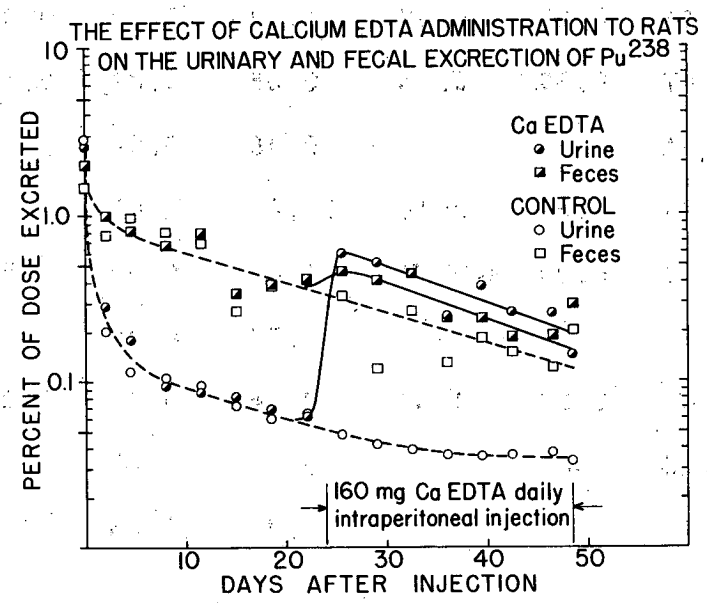
THE EFFECT OF CALCIUM EDTA ADMINISTRATION TO RATS
ON THE URINARY AND FECAL EXCRETION OF Pu238
PERCENT OF DOSE EXCRETED
10
1.0
0.1
Ca EDTA
Urine
Feces
CONTROL
Urine
Feces
160 mg Ca EDTA daily
intraperitoneal injection
10
20
30
40
50
DAYS AFTER INJECTION

Fig. 2

TABLE IV

The Distribution of 0.12 μc of Pu^{238} in the Tissues of the Rat 50 Days After its Intravenous Administration. Values are Expressed as Percent of Dose per Organ and Gram Wet Weight and Corrected to 100% Recovery. The Actual Average Recovery of the 10 Control Animals was 82.3% and 90.0% for the Versene-Treated Animals. The Versene was given Parenterally Starting on the 25th Day Following the Plutonium Administration.

	Control				Versene			
	% per organ	Mean Std. Error	% per gram	Mean Std. Error	% per organ	Mean Std. Error	% per gram	Mean Std. Error
Spleen	0.91	±0.05	1.60	±0.17	0.45	±0.07	0.67	±0.10
Blood	0.15	<0.01	0.01	<0.01	0.14	<0.01	0.01	<0.01
Liver	5.25	0.44	0.63	0.07	4.90	0.67	0.47	0.06
Kidney	0.68	0.07	0.37	0.05	0.65	0.02	0.32	0.01
G.I. Tract	0.29	0.04	-	-	0.16	0.02	-	-
Skeleton	61.2	1.13	3.29	0.16	52.0	1.08	2.74	0.09
Muscle	1.06	0.18	0.01	<0.01	0.80	0.09	<0.01	-
Balance	3.68	0.15	-	-	1.51	0.18	-	-
Skin	0.98	0.04	0.03	<0.01	0.54	0.11	0.02	<0.01
Urine	6.33	0.40	-	-	15.2	0.40	-	-
Feces	19.5	1.11	-	-	24.1	0.68	-	-

was given appeared to decrease the skeletal content to a significant degree and also increase the absorption from the intramuscular site of injection. The latter observation is of interest since a very probable route of exposure of an individual to plutonium might be by subcutaneous or intramuscular introduction into the body. It would be of interest to determine if the parenteral administration of Versene is capable of reducing the plutonium content in the lungs following its inhalation as an aerosol and more particularly in a chemical state which can be absorbed and enter the blood stream, Scott, et al.[5]

A comparison of these studies with those of Cohn, et al[3] are of interest in that the effect they observed was much greater than that noted in the experiments described in this paper. However, it should be kept in mind that in the studies by Cohn, et al[3] the Versene compounds as well as the radio-yttrium were given by intraperitoneal injection. It is entirely possible that in their studies a large fraction of the radio-yttrium was chelated before it was absorbed. Thus their experiments and those described here are not entirely comparable.

With the current information available it would appear that Versene is of very limited practical value for the treatment of chronic plutonium poisoning. The relatively small increase of urinary excretion after this radioelement has been deposited in the skeleton together with the large amount of Versene which appears necessary would seem valid reasons for not giving this substance as a practical therapeutic agent. Moreover, in the rat, the administration of Versene necessary to secure a significant amount of urinary excretion is in the toxic range. The immediate parenteral use of large amounts of Versene following the accidental entry of plutonium into the body through cuts or abrasions would appear to offer some promise. Before such attempts might be made, careful consideration should be given to the problems of acute and chronic toxicity of Versene compounds in man.

Acknowledgments

The authors wish to express their appreciation for the technical assistance of Miss Margaret Gee, Miss Marilyn Hemenway and Miss Helen Johnson. The long and laborious task of maintaining the animals and doing the plutonium assays was their immediate responsibility.

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Long-Term Rat Astatine Studies

Joseph G. Hamilton and Patricia Wallace

On the 29th of April, 1952, four 50-day old Long-Evans female rats whose weight averaged 150 grams and whose range lay between 140 and 100 grams each received 120 microcuries of astatine^{211} by intraperitoneal injection. Since that time these four rats have been maintained on standard laboratory food and tap water. The notable apparent radio toxicity observed in rats injected with astatine^{211} at this dose-level in other animals was not seen. There is always the possibility that an error in the analysis of this radioisotope occurred or not all the material which was present in the syringe was retained in the solution, thus the animals would not receive the amount that they were thought to have received. However, with the precautions taken this does not seem likely.

During the following months, these animals were observed to grow more slowly than rats of the same weight who had not received astatine and otherwise might be considered as normal controls. On January 8, 1953, the animals were weighed and their average weight was 170 grams and they ranged from 140 to 200 grams. Normal females of the same age have an average weight of about 270 grams. The wide range of weight was unexpected. Physically the four animals regardless of their weight appeared to be equally vigorous. However, there are certain differences which may be noted. The hair is slightly ruffled and not as sleek as would be expected in rats of this age and sex. Also the hair appears to be somewhat more coarse. Throughout the experiment the intake of food is less than with the normal animals of the same age. From these observations it would appear reasonable in this pilot experiment to conclude that a sufficient degree of thyroid damage was produced to cause decrease in growth as well as other indications of diminution of function of the thyroid gland.

In conjunction with the program to study the apparent effects of total body beta and alpha irradiation, a larger number of rats will be given astatine^{211} and will be maintained for a long period of time to determine not only the alterations of the morphology of the thyroid gland but also changes in the pituitary gland and other endocrine organs.

The Metabolism of Fluorine

Patricia Wallace

The Effect of Dietary Fluorine on Growth

Fifty-five female rats of the Long-Evans strain approximately 9 weeks old (150 grams in weight) were divided into 11 lots of five and were placed in stock cages. The animals were maintained in two groups, 30 experimental animals that were given sodium fluoride mixed with their food and 25 control animals that were given a ration containing no added flouride. Each lot of five rats received 75 grams of food daily, or an average of 15 grams of food per rat. The composition of the synthetic diet is shown in Tables I and II.

The diet appeared to be adequate since the growth curve of the control animals closely followed the curve for the growth of female rats of this strain obtained by the University of California Institute of Experimental Biology using a diet known to be complete. An analysis of the diet for fluoride was made by the firm of Curtis and Tompkins and showed that it contained 8 p.p.m. of flouride. In the control animals this would amount to an intake of about 0.5 mgm of fluoride per kilo per day, assuming that the daily food intake of each rat was 15 grams. The occurrence of fluoride in the diet is probably due to small amounts present in the chemicals making up the mineral mixture, especially the NaCl and KCL.

The mineral mixture used is a modification of that devised by Hawk and Oser (1931). The most serious modification was the omission of NaF which they add to the mixture so that the final diet contains 10 p.p.m. of added fluoride. The other modification of the salt mixture was the addition of 0.5 grams of $CuSO_4$ to the trace salt mixture.

In the case of the experimental animals sodium fluoride was added to the diet in sufficient quantity so that the average daily fluoride consumption for the group as a whole was 20 mgm/kilo. All of the animals were weighed every 7 to 10 days at approximately the same time of day and the sodium fluoride added to the diet was adjusted for any weight changes in the experimental animals.

Twenty mgm per kilo was the daily fluoride supplement chosen, because it has been shown by other investigators, Lamb et al (1933) and McClure and Mitchell (1931), that a daily intake of fluoride greater than 20 mgm/kilo produces disturbances in the tissues of the rat other than the teeth. Since the main purposes of the experiment were to determine whether or not there are any disturbances of thyroid function or changes in the distribution of radiofluorine following a long period of chronic fluorine intoxication, it was considered desirable to give the animals as much fluorine as possible and at the same time to maintain them in more or less a physiological state.

Figure 3 shows the growth curves for both the experimental and control animals during a period of 4 months. After about 2 months on the high fluoride diet changes were noted in the incisors of nearly all of the experimental animals. The most striking changes were thickening, alterations of normal pigmentation, increased opacity and presence of dark spots on the enamel of the incisors. There appears to be an increased tendency of the incisors to break

TABLE I

Composition of Synthetic Diet

Casein	100 grams
Powdered Skim Milk	60 grams
Whey	40 grams
Brewer's Yeast	40 grams
Hydrogenated Vegetable Oil	60 grams
Wheat	660 grams
Mineral Mixture	40 grams
	1000*

* 2 grams of Vitamin A and D supplement "Nopco Quadrex" added per kilo.

TABLE II

Mineral Mixture for Synthetic Diet

$CaCO_3$	68.6 grams
$CaHPO_4$	112.8 grams
$MgCO_3$	35.2 grams
$Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$	308.3 grams
$MgSO_4$ Anhydr.	38.3 grams
KCl	124.7 grams
NaCl	77.1 grams
$K_2HPO_4 \cdot 3H_2O$	218.8 grams
Trace Mixture	16.2 grams
	1000.0

Trace Salt Mixture

Green Ferric Ammonium Citrate	98.52 grams
KI	0.25 grams
$CuSO_4 \cdot 5H_2O$	0.50 grams
Ammonium Alum	0.59 grams
$MnSO_4 \cdot H_2O$	0.14 grams
	100.00

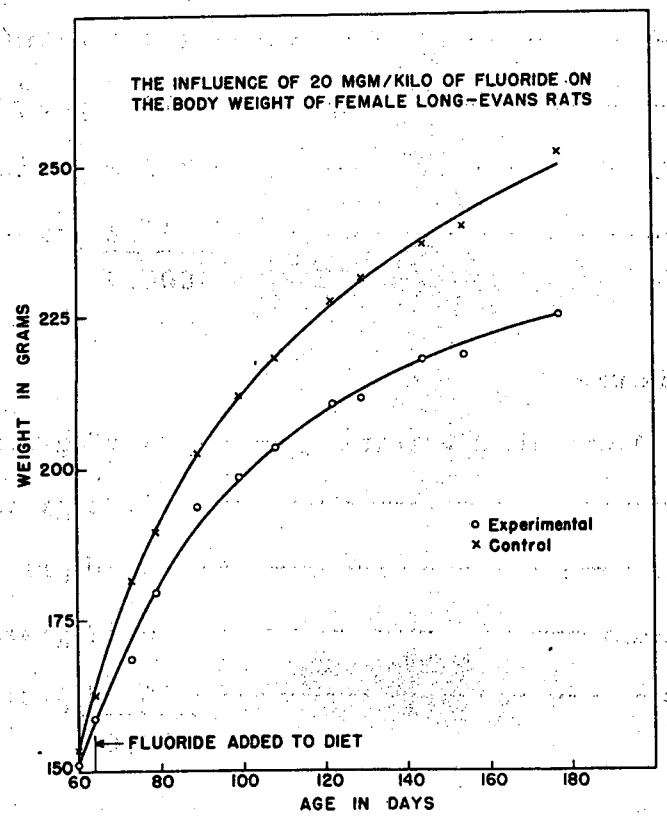
THE INFLUENCE OF 20 MGM/KILO OF FLUORIDE ON
THE BODY WEIGHT OF FEMALE LONG-EVANS RATS
WEIGHT IN GRAMS
250
225
200
175
150
60
80
100
120
140
160
180
AGE IN DAYS
o Experimental
× Control
FLUORIDE ADDED TO DIET

Fig. 3

allowing excessive growth in the opposite incisor. At the end of 4 months the teeth of most of the experimental animals were in such bad condition that the feeding experiments were halted, the animals were given radiofluorine and sacrificed. These results will be reported later.

Although most of the fluorosed animals seemed quite normal except for their teeth, some of them showed other manifestations such as poor coat, an untidy appearance and lassitude. The animals were fed from heavy pyramid shaped porcelain feed cups which effectively prevented spilling and waste, and all of the food provided was consumed over a 24 hour period by the experimental animals as well as the controls despite the handicap of greatly elongated teeth in the case of the experimental animals. Consequently the slower growth rate of the experimental animals seemed to be due to an actual metabolic disturbance rather than to a lower food intake.

The Distribution of Radiofluorine in the Teeth and Bones of Rats

Fifteen female rats of the Long-Evans strain averaging 200 grams in body weight were given carrier-free radiofluorine intravenously and were sacrificed in 3 groups of 5 animals each 1, 4 and 9 hours after injection. Samples of soft tissues as well as excretions were taken to augment the data already reported on the distribution of radiofluorine in rats. In addition to these samples the molars, incisors, mandibles, and the heads and shafts of the bones of the right leg were also taken. The concentrations of radiofluorine in the bony structures for the 3 time intervals are shown in Table III along with their standard errors.

As was stated in the previous report the concentration of radiofluorine in the leg bones reached a peak 4 hours after injection and showed a moderate decline from this peak value at 9 hours. The only bony structures that did not show this peak at 4 hours were the molars for which the highest radiofluorine concentration was found at 1 hour followed by a gradual decline. It seems likely that the appearance of a concentration peak for the molars at this early time interval may be the result of the adsorption on the molar surface of radiofluorine from the saliva, since Volker et al have shown that the saliva contains considerable amounts of radiofluorine in the first few minutes after injection.

The bony structures can be arranged in order of decreasing radiofluorine concentration as follows: mandible, epiphysis, diaphysis, incisors and molars. It hardly seems fortuitous that the same arrangement is obtained when these tissues are placed in order of decreasing vascularity, an observation also reported by Volker et al. Neglecting the high radiofluorine concentration in the molars at 1 hour, it is of interest to note that although the teeth did not accumulate as much radiofluorine as did the other bony structures, neither did they lose what they have accumulated as rapidly as did the other structures investigated. (The teeth retained 90 percent of their peak concentration while the mandible and long bones retained 75 percent.) The slower loss of radiofluorine from the teeth can also be explained on the basis of lower blood supply to the teeth per unit weight.

TABLE III

The Distribution of F^{18} in the Teeth, Leg Bones and Mandible of the Rat 1, 4 and 9 Hours after Intravenous Administration. Values are in Percent of Administered Dose and are Corrected for Deviation of Recovery from 100 percent. Each Value is the Average for 5 Animals ± the Standard Error. (Standard Error = $\pm \frac{d^2}{n(n-1)}$)

	1 hr - 250 μc %/gm	4 hr - 180 μc %/gm	9 hr - 300 μc %/gm
Molars	3.04 ± 0.17	2.75 ± 0.13	2.50 ± 0.13
Incisors	3.14 ± 0.14	3.98 ± 0.32	3.61 ± 0.23
Mandible	5.08 ± 0.33	5.96 ± 0.19	4.61 ± 0.42
Leg bones*			
heads	4.38 ± 0.26	5.20 ± 0.14	3.87 ± 0.22
shafts	3.28 ± 0.20	3.90 ± 0.10	2.90 ± 0.16

* Femur, tibia and fibula of right leg.

Maternal Transfer of Radiofluorine

Transfer of Radiofluorine Across the Placenta of the Rat. Radiofluorine was administered intravenously to 2 pregnant Long-Evans rats 2 days before term. The animals were sacrificed 1 hour after injection and the following tissues and organs taken for assay: blood, liver, kidney, gastrointentinal tract, muscle, bone from the right hind leg, skin, fetuses, placenta, uterus and amniotic fluid and excretions. Despite the fact that only 2 rats were used, the results are considered to be semi-quantitative since sufficient activity was administered to eliminate serious counting errors. At the time of measurement 1 percent of the dose was 370 c/s. The results are shown in Table IV with the distribution of radiofluorine 1 hour after intravenous injection into virgin females shown in the same table for comparison.

The concentrations of radiofluorine in the blood and soft tissues of the pregnant animals were in general higher than those for the corresponding tissues of the virgin animals. The renal excretion of radiofluorine was also greater in the pregnant rats. The radiofluorine concentration in the bone of the pregnant animals was much lower than in the controls. These three observations, high blood level and consequently high tissue levels, low bone uptake and high renal excretion are probably all reflections of the increased circulation rate which accompanies pregnancy.

Although the concentrations of radiofluorine found in the fetuses and the placenta were quite small, less than one-half that of the blood, the ability of fluoride to pass across the placenta even when present in minute amounts seems definitely established, a point which has been quite actively debated for some time, Roholm (1937).

The Secretion of Radiofluorine in the Milk of Rats. Three Long-Evans rats with 16 day old litters (litters were reduced to 6) were given radiofluorine orally by stomach tube. Ordinarily animals receiving material by stomach tube were fasted overnight but fasting was considered unwise in this particular case since lactation alone puts the animals under considerable strain and there was the possibility of the mothers devouring some of their young if food were withdrawn. Although more control data was available for radiofluorine administered intravenously, it was considered advisable to give the material in such a fashion that the animals would not be subjected to the trauma of an operation or the after effects of anesthesis, in which case they might not allow their young to nurse. Excretions were not collected, because the animals were allowed to remain in the stock cages where they had made their nests, again to avoid any circumstances which might discourage the mother from nursing their young.

The mothers and their litters were sacrificed 4 hours after the administration of radiofluorine and the following samples were taken: maternal blood, liver, kidney, gastrointestinal tract, muscle, bone, skin and mammary tissue. Gastrointestinal tracts and samples of leg bone were taken from each nursling and were pooled for each litter. The nursling carcasses were counted separately. The results are shown in Table V. Values are expressed in percent of absorbed dose and are not corrected for recovery.

The mammary tissue showed very nearly the same concentration of radiofluorine as was found in the maternal blood. About 1 percent of the radiofluorine administered to each mother was found in her litter. As was to be

TABLE IV

The Distribution of F^{18} in 19 Day Pregnant Rats One Hour After Intravenous Administration. Values are Expressed in Percent of Administered Dose and are Corrected for Deviation of Recovery from 100 Percent. Each of Two Pregnant Rats Received 500 μc of Radiofluorine. The Average Distribution of Radiofluorine in Five Virgin Females is Shown for Comparison.

	Pregnant		Control	
	%/organ	%/gram	%/organ	%/gram
Blood	2.07	0.12	0.98	0.08
Liver	1.47	0.11	0.49	0.06
Kidney	0.40	0.24	0.27	0.18
G.I. and Contents	3.50	0.19	2.11	-
Muscle	8.98	0.08	6.79	0.08
Bone	42.3	2.16	58.9	3.74
Skin and Mammary Tissue	3.75	0.08	2.24	0.08
Balance	11.2	-	14.7	-
Fetuses	1.76	0.04	-	-
Placenta	0.32	0.06	-	-
Uterus and Amniotic Fluid	0.54	-	-	-
Urine	23.4	-	11.9	-
Feces	0.28	-	1.28	-

expected, more than half of the activity found in the nurslings was present in their gastrointestinal tracts, and the next greatest amount was found in the infant bone.

The results of this study strongly indicate that the fluoride ion present in the maternal blood of the rat even in minute amounts can be secreted in milk. With regard to the possibility of fluoride passing into milk in sufficient quantity to produce dental changes the literature indicates that there may be a species difference. Phillips et al have shown that dairy cows on a high fluorine diet produce milk which contains very little more fluoride than cows on a relatively fluorine free diet. Gaud et al have reported that a sheep with darmous (a dental affliction found in North Africa where the soil and water contain large amounts of fluorine) give milk differing very little in fluorine content from the milk of normal sheep. On the other hand Brinch and Roholm (1934) observed mottled teeth in children who had been nursed for long periods of time by fluorine intoxicated mothers and Murray (1936) showed that young rats suckled by fluorsed mothers contained 30 times more fluorine than those suckled by normal mothers.

Studies on the Appearance of Radiofluorine in the Gastrointestinal Contents of the Rat After Intravenous Administration

When administered intravenously to rats, radiofluorine appeared quite rapidly in the contents of the stomach and intestines. (See the previous Quarterly Report). In order to determine the route of entry of radiofluorine into the contents of the alimentary tract two studies were undertaken. (1) The intravenous administration of radiofluorine to rats 45 minutes after they had received an intraperitoneal injection of ephedrine sulfate. (2) Intravenous administration of radiofluorine to rats in which the cardiac sphincter, pylorus and bile duct had been ligated.

There are several routes by which radiofluorine might enter the gastrointestinal contents: secretion in the saliva with subsequent swallowing, secretion by the stomach mucosa, secretion in the bile, in the pancreatic juice, in the intestinal juice and by ionic diffusion into the entire length of the alimentary tract.

In the first experiment with ephedrine sulfate, a sympathomimetic drug, the following results might be expected according to Goodman and Gilman (1941); a decrease in the motility of the stomach and intestines, a contraction of the sphincters and a reduction of the blood supply to the abdominal region as a whole. These are all effects which would be observed, if an animal received an injection of adrenaline. The effects of ephedrine administration, although less spectacular than those observed with adrenaline, are much more prolonged.

The second experiment on animals with isolated stomachs and ligated bile ducts was designed to eliminate saliva from the gastrointestinal tract, to collect the secretions of the isolated stomachs and to eliminate the flow of bile into the duodenum.

Experimental Procedures.

(1) Five female Long-Evans rats that had been fasted overnight were given 10 mg/kilo of ephedrine sulfate intraperitoneally. Forty-five minutes

TABLE V

The Distribution of F^{18} in Lactating Rats and their Young, Four Hours After Oral Administration. Values are Expressed in Percent of Absorbed Dose. Each of Three Lactating Rats with a Sixteen Day Old Litter of Six Received 600 μc of Radiofluorine. The Average Distribution of Radiofluorine in Five Virgin Females Four Hours After Oral Administration is Shown for Comparison.

	Lactating		Control	
	%/organ	%/gram	%/organ	%/gram
Blood	0.44	0.03	0.15	0.015
Liver	0.27	0.02	0.08	0.01
Kidney	0.10	0.06	0.04	0.04
G.I.	1.85	0.15	3.63	-
Muscle	2.42	0.02	0.87	0.01
Skeleton	39.6	2.02	67.4	5.17
Skin	0.72	0.02	0.20	0.01
Mammary Tissue	0.13	0.04		
Young* Bone	0.28	0.01		
G.I.	0.53	-		
Balance	0.18	-		
Excreta	-	-	27.1	-

* Values shown are totals for litter of six.

later they were given radiofluorine intravenously and were sacrificed one hour after the radiofluorine injection. At autopsy, the following tissues and organs were taken for assay: blood, stomach, small intestines, cecum, large intestine, bone from the right hind leg, muscle and skin. Excreta and carcass were assayed to obtain total recovery. Urine was obtained principally from the bladder. The contents were removed from the stomach and small and large intestines. Assay techniques were the same as those described in the previous report.

(2) Five female Long-Evans rats that had been fasted overnight were put under deep ether anesthesia, a midline incision was made in the abdomen and ligatures were placed around the cardiac sphincter, the pylorus and the bile duct. The operation was performed as rapidly as possible and usually took about 10 minutes. The gut was handled with care and was kept moist throughout the operation. After suturing the abdominal muscle and skin, the animals were given radiofluorine intravenously. One hour after the operation and injection, the animals were sacrificed and the tissues and organs taken for assay were the same as in the first experiment and in addition included liver and kidney. Saliva samples were obtained from the mouths and throats of three of the five rats with cotton swabs. Although there was no appreciable blood loss during the operations, there was a seepage of a pigmented fluid into the peritoneal cavity. The animals were cold to the touch and their fur was ruffled.

Results. At autopsy, it was noted that the stomachs and to a lesser extent, the small intestines of all of the animals of both experimental groups were distended with a considerable amount of watery fluid, pH 6 to 8. The urine from the operated animals was a bright yellow indicating the renal excretion of bile.

The results of both experiments are shown in Table VI which also includes the results obtained from five control animals that have received no treatment other than an intravenous injection of radiofluorine.

On the whole the results obtained from the animals that had received only an injection of ephedrine sulfate would seem to be more reliable than those obtained for the operated animals, since the operated animals were more than likely in primary shock.

Discussion. In the operated animals, the litation of the cardiac sphincter essentially eliminated the flow of saliva into the stomach due to swallowing. Although saliva samples were obtained from 3 of the 5 rats, only 1 of the samples contained significant quantities of radiofluorine.

The same amount of radiofluorine was found in the small intestines of the animals with ligated bile ducts as was found in the animals that were given ephedrine.

The amount of radiofluorine found in the stomach contents did not show any correlation with the treatment that the animals received. The results obtained were comparable for all three groups, treated animals as well as controls. The amount of radiofluorine in the stomach contents seemed also to be independant of the volume of fluid in the stomachs, since it was the same in the two treated groups whose stomachs were filled with fluid as it was in the controls whose stomachs were nearly empty.

TABLE VI

The Distribution of Radiofluorine in Rats Treated with (1) 10mgm/kilo of Ephedrine Sulfate, (2) Ligated Cardiac Sphincter Pylorus and Bile Duct and (3) Controls One Hour after Intravenous Administration. Values are Expressed in Percent of Administered Dose and are Corrected for Deviation of Recovery from 100 Percent. Each Group Consisted of Five Rats.

	Ephedrine		Operated		Control	
	180 μc		122 μc		190 μc	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Blood	1.03	0.07	2.43	0.22	0.98	0.08
Liver	-	-	1.41	0.25	0.49	0.06
Kidney	-	-	0.78	0.60	0.27	0.18
Stomach	0.06	0.05	0.23	0.23	0.05	0.07
Stom. Cont.	0.11	-	0.10	-	0.08	-
Small Intest.	0.19	0.05	0.70	0.22	0.24	0.07
Sm. Int. Cont.	0.58	-	0.50	-	1.26	-
Large Intest.	0.07	0.05	0.23	0.21	0.06	0.07
Lg. Int. Cont.	0.05	-	0.07	-	0.09	-
Cecum and Cont.	0.23	-	0.32	-	0.32	-
Bone	54.9	3.34	54.0	3.84	58.9	3.74
Muscle	5.11	0.06	9.92	0.14	6.79	0.08
Skin	2.65	0.08	4.02	0.16	2.24	0.08
Balance	15.4	-	19.5	-	14.7	-
Urine	19.6	-	6.11	-	11.9	-
Feces	0.08	-	0.004	-	1.28	-

There was only one statistically significant difference ($P \leq 0.05$) between the distribution of radiofluorine in the gastrointestinal contents of the control animals and the two groups of treated animals in which the distributions were essentially the same. This difference was in the contents of the small intestines, which compartment, in the treated animals contained only about one-half as much radiofluorine as in the controls. The range of the experimental values for the contents of the small intestine were as follows: ephedrine group 0.43 to 0.69, operated group 0.39 to 0.54 and control 0.89 to 1.29.

The concentration of radiofluorine in the empty stomach and intestines of the treated groups closely parelleled the blood level as did the radiofluroine concentrations of the skin and muscle in the operated animals. The same paralleling of the blood level is seen in the stomach and intestines and the soft tissues of the controls. In previous work it was found that the same is also true of the pancreas.

Although radiofluorine is concentrated by the salivary glands shortly after its intravenous administration and is subsequently found in the saliva, confirming the findings of Volker et al (1941) and of Wills (1940), the observation that the isolated stomachs of the operated animals contained the same amount of radiofluorine as those animals in which the saliva had free access to the stomach seems to indicate that the saliva plays only a minor role in the appearance of radiofluorine in the alimentary tract.

Since the amount of radiofluorine in the small intestine contents in the animals with ligated bile ducts was the same range it was in the ephedrine treated animals (there is no evidence that the ephedrine inhibits the secretion of bile, the flow of which is continuous in the rat) it would appear that the bile does not contribute to the presence of radiofluorine in the intestines to any great extent.

According to Peters (1935) the secretions of the gastrointestinal tract, despite their chemical diversity and with the possible exception of saliva, are isotonic with the blood. The general iso-tonicity of the gastrointestinal secretions indicates that the digestive glands are unable to perform osmotic work; this does not indicate that these secretions are produced simply by diffusion, however, he also states that certain of the electrolytes, namely sodium and chloride, seem to play an almost indifferent role in these secretions, serving mainly to establish osmotic equilibrium when they are needed and that their mode of passage to and from the gut more nearly suggests diffusion than do any other ions. Even in the stomach where chloride has a specialized function, it is more responsive than any other anion to osmotic changes; while sodium among the bases bears the brunt of all fluctuations of acidity. It therefore seems possible that if radiofluorine can permeate cell membranes, it can pass into the gastrointestinal contents in these secretions without being concentrated by the secreting glands, provided it is also treated by the body as a simple ion.

It has been shown by Everett that the ratio of red blood cell chloride to plasma chloride is 0.51 that in humans this ratio is about the same for bromides. Rall et al (1950), found that the ratio for iodide is 0.67 as determined in in vitro studies on human blood. In an early phase of this work it was found that the red cell to plasma ratio for radiofluorine in the rat blood was about 0.50. This figure is an average of the results obtained from 35 rats sacrificed at time intervals of from 1 minute to 1 hour after intravenous injection of radio-

fluorine. From this observation, it would seem that the behavior of radiofluorine is analogous to the other halides in so far as its partition between red cell and plasma is concerned. A confirmation of this is found in the observation that the concentrations of intravenously administered radiofluorine in the soft tissues was found to parallel the blood level. Therefore, it seems reasonable to conclude that for the most part cells are readily permeable to radiofluorine.

It is most noteworthy that the percent of administered radiofluorine found in the contents of the four major segments of the gastrointestinal tract is the same in both treated groups and that the distributional patterns appear similar. These similarities between the two groups suggest that there is some mechanism which is active in both, despite the radically different treatment they received. One of the major actions of ephedrine is to constrict the peripheral and splanchic blood vessels reducing the blood supply to the skin and to the abdominal region. This same shunting of the blood from the skin and viscera to the more vital organs is seen in animals in primary shock, which invariably follows a major injury or surgical operation. This blood-vascular pattern arises from nervous and vascular responses to pain andpsychic factors according to Best and Taylor (1947). Further substantiating the hypothesis that the sympathetic nervous system had been called into play in the operated animals was the observation that the stomach fluids were alkaline an effect which is observed in experimental sympathetic stimulation. The lower body temperature and ruffling of the fur also suggest intervention of the sympathetic system.

It was found in experiments presented in the previous report that the amounts of radiofluorine in the contents of the various segments of the gastrointestinal tract were the same 1 hour after the injection as they were 15 minutes after the injection. From this it may be assumed that any radiofluorine reaching the contents of the gut does so in the first few minutes after injection when the blood level is high.

The following conclusions may then be drawn:

1. Radiofluorine appears to be secreted in the saliva, but the saliva does not seem to contribute greatly to the presence of radiofluorine in the gastrointestinal contents.

2. The bile does not constitute a major route of entry of radiofluorine into the small intestine.

3. In animals displaying the effects of sympathetic stimulation, whether induced indirectly by drugs or directly by primary shock, the only disturbance in the radiofluorine distribution in the gastointestinal contents is the lowering of the amount found in the small intestines, the most vascular and actively secreting portion of the digestive tract, according to Best and Taylor (1947). Radiofluorine has been shown to be as capable of permeating cell membranes as are the other halides.

4. Visscher et al (1944) showed that there is a forced flow of water and ions across the intestinal membrane simultaneously in both directions to and from the blood, the direction and magnitude of the flow depending on the concentrations of the solutes and rates of flow of the two streams. From Visscher's (1944) work

and from the observations in these experiments, it can be tentatively concluded that radiofluorine enters the gastrointestinal tract by passing from the blood by some process resembling diffusion into the gastric and intestinal secretions, and that the extent to which it enters these compartments depends upon the level of radiofluorine in the blood and the rate of flow of the blood in the secreting organs.

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Inhalation Effects

K. G. Scott and J. G. Hamilton

In June of 1952 a monkey colony of 10 machus rhesus monkeys was established in the animal house. The purpose of acquiring these monkeys was to subject some of them to alpha-particle aerosols and to follow both the short term and long term fate of these aerosols. An interval of three months was felt desirable before doing anything with these animals so that they could be tuberculin tested and treated for gastrointestinal parasites which seemed to be indigenous. Soon afterward, attempts were made to prepare aerosols in the 1 to 5 micron range of inactive substances such as quartz. At the same time various sources were contacted such as Los Alamos and Oak Ridge and after considerable correspondence covering several months the results were rather discouraging. Likewise our attempts to prepare aerosols were unsatisfactory from the practical point of view when an alpha-active source was to be used. A ball mill as well as the use of a mechanically driven mortar and pestle system made it evident that some other procedure must be devised. Accordingly a smoke generator is under design and construction which will employ 1/8th inch carbon rods having a small hole drilled through the center of each rod and these in turn are to be packed with milligram amounts of plutonium metal and graphite powder. These are nearly completed and it is anticipated they will be put to trial in the near future.

The procedure is to anesthetize the animal, insert a trachea cannula which in turn is attached to the smoke generator. The electrodes are connected to 110 volts D.C. The moment the arc is struck the plutonium oxide formed will be inhaled and of the order of 5 percent of the plutonium oxide smoke should be retained by the lungs. There has been considerable experience accumulated in this laboratory with this type of generator. A reference has been made to it by Scott, et al[1] for its use in rats.

Radio-Potassium Studies

K. G. Scott and J. G. Hamilton

A series of studies have been initiated in an attempt to compare the effect of total body beta irradiation to that of total body alpha particle irradiation in mammals. There has been the ever present question as to the relative biological effectiveness of these two types of ionizing radiation. The difficulty in securing an approximation of total body radiation of beta-particles is not too difficult. Radioisotopes of electrolytes such as sodium and potassium are examples of radioelements that may be employed for this purpose. In the case of both electrolytes, even though one is predominantly intracellular and the other extracellular, the range of beta-particles is such as to overcome this problem. Even so there is not a complete uniformity of distribution and thus such data that may be obtained must not be considered to be on a true quantitative basis.

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1 Scott, K. G., et al, Archives of Pathology, 48, 31-64 (1949).

The situation surrounding total body alpha-particle irradiation is far more difficult to acheive. In the past radon has been employed but it would appear that this radioelement will not give anything approaching uniform distribution within the body for several reasons. In the first place, radon tends to become selectively localized in fat which might seriously distort the data obtained. Secondly, some of the radioactive descendents have appreciable half-lives; as for example, radium B with a half-life of 26.8 minutes, which is the radioactive isotope of lead, and radium C which is a radioactive isotope of bismuth with a half-life of 19.7 minutes. Both of these radioisotopes are bone seekers and radium C emits alpha-particles. With but one exception, all other radioisotopes which are alpha-particle emitters show a striking degree of non-uniformity of distribution within the body, such as polonium, radium and the actinide group of elements. Unfortunately, there are no suitable radioisotopes of Francium which is the last of alkali metals and whose metabolism may be predicted to be similar to that of potassium. The one exception is astatine, which is the last of the halogen group of elements. It has already been demonstrated and presented in previous reports that this radioelement shows in most tissues and organs a relative uniform distribution. While by no means ideal from the point of view of obtaining information concerning relative biological effectiveness, it at least approaches uniformity of distribution to a sufficient degree to appear to make it worth while to use this radioelement for a comparison of the relative biological effectiveness of beta-particles and alpha-particles.

Accordingly, a series of experiments have been set up and this report is a presentation of preliminary results which have been obtained during the past three months.

These experiments have been organized in the following manner: The experimental animals employed were female Sprague-Dawley rats weighing from 150 to 185 grams. The radio-potassium used was a mixture of carrier free K^{42} and K^{43} given by intraperitoneal injection. Radio-sodium would have been possibly more satisfactory as a source of internal beta radiation due to its more uniform distribution within the body. However, safety consideration for personnel made it inadvisable to employ radio-sodium rather than potassium. Moreover, the very energetic beta-particles with a maximum energy of 3.58 Mev from K^{42} tended to a considerable degree to reduce the inhomognetity of the irradiation. The average energy in approximately 1.2 Mev and given these beta-particles a range of nearly one centimeter in soft tissue.

Four groups of animals were used; there were ten animals in the first, second and fourth groups and eight animals in the third group. In an attempt to observe possible radiation effects, two lines of approach were taken. The first was to determine what alterations if any might take place in the erythrocyte and leucocyte counts. The second observation was the radio-potassium distribution in the animals which received large amounts of radio-potassium as compared to the animals which were given tracer amounts. Consequently leucocyte counts were taken from five animals of the first three groups one day before they received the tracer dose of radio-potassium and these counts were taken again shortly before the animals were sacrificed. Erythrocyte counts were taken in a similar manner for the first two groups. The same procedure was done for the first two groups of five animals and three animals in the third group, all of which received a large dose of radio-potassium. Erythrocyte and leucocyte counts were secured from the fourth group of animals which received no radio-potassium and these counts were taken six days apart. The first group

of five rats which were given a tracer dose received a sufficient amount of radio-potassium so that when they were sacrificed five days later there appeared to be a depression of the leucocyte count. Accordingly, the second and third groups of tracer animals received a much smaller tracer dose of radio-potassium and were sacrificed two days following the administration of this radioelement. This time interval was considered to be sufficient to permit equilibrium to become established with the stable potassium in the body. The following tissues were removed at the time of sacrifice and assayed wet by the use of a scintillation counter: heart, spleen, erythrocytes, plasma, liver, kidney, stomach, stomach contents, small intestine, small intestine contents, skeleton, balance, skin, lymph nodes, urine and feces. The urine and feces were collected and assayed in a similar manner for their content of radio-potassium. These procedures were done for both the tracer and treated animals in all three groups.

The total erythrocyte, leucocyte, granulocyte, and lymphocyte counts for the four groups are presented in the first four Tables. These data include the counts for the individual animals before and after they received radio-potassium. The average values for the distribution of radio-potassium in the tracer and treated animals are given in Tables V, VI, and VII. These procedures were done for both the tracers and treated animals in all three groups.

The calculated amount of radiation received in the first group, assuming uniform distribution, was 702 rep with the treated animals and 62 rep for the tracer animals. The values for the second group were 690 and 24 rep respectively. and for the third experiment the values were 1200 and 7 rep respectively.

Results

The depression of both total leucocyte count as well as lymphocyte and granulocyte counts may be seen to be quite marked in the treated animals of the first group. A comparative decrease may be noted in the second and third group of treated animals. There was a definite depression of the leucocyte counts in the first group of tracer animals and this presumably arose from the fact that they received quite an appreciable amount of radiation. The second and third groups of tracer animals received a minimal amount of ionizing radiation and in the third group the apparent fall in white count cannot readily be explained. It may be noted that the count taken before radio-potassium was administered in this group was high. No convincing explanation for this is available. Another puzzling value is that for the granulocytes in this group. The leucocyte count is rodents tends to be irregular and the somewhat unusual values noted in the third group may be related to this phenomenon. The observation that the third group, which received a calculated dose of 1200 rep showed a no more marked depression of leucocytes than the first group is of interest. Also there may be noted the differences of the treated groups in Tables I and II.

The tracer studies show relatively little variation in the distribution of radio-potassium in either the tracer or treated animals in all three groups.

Current studies are now in progress using Na^{22} as the tracer rather than $K^{42,43}$.

TABLE I

Erythrocyte and Leukocyte Counts of 5 Rats Each of Which Received 12.7 μc of K^{42} and K^{43} Per Gram of Body Weight by Intraperitoneal Injection and Were Sacrificed 5 Days Later. Blood Counts Were Taken One Day Prior to Administration of Radio-potassium and at the Time of Sacrifice. The Standard Error of the Mean and P Values are Included. Similar Values are Included for the 5 Tracer Animals Each of Which Received 1.13 μc Per Gram of Body Weight.

Treated Group

Rat	RBC millions per mm^3		WBC thousands per mm^3		x 1000 Lymphocytes		x 1000 Granulocytes	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
1	7.71	5.44	13.7	0.85	11.99	0.44	1.64	0.41
2	6.60	5.90	11.45	1.75	8.42	1.07	2.81	0.68
3	7.11	5.05	20.85	1.10	17.72	0.58	2.40	0.51
4	6.76	4.30	24.50	0.80	22.30	0.54	2.20	0.26
5	7.46	7.51	14.05	0.95	12.22	0.68	1.83	0.27
Ave.	7.13 ±0.19	5.64 ±0.54	16.91 ±2.46	1.09 ±0.17	14.53 ±2.44	0.65 ±0.12	2.18 ±0.20	0.43 ±0.26
P	P = <0.01		P = <0.01		P = <0.01		P = <0.01	

Tracer Group

1	6.43	5.73	9.00	3.30	7.74	3.02	0.99	0.25
2	7.61	7.27	11.00	2.75	9.62	1.91	1.38	0.83

TABLE I
(continued)

Rat	RBC millions per mm^3		WBC thousands per mm^3		x 1000 Lymphocytes		x 1000 Granulocytes	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
3	6.72	5.20	12.80	4.90	10.24	3.90	2.43	0.91
4	6.51	5.53	14.75	2.65	12.02	2.33	2.29	0.29
5	6.60	5.45	12.05	3.25	11.21	2.39	0.84	0.86
Ave.	6.77 ±0.24	5.84 ±0.35	11.92 ±0.95	3.37 ±0.40	10.16 ±0.75	2.71 ±0.35	1.59 ±0.32	0.63 ±0.24
P	P = <0.01		P = <0.01		P = - 0.01		P = <0.01	

TABLE II

Erythrocyte and Leukocyte Counts of 5 Rats Each of Which Received 12.5 μc of K^{42} and K^{43} Per Gram of Body Weight by Intraperitoneal Injection and Were Sacrificed 5 Days Later. Blood Counts Were Taken One Day Prior to Administration of Radio-potassium and at the Time of Sacrifice. The Standard Error of the Mean and P Values are Included. Similar Values are Included for the 5 Tracer Animals Each of Which Received 0.36 μc Per Gram of Body Weight and Were Sacrificed 2 Days After Injection.

Rat	RBC millions per mm^3		WBC thousands per mm^3		x 1000 Lymphocytes		x 1000 Granulocytes	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Treated Group								
1	8.64	5.66	17.75	2.05	15.80	1.77	1.78	0.28
2	6.05	6.98	16.00	1.70	14.00	0.99	2.00	0.70
3	5.29	5.53	33.75	1.75	25.99	1.01	7.59	0.74
4	6.74	6.50	10.50	3.70	8.93	0.76	1.42	2.90
5	8.11	7.55	15.55	2.20	13.45	1.21	2.02	0.98
Ave.	6.97 ±0.61	6.44 ±0.40	18.71 ±3.94	2.28 ±0.37	15.63 ±2.82	1.15 ±0.17	2.96 ±1.16	1.12 ±0.46
P	P = 0.19		P = <0.01		P = <0.01		P = 0.02	
Tracer Group								
1	5.83	6.34	11.90	14.15	10.12	12.45	1.67	1.56
2	6.36	5.47	16.30	14.75	14.75	13.42	1.30	1.18
3	6.84	5.53	13.00	9.90	11.11	9.11	1.82	0.79

TABLE II
(continued)

Rat	RBC millions per mm^3		WBC thousands per mm^3		x 1000 Lymphocytes		x 1000 Granulocytes	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
4	5.97	5.06	12.35	7.55	11.36	5.89	0.99	1.59
5	6.62	5.86	8.55	7.95	7.35	6.92	1.20	1.03
Ave.	6.32 ±0.22	5.65 ±0.23	12.42 ±1.24	10.86 ±1.52	10.94 ±1.18	9.56 ±1.47	1.40 ±0.14	1.23 ±0.15
P	$P = < 0.01$		$P = 0.16$		$P = 0.19$		$P = 0.16$	

TABLE III

Erythrocyte and Leukocyte Counts of 3 Rats Each of Which Received 21.9 μc of K^{42} and K^{43} Per Gram of Body Weight by Intraperitoneal Injection and Were Sacrificed 5 Days Later. Blood Counts Were Taken One Day Prior to Administration of Radio-potassium and at the Time of Sacrifice. The Standard Error of the Mean and P Values are Included. Similar Values are Included for the 5 Tracer Animals Each of Which Received 0.1 μc Per Gram of Body Weight.

Rat	RBC millions per mm^3		WBC thousands per mm^3		x 1000 Lymphocytes		x 1000 Granulocytes	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Treated Group								
1	not counted		17.85	1.85	15.71	1.42	1.87	0.36
2	not counted		19.20	1.35	16.03	0.91	3.17	0.42
3	not counted		17.35	0.85	14.49	0.63	2.86	0.21
Ave.	not counted		18.13	1.35	15.41	0.99	2.63	0.33
			±0.60	±0.29	±0.47	±0.22	±0.40	±0.06
P			P = $<$0.01		P = $<$0.01		P = $<$0.01	
Tracer Group								
1	not counted		16.60	7.15	13.45	6.54	3.07	0.36
2	not counted		23.15	12.10	17.59	10.10	5.44	1.69
3	not counted		15.65	10.85	13.30	9.87	2.19	0.76
4	not counted		26.35	5.15	19.50	4.74	6.59	0.39
5	not counted		23.30	8.00	21.79	7.24	1.51	0.72
Ave.	not counted		21.01	8.65	17.13	7.70	3.76	0.78
			±2.08	±1.25	±1.66	±1.03	±0.97	±0.24
P			P = $<$0.01		P = $<$0.01		P = $<$0.01	

TABLE IV

Erythrocyte and Leukocyte Counts of 5 Rats Which Received no Radio-Potassium. Two Series of Counts Were Taken 6 Days Apart.

Rat	RBC millions per mm^3		WBC thousands per mm^3		x 1000 Lymphocytes		x 1000 Granulocytes	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
1	9.65	7.35	11.25	12.45	10.35	10.15	0.79	2.24
2	8.27	7.48	18.95	18.05	14.97	17.51	3.79	0.54
3	8.83	7.77	7.65	13.35	6.66	9.95	0.96	3.34
4	7.94	9.14	11.65	16.15	10.89	14.05	0.76	1.94
5	7.95	6.95	8.70	12.35	8.18	10.62	0.48	1.73
Ave.	8.53 ±0.31	7.74 ±0.37	11.64 ±1.98	14.47 ±1.13	10.21 ±1.41	12.46 ±1.46	1.36 ±0.58	1.96 ±0.45
Range	7.94 to 9.65	6.95 to 9.14	7.65 to 11.65	12.35 to 18.05	6.66 to 14.97	9.95 to 17.51	0.48 to 3.79	0.54 to 3.34

TABLE V

The Deposition of $K^{42,43}$ in the Rat 5 Days After Intraperitoneal Injection of Tracer and Treated Doses. Values are Corrected for Recovery and Excreta and are Expressed in Percent of Administered Dose. Each Tracer and Treated Rat Received 0.2 and 2.0 Millicuries of $K^{42,43}$ Respectively and the Radiation Doses Were 702 and 62 r.e.p.

	Treated		Tracer	
Tissue	% per organ	% per gram	% per organ	% per gram
Heart	0.46	0.64	<0.8	-
Spleen	0.21	0.62	<0.8	-
RBC	5.36	0.99	1.68	0.67
Plasma	0.23	0.05	<0.8	-
Liver	5.04	0.69	4.36	0.53
Kidney	0.78	0.56	<0.8	-
Stomach	0.53	0.39	<0.8	-
Stom. Cont.	1.73	-	<0.8	-
Sm. Int.	3.45	0.62	2.51	0.42
Sm. Int. Cont.	1.96	-	1.20	-
Balance	3.34	-	26.0	-
Skeleton	6.26	0.39	<0.8	-
Muscle	63.3	0.92	46.9	0.59
Skin	7.36	0.35	8.66	0.34
Gonads	<0.22	-	<0.8	-
Lymph Gl.	-	<0.22	-	<0.8

TABLE VI

The Deposition of $K^{42,43}$ in the Rat After Intraperitoneal Injection of Tracer and Therapeutic Doses. Values are Corrected for Recovery and Excreta and are Expressed in Percent of Administered Dose. Each Tracer and Treated Rat Received 0.07 and 2.3 Millicuries of $K^{42,43}$ Respectively, and the Radiation Doses Were 690 and 24 Rep.

	Treated		Tracer	
Tissue	% per organ	% per gram	% per organ	% per gram
Heart	0.43	0.54	0.38	0.49
Spleen	0.45	0.75	0.25	0.59
RBC	4.46	0.64	4.51	0.70
Plasma	0.22	0.03	0.25	0.05
Liver	4.57	0.52	5.30	0.61
Kidney	0.70	0.43	0.70	0.45
Stomach	0.49	0.45	0.47	0.41
Stom. Cont.	0.46	-	0.38	-
Sm. Int.	3.09	0.45	3.25	0.52
Sm. Int. Cont.	1.00	-	1.15	-
Skeleton	5.69	0.31	4.94	0.29
Muscle	65.2	0.73	66.6	0.81
Balance	5.12	-	2.93	-
Skin	8.06	0.27	8.82	0.32
Gonads	0.07	0.42	0.09	0.47
Lymph Gland	-	0.52	-	0.47

TABLE VII

The Deposition of $K^{42,43}$ in the Rat After Intraperitoneal Injection of Tracer and Treatment Doses. Values are Corrected for Recovery and Excreta and are Expressed in Percent of Administered Dose. Each Tracer and Treatment Rat Received 0.017 and 3.4 Millicuries of $K^{42,43}$ Respectively, and the Radiation Doses were 1200 and 7 Rep.

	Treated		Tracer	
Tissue	% per organ	% per gram	% per organ	% per gram
Heart	0.57	0.72	0.54	0.77
Spleen	0.62	0.98	0.21	0.98
RBC	5.61	0.92	4.75	0.88
Plasma	0.17	0.028	0.20	0.036
Liver	6.12	0.75	4.20	0.69
Kidney	1.08	0.67	1.06	0.72
Stomach	0.74	0.69	0.70	0.77
Stom. Cont.	0.13	-	0.31	-
Sm. Int.	3.60	0.78	5.37	0.77
Sm. Int. Cont.	1.11	-	1.27	-
Skeleton	8.35	0.48	8.04	0.51
Muscle	58.4	0.75	63.1	0.91
Balance	4.65	-	-	-
Skin	8.79	0.34	9.14	0.41
Gonads	0.07	0.48	0.07	0.47
Lymph Gland	-	0.71	-	0.75

Curium Tracer Studies

Kenneth G. Scott, Herman R. Haymond,
Marilyn Hemenway and Helen Johnson

The curium experiments which were briefly alluded to in the last Quarterly Report were organized because of the fact that increasing numbers of individuals are being exposed to relatively large amounts of the trivalent actinide elements. The experiments were organized in the following manner: each of 10 rats received 1.17 microcuries of Cm^{242} by intramuscular injection. This solution was isotonic saline and had a pH of approximately 3. During the ensuing 15 days, each of 5 rats received 100 mgm of the calcium salt of Versene by intraperitoneal injection and in a volume of 2 cc. The other 5 animals were given equivalent volumes of saline without the addition of Versene. At the end of 15 days the rats were sacrificed and the following tissues and organs as well as urine and feces were obtained: spleen, blood, liver, kidney, gastrointestinal tract, bone, muscle, skeleton, balance, skin and left leg. The second phase of this experiment was done in the following manner: each of 12 rats received by intravenous administration 1.17 microcuries of Cm^{242}. Twenty-four days later and extended for 24 days each of 6 rats received daily 100 mgm of Versene by intraperitoneal injection in a volume of 2 cc. The remaining 6 rats were given the same volume of isotonic saline by the same route of administration. All of the animals were sacrificed 50 days after the curium had been administered. The same tissues and organs were obtained as in the first experiment as well as the collection of urine and feces.

The samples were counted using the lanthanum fluoride precipitation technique mentioned earlier in this report. Attempts were made to modify the TTA process which was found to be unsuccessful.

Another type of solvent extraction technique was attempted. The purpose of solvent extraction methods is to avoid the presence of excessive amounts of calcium in the ashed specimens, and this has been discussed under the portion of the report devoted to the use of Versene in plutonium treated animals. One gram of rat bone ash was dissolved in 11 cc of concentrated nitric acid. To this was added 88 counts per second of Cm^{242} in 0.5 cc of 2 N nitric acid. This solution was extracted with 22 cc of butyl phosphate by shaking for 20 minutes. The acid phase was permitted to separate out and was set aside. The organic phase was extracted with 45 cc of distilled water being shaken for 20 minutes. Two, 4 cc aliquots were taken from the aqueous phase and dried in porcelain dishes. This entire procedure was done for 4 separate samples and recovery values of 31.6, 33.6, 30.6 and 37.8 percent were observed. The ash residue in the final aqueous phase from 1 gram of ash was calculated to be 80.2 mg. This technique shows some promise and further attempts will be made to ascertain, if it can be of practical value for the assay of trivalent actinide elements.

The tissues, organs and excreta from these two experiments are now being counted and this information should be available for the next Quarterly Report.

RADIOAUTOGRAPHY

C. W. Asling and Gretchen Thilo

There are now available additional histologic specimens from the Astatine treated monkey, an untreated control and presumably a normal animal. The Astatine treated monkey was sacrificed two years after there had been administered 100 microcuires of Astatine. The normal appearance of the thyroid and parathyroid gland from the normal animal is shown in Figure 4. Although the follicles vary somewhat in size they are all large compared to the treated and control animals. At the higher magnification in Figure 5, the epithelium is shown to be of low cuboidal dimensions.

The thyroid gland from the control monkey had a structure quite different from the normal animal. Figure 6, the appearance of this section is of interest because of some similarity of groups of cells found in the experimental Astatine treated monkey. The largest follicles from the control animal were only equal to the smallest of those shown in Figure 4. The greater part of the tissue was densely cellular and contained but little colloid. The organization of this tissue was unusual. Figure 7, the connective tissue structure may be clearly seen. At a higher magnification in Figure 8 there may be seen that the masses of cells were found to vary from small follicles down to clusters of from 2 to 3 cells and some of these contained a droplet of colloid. In height, the cells were approximately 15 microns. There were indications that the tissue was proliferating. Some of the smaller groups of cells appeared to be budding from follicles. Although mitotic figures were rare, there was considerable variability in nuclear staining and many of the nuclei were hyperchromatic. Nuclear structure apparently varied from vesicular to pyknotic in character.

A NTB radioautograph is shown in Figure 9 which demonstrates the uptake of radio-iodine, which was given 24 hours before sacrifice, in the larger follicles. It was possible, by increasing the exposure time, to demonstrate that the smaller groups of cells also accumulated radio-iodine.

It is of interest that the control animal showed no evidence of hyperthyroidism except for the fact that he was possibly somewhat more active and energetic than the average monkey. He was sacrificed because of the fact that the animal developed a respiratory infection which was thought might be simian tuberculosis. It was felt warranted that the animal should not be maintained in view of the hazard to those who took care of it. The all over cytologic picture of the thyroid gland from this animal resembled the findings that occur in prolonged thiouricil treatment, in which the thyroid hormone secretion is believed to be blocked and the pituitary thyrotropic hormone production is increased which leads to hyperplasia of the follicular cells. The cytology of the pituitary of this monkey and the Astatine treated monkey will be presented in a subsequent report.

There were apparently a few areas that contained cells resembling thyroid epithelium in the thin layers adjacent to the parathyroid gland from the Astatine treated animal, Figure 10. There were a few scattered nodules, Figure 11, which contained small follicles and the columnar thyroid epithelium. Another small nodule, shown in Figure 12, indicates the variation in size of the follicular cells and the presence of colloid droplets may be observed. It is

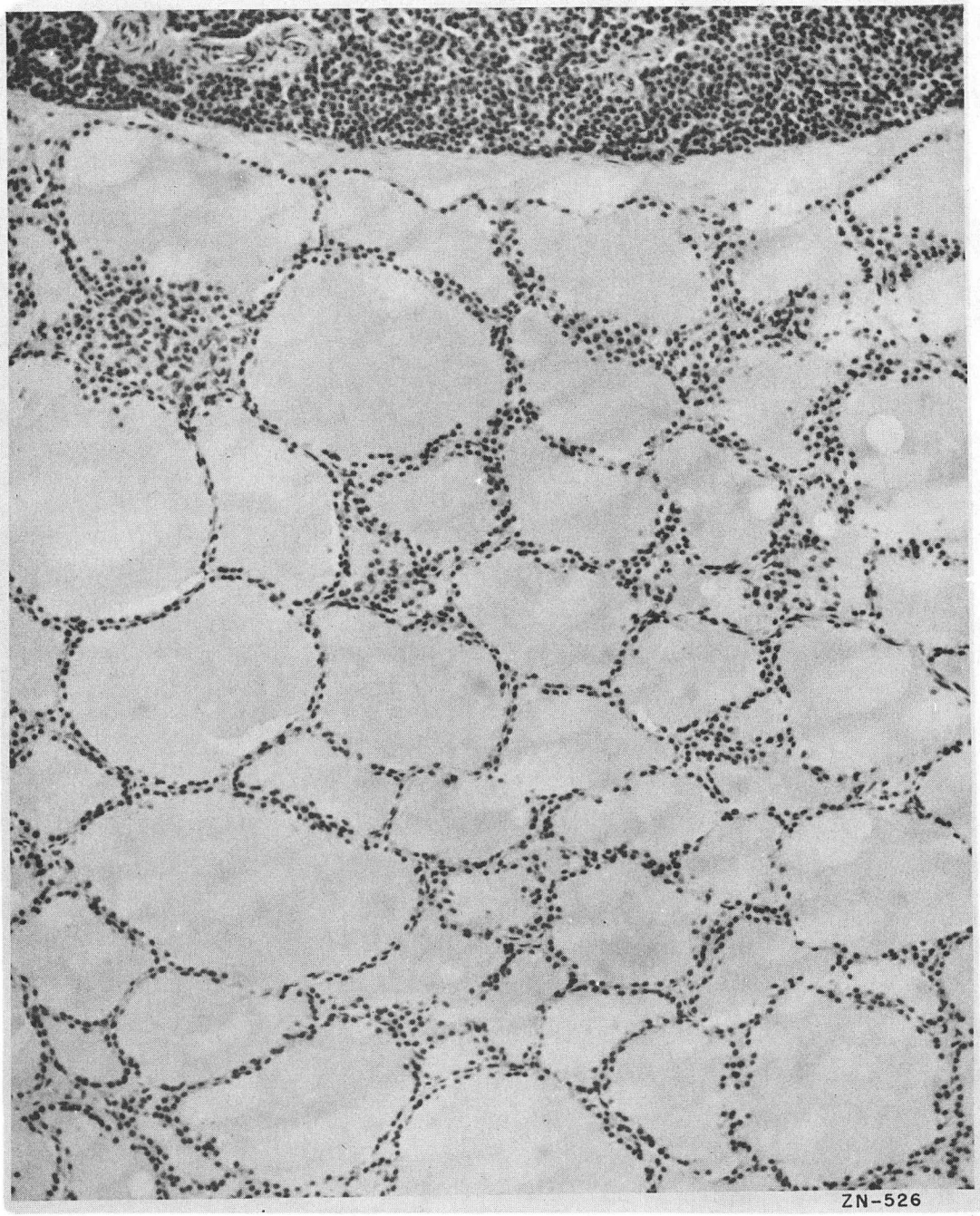

Fig. 4

Normal thyroid and parathyroid of the monkey.
Hematoxylin and eosin stain, x 120.

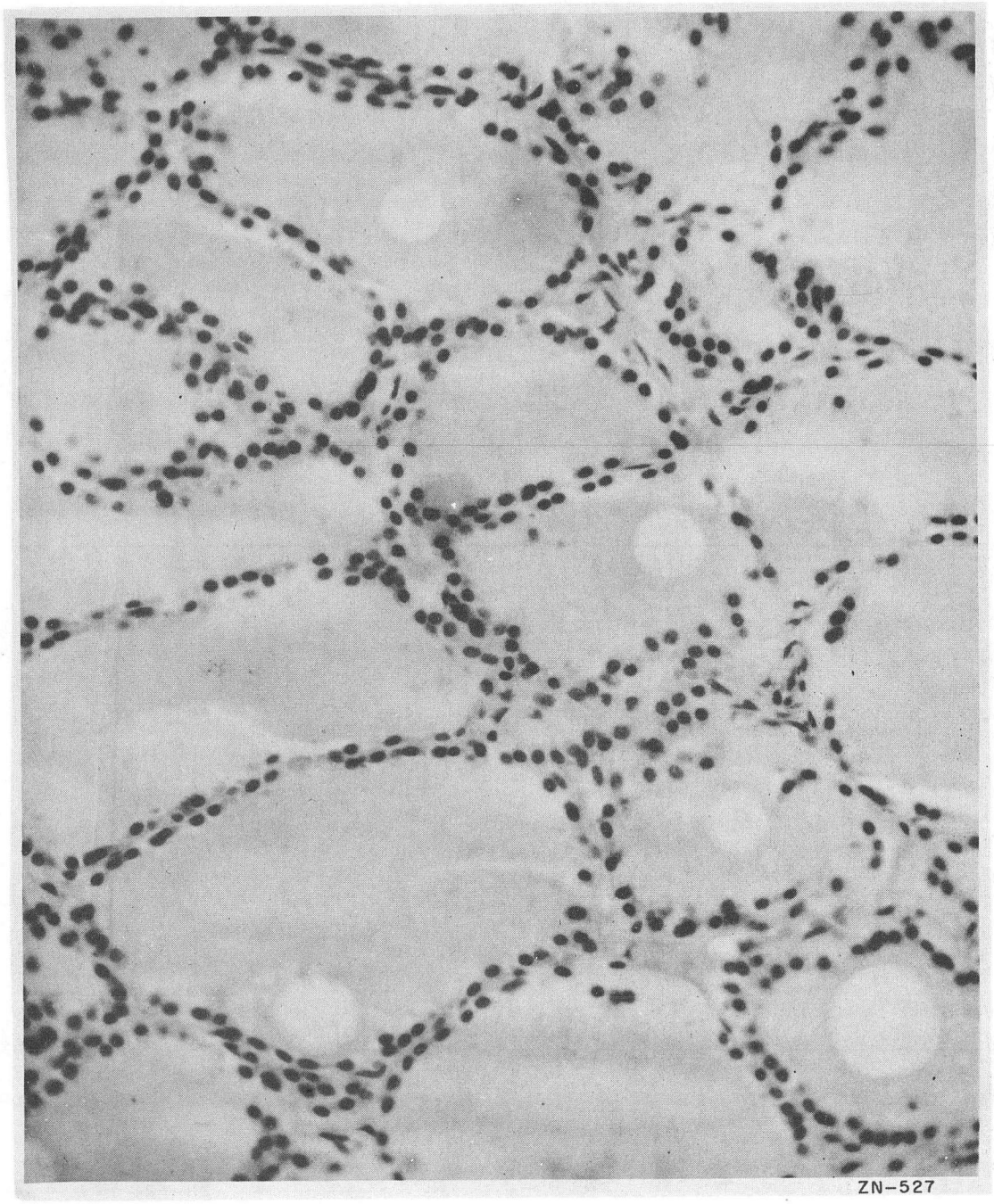

Fig. 5

Higher magnification from the same section showing the low cuboidal character of the epithelial cells. x 300.

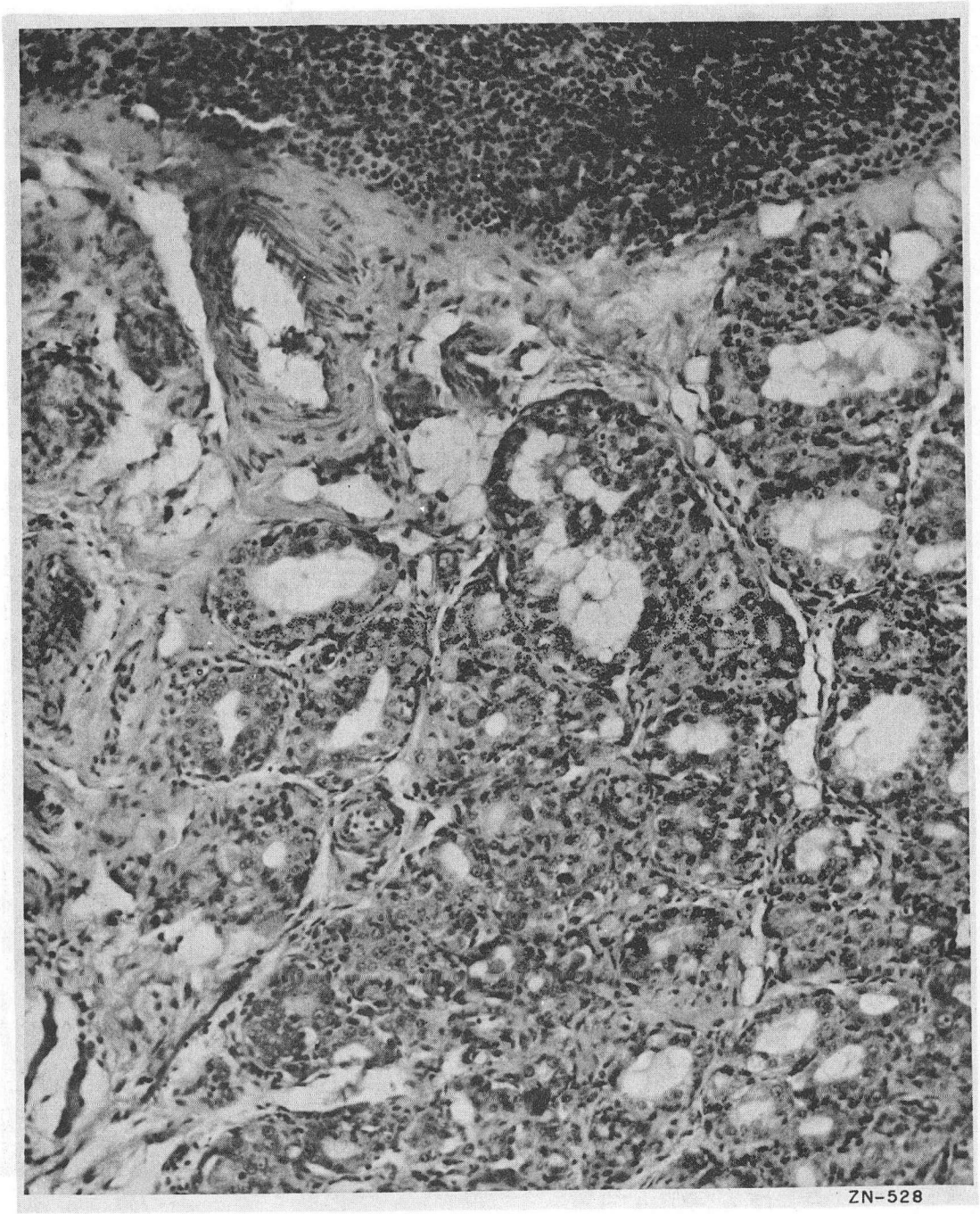

Fig. 6

Thyroid gland and parathyroid gland from the control monkey. Attention should be drawn to the lack of colloid in the over-all appearance of the cellular activity. Hematoxylin and eosin stain. x 120.

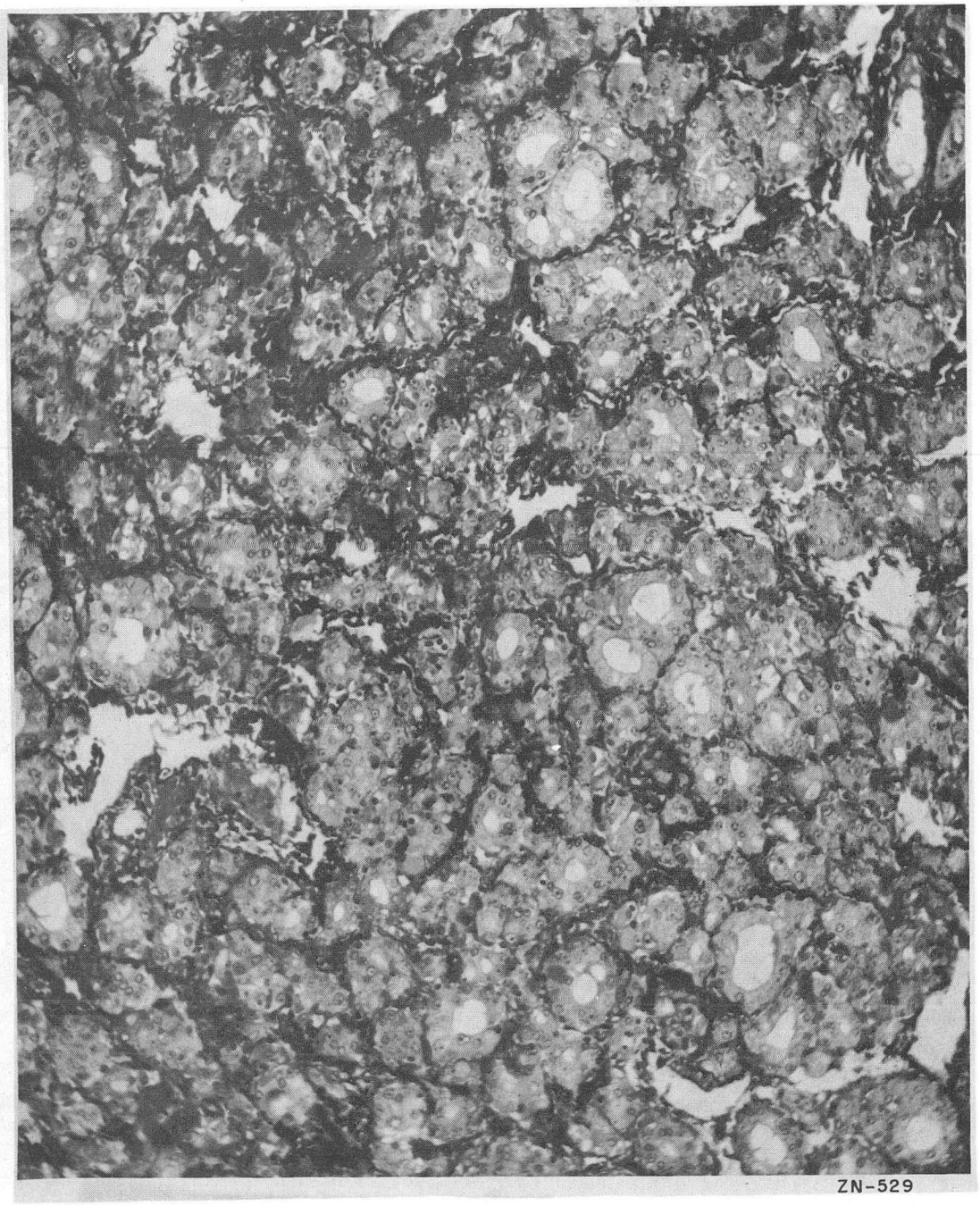

Fig. 7

The connective tissue is shown in this secretion from the same animal and again the hyperplastic nature of the thyroid tissue may be seen. Mallory triple stain. x 120

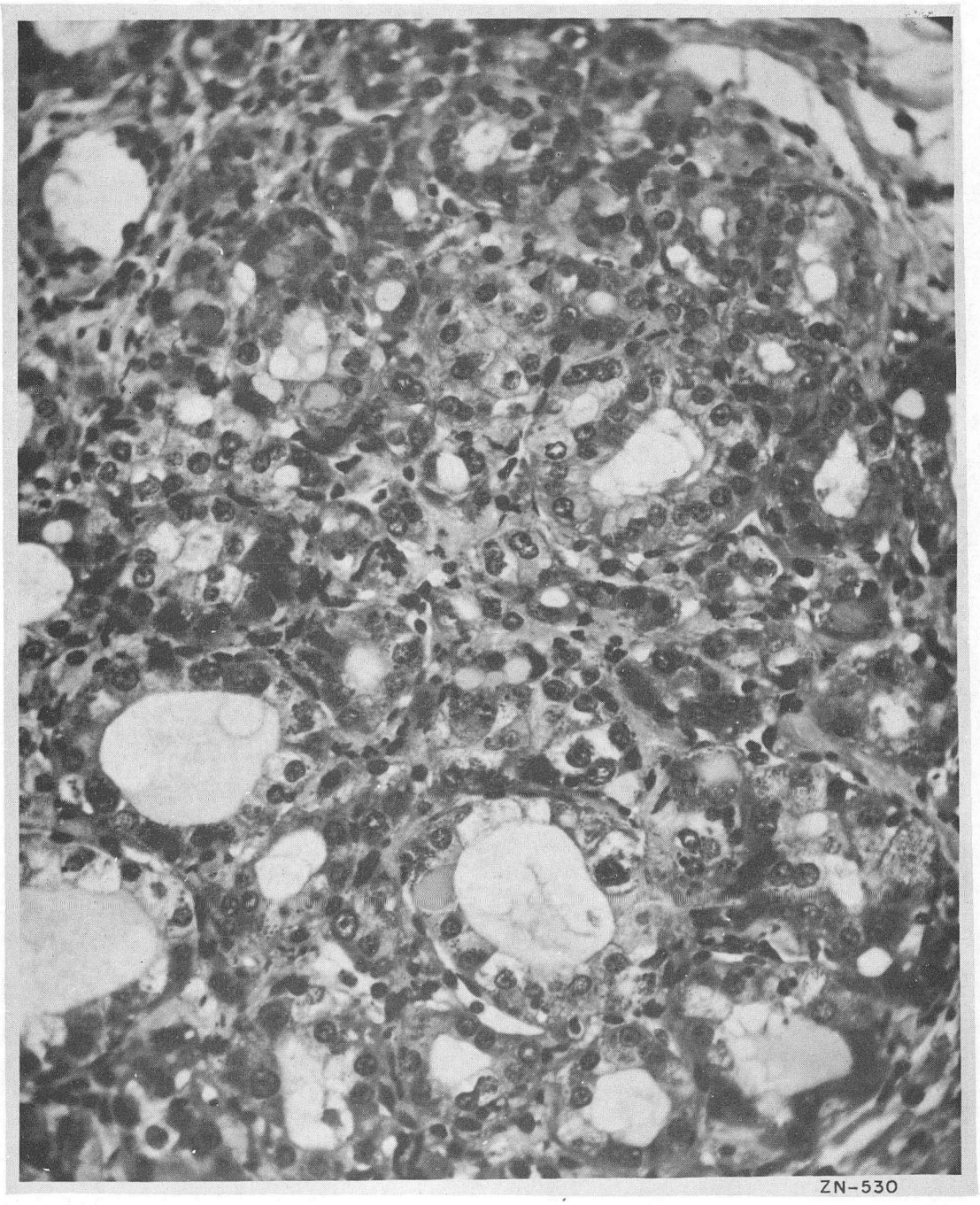

Fig. 8

A higher magnification of the same tissue which demonstrates in greater detail the character of the cellular structure and the presence of the droplets of colloid may be seen in the dense mass of cells. Iron, hematoxylin stain, aniline blue. x 300.

Fig. 9

NTB radio-iodine radioautograph from the control animal which demonstrates the presence of radio-iodine in the larger follicles. Hematoxylin and eosin stain, x 120.

of interest to note that the connective tissue separating the individual follicles as is shown in Figure 12 was either sparse or absent. A higher magnification of an area comparable to Figure 11 shows more clearly the cellular structure, Figure 13. Here may be seen the variations in cell size and in better detail the presence of droplets of colloid. In this respect there is a resemblance to the thyroid gland from the control animal. These observations are attributed to an increase of the level of the thyrotropic hormone which produced excessive stimulation of the follicular cells.

The capacity of some of these regions to accumulate radio-iodine is demonstrated in Figure 14. Figure 15 indicates in greater detail the thin layers of tissue adjacent to the parathyroid gland containing clusters of thyroid epithelial cells. These were rarely organized into follicles but frequently contained droplets of colloid. Figure 16 shows a NTB radioautograph of the same area at a lower magnification which indicates that these atypical thyroid epithelial cells had the capacity to accumulate radio-iodine.

An interesting finding was that in the connective tissue stroma there were occasional localized small round-cell clusters as shown in Figure 17. These areas were apparently formerly occupied by thyroid tissue since these regions were surrounded by either the few remaining follicles or atypical clumps of thyroid epithelium. These cells lacked a reticular frame work and thus presumably were infiltrations of lymphocytes rather than lymphatic nodules.

The results of the administration of Astatine were marked thyroid destruction and atrophy which was not complete. There was no evidence of this tissues' malignant character but the possibility exists that it was pre-neoplastic in character in view of the fact that some of the cellular elements were suggestive of Hürthle cells.

RADIATION CHEMISTRY

Warren M. Garrison

During the last quarter the investigation of chemical changes induced by high energy helium ions from the 60-inch cyclotron has involved the following studies:

Acetic Acid Studies

W. M. Garrison, B. M. Weeks, and H. R. Haymond

In a previous report[1] it was suggested that the production of succinic acid and tricarballylic acid in irradiated acetic acid solutions is most readily accounted for by assuming that the CH_2COOH radical is formed as in intermediate by the reaction

$$\underset{(A)}{CH_3COOH} + OH \longrightarrow \underset{(A')}{CH_2COOH} + H_2O \qquad (1)$$

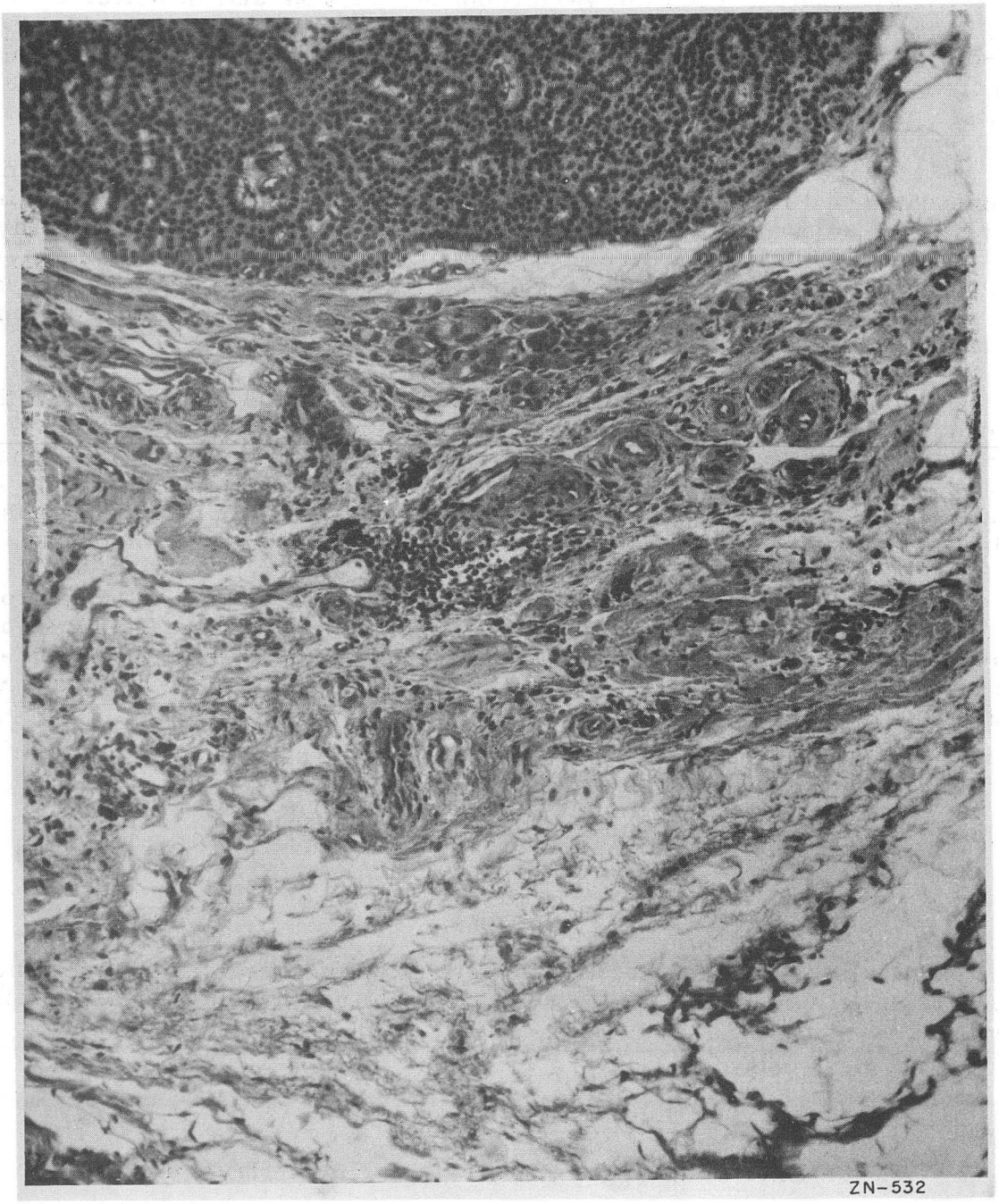

Fig. 10

Thyroid and parathyroid glands from a monkey which had received 100 microcuries of Astatine two years before sacrifice. The obliteration of normal thyroid architecture is evident and the parathyroid gland appears unchanged. Iron, hematoxylin stain and aniline blue. x 120.

Fig. 11

A small group of follicles containing colloid and apparently hyperplastic epithelial cells. Iron, hematoxylin and aniline blue stain. x 120.

Fig. 12

Another small nodule indicating the diminished amount of connective tissue separating individual follicles and cell clusters. Mallory triple stain. x 120.

Fig. 13

A higher magnification of a region comparable to Figure 11 showing in greater detail the variations in cellular structure and the presence of droplets of colloid. Iron, hematoxylin and aniline blue stain. x 300.

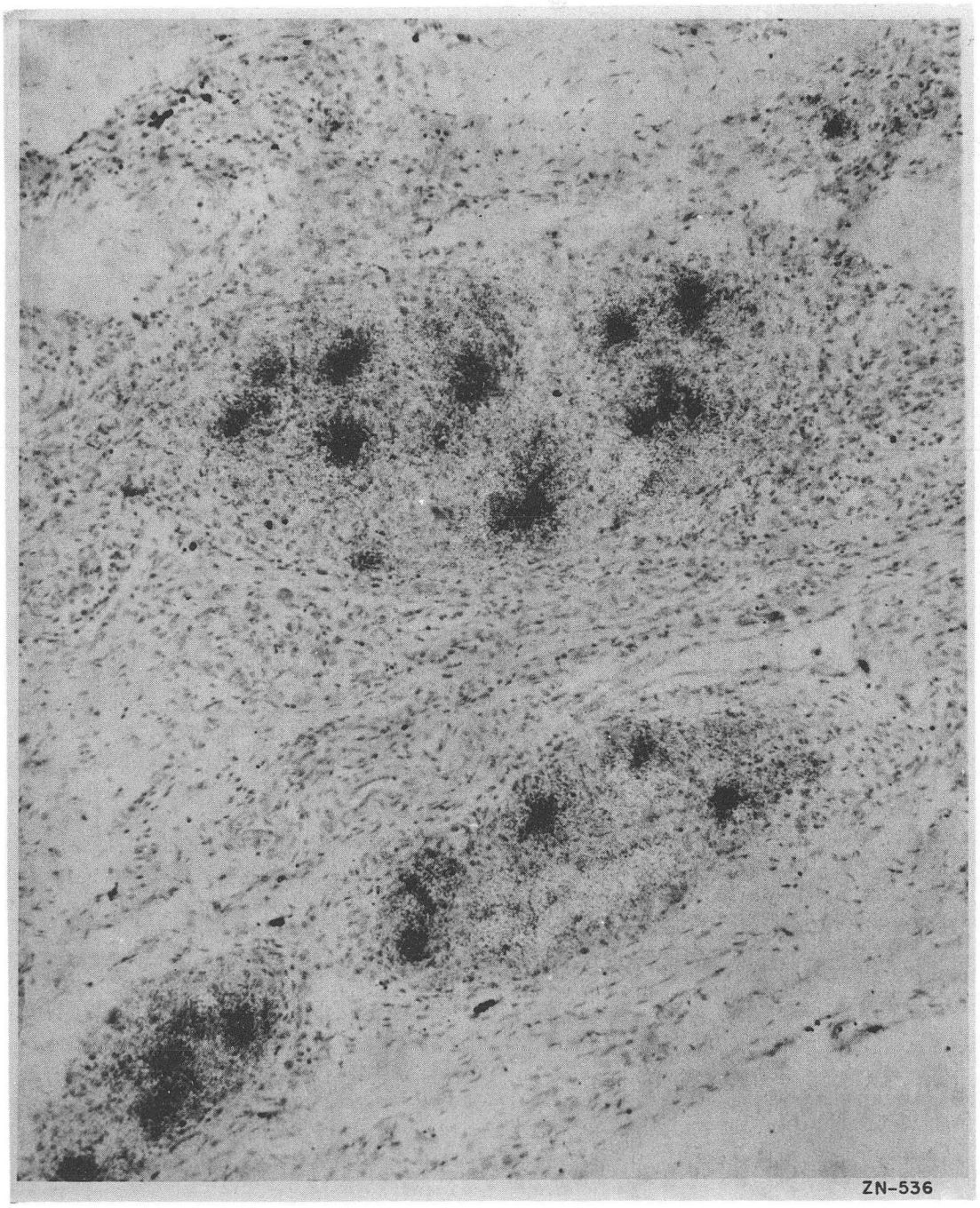

Fig. 14

NTB radio-iodine radioautograph from the thyroid gland of the Astatine-treated monkey. Although the architecture of the thyroid gland indicates a severe degree of injury the accumulation and retention of radio-iodine is demonstrable. Hematoxylin and eosin stain. x 120.

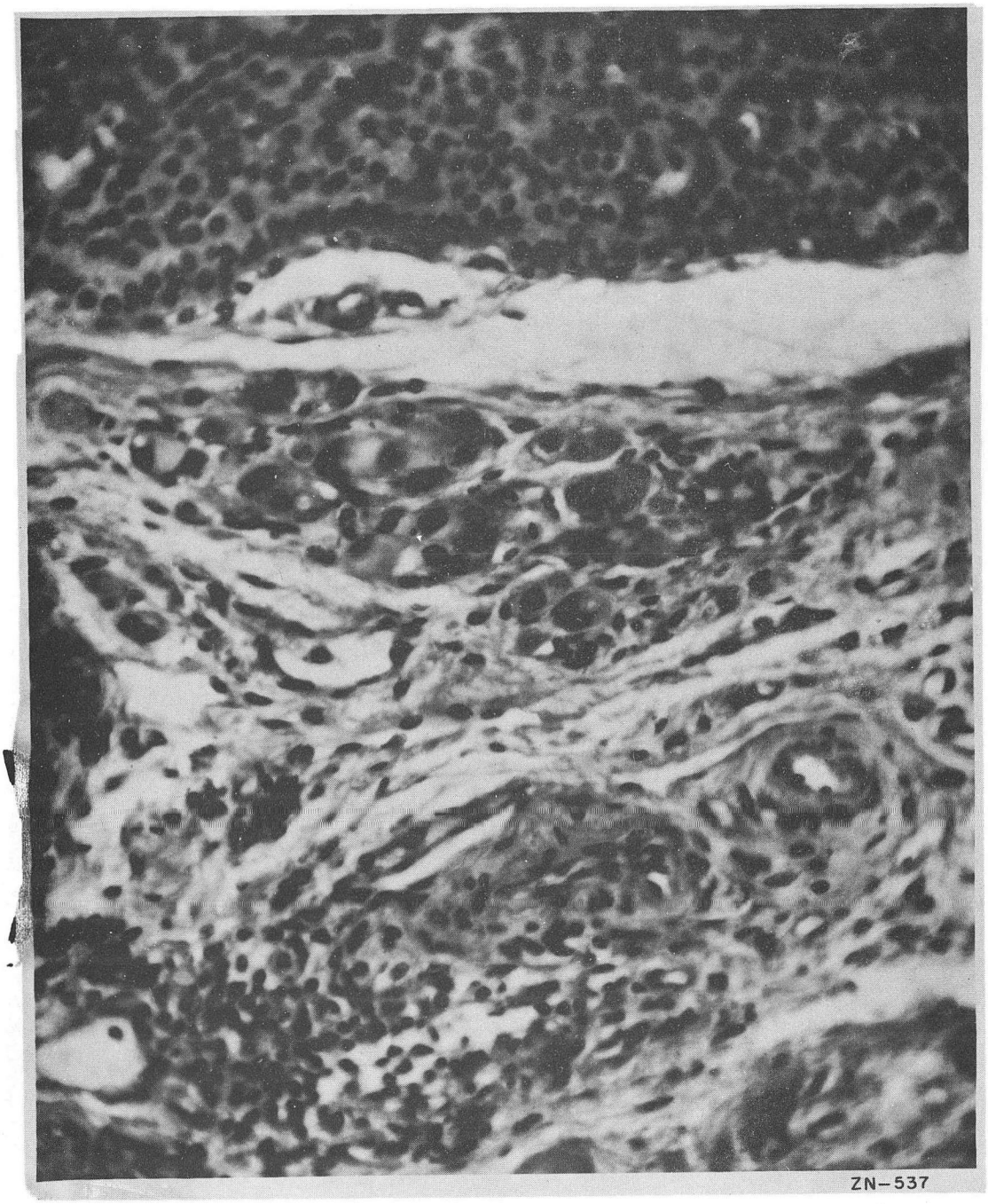

Fig. 15

A higher magnification of thyroid and parathyroid tissue to show details of cellular structure. The lack of organization of thyroid epithelium is evident and a few droplets may be seen. Iron, hematoxylin and aniline blue stain. x 300.

Radioautography

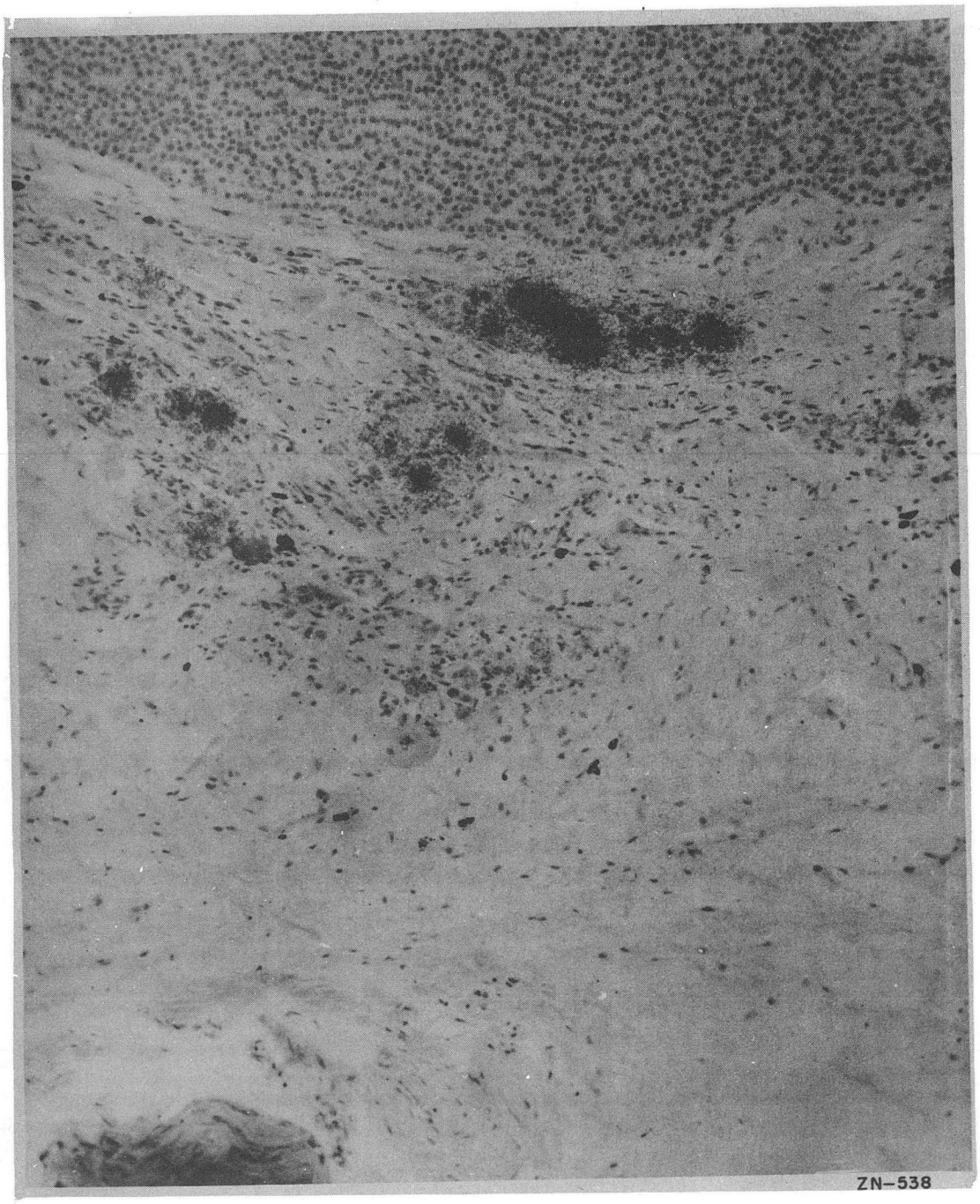

Fig. 16

NTB radioautograph of the same region shown in Figure 15 at a lower magnification. The ability of the clusters of epithelial cells to accumulate and retain radio-iodine is quite evident. Hematoxylin and eosin stain. x 120.

Radioautography

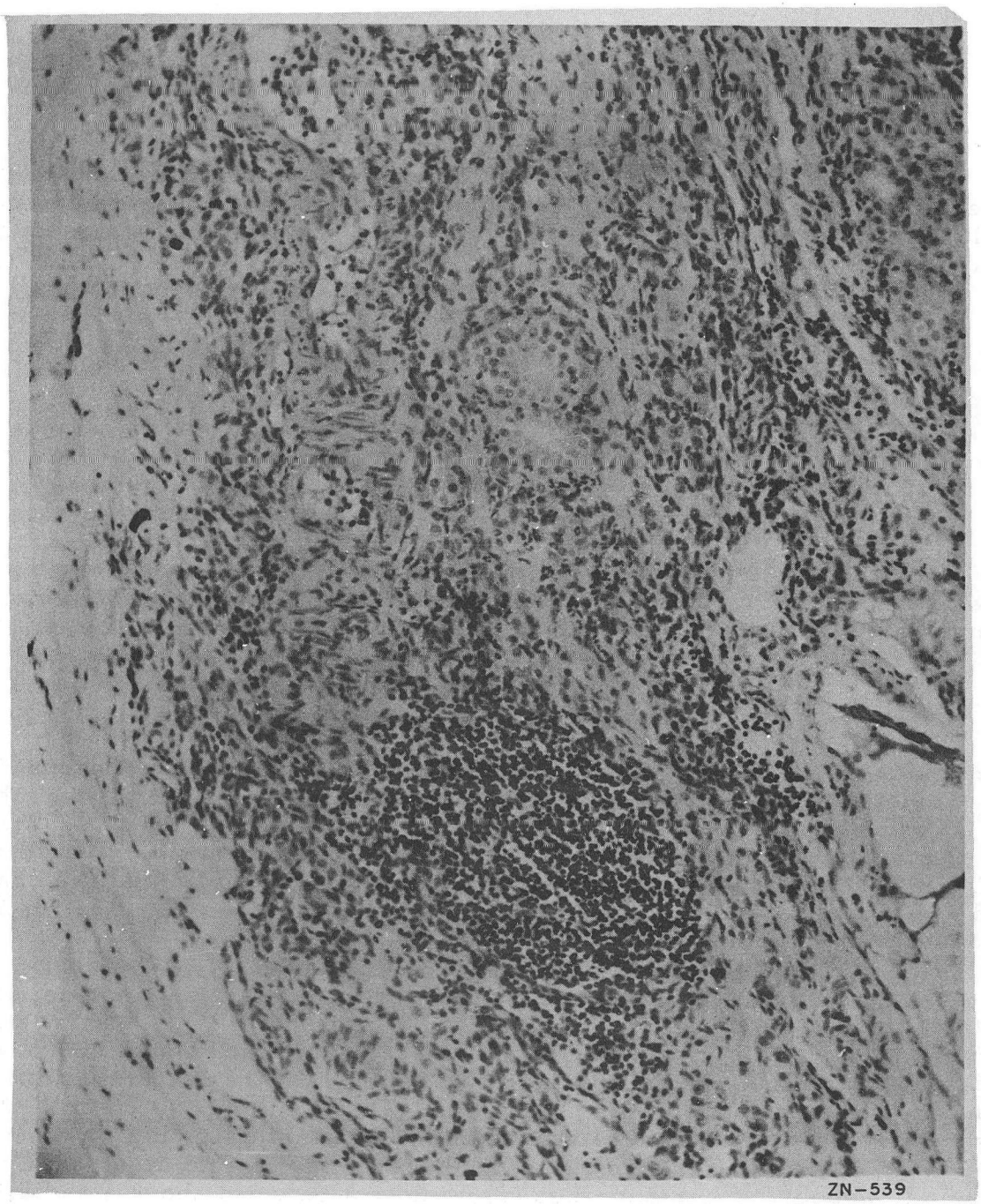

Fig. 17

Focal infiltration in the paratracheal fibrotic tissue of the Astatine-treated monkey. It is presumed that this infiltration occupied areas which had once been normal thyroid tissue. Hematoxylin and eosin stain. x 120.

and that succinic acid and tricarballylic acid are formed by the sequence:

$$2A' \longrightarrow S \qquad (2)$$

$$S + OH \longrightarrow \underset{(S')}{(COOH)CH_2}\text{-}CH\text{-}COOH \qquad (3)$$

$$A' + S' \longrightarrow T \qquad (4)$$

where (A) (S) and (T) represent acetic acid, succinic acid and tricarballylic acid respectively. Since S and T are the principal products below a dose level of 1×10^{21} ev/ml it was possible to apply the usual procedures for deriving a rate law:

$$d(S)/dt = k_2 (A')^2 - k_3 (S) (OH)$$

$$d(T)/dt = k_4 (A') (S')$$

$$k_1 (A) (OH) = 2k_2 (A')^2 + k_4 (A') (S')$$

$$k_3 (S) (OH) = k_4 (A') (S')$$

from which the following expression is obtained for the ratio of the velocity constants for the reaction of OH with acetic acid and succinic acid.

$$k_1/k_3 = \frac{(S)}{(A)} \left[2 \frac{d(S)/dt}{d(T)/dt} + 3 \right] \qquad (5)$$

As the result of an arithmetic error, this expression was shown incorrectly in the previous quarterly report.* Although the change does not appreciably affect the constancy of the calculated k_1/k_3 values, they have been recalculated and are summarized along with the radiation yield values in Table IA, IB and IC. The k_1/k_3 values were calculated using the approximation that the change in acid product concentration $(M_1)-(M_o)$ corresponds to the slope d(M)/dt at the product concentration $(M_o) + [(M_1)-(M_o)]/2$.

According to the mechanism proposed in the derivation of Eq. (5), the rate of tricarballylic acid formation should be initially zero. This is in agreement with the previously reported product identification studies[2] in which the acid products formed at low radiation dose levels were determined by C^{14} counting methods viz. (1) below a dose of 1×10^{20} ev/ml, succinic acid only is formed (2) succinic and tricarballylic acids are the main products in the range 1×10^{20} ev/ml to $\sim 1 \times 10^{21}$ ev/ml and (3) above 1×10^{21} ev/ml other acid products begin to appear. Therefore Eq. (5) would not be valid above a radiation dose of 1×10^{21} ev/ml.

The decrease in radiation yield for both succinic acid and tricarballylic acid with acetic acid dilution (Table IA) indicates that reactions 1 and 3

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* i.e., $k_1/k_3 - \frac{(S)}{(A)} \left[\frac{d(S)/dt}{d(T)/dt} + 2 \right]$

TABLE I

Radiation Yields of Succinic Acid and Tricarballylic Acids. Target Volume, 75 ml; Dose, 0.20 μahr[a]

A. Effect of acetic acid concentration

Beam current, 1 microampere.

Acetic Acid Concen. M	Succinic Acid Concen. M x 10^4	Succinic Acid Yield(G_S) mol/100ev.	Tricarballylic Acid Concen. M x 10^4	Tricarballylic Acid Yield(G_T) mol/100ev.	$k_1/k_3 \times 10^2$ Calcd. (5)
0.0625	8.85	0.049	1.95	0.011	8.54
	9.35	0.052	2.26	0.013	8.44
0.125	17.7	0.098	5.73	0.032	6.50
	16.83	0.093	3.37	0.020	8.75
0.25	26.0	0.144	4.0	0.022	8.32
0.50	40.7	0.226	4.86	0.027	8.08
	37.6	0.209	5.01	0.028	6.80
1.0	49.6	0.274	3.96	0.022	6.97
	49.6	0.274	4.12	0.024	6.73

a With 35.0 Mev helium ions a dose of 0.20 μahr in a 75 ml volume corresponds to an energy dissipation of 1.05×10^{21} ev/ml. Because of small differences in window thickness between target cells, the energy of helium ions absorbed in the solution varied slightly (<3 percent) depending on the particular cell window used. The corrected energies calculated from range-energy data, were used in the calculation of G_S.

B. Effect of intensity

Acetic acid, 0.25 M.

Beam current microamperes	Succinic Acid Concen. M x 10^4	Succinic Acid Yield mol/100ev.	Tricarballylic Acid Concen. M x 10^4	Tricarballylic Acid Yield mol/100ev.	$k_1/k_3 \times 10^2$ Calcd. (5)
1.0	26.0	0.144	4.0	0.022	8.32
0.5	26.8	0.149	4.5	0.025	8.0
	26.2	0.145	4.21	0.0234	8.07
0.2	26.4	0.147	2.97	0.0165	10.89
	25.6	0.143	2.60	0.0145	11.61

C. Effect of added succinic acid

Acetic acid, 0.25 M; succinic acid, 0.003 M; beam current, 1 microampere.

Succinic Acid Concen. M x 10^4	Succinic Acid Yield mol/100ev.	Tricarballylic Acid Concen. M x 10^4	Tricarballylic Acid Yield mol/100ev.	$k_1/k_3 \times 10^2$ Calcd. (5)
48.6	0.102	8.99	0.049	10.51
44.1	0.078	6.26	0.035	11.10

are not the only processes involved in the removal of OH radicals. The falling off in succinic acid yield is apparently not caused by competing secondary reactions involving succinic acid and tricarballylic acid since their radiation products do not appear in appreciable amounts at a dose of 1×10^{21} ev/ml. Furthermore, since the succinic acid yield is independent of intensity (Table IB) and approaches a limiting value at the higher acetic acid concentrations it would appear that the reactions competing for OH removal are not $H + OH = H_2O$ or $OH + OH = H_2O_2$. Although it is not possible at the present time to give a complete mechanism of the reactions involved, it is of interest to note that an explanation of the observed intensity and dilution effects can be obtained in terms of the mechanism proposed by Allen and co-workers[3] for the decomposition of water by ionizing radiation. Two different primary reactions apparently occur when aqueous solutions are irradiated viz

$$H_2O \longrightarrow H + OH \tag{6}$$

$$2H_2O \longrightarrow H_2O_2 + H_2 \tag{7}$$

Reaction 7 represents the formation of products by combination of like radicals in regions of high ionization density along the track of the ionizing particle and 6 represents the production of radicals which escape combination in the track and which are available for reaction with decomposition products or added solute in the bulk of the solution. The relative rates of reactions 6 and 7 are determined by the ionization density properties of the effective radiation. If we consider a competition in which OH radicals produced in 6 react in the bulk of the solution either with products (P) resulting from reaction 7 or with acetic acid (A) we have

$$k_6 I = k_P (P)(OH) + k_1 (A)(OH) \tag{8}$$

where k_6 is the free radical yield of reaction 6, k_P the rate constant for the reaction of OH with P and I the radiation intensity. For low radiation doses the equation for the formation of succinic acid becomes

$$\frac{d(S)}{dt} = \alpha k_1 (A)(OH)$$

where $\alpha \leq 1/2$. Substituting in Eq. (8) and taking (P) as a constant we obtain

$$\frac{1}{G_S} = \frac{1}{\alpha k_6}\left[1 + \frac{C}{(A)}\right] \tag{9}$$

where G_S is the experimentally observed radiation yield for succinic acid and C is a constant involving (P). A plot of $1/G_S$ against $1/(A)$ for the data in Table IA is shown in Fig. 18, (Curve 1). A straight line is obtained which if extrapolated to $1/(A) = 0$ gives $\alpha k_6 = 0.38$.* This corresponds to the case in which all of the OH radicals disappear by reaction with A through reaction 1. Then $k_6 \geq 0.76$

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* We are indebted to Dr. A. O. Allen for pointing out this relationship to us.

which is a minimum value for the free yield of reaction 6. The same relationship between G_S and (A) given in 9 could conceivably be obtained if P were an impurity. This situation is considered unlikely because of the relatively large amount of products formed in comparison to the possible impurity concentration and because the amount of P present would have to be consistently independent of (A).

The above discussion was based on data obtained from acetic acid solutions which were stirred with a stream of oxygen gas during irradiation with approximately 35 Mev helium ions. A similar study of succinic acid and tricarballylic acid yields in evacuated acetic acid solutions has been initiated. Hydrogen peroxide and gaseous products are also being determined quantitatively. The solutions are irradiated in all-glass sealed targets of the type shown in Fig. 19. The targets were handled in the following way (1) aqueous acetic acid solutions were added through (A) which was then sealed to a vacuum mainfold. (2) Air was removed by evacuation until the characteristic "metallic" click could be detected when the target cell was shaken. (3) The cell was then sealed-off and irradiated. (4) After irradiation the cell was again sealed onto the manifold at (B) and gaseous products were removed through the break off seal (B) using a Toepler pump arrangement. The method used in the determination of succinic acid, tricarballylic acid, hydrogen peroxide and gaseous products* were identical with those previously reported.[1,2] The data shown in Table IIA for a constant radiation dose of 0.39×10^{21} ev/ml show the effect of acetic acid concentration on the observed product concentration. Table IIB gives the composition of the corresponding gaseous product. Since the radiation yield of succinic acid (G_S) in oxygen stirred acetic acid solutions has been shown[1,2] to be independant of beam intensity and of dose (Below $\sim 2 \times 10^{21}$ ev/ml), the oxygen effect can be evaluated by a comparison of the data in Tables IA and IIA. It is at once apparent that values of G_S are higher at the lower acetic acid concentrations in the case of the evacuated targets. Apparently, however, the same limiting value of G_S is approached at higher acetic acid concentrations. This may be seen by comparing curves 1 (Table IA) and 2 (Table IIA) in Fig. 18. Furthermore, in the evacuated systems, the yield of hydrogen and hydrogen peroxide increases with G_S. This relationship is most consistently explained in terms of reactions (6) and (7). As pointed by Allen,[3] the reactions:

$$H_2O \longrightarrow H + OH \qquad (6)$$

$$2H_2O \longrightarrow H_2O_2 + H_2 \qquad (7)$$

may be considered as independent primary process. In the absence of added solute the radicals produced in (6) react with H_2O_2 and H_2 from (7) giving rise to a radiation induced back reaction i.e.,

$$H + H_2O_2 \longrightarrow H_2O + OH \qquad (8)$$

$$OH + H_2 \longrightarrow H_2O + H \qquad (9)$$

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* We wish to thank Dr. Amos Newton for the mass spectrometric analysis of the gaseous products.

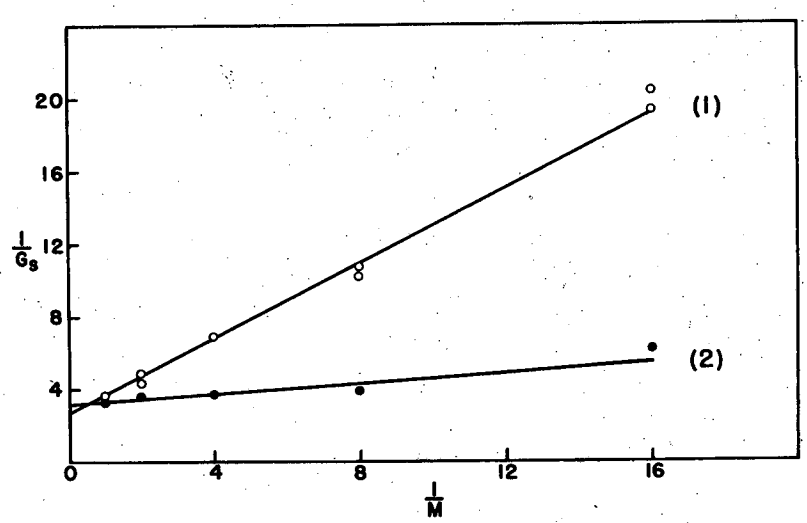

Fig. 18

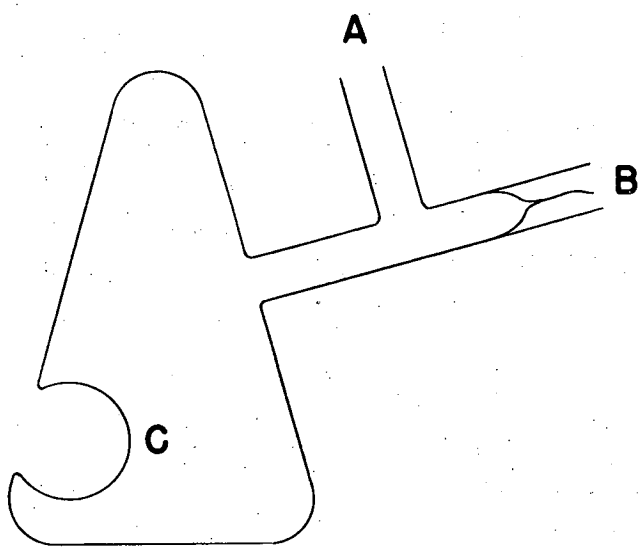

Fig. 19

TABLE II

Effect of Acetic Acid Concentration on Product Yield in Evacuated Solutions. Target Volume, 100 ml; Dose, 0.10 μahr; Beam Intensity, 0.20 μa.

(A)

Acetic Acid	Succinic Acid		Tricarballylic Acid		Hydrogen Peroxide	
M	$M \times 10^4$	G_S	$M \times 10^4$	G_T	$M \times 10^4$	$G_{H_2O_2}$
0.0625	10.7	0.164	2.26	0.035	17.1	0.262
0.125	15.7	0.258	2.98	0.046	18.1	0.277
0.25	17.3	0.266	2.22	0.034	20.8	0.319
0.50	18.4	0.282	2.05	0.031	21.9	0.336
1.00	18.8	0.284	2.50	0.039	22.50	0.346

(B)

Acetic Acid	Total Gas	H_2		CO_2	CH_4
M	CC (STP)	%	G_{H_2}	%	%
0.0625	12.80	91.2	0.760	6.58	1.2
0.125	14.75	88.0	0.839	7.59	1.6
0.25	16.2	86.1	0.905	8.62	2.4
0.50	17.9	79.2	0.916	14.25	3.7

In the presence of added solute (acetic acid) which can react with either H or OH, the back reaction is interrupted and the H_2 and H_2O_2 concentrations increase. A detailed mechanism for the acetic acid system is being developed and will be presented in a subsequent report.

References

1. Quarterly Progress Report, July, August, and September, 1952. UCRL-2001.

2. Quarterly Progress Report, April, May, and June, 1952. UCRL-1922.

3. A. O. Allen, J. Am. Chem. Soc. 56, 575 (1952).

Solutions Containing Initially Both Acetic and Succinic Acids

H. R. Haymond

Additional evidence concerning the ratio of the velocity constants for the reactions of acetic and succinic acids with the OH radical, i.e., the value of k_1/k_3, has been obtained by irradiating solutions of acetic acid which initially contained various concentrations of succinic acid and determining the changes in concentration of succinic acid which occurred. Results of these experiments are shown in Fig. 20, where the change in concentration of succinic acid is plotted as a function of the median concentration of succinic acid during the bombardment. All points in Fig. 20 are for 0.25 M acetic acid, 0.1 microampere hour on 75 ml of solution. Succinic acid was determined in each case by titration of the eluate from a silicic acid column run[1,2] of the non-volatile fraction after irradiation.

From equation 5 of the previous section, when there is no change in concentration of succinic acid $k_1/k_3 = 3(S)/(A)$. This occurs, from Fig. 20, at $(S) = 5.3 \times 10^{-3}$. Since $(A) = 0.25$, a value of $k_1/k_3 = 0.064$ is obtained by this method, which is in fair agreement with other values obtained and previously reported. It should be noted here that the derivation of the equation for k_1/k_3 assumed that CH_2COOH radicals which are formed react to form either succinic or tricarballylic acids. It is known, however, that with an initial concentration of succinic acid of 5.3×10^{-3} M in 0.25 M acetic acid small amounts of other products are formed even in the low irradiations used in these experiments.

References

1. Quarterly Progress Report for July, August, and September, 1952. UCRL-2001.

2. Quarterly Progress Report for April, May, and June, 1952. UCRL-1922.

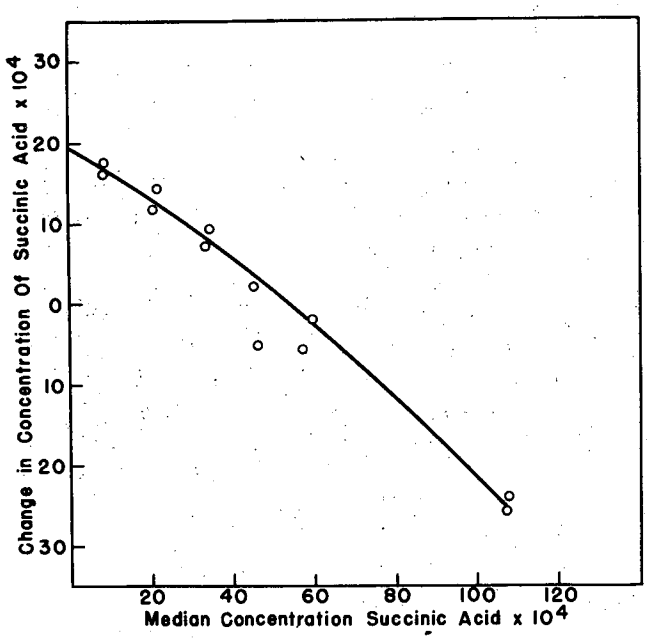
Change in Concentration Of Succinic Acid x 10^4
30
20
10
0
10
20
30
20
40
60
80
100
120
Median Concentration Succinic Acid x 10^4

Fig. 20

Formic Acid Solutions

W. M. Garrison and D. M. Morrison

Recently, as part of a general study of the radiation induced synthesis of organic substances in aqueous solution we reported that in addition to hydrogen and carbon dioxide, formaldehyde, oxalic acid and at least eight other organic compounds are produced by high energy helium ion bombardment of air-free aqueous formic acid solutions. Although a detailed mechanism was not presented for the formation of formaldehyde and oxalic acid it was assumed that the reactions involved were probably the following:

$$H_2O \longrightarrow H + OH$$

$$H + HCOOH \longrightarrow HCO + H_2O$$

$$H + HCOOH \longrightarrow COOH + H_2$$

$$2\ COOH \longrightarrow (COOH)_2$$

$$H + HCO \longrightarrow HCHO$$

However, in investigating the kinetics of the formic acid system it has been found that oxalic acid is apparently not the principle non-volatile acid product at low radiation doses. Partition chromatographic analysis of the non-volatile acid fractions from 0.25 M formic solutions irradiated with 35 Mev helium ions indicate that the principle products have butanol-water distribution coefficients considerably greater than that of oxalic acid. Figure 21 shows a typical elution curve which was obtained by the method of Bulen[1] with a sample of the non-volatile fraction corresponding to a radiation dose of 1×10^{21} ev/ml. Peaks I and II have been tentatively identified as oxalic acid and glycolic acid respectively. Identification of products in region III is now in progress.

Glycine

B. M. Weeks

In the quarterly report covering the period of July, August, and September, 1952 (UCRL-2001), the preliminary steps in a study of the radiation chemistry of aqueous solutions of glycine were described. Since then a detailed program has been worked out and work started on it. The principle work of the radiation chemistry group continues to be devoted to a study of the radiation chemistry of aqueous solutions of acetic acid and it is as a chemical relative of acetic acid that glycine (as well as presumably other 2-carbon chemical species) is now coming under attack. The complete program is divided into several

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1 W. A. Bulen et al, Anal. Chem., 24, 187 (1952).

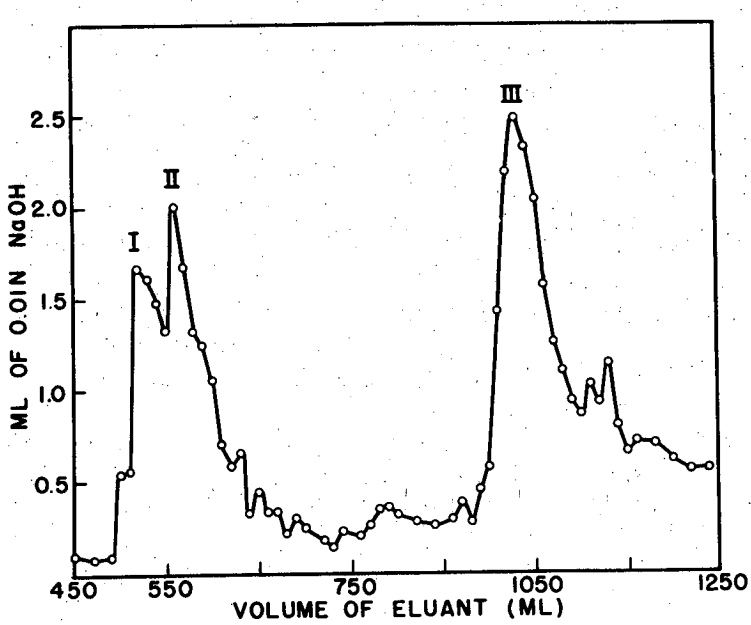
I
II
III
2.5
2.0
1.5
1.0
0.5
ML OF 0.01N NaOH
450
550
750
1050
1250
VOLUME OF ELUANT (ML)

Fig. 21

parts: purification of the target material, glycine and H_2O; bombardment; resolution of the products of bombardment; identification and determination of the resolved products.

Purification

The purification of H_2O for the target solutions is the same as described earlier for the acetic acid program. The purification of the glycine itself imposes different problems. In a procedure[1] which combined the principles of electrical migration and paper chromatography it was shown that ninhydrin-positive impurities were present in commercially available glycine (Nutritional Biochemicals Corporation and Eastman Kodak Company) and that upon bombardment these impurities disappeared when the glycine was used as supplied, (Nutritional Biochemicals Corp.). Although the nature and behavior of these impurities might be worthy of investigation they represented only interference in our study of the radiation chemistry of glycine. Therefore, purification of commercial glycine as well as the C^{14}-labeled glycine used in our experiments was planned. The proposed purification procedure[2] is simply to pass the crude glycine through a cation exchange column and select the portion of the effluent containing only glycine. The capacity of Dowex 50 resin is such that up to 4 grams of the commercial glycine can be purified in one pass through a 1-1/2 x 20 in. column. For the purification of smaller amounts which contain a C^{14}-label, a smaller diameter column is used. The glycine is placed on the column, dissolved in as little H_2O as possible and allowed to drain slowly into the top of the resin bed. A volume of distilled H_2O equal to 2-3 times the hold-up volume of the column is washed through. The glycine is then removed from the column by elution with 1N NH_4OH. The flow rate for both H_2O and NH_4OH is about 2 ml/cm^2 column cross section/hr. Only that part of the effluent is saved which, by radioactivity or ninhydrin reaction, obviously contains the bulk of the glycine. The pure glycine is then recovered from the NH_4OH eluent by evaporating to dryness *in vacuo*. The vacuum is maintained for at least 1 hour after the crystals appear dry to ensure complete removal of NH_3. The glycine thus prepared, if not used immediately, is stored in a sealed, evacuated glass container to prevent the accumulation of moisture and consequent bacterial action. The purity of each target solution is tested by subjecting an unbombarded portion to the procedures described below.

Bombardment

For bombardment 0.25 M solutions of the purified glycine are used and bombardment doses of 1.05×10^{21} ev/ml given. This is the base from which all the departures were taken in the acetic acid study and will be the same for the glycine studies. The C^{14}-labeled glycine is irradiated in the 10 ml evacuated, all-glass rotating cells used in the acetic acid studies and the bombardments of inert glycine are made in the 100 ml, evacuated, shaker cells also described earlier for the acetic acid work. The ion current and average energy are, of course, monitored in the same way as for the acetic acid bombardments.

Resolution of the Products of Bombardment

This part of the glycine work represents the greatest departure from the routine analyses of the acetic acid work, but still it is a paralled in most

respects. After bombardment the following series of steps is performed, if not to each target, at least to the aggregate:

1. After the seal is broken, the gas is drawn off, its volume measured and a portion analyzed on the mass spectrometer.

2. A portion of the solution is analyzed for H_2O_2 by $Ce(HSO_4)_4$ titration.

3. Another portion of the solution is analyzed for NH_3 by the ordinary Kjeldahl titration.

4. The target solution is placed directly on an ion exchange column (Dowex 50-acid cycle) and eluted first with distilled H_2O, then with 2N HCL until the glycine peak is passed, and then with 6N HCL to strip the column as soon as possible of all remaining cations. The flow rate is regulated to about 2 ml/cm^2 column cross section/hr. and the effluent is divided into 3 portions: a. the H_2O eluent; b. the glycine peak in the HCl eluent; c. the rest of the HCl eluent (containing the cationic bombardment products as well as traces of glycine due to overloading of the column with the entire target volume of 0.25 M glycine.)

5a-1. The H_2O eluent is distilled in vacuo and the residue chromatographed on the silica column in the same manner as the non-volatile products from the acetic acid bombardments.

5a-2. A portion of this distillate is titrated to neutrality with NaOH and again evaporated to dryness in vacuo leaving in the residue the Na salts of whatever volatile acids might be present. This residue is either dissolved in 1 ml of H_2O-saturated butanol, acidified with a drop of H_3PO_4, distilled again in vacuo and the distillate containing the free acids in a much smaller volume than before chromatographed on a silica column or the residue is dissolved in 1 ml pure H_2O, acidified with a drop of H_3PO_4, redistilled in vacuo and this distillate run on the mass spectrometer.

5a-3. The portion of the distillate of 5a-2 that was not neutralized with NaOH contains whatever volatile non-acidic products that may be formed. Although no definite plans for the isolation of these substances have as yet been made, it seems likely that standard isotope dilution techniques will prove fruitful.

5b. The glycine portion of the HCl eluent is evaporated to dryness and recovered as described in the section on purification.

5c. The portion of the HCl eluent with most of the glycine removed is evaporated to dryness in vacuo, redissolved in a few ml of H_2O and re-chromatographed on a Dowex 50 ion exchange column. Without the load of glycine which was present in the original target solution a much more precise chromatogram

is possible. The unknown mixture is eluted first with pure H_2O and then with gradually increasing concentrations of HCl from 1.5N to 6N.

Identification and Determination of the Resolved Products

So far work has progressed only far enough to work out the procedure outlined and only a beginning has been made on the identifications and determinations. Although the purification procedure has been worked out, all bombardments made so far have used glycine as supplied. The results of these bombardments served, therefore, mainly as a guide to the setting up of the general procedure. A chromatogram of the cationic fraction (4c) of a C^{14}-labeled glycine bombardment has been obtained which clearly shows several definite peaks besides the original target glycine, but we have not yet proved that these peaks arise solely from glycine in the target solution. Similarly the silica column and mass spectrometer have shown the presence of formic and acetic acids in the volatile acid fraction (5a-2) of the H_2O eluent (4a) from the first pass through the ion exchange column, but the origin of these acids must still be determined. The general problems and procedures involved in identifying the resolved products is identical to those met in the acetic acid studies. Co-chromatographic procedures, isolation of sufficient material for chemical identification, carrier experiments, direct comparison of the behavior of isolated products on the column and in mass spectrometer with authentic compounds suspected of being identical with the products are all possible approached and they will all need to be employed.

References

1. A. Carler, Old Radiation Laboratory, Berkeley, Unpublished.

2. R. Ostwald, Donner Laboratory, Private communication.

Calorimetry

B. M. Weeks

Since the last quarterly report covering the period July, August, September, 1952, another calorimetric determination of the 60-inch cyclotron helium ion beam energy was made. A newly designed accelerating grid ("feelers") on the cyclotron "dee" had been installed to pull the helium ions out of the ionizing arc into their spiral orbit. Also, a repositioning of the ion source (arc) had been made to obtain optimum operating conditions with the new accelerating grid. Thus it was felt that some change in the energy of the helium ions emerging from the target snout might have occurred. This thought was in fact borne out in the measurement. An average energy of 38.6 Mev for the "naked" beam was found. This represented a fall in energy of 1.6 Mev from the previous measurement. Subsequent measurement by absorption in aluminum confirmed this measured fall in energy within the experimental error involved. When the old type "feelers" were replaced and the ion source repositioned, absorption experiments indicated that the helium ion energy returned to approximately its former value of 40 Mev.

Papers Submitted for Publication During the Quarter

Garrison, Warren M. and Rollefson, G. K.: Radiation Chemistry of Aqueous Solutions Containing Both Ferrous Ion and Carbon Dioxide.

Garrison, Warren M., Haymond, H. R., Powell, H., Corum, C., Hamilton, J. G.: A Centrifugal-Pump Target Assembly for the Cyclotron Bombardment of Liquids.

Morrison, D. C., and Crowley, Josephine F.: The Biological Behavior of Organic Compounds Containing Radiophosphorus.

Garrison, Warren M., Haymond, H. R., Morrison, D. C., Weeks, B. M., Melchert, J. G.: High Energy Helium-Ion Irradiation of Aqueous Acetic Acid Solutions.

Hamilton, J. G., Asling, C. W., Garrison, W. M., and Scott, K. G.: The Metabolism and Biological Effects of Astatine in Rats and Monkeys.

II. BIOLOGICAL STUDIES OF RADIATION EFFECTS

John H. Lawrence, M.D. - in charge

Project 48A-1

A Multiple Scintillation Counter In-Vivo Scanner

H. O. Anger

Introduction

A scanning device employing ten scintillation counters is described in this article which maps the location of gamma-emitting isotopes present in the entire body of a patient. The purpose of the instrument is to locate and follow the path of gamma-emitting tracers administered to patients and laboratory animals.

Scanners employing a single scintillation counter have been built by Cassen[1] and Mayneord.[2,3] These instruments have been used to scan the thyroid gland of human patients in-vivo. Previously, it was necessary to measure counting rates at various positions and from this data draw a map in order to form an accurate idea of the distribution of radioactive material.[1,4] Another instrument for showing the distribution of radioactivity is the gamma-ray pinhole camera which uses a large flat scintillation phosphor with a photographic plate in contact to record the image obtained.[5] However, the sensitivity of this instrument is very low.

The principle point of difference between the previous scanners and the one described here is that ten scintillation counters are used in the new instrument together with a different scanning and indicating method. The use of ten counters allows a subject to be scanned in one-tenth the time that was necessary previously. Therefore, it becomes practical to scan a patient from head to toe in a routine manner.

The instrument can be applied both to tracer research and clinical diagnosis. It has been in use for the past 8 months to locate thyroid carcinoma metastases and residual thyroid tissue in human patients. Iodine 131 is used as a tracer in these cases. It has also been used experimentally to show the size and shape of the liver and spleen in patients using colloidal Au^{198} as a tracer. Other possible clinical applications, to mention just a few, are mapping the size and shape of the thyroid gland in human patients, determining the presence of thyroid nodules, and locating other lesions such as brain tumors with the aid of suitable tracer compounds. The potential uses in tracer research are considerable, since the instrument can be used in general to locate and follow changes in the location of any gamma-emitting activity in-vivo in a human subject or laboratory animal.

Apparatus

The scanner produces a picture of the distribution of the gamma-emitting activity present in a small area by means of ten directional scintillation counters in a lead shield, together with an amplifier, ten argon glow lamps, and a camera.

A diagram of the instrument is shown in Fig. 1. The scintillation counters are positioned so that the sensitive parts of the counters, the phosphors, lie along a straight line. The counters are shielded by the lead shield on all sides except for an aperture beneath each phosphor. Apertures which are three-eights inch in diameter are usually used and they point straight down. They are spaced 11/16 inch apart and lie along a straight line about 7 inches long. To scan an area for radioactivity, the counters are moved slowly by means of a motor in a direction perpendicular to the line along which the counters are located. Thus a strip about 7 inches wide and any length, usually 24 inches, is covered by each scan.

When scanning a human subject, the subject lies on a table and the counters move over him from right to left for a distance of 24 inches and then back to the starting point. To cover the whole subject, about 10 or 11 scans are taken and the resulting pictures are joined together to form a composite head-to-toe picture. For instance, the first scan covers a horizontal strip 7 inches wide and 24 inches long centered over the subject's head, the second scan covers the adjoining area over his neck, the third covers his upper chest, and so on. Four minutes are required for each scan, and about 45 minutes are required to scan in this manner from head to toe.

The distribution of activity is recorded by means of the ten argon glow lamps and the camera. The amplifier and glow lamps shown in Fig. 1 are connected so that each gamma ray counted by any of the scintillation counters produces a flash of light in a corresponding glow lamp. Gamma rays detected by the first scintillation counter produce flashes in the first glow lamp, and so on. The glow lamps and the carrier to which they are attached are contained in a dark box. While the counters are scanning over the subject, the glow lamps also scan, moving perpendicular to the line along which they are located. They travel at a rate of speed proportionate to the ratio of the spacing between glow lamps and counter apertures.

During the scanning period, the camera takes a time exposure of the glow lamps and records each flash as a dot on a photographic film. Where the activity is greatest in the subject, the greatest number of dots appears on the photograph. Therefore, a map of the distribution of radioactivity is obtained with concentrations of white dots appearing where the activity is the greatest. The natural background due to cosmic rays and stray radioactivity appears as a few dots randomly distributed over the print.

A rectangular aperture is placed in front of each glow lamp so that each dot appearing on the photograph has the shape of a short vertical line when scanning in the horizontal direction. This has been found by experiment to yield pictures with the best apparent definition. The range of intensity of radioactivity that can be recorded is limited at the present time because when the activity at a given point is high, the dots pile on top of one another, and a horizontal white line is produced over that area, regardless of whether the activity is moderate or very high at that point. If the activity is not high enough to produce a solid line, the amount of activity is indicated by the relative concentration of the dots.

The camera used to record the image is a Polaroid-Fairchild oscilloscope camera. This camera develops and delivers a finished print one minute after each scan is taken. The use of this camera with its rapid and easy development of the pictures is a considerable help in the operation of the scanner.

Interlacing

A single scan results in a picture with ten rows of dots having ten line definition. In practice, increased definition is obtained by interlacing scans. An interlaced scan is taken in the following way. The scanner first moves from right to left over the subject. Then the shield containing the counters is moved one-half the distance between the apertures in a direction parallel to a line through the ten apertures. The subject is then scanned from left to right over the same area as previously. At the same time, the images from the glow lamps are interlaced optically by changing the angle of a one-half inch thick lucite plate in front of the camera lens. This results in a scan with 20 line definition. Additional interlacing is used when small animals which can be placed close to the apertures are scanned. Additional resolution is obtained by taking 40 line interlaced scans in this case providing small apertures are being used. For large subjects, the actual resolution is not increased appreciably by taking a 40 line scan since the sources of radiation are usually further away from the apertures and the resolution of each aperture falls off as its distance from the source of radiation in the subject increases. Therefore, 20 line interlaced scans are usually taken on human subjects.

Sensitivity and Definition

The scanner in its present form will detect one microcurie of I^{131} when the activity is spread over a one inch diameter circle or less at a distance two inches from the apertures. This sensitivity is for a 20 line interlaced scan taken with three-eights inch diameter apertures at a scanning speed of 11 inches per minute, and with two inches of tissue equivalent material between the scanner and the source. Greater sensitivity can be obtained with no loss in definition by scanning at a slower speed or by taking repeated scans of the same area, each scan being recorded on the same print.

Better definition but lower sensitivity is obtained by using smaller apertures. Three-eights inch diameter apertures are usually used when scanning human subjects. One-eighth inch apertures are usually used when scanning small animals if the activity present is great enough. The aperture size can be changed easily by replacing the bottom plate of the shield with a plate having different apertures.

Test images obtained with the scanner are shown in Fig. 2. The scans were taken of a radioactive test pattern made by filling an X-shaped groove in a lucite block with I^{131} solution. The grooves in the test pattern were one-fourth inch wide and about seven iches long. A 10 line image made by a single scan is shown at (a), a 20 line interlaced image at (b), and a 40 line interlaced image at (c). The increase in definition obtained by interlacing scans is illustrated. The test pattern contained a total of 10 microcuries of I^{131}, or about one-fourth microcurie per square centimeter. It was located one inch from the scanner shield, and three-eights inch diameter apertures were used. The increase in definition from interlacing scans is greater when smaller apertures are used.

Figure 2 (d) and (e) show the loss in definition as the distance from the test pattern to the scanner is increased. The test pattern was one-fourth inch from the shield in (d) and three inches in (e).

Figure 2 (e) and (f) show the result of increasing the scanning time without changing the interlacing. Figure 2 (e) was made with a four minute 20 line interlaced scan and 2 (f) was made with an eight minute 20 line scan. The image at (f) is more definite than the image at (e) due to the larger number of counts recorded.

Electronic Circuit

A diagram of one channel of the ten channel amplifier and discriminator circuit is shown in Fig. 3. The signal from each phototube is amplified by a dual triode amplifier, after which it is fed to the input of a one-shot multivibrator circuit. The argon glow lamp is connected in series with the plate load resistor of the second triode unit of the multivibrator circuit. This triode unit remains in a non-conducting condition until a large enough pulse appears at the input of the multivibrator at which time the tube begins to conduct current and remains in a conducting state for about 1/50 second. This produces the required flash in the glow lamp. Then the tube remains non-conducting until the next large pulse appears from the phototube. The multivibrator circuit acts as a discriminator since weak pulses will not trigger it. Phototube noise is discriminated against at this point since most noise pulses are weak.

The Scintillation Counters

The scintillation counters each consist of an RCA 1P21 photomultiplier tube and a thallium activated sodium iodide phosphor. The phosphor in each counter is a 3/8 inch square by 11/16 inch long crystal of thallium activated sodium iodide. The crystal is unpolished and is contained in a holder filled with Monsanto Aroclor to exclude moisture from the crystal. The crystal holder is painted white on the inside to reflect as much light as possible from the phosphor to the phototube. The phosphor is held to the side of the photomultiplier tube next to the photocathode.

When detecting I^{131}, the counters have a plateau of 200 volts with a slope of about 5 percent per one hundred volts applied to the phototube dynode voltage divider. The voltages applied to the ten phototubes are obtained from a single regulated supply. A variable resistance is connected between the supply and the individual phototube dynode voltage dividers so that each counter can be made to operate approximately at the center of its plateau.

Scanner Pictures of a Rabbit

The first in-vivo pictures taken with the equipment were of a rabbit which was injected intravenously with Au^{198} in the form of gold colloid. Most of this colloidal material, when it is administered intravenously, is removed by the reticulo-endothelial system, hence the major portion goes to the liver and spleen. Smaller amounts go to the bone marrow. Pictures were taken at intervals of time as the activity decayed with the 2.7 day half life of Au^{198}. Figure 4 shows a picture taken after 0.65 millicurie remained. The rabbit is lying on its side. The large white spot outlines the liver and spleen. These two organs are not distinguishable from one another since they lie close together. The fainter lines outline the spine, neck, pelvis, femur, and rib cage. The

picture is a composite made by joining together three separate adjacent scans of the rabbit. Each separate scan is a 40-line interlaced image. The entire picture was taken in about 15 minutes.

Scanner Pictures of a Thyroid Carcinoma Patient

Complete head to toe scanner pictures have been taken of eight patients with known or suspected thyroid carcinoma. The results obtained with one of the patients are shown in Fig. 5. This patient's thyroid had been removed surgically two years previously and he had a large metastasis near his left elbow for which he had been given large therapeutic doses of I^{131}. No other metastases were evident before the scanner pictures were taken.

The patient was given an oral dose of 5 millicuries of I^{131} and the following pictures were taken with the scanner. Figure 4 (a) was taken one hour after the dose was given and shows that the I^{131} was distributed throughout his body. A complete outline of the patient is shown in this picture. Figure 4 (b) was taken 2 days after the dose was given. An outline of the patient's body is still visible because of the slow disappearance in this patient of iodine from the blood stream and intercellular space. The points of concentration are inconclusive at this time except for the elbow because of the high body background. Figure 4 (c) was taken 4 days after the dose was given. There are six points where activity is shown to be taken up. They are located at the sternum, the right pelvis region, the right lower chest, the left arm near the shoulder, the left elbow, and the jaw region near the front teeth. The locations are shown in Figure 4 (d).

This subject was scanned at two other times, 3 and 6 months previous to the time at which the above pictures were taken. The first time the active point on the sternum was found, and the second time the point in the pelvis region was found.

After finding these points with the scanner, the patient was explored with a directional in-vivo scintillation counter to confirm the presence of the points of uptake and locate them more accurately. All of the active points found with the scanner were confirmed by obtaining high counting rates over the same points with the directional in-vivo counter.

X-ray radiographs were taken of the sternum and pelvis regions. On the sternum near the third rib, a calcified area was found, and in the pelvis region a small spur of bone was found on the ilium. Neither of these lesions as seen on the x-ray plate was abnormal enough to be considered as the probable site of a metastasis on the basis of the x-ray evidence alone. However, on the basis of the findings with the scanner and the in-vivo counter, all six points with the possible exception of the jaw region can be identified with relative certainty as the site of a thyroid metastasis.

The use of the scanner in combination with x-ray radiography provides maximum information in locating thyroid metastases since the x-ray plates, if the lesion is visible on them at all, show how deep within the body it is located. With the help of the scanner, slight abnormalities that are barely visible on an x-ray plate can be identified with relative certainty as metastatic thyroid lesions.

Future Development

A new model of the scanner will soon be built with increased sensitivity as the main object. It will have larger thallium activated sodium iodide crystals so that larger apertures can be profitably used if desired. Also the thickness of lead shielding will be increased so that isotopes with higher energy gamma rays can be employed.

In addition more counters may be used in order to decrease scanning time or increase the effective sensitivity.

Summary

A description of a scanning device employing 10 scintillation counters is given which in a relatively short time provides a picture of the sources of gamma emitting activity present in a small area. Human subjects can be scanned from head to foot in about 45 minutes, and laboratory animals in a proportionately shorter time. The active areas in the subject are shown as white areas on the resulting gamma ray picture.

The instrument has wide applications both in tracer research with gamma emitting isotopes and in clinical diagnosis of thyroid lesions and other conditions.

Acknowledgements

The author wishes to thank Dr. C. A. Tobias for his help and encouragement in this work. The assistance of Mr. Don Wehner in constructing the equipment is appreciated. The cooperation of Dr. Frank Pierce and Dr. John H. Lawrence in supplying patients is gratefully acknowledged. This work was done in the Biophysics group of the Donner Laboratory and was supported by the Atomic Energy Commission.

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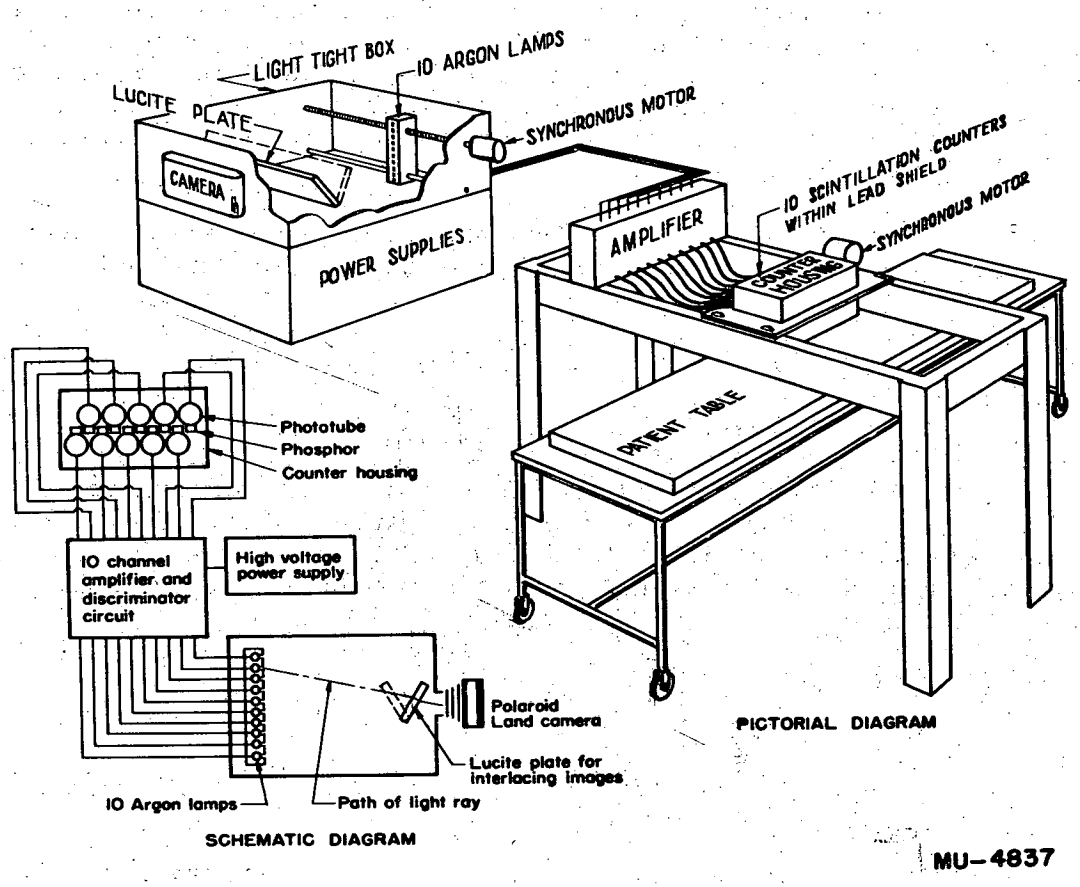
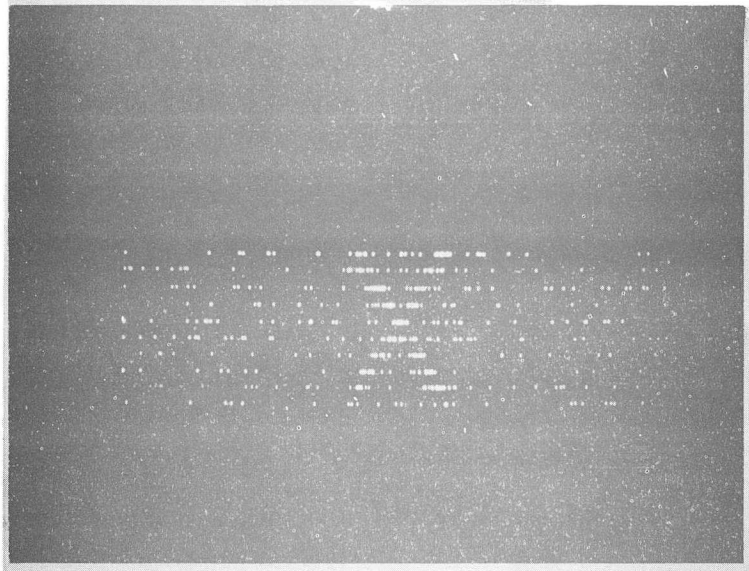
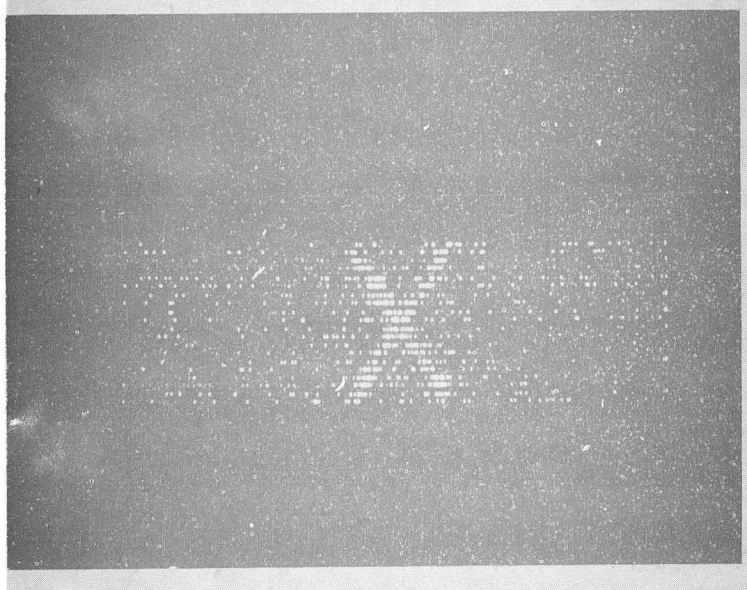

LIGHT TIGHT BOX
10 ARGON LAMPS
LUCITE PLATE
SYNCHRONOUS MOTOR
CAMERA
POWER SUPPLIES
10 SCINTILLATION COUNTERS WITHIN LEAD SHIELD
AMPLIFIER
COUNTER HOUSING
SYNCHRONOUS MOTOR
PATENT TABLE
Phototube
Phosphor
Counter housing
10 channel amplifier and discriminator circuit
High voltage power supply
Polaroid Land camera
Lucite plate for interlacing images
10 Argon lamps
Path of light ray
SCHEMATIC DIAGRAM
PICTORIAL DIAGRAM
MU-4837

Fig. 1



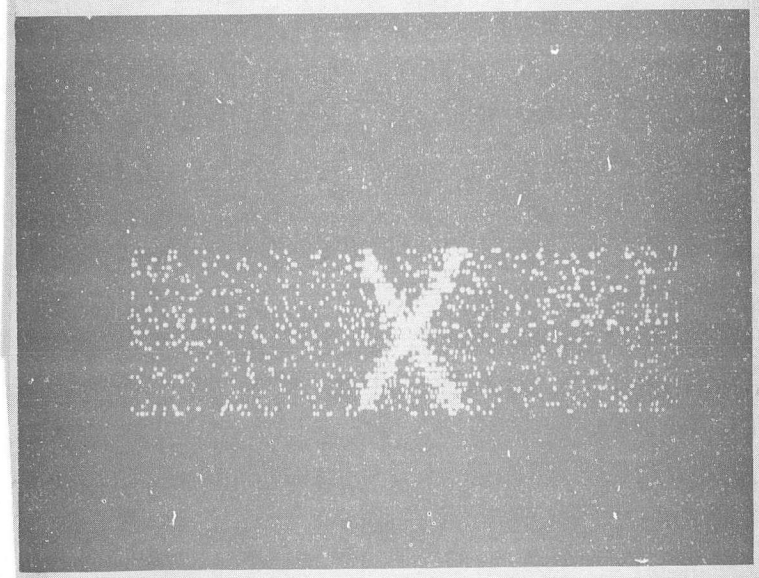
10 line image. Two minute scanning time. Ten microcurie sample 1 inch from counter housing.

(a)



20 line interlaced image. Four minute scanning time. Ten microcurie sample 1 inch from counter housing.

(b)

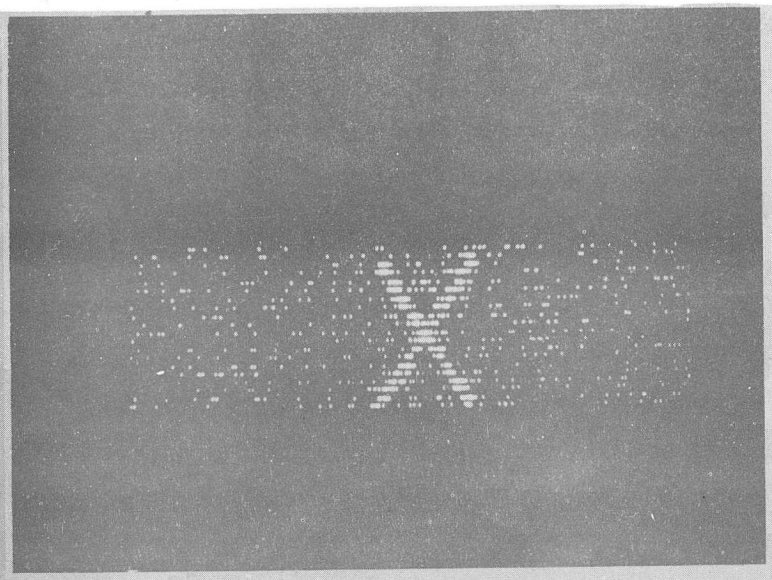


40 line interlaced image. Eight minute scanning time. Ten microcurie sample 1 inch from counter housing.

(c)

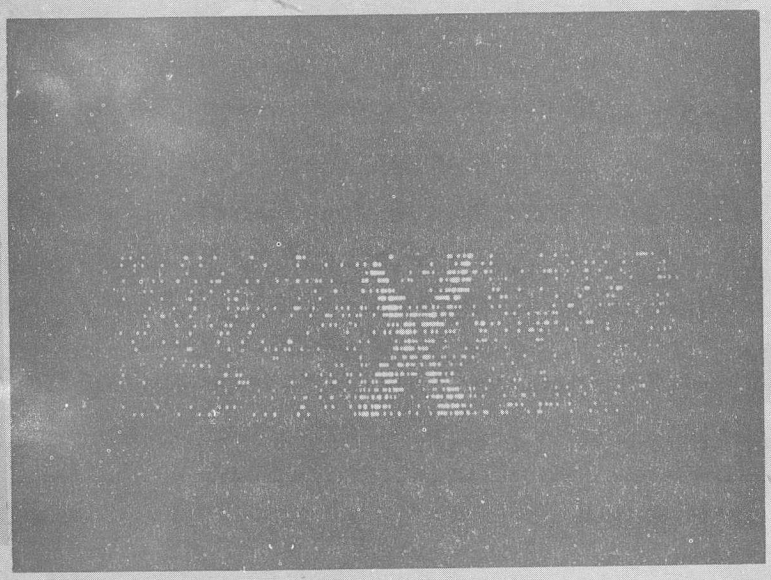
ZN-550

Fig. 2



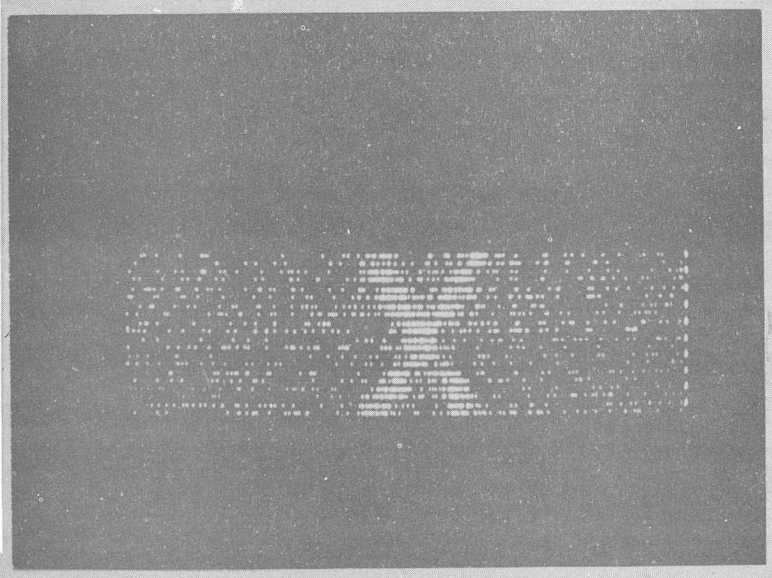
Ten microcurie sample 1/4 inch from counter housing. 20 line interlaced scan. Four minute scanning time.

(d)



Ten microcurie sample three inches from counter housing. 20 line interlaced scan. Four minute scanning time.

(e)

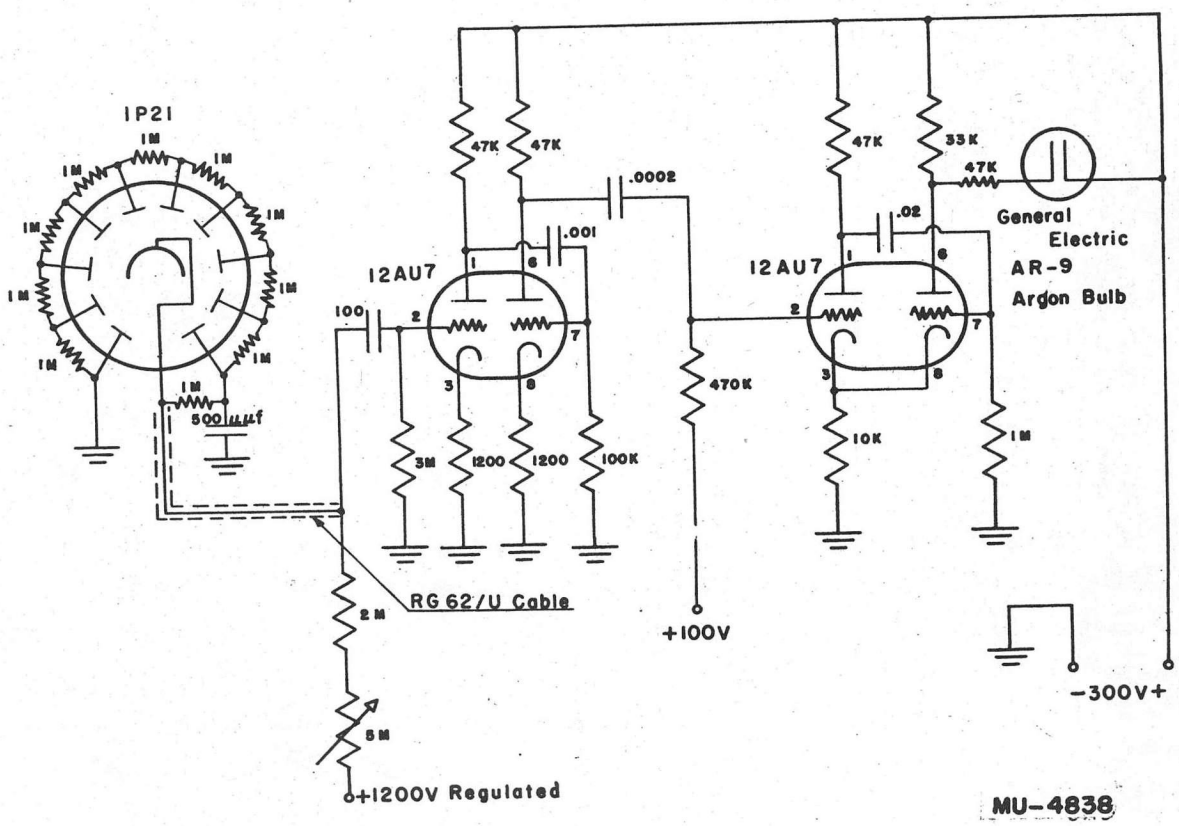


Ten microcurie sample three inches from counter housing. 20 line interlaced scan repeated once. Eight minutes total scanning time.

(f)

ZN-551

Fig. 2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12AU7
100
47K
.0002
.001
3M
1200
100K
470K
+100V
RG 62/U Cable
2M
5M
+1200V Regulated
33K
.02
10K
General
Electric
AR-9
Argon Bulb
-300V+
MU-4838

Fig. 3

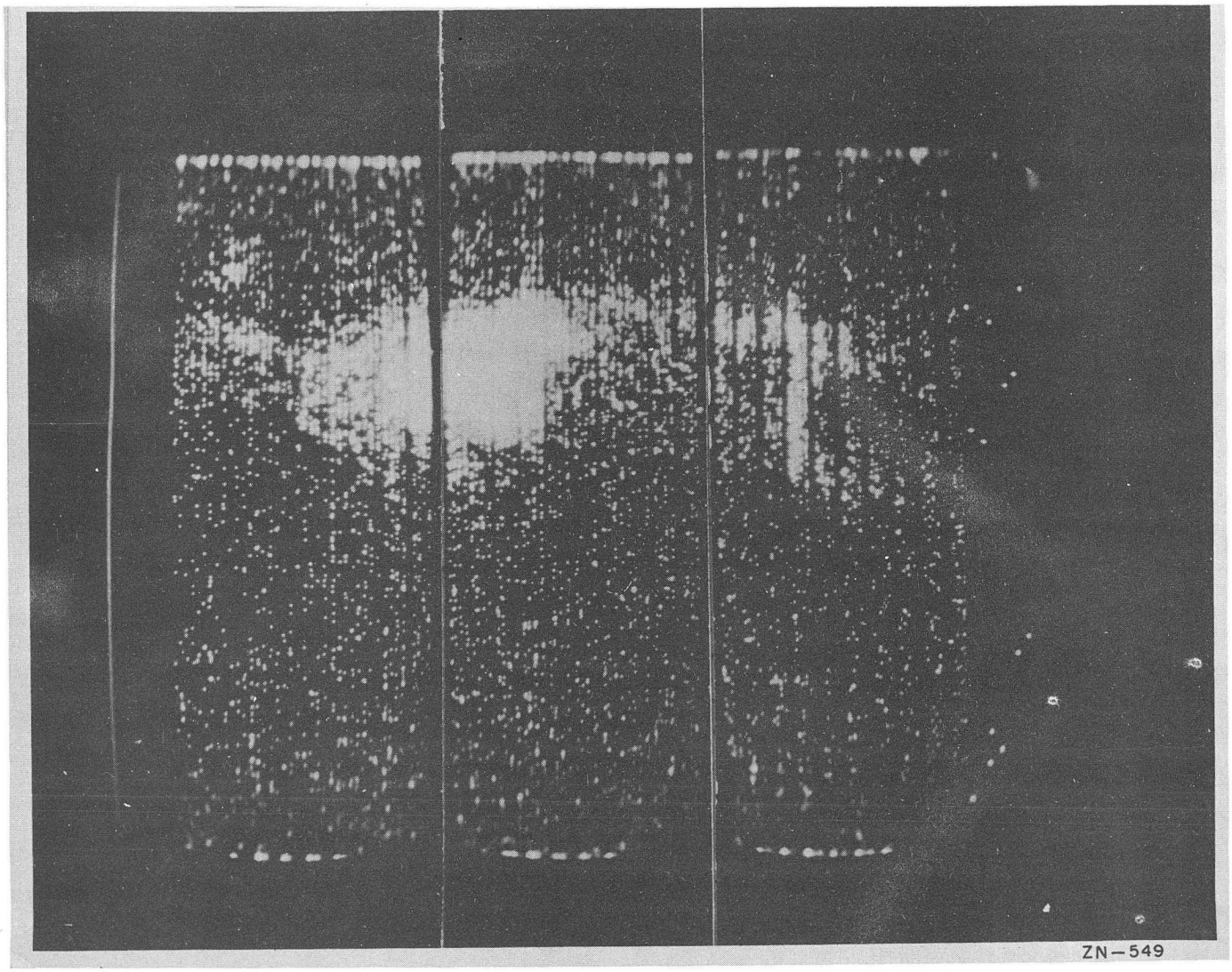

Fig. 4

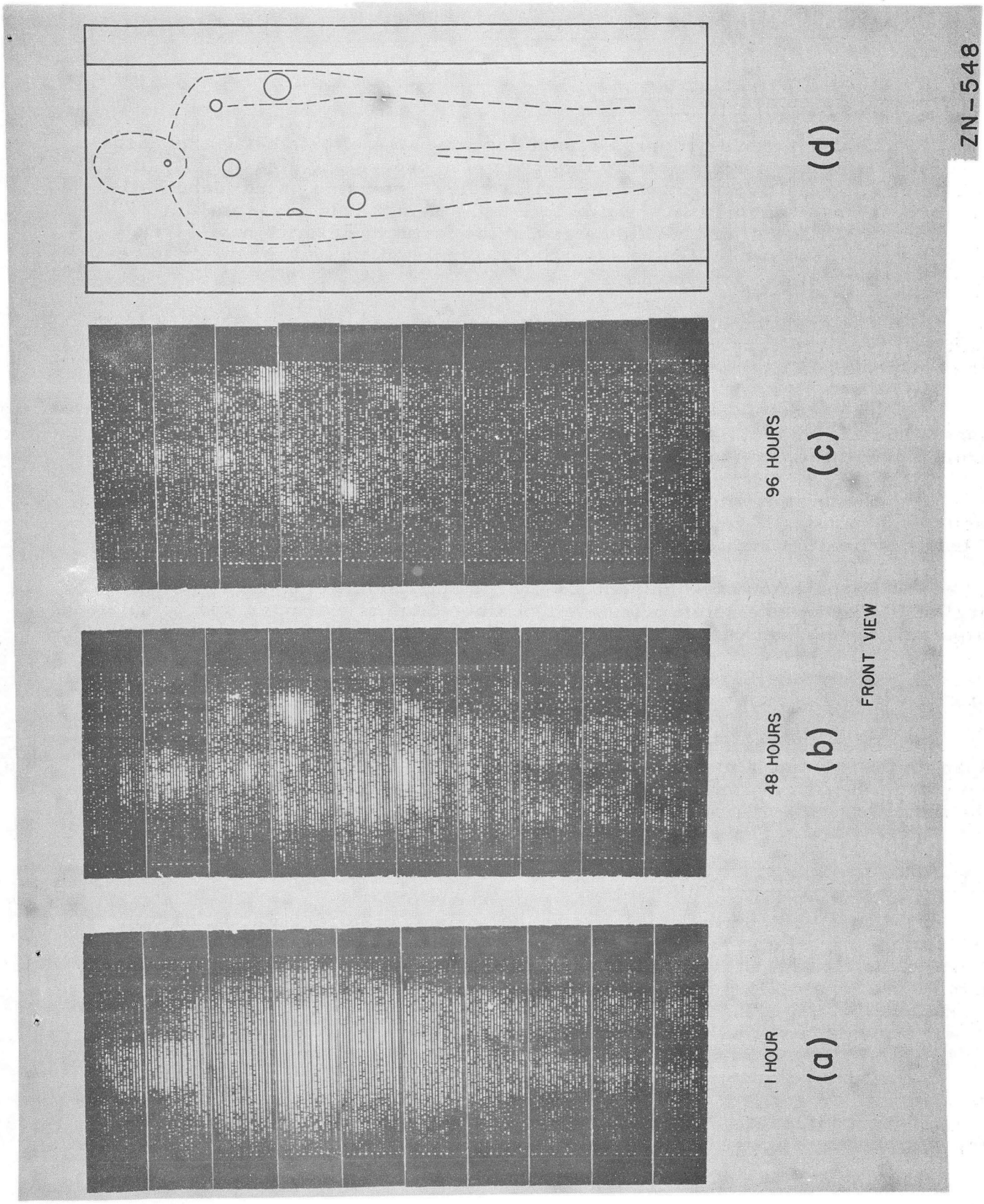

Fig. 5

III. HEALTH CHEMISTRY AND PHYSICS

Health Chemistry

N. B. Garden

Equipment Development Group

Last quarter's progress report described the efforts being expended on the construction of equipment by this group for processing high activity (multi-curie alpha and multi-curie beta-gamma) samples irradiated in the Idaho Falls reactor. It was reported that the two-inch and six-inch lead caves in Bldg. 5 were being readied for this work, details of the features of which have already been given. These two units are essentially completed and will be ready to receive the first sample this month.

The creation of shipping containers for these high-level samples from Idaho has required considerable attention and fabriction effort, and arrangements for transportation to and from the pile have been made by Health Chemistry.

The one-hundred-twenty-curie cobalt source was placed in its permanent housing in Bldg. 4A; the cobalt pellets were loaded into their container through use of the six-inch lead cave in Room 107, Bldg. 5.

Numberous small items created for use in radioactive chemical processing and handling have been made as usual during this period and are described in detail in Health Chemistry files.

Preparation of equipment for processing the larger quantity (500 gallons) of Hanford waste solution from which americium is extracted was completed; shipments of this material are expected to begin arriving in the very near future.

Forty-one Berkeley Boxes were assembled and fitted for radiochemical jobs during this period.

Special provisions were made for aerosol innoculation of high level alpah emitters using a monkey. This experiment was carried out satisfactorily and without contamination, using special boxes and equipment provided by Health Chemistry.

Activity Handling Group

The Pit Room in Bldg. 5, where the Central Storage Facilities for radioactive materials are located, has been renovated by repainting, and the doors to the pit have been modified for easier movement. To afford more room in this area, the milling machine assigned for milling radioactive materials, heretofore located in the Pit Room, has been moved to Health Chemistry's temporary storage facilities in the basement of Bldg. 29.

During this period a small bucket of uranium turnings, only partially covered with oil and left standing for several days in the Machine Shop, Bldg. 7, ignited spontaneously; although no damage occurred because of the fire, sufficient amounts of active oxides were deposited to necessitate the decontamination

of the entire surface of the shop and its contents. All groups in Health Chemistry, with other assigned help, accomplished this decontamination in several days without serious loss of work time in the shop. The Safety Group and Health Chemistry wrote up and distributed more detailed instructions on handling of uranium and thorium chips, calling attention to the fact that the test of commercial materials showed Pyrene G-1 (graphite and talc) to be satisfactory for quenching a fire of this type.

The uranium chips and turnings described in the last quarterly report are still being held pending instructions from the Atomic Energy Commission as to shipment to other sites where setups for processing this type of material are already in existence--likewise the uranium already converted to the oxide.

Active waste for disposal at sea continues to increase. Work has slowed down on the development of the process for solidification of active liquid wastes because it was necessary to divert manpower to UCRL-Livermore.

Monitoring

Regular routine monitoring functions have remained essentially unchanged, with the possible exception of the introduction of additional large quantities of alpha emitters into Bldg. 50 during this period. Routine use is now being made of the Jordan meters described last quarter. These are gamma survey meters whose range is 20 mr/hr to 500 r/hr; they will probably eventually replace the Zeus meters now in use. In response to our request the manufacturer has modified than for beta as well as gamma surveying.

Airborne Activity Control Group

The scrubber-aspirator (described in the last quarterly report) proved successful as a liquid transfer, off-gas neutralizer and condenser receiver exhauster. The flow rate is, however, too low for ventilation purposes above 2 cfm. The present limitation in the apparatus appears in the liquid pump and in the foaming of NaOH solutions.

A gas scrubber in which the scrubbing liquid is circulated through a column composed of saran fibers and driven by an air-lift has been devised and tested up to about 8 cfm with better than ninety percent recovery against HCl evaporations. Four units have been ordered for installation at Livermore. No pump is required for this installation, and dimensions represent a considerable space saving over the equipment described in the previous paragraph.

In view of the fact that the two portable 600 cfm emergency blower units already on the project have been in constant demand--in the Decontamination House, during the burning of uranium chips, during machining of uranium and in many other areas--a third portable unit, with a few minor improvements, has been ordered.

A box manifold exhaust system was designed and installed in the basement of Bldg. 29, which has been given to Health Chemistry for the temporary storage of active gloved boxes not currently but presently to be in use.

A long-term attempt to locate low-level airborne alpha contamination in Bldg. 5 was successful when a cave hood leak due to failure to close a filter plenum door was discovered. About 300 square feet of attic carpentry was contaminated. Decontamination by vacuum cleaning reduced the level to about 2000 c/m per 120 square centimeter, and this was painted down.

Considerable time was devoted to Livermore Bldg. 161 ventilation and airborne contamination problems in conferences with architects and chemistry personnel, together with conferences on other prospective structures there.

General

Increase in work at UCRL-Livermore has necessitated the dispatching of more Health Chemistry personnel to that area. New members are being employed, and when clearance is received, they will be trained expressly for this purpose. In the interim the experienced members are carrying out their activities in both areas; in many cases requiring double shift work.

Nelson Garden and Elmer Nielsen attended a conference on engineering for the control of radioactive materials at Oak Ridge during October, where a talk was presented by Dr. Nielsen.

Health Physics

B. J. Moyer

Statistical Summary of Monitoring Program

Survey Instruments Maintained

1.	B-γ Ionization Chamber	43
2.	Victoreen 263 Meters	19
3.	I.D.L. Portable Survey Instruments	20
4.	Cutie Pies	3
5.	Recording γ-Intensity Meters	20
6.	Victoreen Proteximeter	3
7.	Fast Neutron Proportional Counters	6
8.	Slow Neutron Proportional Counters	15
9.	Fast Neutron Proportional Counter (Portable)	6
10.	Slow Neutron Portable Unit	3

11. Balanced Chamber - Fast Neutron - Portable 1

12. Special Tissue Wall Survey Instrument 1

Personnel Meters in Use

1. Total Personnel Covered with Film Badges 2,150

2. Total Man Days Coverage with Pocket Chamber 2,810

3. Total Man Days Coverage with Pocket Dosimeters 3,012

4. Total Man Days Coverage with Pocket Chambers (S.N.) 3,001

Cases of Weekly Exposure above 0.3r

Weekly Film Expos. above	184" Area	60" Area	Lin. Acc.	Chem.	Other	Total
0.3	1	11	9	29	8	58
0.5	0	5	3	16	2	26
1.0	0	2	0	5	0	7
1.5	0	1	0	1	0	2
2.0	0	1	0	1	0	2
2.5	0	0	0	0	0	0

Portable Fast and Slow Neutron Survey Meter

A portable fast and slow neutron survey meter was recently put into operation. The instrument employes two counters; a polyethylene walled proportional counter and a BF_3 proporational counter. With a simple switching arrangement each counter output may be read on a count rate meter. The counter high voltage is supplied by a vibrator-type supply. Three range positions are provided on the count rate meter indicating from 25 to 40,000 slow neutrons/cm^2sec and from 5 to 4,000 fast neutrons/cm^2sec (Po-Be neutrons).

Information Division
2-26-53 bw