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July, August, September, 1953

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MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

July, August, September, 1953

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MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

July, August, September, 1953

THE METABOLIC PROPERTIES OF VARIOUS MATERIALS

Joseph G. Hamilton, M.D.

TRACER STUDIES

LANTHANIDE AND HEAVY ELEMENTS

J. G. Hamilton, Patricia Durbin, Marilyn Hemenway, Maggie Gee, Ruth Newman and Gretchen Thilo

Tracer studies are in various stages of progress employing 275-day cerium^{144} in the carrier-free state, 120-day thulium^{170} of high specific activity, 11.2-day radium^{223} and the 18.6-day thorium^{227} obtained from an equilibrium mixture of actinium^{227} and the 45-day iron^{59} of relatively high specific activity. The iron^{59} and thorium^{227} studies have not been completed to the point where it is felt that they should be considered in this report in any detail. However, the cerium^{144}, thulium^{170} and radium^{223} studies are well under way and will be discussed here.

The investigation of representative members of the light and heavy members of the lanthanide series has arisen because of several considerations: first, the early experiments in this laboratory employed relatively small groups of animals, and in reviewing the data from these experiments we noted several rather large inconsistencies; and second, in many instances the radioelement employed was of a very low degree of specific activity, and 100 micrograms or more often had to be administered. Moreover in many cases no complexing agent was used, and the amount left unabsorbed from the site of the injection was often considerable, thus obscuring the picture of the actual metabolism of these radioelements.

Existing data indicate that the metabolism of the lanthanide rare earths follows the pattern often called the "lanthanide contraction". This phenomenon is a result of the decreasing basicity of the rare earth hydroxides, and in the region of terbium one may expect the metabolism of these earths to be quite like that of carrier-free yttrium, zirconium, thorium and plutonium. The last-mentioned element presumably remains in the body in the tetravalent state. Since large amounts of rare earths now in use in other laboratories may very well include some of the heavier members of the series, and consequently may present a potential health hazard, it was felt that a more careful and accurate study of their metabolic properties might prove fruitful.

Certain members of the actinide group, notably americium, curium and actinium, the metabolic characteristics of which are almost indistinguishable from one another, are potential health hazards. The fact that these three trivalent elements are metabolically indistinguishable from the lighter members of the lanthanide series is a factor that has prompted us to undertake studies of the relative biological effects of the alpha and beta emitters.

All the radioelements for which data appear were administered by the intramuscular route into the left hind leg. The cerium^{144}, thulium^{170} and thorium^{227} were given in the form of citrate complexes. The radium^{223} was administered presumably as the chloride, since it was anticipated that the absorption of radium from the injection site would be nearly complete. The animals used in these experiments were females of the Sprague-Dawley strain and ranged in age from 80 to 100 days and in weight from 200 to 250 grams. At least five rats were used for each time interval. Larger numbers of animals would have been desirable from the statistical point of view, but at the present time space, facilities and personnel are not available to permit experiments involving larger numbers of animals.

Cerium

The time intervals investigated in the carrier-free radiocerium studies were 1, 4, 64 and 256 days postinjection. Each animal received 30 microcuries of radiocerium complexed with sodium citrate in isotonic saline. With the exception of the one-day studies in which all the rats were placed in one metabolism cage, the animals were maintained in groups of two and three in order to obtain duplicate determinations on the excretion rate. Urine and feces were separated by the usual means and were collected every three to four days for the first month and once a week thereafter. It will be noted that in general the procedures employed in the radiocerium studies are the standard procedures employed in this laboratory and apply to the other tracer studies as well as to the radiocerium.

The tissues and excreta were ashed in a muffle furnace at 500° for 25 hours. The tissues and organs taken were those listed in Tables I, II and III. As in other experiments the "balance" represents the skeletonized, skinned, partly eviscerated carcass from which has been subtracted the calculated values for the total blood and muscle. The blood volume is assumed to be 4.6 percent of the total body weight, and the muscle mass to be 45 percent of the total body weight. These same values were used in the other tracer experiments employing radiothulium and radium^{223}.

The samples were counted with a sodium-iodide crystal gamma counter, thus avoiding the necessity of self-absorption corrections. Also included in Table I are the actual measured recoveries and the percent of the administered dose unabsorbed from the injection site.

Results

The results agree quite well with those observed in earlier experiments, but the reliability of the observed values has been considerably increased. Attention should be drawn to the fact that the concentration of radiocerium in the spleens of both the 1- and 4-day groups was on a percent per gram basis only about 1/50 that of the concentrations in the liver. This would certainly seem to rule out the possibility of the presence of any of the radiocerium as a radiocolloid. Almost invariably when radioelements that form highly insoluble hydroxides are given intravenously as halide salts there tend to be comparable concentrations per gram of the radioelement in the spleen and liver. It is felt that when these radiocolloids are formed, the true picture of the metabolism of the material is greatly distorted.

Table I

The Deposition of Carrier-Free Cerium^{144} Complexed with Sodium Citrate in the Rat at 1 and 4 Days Following Intramuscular Injection. Values are Corrected for 100 Percent Recovery and Expressed in Percent of Administered Dose. The Standard Error of the Mean for the Liver and Skeleton is Shown at the Bottom of the Table. Each Rat Received 20 Microcuries of Cerium^{144} and 3.0 Milligrams of Sodium Citrate.

Tissue	1 day		4 days	
	% per organ	% per gram	% per organ	% per gram
Spleen	0.08	0.17	0.10	0.11
Blood	0.06	<0.01	<0.01	-
*Liver	53.7	7.42	51.0	6.57
Kidney	1.60	0.99	1.45	0.76
G.I. Tract	0.76	0.15	1.01	0.11
G.I. Contents	0.63	-	1.77	-
**Skeleton	28.5	1.52	27.7	1.40
Muscle	1.56	0.02	1.78	0.02
Balance	4.99	-	2.01	-
Skin	1.26	0.04	1.01	0.04
Urine	5.77	-	5.95	-
Feces	1.03	-	6.21	-
*Mean Std. Error	±3.1	±0.50	±0.67	±0.18
**Mean Std. Error	±1.9	±0.08	±0.9	±0.07
Injection Site	10.2		6.15	
Actual Recovery	98.2		89.6	

At the 1- and 4-day intervals the only tissue, apart from the liver and skeleton, that shows a relatively high concentration of radiocerium is the kidney. From previous experience it is predictable that the concentrations in both the kidney and liver will drop fairly rapidly, while the radiocerium deposited in the skeleton will remain relatively constant.

The absorption from the injection site was quite rapid when radiocerium was complexed with sodium citrate; 90 percent of the dose was absorbed in 24 hours. This is gratifying, and the results of these experiments should give a clear concept of the metabolic properties of this element. Another interesting point is that the urinary excretion occurs almost entirely in the first 24 hours; at the 4-day interval the total amount that has been excreted by this route is little more than it was on the first day. However, during the interval from the first to the fourth day postinjection the fecal excretion rises rapidly. Presumably the radiocerium that is excreted by the gastrointestinal tract entered that compartment from the liver via the bile duct.

Thulium

The thulium studies are being carried out in much the same fashion as the radiocerium studies discussed above, except that only 3 microcuries was given to each animal so as to reduce the amount of stable thulium. The solution contained 3 milligrams of sodium citrate and was isotonic. Six microcuries of thulium^{170} complexed with sodium citrate was given to the 8-month group. The gamma activity associated with its radioactive decay is so weak that it was necessary to measure the beta activity of the samples. Accordingly the appropriate self-absorption corrections have been applied.

Results

Several pertinent observations may be made for the results given in Table II. The initial uptake by the liver is approximately 1/15 that observed for cerium and the skeleton uptake is nearly twice as great as that of the radiocerium. The high urinary excretion is of interest, and very possibly a part of what was found in the feces could be urinary contamination. The other tissues and organs did not show any striking differences from those observed for cerium. The possibility of the presence of radiocolloid was not likely in view of the low concentration of radiothulium in the spleen. Another consideration is that the absorption from the site of injection was approximately the same as in the case of radiocerium. The possibility exists that the citrate ion complexed thulium more tightly than it did radiocerium, and some may have been excreted in the complexed state. It is not deemed sufficiently significant to distort the experiment, since most of the urinary excretion occurred during the first 24 hours.

ACTINIUM SERIES

A program to investigate the metabolism and radiotoxicity of actinium^{227} has been felt of such interest that it is worth the expenditure of a considerable fraction of our effort. The early tracer studies done here with actinium, which have been presented in previous reports, are reasonably adequate; however, certain points were not taken into account. One of the reasons for embarking on the present program is that in the study of the radiation effects of a member of a radioactive series that decays to give daughter activities of appreciable half-lives, some attention should be given to the daughter products as well.

Table II

The Deposition of Thulium^{170} Complexed with Sodium Citrate in the Rat 1 and 4 Days After Intramuscular Injection. Values are Corrected for 100 Percent Recovery and Expressed in Percent of Administered Dose. The Standard Error of the Mean for the Liver and Skeleton is Shown at the Bottom of the Table. Each Rat Received 0.4 Micrograms of Thulium.

Tissue	1 day		4 days	
	% per organ	% per gram	% per organ	% per gram
Spleen	0.12	0.20	0.13	0.22
Blood	0.16	0.02	0.02	<0.01
*Liver	3.40	0.46	1.86	0.24
Kidney	1.43	0.92	1.07	0.64
Gastrointestinal	0.81	0.12	0.59	0.10
G.I. Contents	1.28	-	0.50	-
**Skeleton	64.8	3.09	64.1	2.72
Muscle	1.96	0.02	1.59	0.01
Balance	3.63	-	1.98	-
Skin	1.53	0.03	1.33	0.03
Urine	16.9		22.1	-
Feces	4.00		4.66	-
*Mean Std. Error	±0.10	±0.01	±0.18	±0.04
**Mean Std. Error	±0.5	±0.06	±0.3	±0.09
Left Leg	5.31		5.15	
Actual Recovery	96.6		95.0	

In this case the daughter activities of importance are the 11.2-day radium^{223} and the 18.6-day thorium^{227}. When actinium^{227} is introduced into the circulation in a noncolloidal state, roughly 25 percent is initially deposited in the skeleton and over 50 percent in the liver. In the cases of thorium^{228} and radium, nearly 60 percent is deposited in the skeleton and very little in the liver. Both the actinium^{227} and thorium^{227} concentrations in the skeleton remain fairly constant over long periods of time, whereas the actinium^{227} in the liver is lost quite rapidly. Consequently the probability of translocation of the daughter products from the initial site of the deposition of actinium^{227}, in other words from the liver to the skeleton, must be considered. Actinium^{227} is a highly dangerous radioelement, as has been shown by a large-scale investigation at the Los Alamos Laboratories, since most of its decay products are alpha emitters and a total of nearly 30 Mev of energy is released before a stable isotope of lead is reached. Accordingly it was deemed necessary to perform some tracer studies employing the thorium^{227} and radium^{223} daughters so that accurate information on the metabolism of these two elements might be obtained with carrier-free material. These studies are now well under way, and when they are completed a study on the possible translocation of the daughter products from the initial site of a deposition of actinium will be initiated. Translocation has been suspected for some time, but up to the present has not actually been investigated.

The customary procedure when actinium^{227} is employed in animal investigations is to inject actinium^{227} as the equilibrium mixture, sacrifice the animals at various time intervals, ash the tissues, and then wait until the daughter product initially present has decayed away and a new equilibrium has been established. In the contemplated studies pure actinium^{227} will be administered, the animals will be sacrificed at intervals ranging from a few days to several months, the actinium^{227}, thorium^{227}, and radium^{223} will be separated by chemical means from the tissue samples, and a mathematical analysis will be employed in order to determine whether the daughter products remain at the site of their formation or, once formed, follow their own distinct metabolic patterns.

Radium^{223}

Radium^{223} was separated from actinium^{227} and thorium^{227} by the use of an ion exchange column employing Dowex 50, and 2N nitric acid was employed as the eluant. After the eluant had been collected sodium chloride was added and the mixture was evaporated to remove the nitric acid and finally redissolved in distilled water for injection at pH 3.5 and was isotonic. The radiochemical purity was established by determining decay curves, which ruled out the presence of any more than insignificant quantities of either actinium^{227} or thorium^{227}.

These tracer studies were done in a manner similar to the radiocerium and radiothulium studies, except that no sodium citrate was used except in the 64-day group of animals, which have not as yet been sacrificed. The 1-, 8-, and 16-day groups of animals did not receive sodium citrate, for it was believed at the time these experiments were initiated that the radium chloride would be rapidly absorbed from the site of injection, particularly as the isotope employed has such high specific activity.

The samples, after being ashed were counted with the sodium iodide crystal counter, as some of the short-lived decay products emit energetic gamma rays. There are no daughter products of radium^{223} having sufficient half-lives to present any problems of translocation. The good recoveries indicate that no significant loss by exhalation of the 3.9-second emanation^{219} occurred.

Results

The data displayed in Table III for the first three time intervals show the relatively rapid loss of radium from the soft tissues and its selective location in the skeleton. The relatively high level of material at the injection site was unexpected. The 64-day group of animals received the radium in a solution of 1.5 milligrams of sodium citrate in isotonic solution. Even though the data were corrected for the material remaining at the site of injection, there appeared to be a fairly rapid rate of elimination from the skeleton by way of the kidney and digestive tract for the first 16 days. A further evaluation of this information must await later tracer experiments. There is the possibility that, if this effect is real, it may be related to the high specific activity of the isotope employed as compared to radium^{226}. The specific activity of the radium^{223} used in these experiments is nearly 50,000 times that of radium^{226}.

Table III

The Deposition of Radium^{223} in the Rat at 1, 8 and 16 Days Following Intramuscular Injection. Each Rat Received 1.5 Microcuries of Radium^{223}. Values are Corrected for 100 Percent Recovery and Expressed in Percent of Administered Dose. The Standard Error of the Mean for the Liver and the Skeleton is Shown at the Bottom of the Table.

Tissue	1 day		8 day		16 day	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Spleen	0.05	0.08	0.01	0.02	0.01	0.02
RBC	0.10	0.01	<0.01	-	<0.01	-
Plasma	0.21	0.03	<0.01	-	<0.01	-
*Liver	0.27	0.04	0.08	<0.01	0.07	0.01
Kidney	0.44	0.25	0.08	0.07	0.05	0.02
G.I. Tract	1.72	0.17	0.22	0.02	0.08	<0.01
G.I. Contents	8.21	-	1.17	-	0.44	-
Muscle	1.97	0.02	0.39	<0.01	0.68	<0.01
Femur	-	2.75	-	2.67	-	2.18
Tibia	-	3.00	-	3.54	-	2.79
Cranium	-	-	-	-	-	2.18
Costochondral Junction	-	0.48	-	0.44	-	-
**Skeleton	63.4	3.41	53.3	2.83	46.7	2.27
Balance	1.98	-	1.14	-	0.45	-
Skin	0.89	0.03	0.22	<0.01	0.13	<0.01
Urine	8.65	-	15.0	-	17.6	-
Feces	12.1	-	28.3	-	33.6	-
*Mean Std. Error	±0.02	±0.006				
**Mean Std. Error	±0.6	±0.05	±0.76	±0.08	±0.55	±0.06
Injection Site	42.2		14.4		8.90	
Actual Recovery	103.2		99.1		95.0	

ASTATINE^{211}

J. G. Hamilton, Marshall Parrott, Muriel Johnston and Patricia Durbin

Recently it was suspected that some of the preparations of astatine^{211} contained colloidal material, and it could be confirmed by the Tyndall effect. Subsequently this problem was approached from several directions. The suspension was spun down in a centrifuge and subjected to spectrographic analysis. This analysis revealed some surprising results: both silicon and beryllium were found to be present; also, much of the astatine was lost in the precipitate. The isolation of astatine from biological material has been described in a paper by Garrison et al, in Analytical Chemistry 23, 204, 1951. However, neither in this paper nor in the more extensive report, "Accumulation, Metabolism and Biological Effects of Astatine in Rats and Monkeys", Hamilton et al, U.C. Press, 1953, was there discussion of actual details of the isolation of astatine from a bismuth target following its bombardment with alpha-particles from the 60-inch cyclotron at the Crocker Laboratory. Such details are offered here.

In brief, an all-quartz, oxygen-free system is used; it is continuously evacuated by a mechanical pump, and a small amount of inert gas such as nitrogen, argon, or helium is allowed to flow through. The astatine is collected on a quartz finger, inserted into this sealed system, that is cooled by liquid nitrogen. The astatine is volatilized from the bismuth target by an electric heating furnace and then accumulated on the cold finger. In order to protect the quartz from the bismuth target, mica had been used as an insert. After it had been discovered that beryllium was present in the astatine preparation, the mica was no longer employed; a quartz boat was used instead. Silicone stopcock grease had been used on several of the ground joints in the system, but when silicon was found in the final preparation of astatine use of this grease was also abandoned. Under these conditions, and with no possibility of oxidation of bismuth in the system, very clean preparations may be obtained. The difficulties caused by contamination were not encountered in the early work, as no attempts were made to secure large quantities of astatine and the temperature was kept below 350° C. In recent experiments the temperature was increased to approximately 600° C, which increased the yield by a factor of five or more, but also increased the probability of contamination or formation of a colloid.

In view of these problems, which were faced and finally solved, a series of tracer studies was done. When high temperatures, which approached the volatilization point of bismuth, were employed and the mica insert and the silicone grease were used, approximately 2 percent per gram of the intravenously administered dose appeared in the liver and spleen after four hours, which suggested that something of the order of 20 percent of the astatine was in the colloidal state. When lower temperatures were used--in the range of 350° C--the uptake in the liver was in the range of 0.3 percent per gram, and a corresponding value was observed in the spleen. It was of interest to note that the accumulation of astatine by the thyroid gland was in the range of one percent in either situation.

The possibility that part of the astatine may be colloidal is naturally of concern if we attempt to make significant comparisons between biological effects of astatine--an alpha-emitter--and of a noncolloidal beta-emitter or

x-rays. In view of this possibility, we prepared radioautographs of the liver 18 hours after administration of material prepared in a manner that produces the colloidal effect. Figures 1 and 2 show alpha-particle stars that must represent colloidal aggregations containing many hundreds of atoms of astatine. Since the tissue sections were only 5 microns thick, the small number of alpha-particle tracks seen represents only a small portion of the disintegrations that took place after the 18-hour period. Moreover, by the time the sections were mounted on NTA film nearly three half-lives of astatine^{211} had elapsed, so that the remaining activity was but a little more than one-eighth of that originally present. Thus a far greater number of astatine disintegrations took place than can be observed in the stars in the film. It is apparent that possible radiation effects of astatine^{211} in a colloidal state--which seems to concentrate in the liver, spleen, and bone marrow--cannot be compared to those of a radioelement not in a colloidal state, which should be quite differently distributed throughout the body.

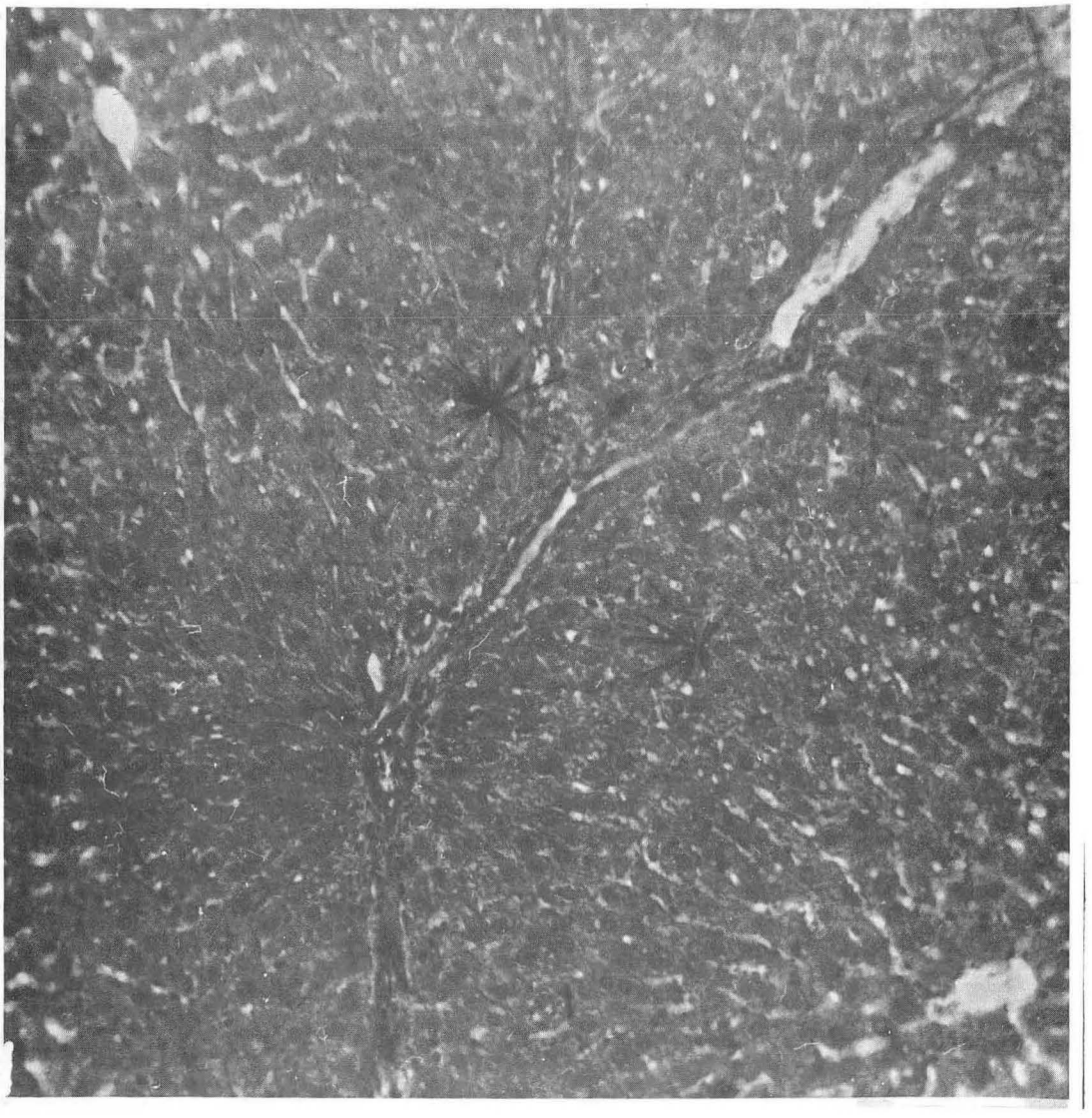

Fig. 1 An NTA radioautograph of astatine^{211} in the liver. Two stars may be seen, which are evidence of astatine in the liver in the colloidal state. (Magnification x 92).

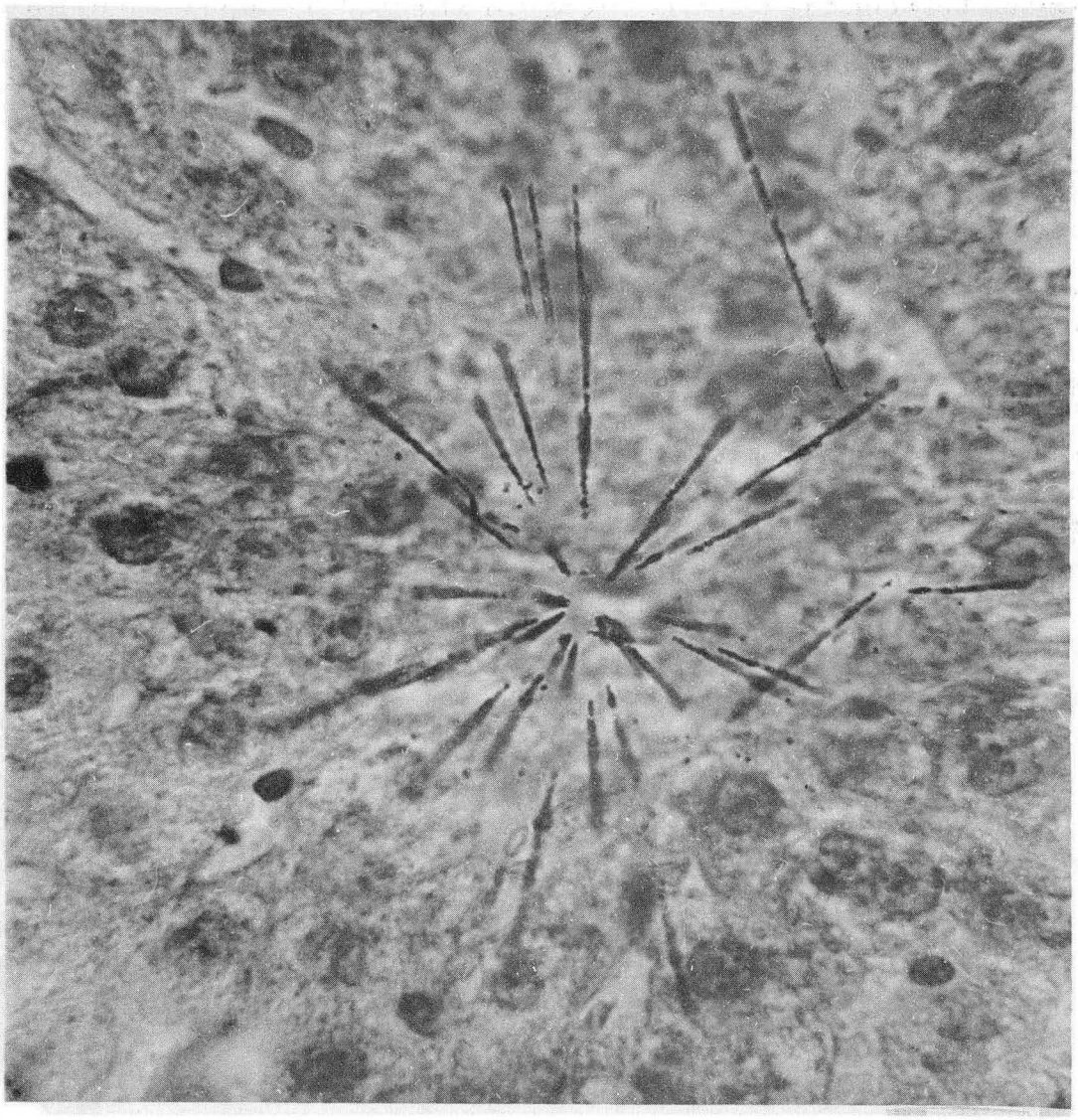

Fig. 2 A higher magnification of the alpha-particle tracks from an invisible colloidal particle containing astatine from the liver of a rat. (Magnification x 300).

COMPARISON OF THE RBE OF ALPHA- AND BETA-PARTICLE IRRADIATION OF THE HEMATOPOIETIC SYSTEM

J. G. Hamilton, Patricia Durbin and Nylan Jeung

A by-product of the experiments with actinium and its daughter products just described will give an opportunity, using radium^{223}, to make a direct comparison between the relative biological effect of the alpha-particles emitted by this isotope and the beta-particles of barium^{140}. The comparison should be a very interesting one, since these two isotopes have very nearly the same half-lives (radium^{223}, 11.2 days and barium^{140}, 12.8 days), and very similar metabolic patterns.

Groups of ten female Sprague-Dawley rats approximately 100 days old have been injected intramuscularly with radium^{223} in a sodium citrate solution at dosage levels of 0.035 and 0.07 microcuries of radium^{223} per gram of body weight. Red-cell counts, hemoglobin and hematocrit determinations, white-cell counts, and differential white counts are being made weekly. The animals are also being weighed periodically. When barium^{140} is available, a parallel experiment using the barium^{140}-lanthanum^{140} mixture will be undertaken in which the doses administered will be based on the fact that in its decay barium^{140} produces only 1/37 of the amount of energy produced in the decay of radium^{223}. Translocation of the lanthanum daughter will also be investigated. The members of the chain decay of radium^{223}, with the exception of 36-minute lead^{211}, have such short half-lives that translocation can be neglected. For lead^{211} translocation should not be of significance, for even if translocation should take place, emitted beta-particles would have an approximate average energy of 0.45 Mev.

The details of the results of the radium^{223} experiments will appear in the next quarterly report.

After the translocation studies with actinium^{223} have been evaluated, then if this effect is small, a direct comparison of the radiobiological effects of this alpha-emitting radioisotope and its radioactive descendants may possibly be made using promethium^{147}, which is a beta-emitter.

HISTOPATHOLOGICAL EFFECTS OF ASTATINE^{211}

J. G. Hamilton, C. W. Asling, Muriel Johnston and Patricia Durbin

Rats

Autopsies were performed on four female Long-Evans rats who had each received 0.70 microcuries of astatine^{211} per gram by intraperitoneal injection a year before the time of sacrifice. The animals had been approximately 6 weeks of age at the time of injection. The objective of this experiment was a survey to determine long-term histopathological alterations that might have resulted as radiation effects from the astatine^{211}, and also to note what changes had developed in consequence of the long period of presumptive hypothyroidism. Brief reference was made to these animals in past reports, but none of the details of the pathological changes were then available.

The following tissues and organs were secured: heart, aorta, stomach, intestine, salivary gland, thyroid and parathyroid glands, lacrimal gland, lymph nodes, spleen, liver, thymus, kidney, pituitary, adrenals, skin and skeleton. For the skeleton, x-rays were made of the entire body in an attempt to evaluate possible changes that might have occurred.

The spleen revealed some reduction in size of the malpighian corpuscles, and there appeared to be a reduction of lymphocytes in the spleen in one animal. The livers of most of the four animals showed small areas of cellular degeneration and a porous replacement by some lymphocyte infiltration. These areas were usually less than half the diameter of magnification at 300X, and these were frequently located about midway between portal space and central vein. The significance of these areas is not clear, but they may have been due to the presence of astatine in a colloidal state. The thymus was atrophic, but this is probably not of significance when the age and endocrine deficiency of the animals are considered. By and large the kidneys were apparently normal, but about one-fourth of the glomeruli appeared avascular, fibrotic and shrunken, a circumstance that may have been due to radiation damage, but--in view of the small number of animals--cannot positively be ascribed to radiation injury.

In an attempt to evaluate the physiology of the thyroid gland, each of the four rats received 5 microcuries of carrier-free radioiodine by intraperitoneal injection 24 hours before sacrifice. In addition to the regular histopathologic preparations, NTB and NTA radioautographs also were prepared from the thyroid tissue of these four animals. Total accumulation of radioiodine by the thyroid gland ranged from 0.009 percent to 0.17 percent of the administered dose. The normal accumulation of carrier-free radioiodine in the rat given stock diet and tap water is usually at the level of 10 to 20 percent of the administered dose at the end of 24 hours. In three of the four animals thyroid tissue was not recognizable as such. It had been almost totally replaced by fibrous connective tissue, or tissue which appeared to be adipose in character and full of minute fat vacuoles. Some of the cells that resembled connective tissue appeared to accumulate radioiodine, as demonstrated by the radioautographic technique. The animal in which the thyroid accumulation was the greatest showed a few small fragments of recognizable thyroid epithelium. No cells were seen to resemble Hürthle cells, which might have

appeared as a result of the intense and brief radiation that took place followed by an observation period of a year. Obviously this point cannot be evaluated until more data are obtained.

The parathyroid tissue in all four animals appeared to be normal. The pituitary gland in all four animals showed definite change; the eosinophiles had lost their normal granular structure, which is a typical "thyroidectomy" response. In all, with the exception of one animal, the basophiles showed degeneration, and cells were found which were large vacuolated basophiles and were filled with basophilic colloid. The adrenals showed some changes in that the cortex was thinned, and strands of non-fat-containing cells invaded the fas ciculata from the glomerulosa.

Examination of the skin indicated that the epidermis was thinned more than was normal, as might be expected in hypothyroidism. Here again evaluation is not easy, as the epidermis varies markedly and depends on the location from which skin specimens are taken.

Histopathological studies are still in progress, using celloidin for representative regions of the skeleton. As indicated earlier, total-body x-rays were taken and changes may be seen that may prove significant. A careful study of the x-ray pictures of the skeleton indicated a bone age of from 80 to 120 days as contrasted to the chronological age of over 400 days. This evaluation is based upon a patent epiphyseal plate of the calcaneus, partial fusion of the metatarsal epiphysis, a patent epiphyseal plate at the proximal end or the radius, and epiphyseal fusion of the metacarpals. These changes seem to be a result of hypothyroidism rather than of direct radiation effects.

In an extension of the preliminary long-term histopathological experiments, Female Sprague-Dawley rats weighing 110 to 130 grams are being used, fed on standard laboratory chow with access to tap water ad libitum. They are used in groups of 10. The attempt was to obtain approximately an MLD_{50} dose of $astatine^{211}$ at 30 days, and then allow the survivors to live to from 6 to 12 months to observe some of the long-term effects. It seems, however, that they either die in a much shorter time or survive.

The usual procedure is to administer the astatine in the -1 valence state by either the intraperitoneal or the intravenous route. Several dose levels have been selected in the range of from 1.1 to 1.85 microcuries per gram. Three have been used: 0.83, 1.1, and 1.83 microcuries per gram. The weight changes for 0.83 and 1.83 μc/g in contrast to the control group are shown in Fig. 3. From the present information it would appear that the MLD_{50} is in the range of approximately 1.5 microcuries per gram, although some allowance must be made for the fact that approximately 20 percent of the astatine used for the group was presumably in the colloidal state; experiments that are so far more satisfactory are now being done, using astatine in which there is presumably no colloid present.

From these studies a number of interesting observations have already been made. The first is that if the animals survive past the tenth day the signs of radiation sickness--such as loss in weight, ruffled hair, and diarrhea--rapidly vanish and they begin to gain weight again, although slightly less rapidly than the controls.

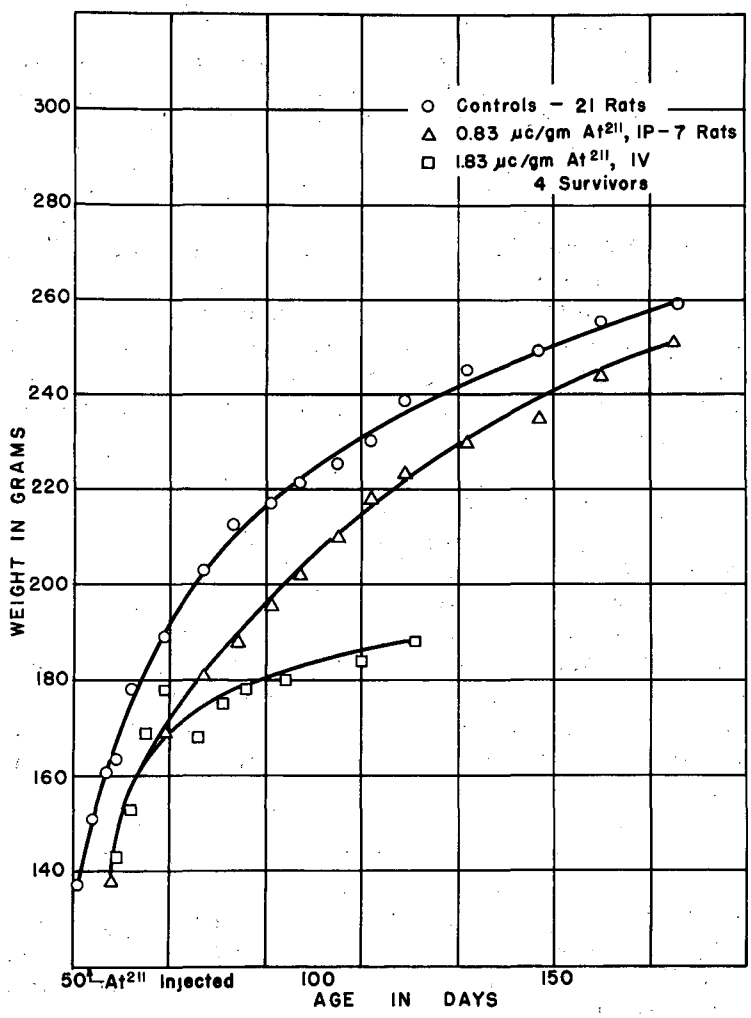

Fig. 3 Growth curves showing the effect of parenteral administration of astatine in Sprague-Dawley rats at two dose levels.

Another aspect of these studies was to dissociate the long-term endocrine changes from those due to irradiation, since the thyroid is virtually destroyed at high dose levels. After an extended period of time several effects are to be observed in the pituitary gland and other endocrine organs. Accordingly, animals in groups of ten received potassium iodide intraperitoneally daily for 6 days at a level of 40 milligrams per kilogram to reduce the uptake of astatine by the thyroid gland, and on the sixth day they also received astatine.

Ten animals that received 1.83 microcuries of astatine per gram and no potassium iodide showed six fatalities within 10 days; at six weeks none of the four survivors has died. The 10 animals that received potassium iodide as well as astatine died within a week. Even though some of the astatine may have been colloidal, this should not have been of great importance, since both groups received the same amount of astatine in the same physical state.

Fortunately, it was possible to secure histopathological specimens from several animals of both groups when it became evident that the animals would survive only a few more hours. In both groups of animals marked changes in the spleen and the lymph nodes were observed; the normal structure of these organs was virtually wiped out and extensive hemorrhage was to be noted. The mesentery lymph nodes were damaged more than the cervical lymph nodes. The thyroid glands from those animals which had received potassium iodide appeared to be normal, which is an indication of the protective effect of stable iodine to the thyroid gland.

No correlation could be established in the lymphatic tissue and the spleen between the group receiving potassium iodide and the other group of astatine-treated animals. Apart from the thyroid gland, where the differences under the two circumstances was so striking, no significant correlation could be established in other tissues and organs. Consideration must be given to the small number of animals whose tissues were available for histological study. It might be mentioned that other tissues investigated included the liver, stomach, small intestine, lacrimal gland, salivary gland, adrenal and pancreas. Representative photomicrographs of the tissues which are interesting will be presented in the next quarterly report.

As has already been shown,* astatine is not uniformly distributed throughout the tissues, organs and body fluids. However, it is more so than any other alpha-particle emitter that is suitable for such an investigation. However, radioiodine has a distribution which in many respects is quite similar to that of astatine, and accordingly we plan several similar experiments employing radioiodine at a dose level that will permit approximately 50 percent survival.

A preliminary study employing total body radiation has been initiated, using a radiation source of 220-kv x-rays at a 50-cm target distance. In this preliminary study 20 animals were used, 10 pretreated with potassium iodide; all 20 received a dose of 740 r. of x-rays. Little effect was seen in that the LD_{100} was six days for the potassium-iodide-treated animals and seven days for the untreated controls. Later these experiments were repeated using a

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*J. G. Hamilton, C. W. Asling, W. M. Garrison, and K. G. Scott, The Accumulation, Metabolism, and Biological Effects of Astatine in Rats and Monkeys, Univ. of Calif. Publ. Pharmacol. 2, No. 21, 283 (1953).

dose level of 640 r. to see if the potassium iodide effect does or does not exist with total body irradiation. At the present time there appears to be a real effect of the potassium iodide pretreatment in enhancing the mortality of this group. More data must be accumulated before we can be certain of this phenomenon. The same age, sex and strain of rats were used in this experiment as were used in the astatine studies.

The machine was carefully calibrated using a 0.5-mm copper foil and 1.0-mm aluminum foil as filters; the dose administered was in the range of 50 r. per minute and at a distance of 50 cm.

In the over-all conclusions of this particular radiation biology problem, consideration must be given not only to the factor of specific ionization, but also the period of irradiation. In the case of x-ray irradiation the rate is very high. Fortunately radioiodine, although it has a half-life of 8 days, has quite a short effective half-life in the body (owing to excretion), and in the case of the rat it is approximately 18 hours. It is not felt that the difference of the short half-life of astatine of 7.5 hours as contrasted to the biological half-life of 18 hours for radioiodine is of too great importance in this respect to the rate of irradiation.

Monkeys

Chronic astatine toxicity experiments are now under way employing four astatine-treated monkeys (two males and two females) and two normal male controls. The first two animals (males) received 0.96 millicuries of astatine^{211}, which was approximately 420 microcuries per kilogram. At the time of injection one weighed 2.4 kilograms and the other weighed 2.2 kilograms. A second group of two female monkeys each received 2.0 millicuries of astatine^{211} by intraperitoneal injection, May 26, 1953. The first animal weighed 2.4 kilograms and the second weighed 2.6 kilograms. The previous reports have indicated the changes in weight and alterations of the nature of the blood. These are being continued, and further x-ray studies will be presented in a later report to give a more adequate correlation between the treated and the control animals. One animal is showing evidences of what may be interpreted as hypothyroidism, as manifested by a marked decrease of food intake, thinning of hair, and general lack of responsiveness.

RADIATION CHEMISTRY

Warren M. Garrison, H. R. Haymond, Boyd Weeks and Winifred Bennett

In previous quarterly reports and in other publications we have presented kinetic studies of radiation-induced reactions in aqueous solutions of carbonic acid, formic acid, acetic acid, and glycine. As we have pointed out previously, these studies are closely interrelated from the standpoint of the nature of the radiation-induced oxidation-reduction reactions involved. The present work is a continuation of our radiation chemical studies of (1) dilute aqueous solutions of organic acids in which radiation-induced chemical change results from reactions of H and OH radicals formed from water in the primary radiation act, and of (2) more concentrated solutions in which the direct action of the radiation with the solute molecule is involved.

Radiation chemical studies during the last quarter have involved primarily (1) a consideration of the mechanism of heavy-particle radiolysis of acetic acid solution from 0.0625 M to 16 M and interpretation of the experimental data in terms of kinetic considerations, (2) a further investigation of the effect of oxygen on the radiolysis of acetic acid and the development of adequate analytical procedures for the determination of anticipated products (i.e., glycolic and glyoxylic acids), (3) a continuation of radiation chemical studies of aqueous glycine, including the development of analytical techniques for product identification, and (4) further study of radiation-induced chemical change in formic and oxalic acid solutions.

Acetic Acid Studies

In previous studies it has been shown that succinic acid is the principal nonvolatile product formed by heavy-particle irradiation of aqueous acetic acid at radiation dose levels below $\sim 3 \times 10^{20}$ ev/cc. The radiation yields (G) of succinic acid, hydrogen, and hydrogen peroxide increase with acetic acid concentration in the range 0.0625 M to 1.0 M and approach a limiting value at the higher concentration. Above 1-2 M the radiation yield of these products decreases almost linearly with increasing acetic acid concentration. The carbon dioxide and methane yields, however, increase linearly with acetic acid up to 16 M. Results with 35-Mev helium ions and 18-Mev deuterons in the acetic acid concentration range of 0.0625 M to 1 M are reproduced from a previous quarterly report in Fig. 1A and 1B. Figure 2 shows the radiation yields obtained with 35-Mev helium ions from 0.0625 M up to 16 M acetic acid.

It has been suggested that the radiation yield (G) of succinic acid, hydrogen, and hydrogen peroxide in dilute acetic acid solution would be expected to increase as shown in Fig. 1A and 1B if we assume[1,2] that two primary radiation processes occur when dilute aqueous solutions are irradiated.

$$H_2O \rightsquigarrow H + OH \qquad k_1 \cdot I \qquad (1)$$

$$H_2O \rightsquigarrow H_2 + H_2O_2 \qquad k_1' \cdot I \qquad (1')$$

[1] A. O. Allen et al, J. Phys. Chem. 56, 575 (1952).
[2] E. J. Hart, J. Am. Chem. Soc. 73, 68 (1951).

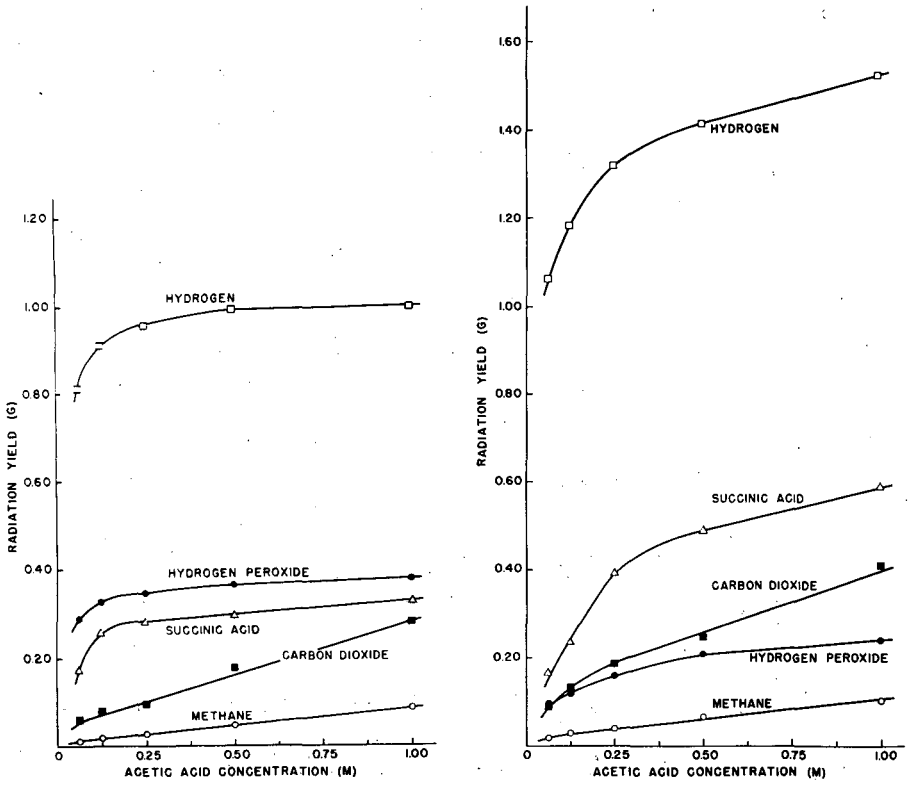
1.20
1.00
0.80
0.60
0.40
0.20
RADIATION YIELD (G)
HYDROGEN
HYDROGEN PEROXIDE
SUCCINIC ACID
CARBON DIOXIDE
METHANE
0
0.25
0.50
0.75
1.00
ACETIC ACID CONCENTRATION (M)
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
RADIATION YIELD (G)
HYDROGEN
SUCCINIC ACID
CARBON DIOXIDE
HYDROGEN PEROXIDE
METHANE
0
0.25
0.50
0.75
1.00
ACETIC ACID CONCENTRATION (M)

Fig. 1A, 1B

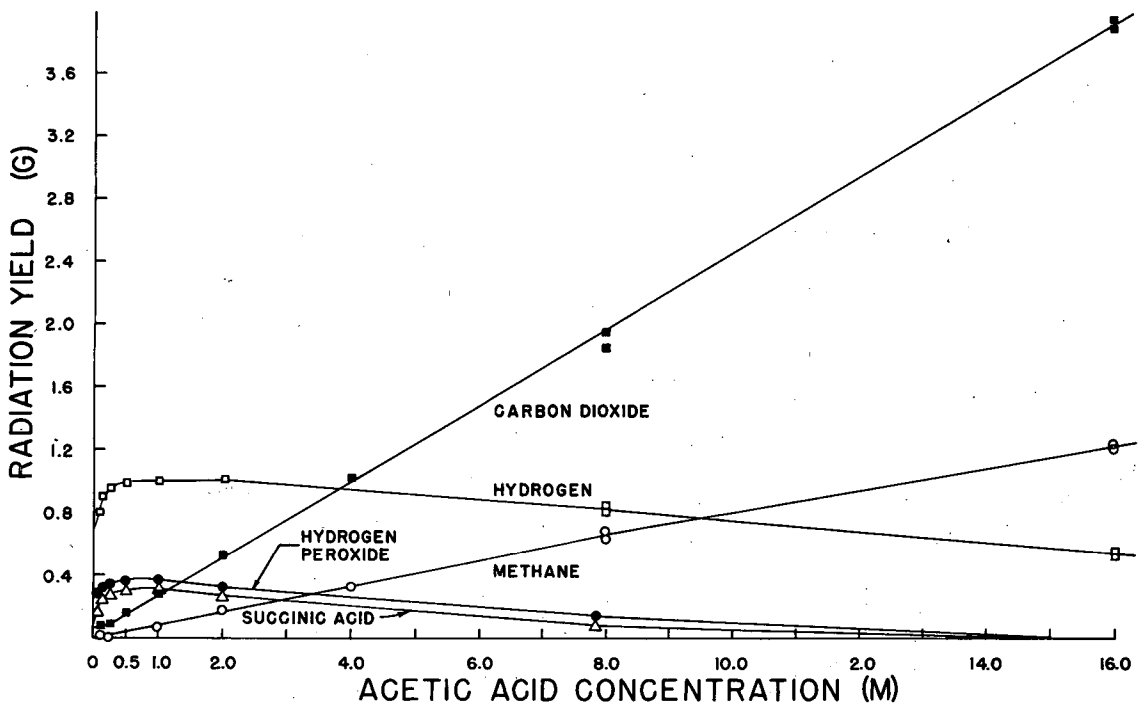
RADIATION YIELD (G)
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
CARBON DIOXIDE
HYDROGEN
HYDROGEN PEROXIDE
METHANE
SUCCINIC ACID
0 0.5 1.0 2.0 4.0 6.0 8.0 10.0 2.0 14.0 16.0
ACETIC ACID CONCENTRATION (M)

Fig. 2

In the absence of added solute or at low solute concentration the radicals produced in (1) react with H_2O_2 and H_2 from (1') giving rise to the radiation-induced back reactions

$$OH + H_2 \longrightarrow H_2O + H \qquad k_2 , \tag{2}$$

$$H + H_2O_2 \longrightarrow H_2O + OH \qquad k_3 , \tag{3}$$

In the presence of acetic acid we have the possible competing reaction

$$OH + A \longrightarrow A' + H_2O \qquad k_4 , \tag{4}$$

which results in the formation of succinic acid via

$$2A' \longrightarrow S \qquad k_5 . \tag{5}$$

At the higher acetic acid concentration all of the OH would react with A and the radiation yield for G_S would be represented by

$$G_S = \frac{k_1}{2} \qquad I$$

when k_1 is the radical pair yield for the effective radiation. Hydrogen would be formed by (1') or by

$$2\,H \longrightarrow H_2 . \tag{6}$$

Although this reaction scheme accounts for the observed relative dependence of G_{H_2}, $G_{H_2O_2}$ and G_S on acetic acid concentration, it does not account for the observed values of G. On the basis of Hart's work on the formic acid-oxygen system it appears that the total number of water molecules decomposed via (1) and (1') above may be considered a constant regardless of the type of radiation used, i.e., the ionization density determines only the relative number of molecules decomposing via (1) or (1'). The total is a constant and equal to 3.2 to ~3.6. Therefore the observed G for hydrogen as the basis of the above series of reactions should approach 1.8 for both helium ions and deuterons.

A mechanism in accord with observation is obtained if it is assumed that H reacts via (3) and (7) above and also with A' via

$$A' + H \longrightarrow A . \tag{7}$$

For dilute acetic acid solution in which the energy is absorbed primarily in water, we can set up the following equations to represent the production of hydrogen peroxide, hydrogen, and succinic acid (S).

$$\frac{d(H_2O_2)}{dt} = k_1'I - k_3(H)(H_2O_2) \qquad I$$

$$\frac{d(H_2)}{dt} = k_1'I + k_6(H)^2 - k_2(OH)(H_2) \qquad II$$

$$\frac{d(S)}{dt} = k_5(A')^2 \qquad \text{III}$$

By making the usual steady-state assumptions for radical intermediates we can write

$$k_1 I + k_2(OH)(H_2) = k_3(H)(H_2O_2) + k_7(A')(H) + 2\,k_6(H)^2, \qquad \text{IV}$$

$$k_1 I + k_3(H)(H_2O_2) = k_2(OH)(H_2) + k_4(A)(OH), \qquad \text{V}$$

$$k_4(A)(OH) = k_7(A')(H) + k_5(A')^2. \qquad \text{VI}$$

Solving these equations by conventional methods, we obtain the following expression for the limiting yield (G_S) for succinic acid at the higher acetic acid concentrations in which case all of the OH produced via (1) or (3) reacts with A.

$$G_S = \frac{k_1 + k_1' - G_{H_2O_2}\,\frac{k_7}{2\sqrt{k_6}}}{\frac{k_8}{\sqrt{k_5 k_6}} + 2}$$

where $(k_1' - G_{H_2O_2})$ represents the increase in OH from reaction (3). This reduces to

$$G_S = \frac{k_1}{2}$$

if the contributions of reaction (3) and (7) are neglected.

At higher acetic acid concentration the values for G_{H_2}, $G_{H_2O_2}$ and G_S decrease linearly with increasing acetic acid. The values for G_{CO_2} and G_{CH_4} increase linearly over the entire concentration range from 0.0625 to 16 M acetic acid. This linear dependency for CO_2 and CH_4 suggests that these products are formed by direct interaction of the effective radiation with the acetic acid molecules via reactions not involving radicals. The falling off of G_{H_2}, $G_{H_2O_2}$ and G_S at the higher acetic concentrations corresponds to the fractional decrease in radiation energy observed in water. If radical reactions were involved in the production of CH_4 and CO_2 it would seem logical to assume that these reactions would compete with others involving H and OH or CH_2COOH. The observed shape of the product-yield curves would, however, indicate that such processes are notinvolved. One possibility which would account for the linearity in CH_4 and CO_2 is that direct interaction of radiation with acetic acid results in the formation of an electronically excited species A* which could decompose via

$$A^* \longrightarrow CH_4 + CO_2$$

or react with another acetic acid molecule.

$$A^* + A \longrightarrow CO_2 + \text{product.}$$

The main point here is that if the excitation energy corresponding to a process

$$A \longrightarrow\!\!\!\!\sim\!\!\sim\!\!\sim\!\!\!\!\longrightarrow A^*$$

is less than that required for excitation of water molecules, A* could react bimolecularly with A before fluorescence occurs. Both the unimolecular reaction and the bimolecular reaction give the observed linear dependence on concentration. These processes will be discussed in detail in a forthcoming report.

Previously reported radiation studies of oxygen-aerated acetic acid solutions have shown that the effect of dissolved oxygen is to decrease G_S and to increase $G_{H_2O_2}$. It has been suggested that these results indicate that both H and CH_2COOH react with oxygen

$$H + O_2 \longrightarrow HO_2$$

$$CH_2COOH + O_2 \longrightarrow O_2CH_2COOH\ ,$$

and that CO_2 is produced by subsequent oxidation of the O_2CH_2COOH radical via glycolic, glyoxalic, and oxalic acids. Since glycolic and glyoxalic acids are not recovered with succinic acid in the nonvolatile acid fraction, other analytical procedures have been developed. These are described in the following section.

Determination of Glyoxylic Acid and Glycolic Acid

H. R. Haymond and Winifred Bennett

Glyoxylic Acid

The following method, which is similar to a method for the determination of pyruvic acid,[3,4] has been used for the determination of glyoxylic acid in irradiated aqueous solutions of acetic acid.

1. To 1 ml of target solution, not more than 0.50 M acetic acid, after removal of H_2O_2 by the addition of platinum black, add 1 ml of 0.1% 2,4-dinitrophenylhydrazine in 2N HCl. Heat at 50° C for 30 minutes. Cool.

2. Extract three times with 2 ml ethyl acetate. Combine the ethyl acetate fractions and set aside the aqueous phase for determination of glycolic acid.

3. To the ethyl acetate phase, containing the 2,4-dinitrophenylhydrazones of ketones, aldehydes, and keto acids, and the excess 2,4-dinitrophenylhydrazine reagent, add excess $CaCO_3$ to neutralize. Centrifuge and wash with ethyl acetate. Combine wash with supernatant.

4. Extract four times with an equal volume of 10% sodium carbonate solution. Combine aqueous phases.

5. To 2 ml of aqueous phase add 2 ml of 10% KOH in 20% water - 80% methanol, carbonyl-free. After 10 minutes measure optical density at 480 mμ. Compare with simulated target solution containing a known amount of glyoxylic acid which has been treated simultaneously by this process.

In the determination of glyoxylic acid at concentrations near 10^{-3} molar, acetic acid does not interfere if its concentration is less than 0.50 M. Glycolic acid, acetone, and formaldehyde do not interfere in concentrations about the same as the glyoxylic acid concentration.

Glycolic Acid

The procedure for the determination of glycolic acid in aqueous acetic acid solutions is essentially that reported by Calkins[5] and by Pereira.[6]

Into a 15-ml centrifuge tube are pipetted 20 λ of concentrated sulfuric acid, 200 λ of sample solution, and 2.0 ml of 0.01% 2,7-dihydroxynapthalene in concentrated sulfuric acid. The tube is kept in a cold water bath during the addition of the reagent, then the mixture is made homogeneous by rotating the tube between the hand. After being heated for twenty-five minutes in an oil bath at 100 degrees centigrade, the solution is allowed to cool and is

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[3] E. M. Case, Biochem. J., 26, 753 (1932).
[4] G. D. Lee, Biochem. J. 33, 249 (1939).
[5] Ind. and Eng. Chem., Anal. 15, 762 (1943).
[6] Mikrochemie, 36-37, 398 (1950).

diluted with 4.0 ml. of 2 M sulfuric acid. The liquid is made homogeneous and the optical density is read at 540 mμ in the Beckmann DU spectrophotometer.

By combining the above analytical procedure with the standard chromatography method now in use for the determination of certain acidic products from irradiated aqueous acetic acid solutions, it should be possible to establish either the presence or absence of glycolic acid as a product of irradiation. Glycolic acid steam-distills with acetic acid in the process previously used to separate the reaction products from the solvent and the original solute. However, six extractions with twice the volume of ethyl ether have been found to remove 97 percent of the acetic acid from a 0.25 M aqueous solution without decreasing the glycolic acid concentration by more than one percent. The acidic portion remaining in the water phase can be separated by neutralization and vacuum distillation to give a residue that can be put through an ion-exchange column and a silicic acid column to give positive identification of the volatile acid without interference by the original acetic acid.

The Effects of Dissolved Oxygen in the Irradiation of Aqueous Acetic Acid Solutions

H. R. Haymond

A detailed study of the effects of dissolved oxygen in the irradiation of aqueous acetic acid solutions has been started. Following is a summary of results obtained from several targets run with efficient oxygenation during irradiation, i.e., the solution was mechanically stirred directly behind the irradiation zone and oxygen was fed into the solution through a fritted glass disc. All targets received approximately 5×10^{20} ev/ml irradiation with 35-Mev helium ions. Over the range 0.125 to 1.0 M the yield of hydrogen peroxide is approximately constant with a G value of 1.0 molecule per 100 ev. The succinic acid yield in this same concentration range is less than G = 0.07. The G values for glycolic acid are approximately 0.1 in 0.2 M acetic acid and 0.15 in 1.0 M acetic acid. Addition of hydrogen peroxide to the target solution before irradiation in a concentration approximately equivalent to its normal concentration at the end of irradiation has no effect on the yield of glycolic acid in irradiation of 0.25 M acetic acid.

Glycine

Boyd Weeks

In previous quarterly reports beginning with the report for October-December, 1952, the present study of the radiation chemistry of aqueous solutions of glycine has been described in detail. In the last report (July-September, 1953) problems associated with purification of glycine for bombardment, analysis of volatile base products, and the separation of the cationic products on a Dowex 50 ion-exchange column were discussed. Since then a new batch of glycine, recrystallized four times from doubly distilled H_2O, was prepared and three bombardments made of this glycine. Three other bombardments of previously prepared glycines were also made. Radiation yields for H_2, CO_2, H_2O_2, and total acid have been determined for all these and check with one another satisfactorily.

Work is now in progress, as outlined in the previous report, for the separation and determination of the individual components of the total acid and total cationic portions of each of these six bombardments. Detailed discussion of radiation yields already determined will be reserved until a more comprehensive discussion is possible. Investigation of possible carbonyl and other neutral products will also be carried out for these six bombardments, as described already in connection with the acetic acid work.

SEARCH FOR AN EFFECT OF HYDROSTATIC PRESSURE ON X-RAY INACTIVATION OF YEAST

Victor W. Burns

Donner Laboratory and the Radiation Laboratory
University of California, Berkeley

The nature of the molecular alteration leading to mutation or chromosome breakage remains obscure, although many theories have been proposed to account for these phenomena. The use of infrared as a potentiating agent for X-ray-induced chromosome aberrations in Tradescantia has produced experimental evidence for the formation of metastable activated states in chromosomes.[17] Hydrostatic pressures of 5,000-10,000 psi have been used to alter the mutation frequency of Neurospora after treatment with U.V. or nitrogen mustard[13,14,15]; the results indicated that the transition from "activated" gene to mutant gene involved a large molecular volume change. A reaction involving a large molecular volume change in the rate-determining step will exhibit pressure-dependence according to the rate theory of Eyring.[2,3] Studies on the denaturation and hydrolysis of proteins and on enzyme-catalyzed reactions have generally shown the occurence of a large molecular volume change during these reactions,[4,5,6,7,8,9,10,11,12] and a dependence of reaction rate on pressure.[3,6,7,8,9,10,11] X-rays are known to denature certain proteins in vitro.[1,16] It is to be expected that, if mutation involves one of the above reactions,[14,16] mutation rate should be affected by high pressures.

In the work on the effect of pressure on mutation rate cited above, pressure was applied after irradiation. McElroy has suggested that a large number of radiation-induced mutations go to completion during irradiation, and hence are not affected by postpressure treatment.[14]

The experiments described in this paper were designed to provide the following information:

1. The variation of the radiosensitivity of haploid yeast with pressure. The inactivation of haploid S. cerevisiae as a function of X-ray dose has been investigated experimentally and theoretically by Tobias.[18] Inactivation corresponds to lethal mutation.

2. The effect of pressure applied during irradiation, as well as after irradiation.

3. The effect of temperature during pressure treatment.

Methods and Materials

A haploid strain of Saccharomyces cerevisiae, SC7, was used throughout this work, except for some exploratory experiments with a tetraploid derivative. The LD50 of SC7 is 3100 r. The yeast was harvested from nutrient agar cultures, suspended in phosphate buffer, and diluted until a concentration of approximately 10^6 cells/ml was obtained. Less than 1% of the cells were in the process of budding. Thin-walled polyethylene microcells of 0.03 ml capacity were constructed to contain the yeast suspension in the pressure chamber. These microcells were filled and capped in a manner such that air bubbles were excluded, in order to prevent decompression death.

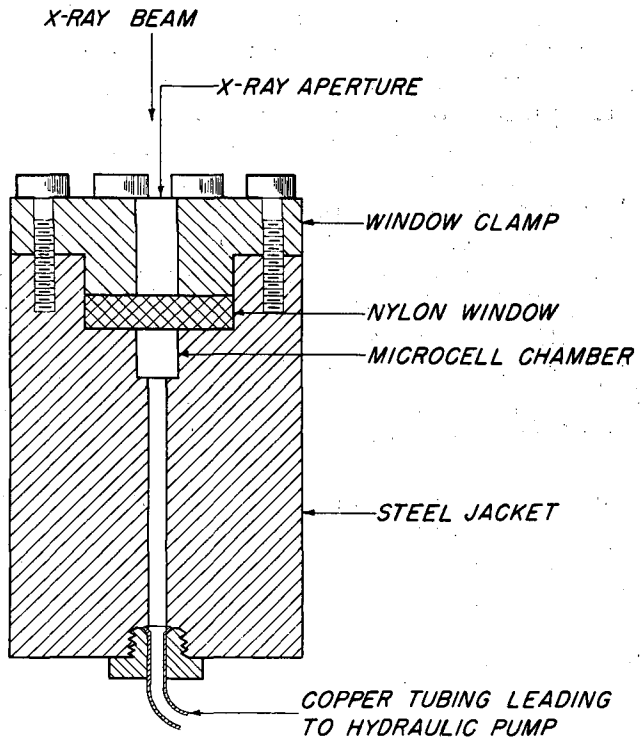
X-RAY BEAM
X-RAY APERTURE
WINDOW CLAMP
NYLON WINDOW
MICROCELL CHAMBER
STEEL JACKET
COPPER TUBING LEADING
TO HYDRAULIC PUMP

Fig. 1

A steel chamber with a plastic window and microcell holder at one end and a flexible connection to a hydraulic pump at the other end was used for application of hydrostatic pressure (Fig. 1). The plastic window could withstand pressures of 10,000 psi, but it did not seriously attenuate the X-radiation employed. X-radiation was supplied by a Machlett OEG-60 tube operated at 50 KV. The dose rate delivered to a microcell was 221 r/sec.

The contents of each microcell were assayed by spreading a suitable dilution on nutrient agar; after 48 hours' incubation at 30° C, macrocolonies were counted, and the count compared to an unirradiated control to determine percentage survival. The percentage mortality obtained in this way is a measure of lethal mutation.

All experiments were designed to give maximum information concerning the effect of high pressure as contrasted to standard pressure at a given dose of radiation. With this end in mind, the microcells used in each experiment were always paired. One member of each pair was subjected to high pressure, the other to standard pressure, the conditions of radiation (geometry, absorption, etc.) remaining exactly the same in both cases.

Pressure was applied during irradiation to determine its effect on immediate radiation damage; since it is likely that some ionization products persist for a time after irradiation and also that metastable activated states of the genetic material may be formed, pressure would be expected to have an effect if applied after the termination of irradiation. Hence, pressure was continued for five minutes after irradiation, except for some tests to determine if an extension to 30 minutes caused an effect.

Results

For preliminary experimentation it was necessary to choose informative combinations of the following variables: temperature, pressure, and yeast ploidy. The following combinations were tried:

1. 20° C, 2000-5000 psi, haploid yeast
2. 20° C, 8000-10,000 psi, haploid yeast
3. 20° C, 8000-10,000 psi, Tetraploid yeast
4. 50° C, 8000-10,000 psi, haploid yeast
5. 0° C, 8000-10,000 psi, haploid yeast

Experiments conducted under each set of conditions gave no indication that high pressure affected the survival of yeast under X-radiation, even for 1% survival. It was further ascertained that high pressure alone did not cause death unless it was applied for several hours, and that pressure applied before irradiation had no effect.

The second set of conditions above, i.e., haploid yeast at room temperature with 8000-10,000 psi applied, was most favorable for extensive experiment and analysis, so a series of determinations was made in the survival range from 1% to 70%. The survival of haploid yeast is described by the formula

$S = e^{-kD}$ where D = dose, and k = radiosensitivity at standard pressure. Let S_p = survival at pressure p. Then $S_p = e^{-k_p D}$, where k_p = radiosensitivity at pressure p. Then, for a given dose, $\log S_p/\log S = K_p/K$. In Fig. 2 the experimental values of this ratio are plotted versus dose. The average value of the ratio is 0.994 ± 0.028, so that the radiosensitivities are equal within the 3% error quoted, and we must conclude that X-ray damage to nondividing haploid yeast is not a function of pressure.

Discussion

It is of interest to interpret the results obtained in terms of the maximum volume change that could occur in the reaction between genetic material and ionization products and yet remain undetectable by the present method. Applying the chemical reaction rate theory of Eyring, the probability of reaction for the reaction between ionization products and genetic material is proportional to $e^{-\Delta F^{\pm}/RT}$, where $\Delta F^{\pm}$ is the free energy of activation. The pressure-dependent factor of this function is $e^{-p\Delta V^{\pm}/RT}$, where $\Delta V^{\pm}$ is the volume change during activation (the identification of $\Delta V^{\pm}$ with a particular rate constant of an enzyme reaction is difficult).[11] The radiosensitivity k is proportional to the reaction probability for lethal mutation. Then $k_p/k = e^{-p\Delta V^{\pm}/RT}$ where p = gauge pressure (absolute pressure minus 14.7 psi.). (This formula may be derived, in a more sophisticated fashion, from the diffusion theory interpretation of the radiosensitivity constant). Experimentally, we found $k_p/k = 0.994 \pm 0.028$. Hence $e^{-p\Delta V^{\pm}/RT} = 0.994 \pm 0.028$, or to find the maximum $\Delta V^{\pm}$ that could occur and yet remain undetectable to an error of less than 0.05, two standard deviations may be taken;

$$e^{-p\Delta V^{\pm}/RT} = 0.994 \pm 0.056$$

Letting p = 10,000 psi, we can solve for $\Delta V^{\pm}$:

$$-1.7 \text{ cc/mole} < \Delta V^{\pm} < 2.4 \text{ cc/mole}$$

The molecular volume changes found for protein denaturation, hydrolysis, and several enzyme reactions are much larger than these limiting values for $\Delta V^{\pm}$[3,4,5,6,8,11,12]. Thus we are led to the view that none of these processes is primary in the X-ray lethal mutation of yeast. Perhaps this type of mutation involves no immediate molecular unfolding, but rather an alteration of a surface prosthetic group, analogous to the action of the Type I inhibitors of bioluminescence.[6] It has been tacitly assumed that the unfolding of a macromolecule due to a direct hit or indirect chemical action would be affected similarly by pressure; work with protective agents indicates that at least half the radiation effect is indirect, so the values of $\Delta V^{\pm}$ given above would be no more than doubled if the direct effect is presumed to be pressure insensitive, even though it may involve large volume changes.

Summary

Evidence has been presented to show that the X-ray-induced lethal mutation frequency of haploid S. cerevisiae is not altered by hydrostatic pressures reaching 10,000 psi. From these results it is inferred that this mutation process does not initially involve macromolecular unfolding.

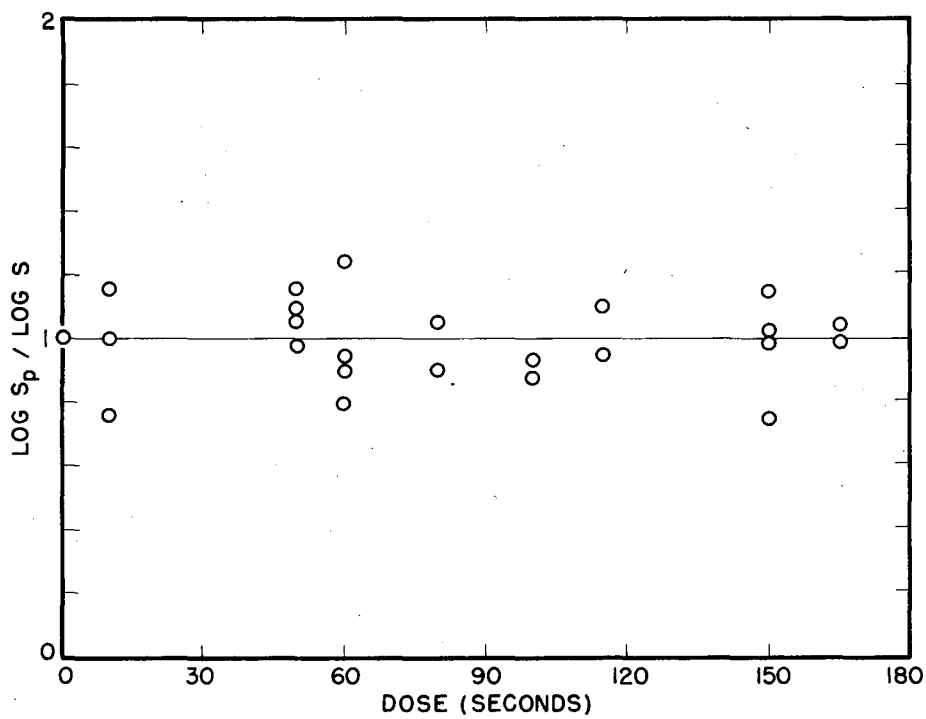

Fig. 2

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HEALTH CHEMISTRY

N. B. Garden

PROGRESS REPORT

An extensive program taking place in Bldg. 106 at Livermore--equipment for which was designed, built and set up by the Health Chemistry Equipment Development and Airborne Activity Control groups, with assistance from other Health Chemistry personnel--has absorbed almost 100 percent of the creative efforts of the Health Chemistry group. The program involves processing of large quantities of heavy-element alpha-emitters in a completely air-tight system. In view of the attention given to this program and of the fact that the summer months constitute a relatively quiet period in the chemistry laboratories at Berkeley, the progress report for this quarter is therefore of limited content.

During this period a completely revised flow sheet for the processing of the special soils by Dr. S. G. Thompson's group has been made, necessary because material of different character is being treated; new and modified equipment has been set up and is in use.

Continued investigation of the solidification of liquid radioactive waste has taken place, with promising results; the research has involved use of jelling agents in conjunction with Portland cement.

Successful decontamination of the large quantities of the low-level liquid waste from Livermore has been effected through use of an iron hydroxide scrubbing operation.

A unit for carrying the pistol-grip target holders, with their bombarded targets (usually large quantities of heavy-element alpha-emitters), from the 60-inch cyclotron to the respective laboratories without possibility of contamination has been completed and successfully used. This unit, located on a dolly, is comprised of lead shielding and a gloved box, into which the target assembly, with its air line filter and hoses, cooling-water hoses and vacuum-tank lines, is moved, with the lines sealed; these lines are broken only within the gloved box at the laboratory where the target material is to be processed.

Four leaking radium-beryllium sources were successfully packaged for shipment to a repair center in New York.

A 1500-pound container was built for transporting highly active irradiations from Idaho Falls; this container has features for the shielding of both gamma radiation and neutrons.

A container for transporting a 50- to 150-curie Ba^{140} source from Los Alamos to Berkeley was fabricated and made ready for use.

The Health Chemistry group continues to perform the accountability for the source and fissionable material held at Berkeley and Livermore, together with the preparation of manuals for this function, as prescribed by the Atomic Energy Commission.

HEALTH PHYSICS

Burton J. Moyer

STATISTICAL SUMMARY OF MONITORING PROGRAM

Survey Instruments Maintained

1.	B-γ Ionization Chamber	52
2.	I. D. L. Portable Survey Instruments	20
3.	Cutie Pies	3
4.	Recording γ-Intensity Meters	22
5.	Victoreen Proteximeter	3
6.	Fast-Neutron Proportional Counters	8
7.	Slow-Neutron Proportional Counters	15
8.	Fast-Neutron Proportional Counter (Portable)	9
9.	Slow-Neutron Portable Unit	4
10.	Balanced Chamber - Fast Neutron - Portable	2
11.	Special Tissue Wall Survey Instrument	1

Personnel Meters in Use

1.	Total Personnel Covered with Film Badges	2,850
2.	Total Man Days Coverage with Pocket Chamber	3,173
3.	Total Man Days Coverage with Pocket Dosimeters	3,173
4.	Total Man Days Coverage with Pocket Chambers (S.N.)	2,556

Cases of Weekly Exposure above 0.3r

Weekly Film Expos. above	184" Area	60" Area	Lin. Acc.	Chem.	Other	Total
0.3	1	13	8	34	5	61
0.5	1	7	0	11	3	22
1.0	0	0	0	2	0	2
1.5	0	0	0	0	0	0
2.0	0	0	0	0	0	0
2.5	0	0	0	0	0	0

Information Division
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