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MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

April, May, June 1954

July 29, 1954

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MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

April, May, June, 1954

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MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

April, May, June 1954

Radiaton Laboratory, Department of Physics
University of California, Berkeley, California

July 29, 1954

BIOLOGICAL STUDIES OF RADIATION EFFECTS

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

POSTIRRADIATION TIME AND DOSE RESPONSE STUDIES ON THE INCORPORATION OF PHOSPHORUS-32 INTO DNA OF MOUSE TISSUES*

Lola S. Kelly, J. Dorothy Hirsch, Genevieve Beach, and Anita H. Payne

The pioneering studies of Hevesy's group[1], demonstrating that irradiation inhibits desoxyribose nucleic acid (DNA) synthesis in the organs of rats, have evoked considerable interest and speculation among radiobiologists. The original observations have been substantially confirmed by numerous workers utilizing the varied DNA precursors now available, such as phosphorus-32,[2,3,4,5,6,7] glycine-1-C^{14},[8] adenine-4-6-C^{14},[9] and formate-1-C^{14}.[10,11] The experiments to be reported here were undertaken in order to study the dose response and the time response of the inhibition. The short-term incorporation of P^{32} into DNA normal and tumor tissues of mice was measured at varying times after 300, 800,** and 2500 r of total body x-irradiation. It was hoped that this more detailed study might clarify some of the mechanisms involved and help explain the morphologically observed difference in radiosensitivity of organs.

Materials and Methods

Throughout this experiment male Heston A strain mice*** 2-1/2 to 4 months of age were used. They were irradiated by a G.E. x-ray machine operated at 220 kv, 15 ma with added filtration of 1 mm Cu and 1 mm Al at a target distance of 65 cm which produced a dose rate of approximately 13 r per minute. During irradiation the mice were housed in a wooden box with a nylon screen top and were free to move about. The radiation dose, which included approximately 15% backscatter, was measured with a Victoreen r meter placed in the box with the mice. In most of the experiments 40 mice were irradiated simultaneously.

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*Preliminary report presented at the 1953 meeting of the Federation of American Societies for Experimental Biology.

**800 r is usually a lethal dose under these conditions of irradiation, with the majority of the mice dying between days 9 and 15. Occasionally, however, an appreciable number of mice have survived this dose.

***The mice were kindly supplied by Dr. Kenneth DeOme.

They were used for two different time intervals after irradiation, together with 20 sham-irradiated controls.

At various times after irradiation the mice were injected intraperitoneally with a tracer dose of $Na_2HP^{32}O_4$ in isotonic saline. They were sacrificed exactly 2 hours thereafter for points up to one day postirradiation, and 4 hours thereafter for the later points.

The mice were dissected as rapidly as possible and DNA was isolated from the following tissues: liver, spleen, kidney, lung, small intestine, and "carcass".* To obtain enough DNA for satisfactory purification from spleens, kidneys, and lungs, the organs from ten animals were pooled. For the other tissues the organs from two animals were pooled.

The tumors investigated were transplants of a mammary adenocarcinoma and a lymphosarcoma. The mice were irradiated ten to twelve days after bilateral transplantation when the individual tumor weights were of the order of half a gram. The other tissues of tumor-bearing mice were not included in the results on normal tissues since differences in DNA synthesis had been noted earlier[12].

The method for the isolation of DNA and the determination of its specific activity has been published previously[13]. It has been retained in this laboratory, in spite of the recent publication of other methods which give better yields, because of its convenience for handling large numbers of samples and because of the reproducibility of the results.

In the experiments on mammary carcinoma the specific activity of the cytoplasmic ribose nucleic acid (RNA) was also determined. The procedure used for the isolation of RNA has been published previously[12].

Results and Discussion

It is evident from Fig. 1 that all tissues examined show a decreased DNA specific activity after irradiation**, but that there is wide variation in the ultimate percentage depression, the time at which recovery sets in, and the dose dependence. For this reason each tissue will be discussed separately, but a few general points should be considered first.

It seems to be well established now that P^{32} is incorporated into DNA only at the time that the cell is synthesizing new DNA and that this probably occurs at some time during interphase.

---

*"Carcass" was prepared by skinning and decapitating the mouse after the liver, spleen, sex organs, kidneys, lung, stomach, small and large intestine, and heart had been removed. The remainder was ground in a Waring Blendor before the DNA isolation was begun.

**In one group of mice, that was not included in the graphs, P^{32} was injected just before the beginning of irradiation, and the mice were sacrificed one hour after the end of irradiation (800 r). The specific activities were all intermediate between the control and 2-hour values, indicating that there is no major depression of DNA formation at the time of irradiation.

The quantity that is actually measured in this experiment is the specific activity of the DNA two or four hours after the administration of $Na_2HP^{32}O_4$. This could be influenced by a number of events other than a change in the ability of an individual cell to synthesize DNA, and the results must therefore be interpreted with caution. First of all, any decrease in the specific activity of the phosphate precursors, as a result of irradiation, would give a lowered DNA specific activity. The immediate precursor has not yet been identified, but no very marked decrease has been found in the specific activity of the inorganic phosphate or various acid-soluble phosphorus compounds after irradiation.[1, 5, 14] Furthermore, depression of DNA specific activity has been measured using a second precursor, adenine-4-6-C^{14}, under the same conditions that were used for the present experiments, and the results agree with the data obtained with P^{32}.[9] This suggests that the changes observed are not primarily due to precursor changes.

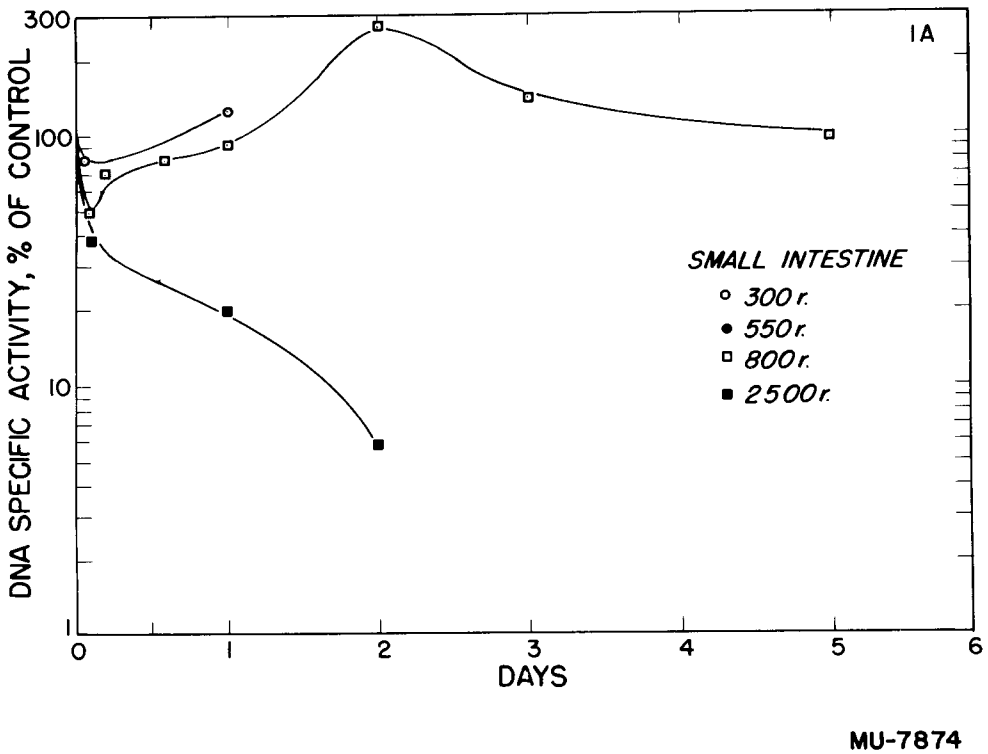
A second factor which must be considered is that each tissue measured is composed of several types of cells and, in some tissues at least, a marked shift in cell population is a well-known postirradiation response (e.g. spleen, bone marrow). These different cell types may have entirely different rates of DNA renewal, and therefore one might observe an increased or decreased DNA specific activity, depending on which cell type is depleted. Furthermore, dead or dying cells, which can be observed in certain irradiated tissues even shortly after irradiation, could cause a decrease in the DNA specific activity. This decrease would occur, despite the fact that the remaining viable cells might be synthesizing DNA normally, if the DNA isolation procedure is such that DNA from dead and disintegrating cells is included in the sample.

Small Intestine

Figure 1A represents the DNA specific activities observed at various time intervals after irradiation. There is a marked difference in response to the three doses measured. After 300 r there is a slight depression and overcompensation. After 800 r the minimum specific activity is observed in mice injected 2 hours after irradiation, and from this time on recovery occurs. The specific activity at 2 days is 260% of normal, and then there is a gradual return to normal. Early mitotic recovery with occasional overcompensation has been noted in this dose range by several authors.[15, 16]

The interpretation of the postirradiation specific activity curves is complicated by the fact that the small intestine is composed of cells with highly different rates of division. It has been shown that essentially all the DNA synthesis occurs in the crypts and that the cells of the villus epithelium are continually replaced by cells that have been newly formed in the crypts.[17]

The total DNA content of the small intestine observed 24 hours after 800 r is reduced to 65% of normal,[18] but it is not known which cells contribute to this loss. It is possible that the crypt cells may be synthesizing DNA at a reduced rate for some time after 800 r, and the early rise in specific activity may, to some extent at least, be caused by a loss of inert DNA. On the other hand many dead and dying cells are seen in the crypts,[19] and the DNA content and cell number per crypt are markedly reduced[20] very soon after irradiation. Therefore, the initial depression of incorporation might be due to death of cells that would normally be synthesizing, rather than to a direct inhibition of synthesis. After 2500 r the specific activity decreases progressively, reaching 6% of normal after 2 days. Measurements were not carried beyond this point to determine whether there is an attempt at recovery before death intervenes.[21]



Figs. 1A–E. Incorporation of P^{32} into DNA after various doses of x-irradiation. Ordinates: DNA specific activity 2 hours after P^{32} injection as percent of control. Abscissas: time from beginning of irradiation to injection of P^{32}. Each point represents the mean from at least twenty mice. With the exception of spleen values the standard error of the mean for each group was never greater than 10%.

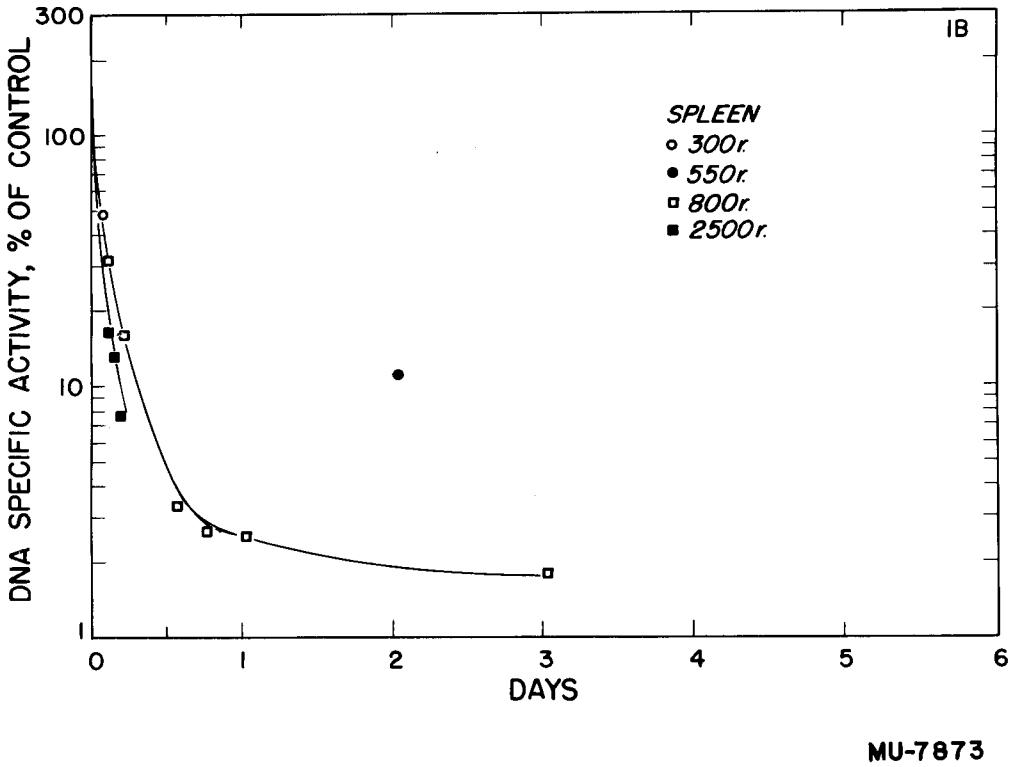


Figure 1B

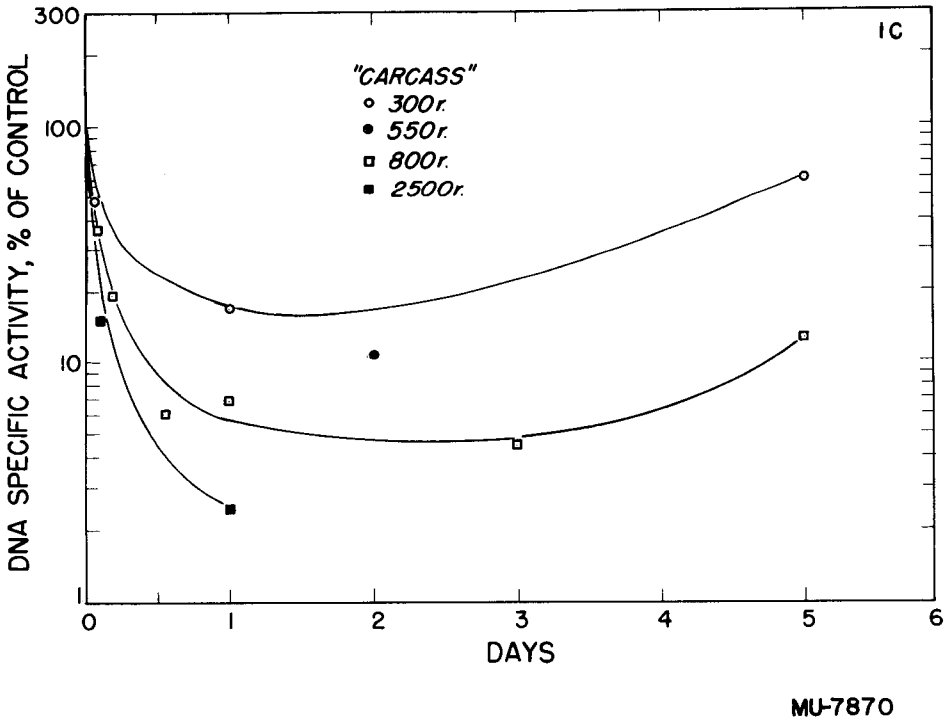


Figure 1C

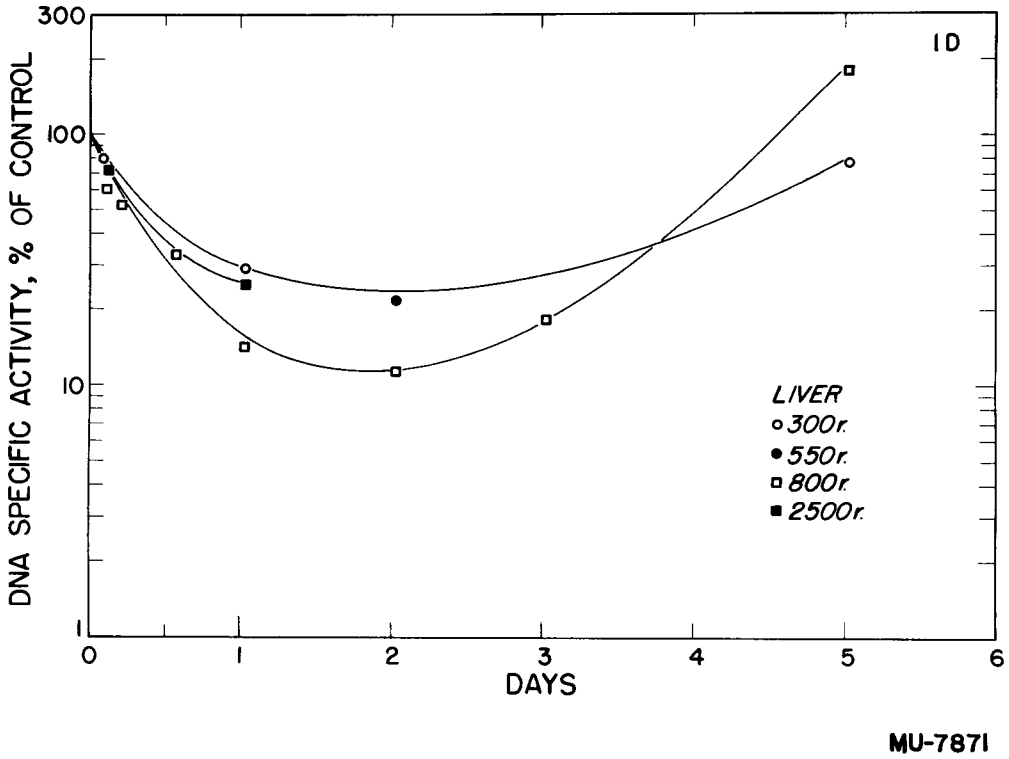


Figure 1D

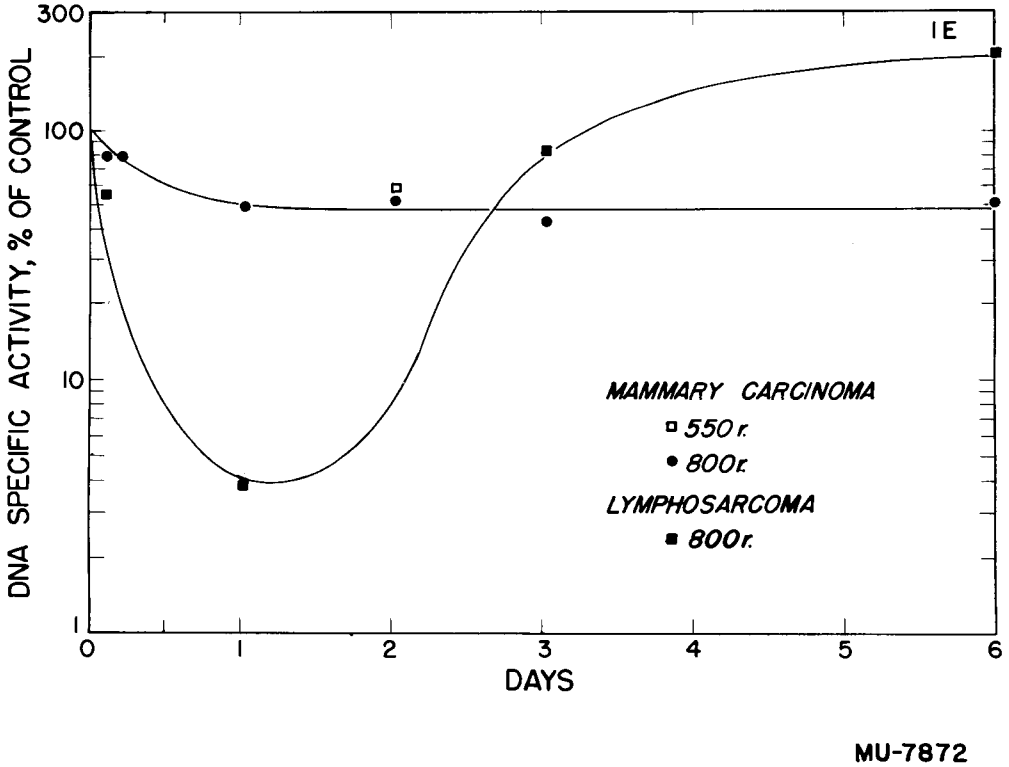


Figure 1E

Spleen

The data on spleen specific activities are sparse, because the marked involution of this organ, with concomitant loss of DNA, made it difficult to isolate enough material to measure. Frequently only one value was obtained from 20 spleens. Some of the points in Fig. 1B were taken from experiments done for a different purpose, in order to make the data as complete as possible. It is evident from Fig. 1B that the incorporation of P^{32} into spleen DNA drops precipitously and, at the early time intervals at least, is correlated with the radiation dose. A small group of survivors measured 14 days after 800 r showed recovery of incorporation to a level of 30% of normal.

It is known from histological observations[21, 22] that there is a great deal of cell death shortly after irradiation, involving primarily the lymphopoietic and myelopoietic cells. This leads to a rapid change in the cellular composition of the organ. Biochemical evidence for this effect has been presented by Cole et al.[23] by measurements of marked changes in DNA composition. The very high specific activity of DNA in normal mouse spleens is undoubtedly associated with the rapid cell division in the lymphopoietic and myelopoietic cells. Since these cells are essentially eliminated by the radiation doses used in these experiments, it appears that the data presented here can be interpreted primarily as as reflection of the changed cell population.

"Carcass"

DNA from this fraction was studied in an attempt to develop an easy method for following the recovery of bone marrow and lymphoid tissue in mice as a tool in radiation-protection experiments. From our experience with rabbits and rats and from the results of other investigators, it is clear that the incorporation of tracers into DNA of adult muscle is negligible, and muscle DNA therefore serves essentially as an inert diluent of the normally very active DNA of bone marrow, thymus, and diffuse lymphoid tissue. It is of interest that in normal mice the specific activity of this mixed "carcass" DNA is as high as small intestine DNA 2 hours after the injection of P^{32} and that it is very reproducible.

Figure 1C indicates that the incorporation of P^{32} into "carcass" DNA drops rapidly after irradiation and correlates well with the radiation dose. At 5 days the 300 r group shows fairly good recovery while the 800 r group is still very depressed. A small number of survivors measured 14 days after 800 r had a specific activity of 25% of normal.

Histological[24] as well as biochemical observations[25, 26, 27] on bone marrow in mice, rats, and rabbits have shown that there is rapid death and depletion of the hematopoietic cells, the early forms being affected more severely. Similarly, early pyknosis and death of cells have been observed by many investigators in lymphoid tissue.[22, 28] Here as in the spleen, drastic changes in cell population are thus known to occur shortly after irradiation. The inhibition of incorporation into "carcass" DNA may therefore again be due to death of the cells that would normally be synthesizing DNA or to interference with the ability of the cells to synthesize DNA. It is unfortunately not possible to decide at the present time what the relative contribution of these two proposed mechanisms is.

Liver

Figure 1D summarizes the data on liver DNA specific activities. There is no apparent dose dependence, and recovery occurs at four to five days. It should be emphasized that normal liver DNA has an extremely low specific activity compared to the other phosphorus compounds, and therefore the chemical purification is critical. After irradiation the incorporation of P^{32} is even lower, and it is possible that at late time intervals a dose dependence is marked by traces of contaminants.

Histological observation of irradiated rat and mouse livers[29] revealed no damage with the exception of some transient intranuclear vacuolization[30]. Paigen and Kaufman[31] reported a decrease in the amount of liver DNA after 600 r which, however, we have been unable to confirm. Thus it would appear that the lowering of the liver DNA specific activity after irradiation is due to a true interference with the ability of the cell to synthesize DNA. An alternative explanation can be made by postulating a special sensitivity to death of just the few cells that are at the point of synthesis.

An experiment on irradiation of mouse livers regenerating after carbon tetrachloride poisoning[32] indicates that liver cells are able to incorporate P^{32} into DNA normally after doses as high as 2000 r if the radiation is given between 24 and 60 hours after the drug, while irradiation either before or after this time interval depresses the incorporation markedly. At the moment there is no explanation for this effect, but it does show that under certain circumstances high radiation doses leave the DNA synthetic ability of liver cells intact.

Kidney and Lung

In the 800 r series kidney and lung DNA specific activities were measured. The data have been omitted from the graphs because they are limited and not very reliable. Both tissues normally have low DNA specific activities, and many mice have to be pooled to obtain a large enough sample to measure. However, it can be stated that the percentage depression of incorporation into kidney DNA follows that of liver closely, while the data on lung resembles the "carcass" fraction.

Tumors

The mammary carcinoma and lymphosarcoma were chosen for study as examples of "radioresistant" and "radiosensitive" tumors respectively. It is evident from Fig. 1E that they also respond very differently to irradiation with respect to their ability to incorporate P^{32} into DNA. After 800 r of total body irradiation the DNA specific activity of the mammary carcinoma drops to approximately 50% of normal and remains there for several days, while the lymphosarcoma DNA specific activity decreases to 3.8% 24 hours after irradiation, but by three days it has returned to normal. It is tempting to speculate that the ability of the mammary carcinoma to continue its DNA synthesis after irradiation is the reason for its radioresistance; however, at this time there is not enough information to interpret the 50% DNA specific activity in terms of events in the individual cells. Vermund et al.[5] observed a similar inhibition of P^{32} incorporation into DNA to 50% of normal after local irradiation of mammary carcinoma with doses as high as 4500 r and 9000 r. They suggest that irradiation inhibits "synthesis" but leaves "turnover" of DNA intact.

It should be mentioned that the mammary carcinoma and the lymphosarcoma used in this experiment had approximately the same growth rate, mitotic index, and short-term incorporation of P^{32} before irradiation. After 800 r the weight of the mammary carcinomas did not show any appreciable change over the six-day observation period. The lymphosarcomas, on the other hand, involuted markedly; 3 days after irradiation they were barely visible, and at 6 days they had only grown slightly. Histological observation of the mammary carcinomas revealed relatively little difference between control and irradiated tumors, while the irradiated lymphosarcomas at 1 and 3 days appeared to be a mass of cell debris.

Mitotic counts* made on control mammary carcinomas gave an average value of 10.3 ± 0.8 mitoses per 10 fields. Tumors examined for the first 4 hours after 800 r showed essentially no mitotic figures, while tumors examined between 1 and 6 days postirradiation gave an average value of 5.0 ± 0.7 mitoses per 10 fields. The close agreement between the percentage reduction in incorporation of P^{32} into DNA and the percentage reduction in mitoses (50% in both cases) is undoubtedly fortuitous, since even the control tumors showed considerable necrosis. Such agreement, however, does indicate that under our conditions of irradiation there is no evidence for an independent "turnover" of DNA as Vermund et al. had suggested. The lack of tumor growth after irradiation, in spite of the considerable mitotic activity, is presumably due to increased cell death. On the basis of the present information it is not possible to decide whether the reduction in mitotic activity and in DNA specific activity is due to a shift in cell population, or a doubling of the intermitotic time of each cell, or a complete inhibition of half the cells with a relatively normal process in the other half. It is hoped that radioautographic techniques will shed more light on the mechanisms involved.

In the irradiated lymphosarcoma mitotic counts were not possible because of the large amount of cell debris. The low DNA specific activity at 1 day, with recovery at 3 days, is undoubtedly partly due to dilution of viable cells with dead cells and debris, which diminishes with time. Whether, in addition to this, there is a direct inhibition of DNA synthesis with subsequent recovery in some cells is not known.

RNA

The incorporation of P^{32} into the cytoplasmic RNA of the mammary carcinomas was measured on the same tumors as the DNA up to 2 days after irradiation.** The percentage depression was very similar for both nucleic acids. This does not agree with the results of Vermund et al.,[5] who found no change in the specific activities of various cytoplasmic RNA fractions of irradiated mammary carcinomas, and there is no explanation for the divergent results.

The effect of irradiation on the incorporation of precursors into RNA appears to vary from tissue to tissue. Abrams[8] reported a marked inhibition

*A minimum of 10,000 cells were examined per tumor and only metaphases recorded. There were approximately 100 cells per field. Sections were cut 6μ thick and stained with H & E. We are indebted to Mrs. W. Palmer for the mitotic counts.

**A limited number of measurements of acid-soluble phosphorus specific activity revealed no difference between control and irradiated tumors.

of glycine-1-C^{14} incorporation into bone marrow and intestine RNA, Thomson et al.[33] found an inhibition of P^{32} incorporation into thymus RNA, and Bennett et al.[9] found a lowered incorporation of adenine into "carcass" RNA. On the other hand Payne et al.[4] reported a slightly increased P^{32} incorporation into cytoplasmic liver RNA, and Thomson et al.[7] found a similar increase in regenerating liver. It is possible that this difference in response among the various tissues is again dependent on shifts in cell population.

In summary, most of the observations reported in this paper on the incorporation of P^{32} into DNA after irradiation could be explained on the basis of a changed cell population. This does not rule out the possibility of a specific inhibition of DNA synthesis. However, in view of the fact that, in the dose range studied, the decrease in the incorporation of P^{32} into DNA is gradual, one would have to postulate an effect on cells that have not yet entered synthesis rather than an immediate arrest of synthesis. Howard and Pelc,[34] by means of a radioautographic method, have presented evidence that in irradiated Vicia faba root meristem the number of cells synthesizing DNA is normal for the first six hours and then drops below control values.

Harrington and Lavik[35] suggest a specific inhibition of P^{32}, formate, and orotic acid incorporation into irradiated (100 r) rat thymus DNA. This is derived from the fact that radiation depresses these precursors while they find no inhibition of adenine incorporation. Bennett et al.[9], on the other hand, find no difference between adenine and P^{32} incorporation after 800 r in several mouse tissues other than thymus.

Clearly definitive evidence for a specific inhibition of DNA synthesis may not be obtainable from mammalian experiments owing to the mixed cell populations that are always present in mammalian tissues. However, careful measurements of microorganisms at very short time intervals after irradiation may yield the desired information. Some results on DNA content of ultraviolet-irradiated bacterial cultures are available now[36, 37], but the inhibition was found to be short-lived even after massive doses, and the possibility of an initial loss of DNA due to cell death was not ruled out.

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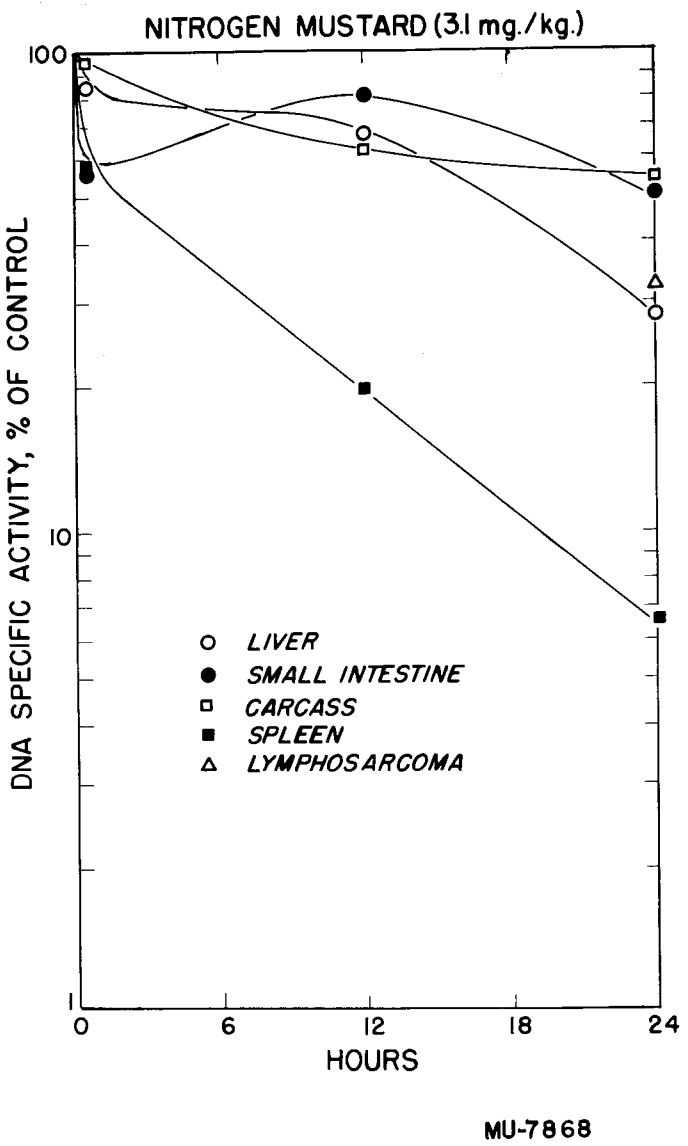

Fig. 2. Incorporation of P^{32} into DNA after the intraperitoneal injection of nitrogen mustard. Ordinate: DNA specific activity 2 hours after P^{32} injection as percent of control. Abscissa: time from injection of nitrogen mustard to injection of P^{32}.

INFLUENCE OF NITROGEN MUSTARD AND COLCHICINE ON THE INCORPORATION OF PHOSPHORUS-32 INTO DNA

Lola S. Kelly, J. Dorothy Hirsch, and Genevieve Beach

Nitrogen mustard and colchicine have been termed "radiomimetic" drugs because their morphological and physiological effects are in many respects similar to those of total-body irradiation. Philips,[1] Friedewald,[2] Levine,[3] Dustin,[4] and others have reviewed the field recently. It seemed of interest to compare the effect of these drugs to that of x-irradiation on the incorporation of P^{32} into DNA, particularly since it has been postulated that nitrogen mustard specifically interferes with DNA synthesis.[5] The experiments to be reported were intended as a preliminary survey.

Methods

Heston C-strain mice were injected intraperitoneally with 3.1 mg/kg nitrogen mustard (Mustargen Hydrochloride, Merck & Co.) dissolved in isotonic saline, a dose which is in the lethal range. At various times after mustard administration (1/2, 11, or 24 hours) $Na_2HP^{32}O_4$ was injected intraperitoneally. In one group of mice the nitrogen mustard was injected intravenously and P^{32} given 1/2 hour later intraperitoneally. To test the effect of nitrogen mustard on a tumor, Heston A mice bearing a transplanted lymphosarcoma were injected with the drug intraperitoneally 14 days after the transplanting and P^{32} was given 24 hours later.

To test the effect of colchicine, 1.5 mg/kg dissolved in isotonic saline was injected subcutaneously and P^{32} given intraperitoneally 1/2, 3, or 24 hours later. One group of mice was injected with 3 mg/kg of colchicine and P^{32} 3 hours later. Heston A mice bearing a transplanted lymphosarcoma were injected with 1.5 mg/kg of the drug and P^{32} 3 hours or 24 hours later.

Twenty experimental mice were run in each group together with twenty saline-injected controls, and the mice were sacrificed exactly two hours after the injection of P^{32}. All the other procedures employed have been described in the preceding section on radiation effects.

Results and Discussion

Figure 2 summarizes the DNA specific activities obtained at various times after the injection of nitrogen mustard. It is clear that the high dose of nitrogen mustard used depresses the incorporation of P^{32} into DNA of all the tissues measured, and that the maximum depression in spleen, liver and "carcass" does not occur until many hours after injection of the drug. Since it has been shown that the drug is fixed to tissue constituents very rapidly after injection,[6] the data suggest that at least a part of the inhibition of DNA synthesis is a secondary effect. In order to compare in detail the results of the drug and of x-irradiation, it would be necessary to run the experiments for longer time intervals and to repeat them using different dosages. However, the "carcass"

fraction seems to be relatively less sensitive to nitrogen mustard than to irradiation if it is compared with the other tissues measured. In one experiment the drug was injected intravenously and P^{32} given 1/2 hour later. In this case the carcass showed a greater response (66%) than after the intraperitoneal injection, suggesting that some of the "insensitivity" observed using the latter route may have been due to a low availability of the drug. In the other tissues no difference in response could be seen between the two modes of administration of the drug.

In addition to the tissues represented in Fig. 2, skin, kidney, and lung specific activities were measured. The skin was depressed to 53% of normal 11 hours after nitrogen mustard (the only time interval observed). The data for kidney and lung are not very reliable, owing to the small number of samples obtained, but both tissues showed a lower incorporation of P^{32} into DNA at the later time intervals.

Figure 3 summarizes the DNA specific activities obtained at various times after the injection of colchicine. Each tissue apparently has a different response pattern to this drug and at the present time there is no adequate explanation for the mechanism by which colchicine affects the incorporation of P^{32} into DNA. Classically, the principal effect of colchicine in the dose range employed is a temporary arrest of mitotic cells in metaphase because of interference with spindle formation.[3] However, several investigators have noted the extensive damages produced by the drug in spleen, lymphatic tissue, and bone marrow. Dustin,[4] for example, states "(in thymus) the extent of the cellular destruction which may be found within less than 24 hours suggests that more cells are destroyed than would have divided in this period of time". It seems reasonable to assume, therefore, that the marked effect of colchicine on the incorporation of P^{32} into DNA of spleen and "carcass" is a result of cellular destruction rather than a direct inhibition of DNA synthesis.

Colchicine had essentially no effect on the DNA specific activity of small intestine, although intestines examined 5 hours after administration of the drug showed metaphase arrest. It is possible that the time intervals studied were poorly chosen or that the dose used was not large enough to cause any marked amount of cellular destruction.

The incorporation of P^{32} into liver DNA is 690% of normal 24 hours after the injection of colchicine.* It has been found by Teir[7,8] that there is a marked increase in mitotic activity of rat livers one or two days after colchicine. Apparently the drug causes liver damage and subsequent regeneration. The increased DNA specific activity occurs somewhat earlier than after carbon tetrachloride poisoning,[9] but the time at which regeneration begins may be influenced by the amount of liver tissue destroyed.

When a larger dose (approaching the lethal range) of colchicine was used (3mg/kg), with P^{32} 3 hours later, the changes observed were the same as with the smaller dose, although somewhat more pronounced.

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*Liver inorganic phosphate specific activities measured on a limited number of samples showed a slight elevation above controls, which was of doubtful significance.

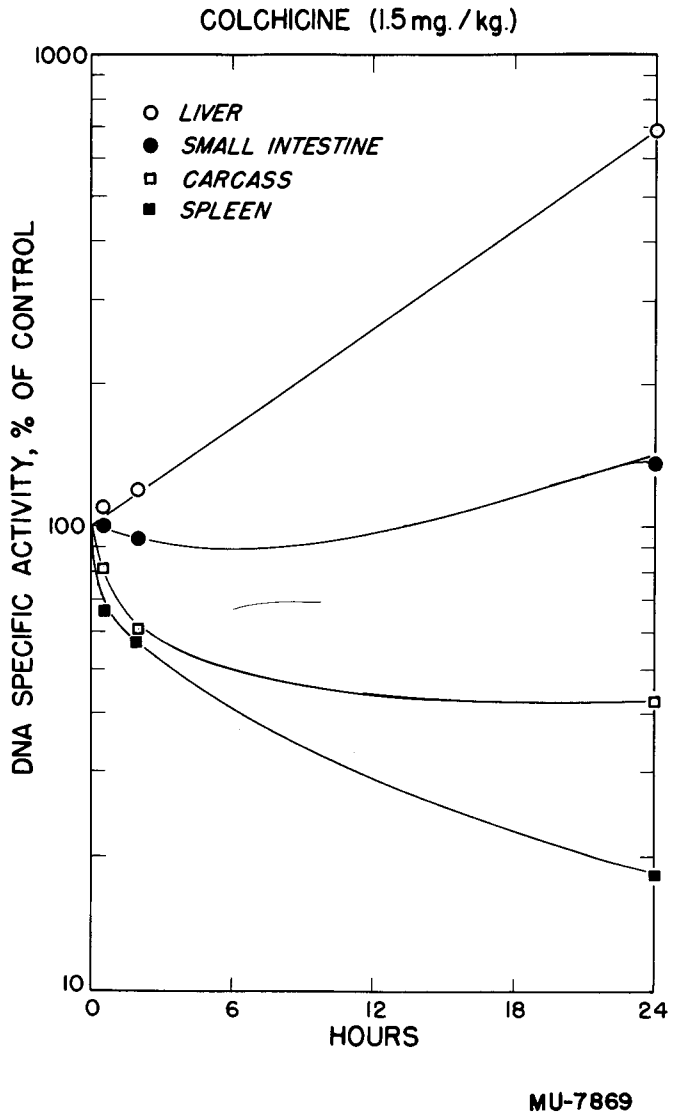

Fig. 3. Incorporation of P^{32} into DNA after the subcutaneous injection of colchicine. Ordinate: DNA specific activity 2 hours after P^{32} injection as percent of control. Abscissa: time from injection of colchicine to injection of P^{32}.

In one experiment 1.5 mg/kg of colchicine was injected into mice bearing a lymphosarcoma. The tumor DNA specific activities were approximately normal (94% of controls) for mice injected with P^{32} 3 hours after the drug, but considerably depressed (18% of controls) when the mice were injected with P^{32} 24 hours later.

In summary: x-irradiation and the two "radiomimetic" drugs, nitrogen mustard and colchicine, have similar effects on the incorporation of P^{32} into DNA of some tissues (e.g. spleen, lymphosarcoma) while in other tissues (e.g. liver, small intestine) they show marked differences. Most of the changes observed in DNA synthesis may well be secondary to cell death or cell damage produced by mechanisms that are not necessarily the same for the three agents.

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THE METABOLIC PROPERTIES OF VARIOUS MATERIALS

Joseph G. Hamilton, M.D.

Tracer Studies

LANTHANIDE AND HEAVY ELEMENTS

Joseph G. Hamilton, Patricia W. Durbin, Marilyn Williams, Ruth Newman, and Margaret Gee

Tracer studies with the lanthanide rare earths are continuing. Quite detailed and accurate information concerning the distribution, retention, and excretion following parenteral administration to female Sprague-Dawley rats has been completed for cerium-144, thulium-170, and lutetium-177. In the case of lutetium-177 the time intervals of the studies were restricted, owing to the short half life of the only available isotope of high specific activity. The thulium and cerium studies have been completed through 256 days, and the work on thulium-170 appears in this report. There remain to be done the 40-hour lanthanum-140, the 12-day praseodymium-143, the 40-hour samarium-153, the 60-hour dysprosium-166, the 27-hour holmium-166, the 9-day erbium-169, and the 4-day ytterbium-175. The terbium, europium, and promethium experiments are being carried on currently between the 64- and 256-day intervals, as in the case of actinium.

Promethium-147

The tracer studies including the excretion values are presented in Table I for promthium-147. Since this is a fission product it its essentially carrier-free. Attention should be directed to the fact that accumulation in the liver initially is approximately 45% and declines fairly rapidly thereafter. This value is signigicantly lower than for cerium-144, where the initial uptake by the liver was 54%. The skeletal deposition and retention of promethium-147 were greater than found for cerium-144. At the 64-day interval the organs showing appreciable concentration were bone, kidney, liver, and spleen, in that order. It is of interest to note that the promethium recovered from the injection site did not change significantly between the first and sixty-fourth day. This would suggest that complexing with sodium citrate was most effective.

Thulium-170

A comparable experiment is presented in Table II for thulium-170, which is the twelfth member of the lanthanide series. Here there would appear to be very definite evidence of the effect of the lanthanide contraction, since the liver uptake was only 3.4% on the first day, whereas the skeleton contained nearly 65%. At the end of 256 days it may be noted that the skeleton content had decreased only slightly, from an initial value of 60% to 54 %. This is certainly an indication of slow release of thulium-170, under the conditions of these experiments, from the skeleton. Even in the case of the light rare earths, for which cerium-144 serves as an example, the apparent relative loss from the

Table I

The Deposition of Promethium-147 complexed with Sodium Citrate in the Rat 1, 4, and 64 Days after Intramuscular Injection. Values are Corrected for 100 Percent Recovery and are Expressed in Percent of Absorbed Dose. Each Rat of the 1- and 4-Day Group Received 10 Microcuries of Promethium-147 and 3 Milligrams of Sodium Citrate; Each Rat of the 64-Day Group Received 20 Microcuries of Promethium-147 and 6 Milligrams of Sodium Citrate. The Standard Error of the Mean for the Liver and Skeleton are Shown at the Bottom of the Table.

Tissue	1 day		4 days		64 days	
	%/organ	%/gram	%/organ	%/gram	%/organ	%/gram
Spleen	0.09	0.19	0.07	0.15	0.04	0.07
Blood	0.05	0.006	<0.01	<0.001	<0.01	<0.001
Liver	43.8*	6.37*	41.4*	6.12*	1.70*	0.19*
Kidney	1.76	1.17	1.44	1.04	0.46	0.26
G.I. tract	1.36	0.20	0.63	0.09	0.25	0.04
G.I. contents	1.75	-	1.45	-	0.09	-
Muscle	1.98	0.02	0.73	0.008	0.86	0.008
Skeleton	34.3**	1.82**	36.4**	2.13**	28.65**	1.28**
Balance	2.55	-	2.32	-	1.01	-
Skin	1.63	0.05	0.73	0.03	0.45	0.01
Urine	9.79	-	9.61	-	14.5	-
Feces	0.86	-	5.07	-	49.8	-
Injection site	4.24	-	4.75	-	3.21	-
Actual recovery	97.6	-	94.0	-	91.2	-
Standard error of mean *	±1.95	±0.35	±2.4	±0.35	±0.32	±0.03
Standard error of mean **	±1.6	±0.12	±2.3	±0.28	±1.19	±0.08

skeleton is not much greater, although the skeletal deposition was half that of thulium-170. The only soft tissues that showed appreciable concentration at the end of 256 days were the kidney and spleen. These both were much below that of the skeleton, but it is of interest to note that there is a presistant retention of thulium in the kidney. This is probably related to the fact that the kidney is the principal channel of elimination of this radioelement. The small amount seen in the spleen is probably due to a very insignificant colloid effect, although this consideration is purely speculative in character. It is interesting to note that the initial urinary excretion was quite high and appeared significantly greater that that observed at the 24-hour interval for promethium-147, which in turn was greater than that seen with cerium-144.

Actinium-227

There is presented in Table III a comparable tracer study for actinium-227. This radioelement was administered by parenteral injection to Sprague-Dawley female rats and was complexed with sodium citrate, as all the lanthanide rare earths have been. In general the recovery, while good, was not so high as in the case of the lanthanide rare earths. This was because of the high degree of toxicity of actinium-227 and its radioactive descendants, which made it necessary to administer a very small amount of actinium-227 and to count the ashed samples by measuring their alpha-particle activity. The problem of self-absorpiton was always present, and to evenly distribute an ashed sample on a counting dish and avoid self-absorpiton is extremely difficult. These samples for the three time intervals--namely 1, 4, and 64 days--were all allowed to age for at least three months so that complete equilibrium existed between actinium-227 and its daughter products.

The work with the lanthanide rare earths should be completed within the next four months, and will be written up as a monograph. Special attention will be directed to the chemical properties of the lanthanide group in an attempt to correlate this with their metabolism. In our opinion this is more than an exercise in experimental philosophy, for it would appear that the more information we can gain concerning the possible correlation between the chemical properties of elements and their metabolism, the more effectively can their health hazards be understood.

Radium-223

Lethality studies of a single intramuscular injection of radium-223 were initiated 7-1/2 months ago. Sprague-Dawley female rats 100 days of age were used in these studies, and the radium was complexed with sodium citrate after its isolation from actinium-227. Radium-223 emits alpha particles and there is a series of short-lived alpha-particle descendants with a total energy of approximately 20 Mev, including the alpha-particle energy from radium-223 itself. There are two beta emitters with an average energy of 0.5 Mev each.

In each instance 9 or 10 animals were employed. The lethality of this 11.2-day radium isotope as shown in Table IV was surprisingly high, as evidenced by some deaths at the two higher dose levels within a month after the injection. Quite a number of the animals succumbed to pneumonia at a later time, three to six months after the injection. Our rat colony is relatively

Table II

The Deposition of Thulium-170 Complexed with Sodium Citrate in the Rat 1, 4, 64, and 256 Days After Intramuscular Injection. Values Are Corrected for 100 Percent Recovery and Expressed in Percent of Absorbed Dose. Each Rat of the 1-, 4-, and 64-Day Groups Received 3 Microcuries of Thulium-170, 0.08 Micrograms of Thulium, and 3 Milligrams of Sodium Citrate; Each Rat of the 256-Day Group Received 12 Microcuries of Thulium-170, 0.32 Micrograms of Thulium, and 12 Milligrams of Sodium Citrate. The Standard Errors of the Mean for the Liver and Skeleton Are Shown at the Bottom of the Table.

	1 day		4 days		64 days		256 days	
Tissue	%/organ	%/gram	%/organ	%/gram	%/organ	%/gram	%/organ	%/gram
Spleen	0.12	0.20	0.13	0.22	0.09	0.12	0.04	0.06
Blood	0.16	0.02	0.02	<0.01	<0.01	<0.001	<0.01	<0.001
Liver	3.40*	0.46*	1.86*	0.24*	0.25	0.03	0.16	0.01
Kidney	1.43	0.92	1.07	0.64	0.35	0.17	0.26	0.12
G.I. tract	0.81	0.12	0.59	0.10	0.18	0.02	0.07	<0.01
G.I. contents	1.28	-	0.50	-	0.03	-	<0.01	-
Muscle	1.96	0.02	1.59	0.01	1.08	0.01	0.57	0.004
Skeleton	64.8**	3.09**	64.1**	2.72**	61.7**	2.57**	54.0**	2.00**
Balance	3.63	-	1.98	-	0.78	-	0.59	-
Skin	1.53	0.03	1.33	0.03	0.39	0.01	0.16	<0.01
Urine	16.9	-	22.1	-	25.3	-	32.7	-
Feces	4.00	-	4.66	-	9.88	-	11.4	-
Injection site	5.31	-	5.15	-	1.63	-	2.45	-
Actual recovery	96.6	-	95.0	-	98.8	-	86.1	-
Standard error of mean *	±0.10	±0.01	±0.18	±0.04	-	-	-	-
Standard error of mean **	±0.1	±0.06	±0.3	±0.09	±0.8	±0.07	±1.6	±0.07

Table III

The Deposition of Actinium-227 Complexed with Sodium Citrate in the Rat 1, 4, and 64 Days After Intramuscular Injection. Values Are Corrected for 100 Percent Recovery and Expressed in Percent of Absorbed Dose. Each Rat Received 0.25 Microcuries of Actinium-227 and 6 Milligrams of Sodium Citrate. The Standard Error of the Mean for the Liver and Skeleton Are Shown at the Bottom of the Table.

Tissue	1 day		4 days		64 days	
	%/organ	%/gram	%/organ	%/gram	%/organ	%/gram
Spleen	0.06	0.11	0.05	0.09	0.05	0.12
Blood	<0.01	<0.001	<0.01	<0.001	<0.01	<0.001
Liver	59.0*	10.6*	56.4*	7.00*	4.02*	0.52*
Kidney	0.97	0.73	0.56	0.36	0.34	0.22
G.I. tract	0.85	0.07	0.38	0.04	0.15	0.03
G.I. contents	1.25	-	2.11	-	0.09	-
Muscle	0.70	0.01	0.87	0.01	0.22	0.002
Skeleton	29.9**	2.22**	26.8**	1.94**	20.6**	0.93**
Balance	2.40	-	1.36	-	1.01	-
Skin	1.07	0.05	0.86	0.03	0.34	0.01
Urine	3.69	-	4.78	-	6.37	-
Feces	0.31	-	5.82	-	65.4	-
Injection site	7.15	-	2.74	-	0.60	-
Actual recovery	85.4	-	91.4	-	88.3	-
Standard error of mean *	±1.3	±0.5	±1.3	±0.09	±0.57	±0.05
Standard error of mean **	±1.3	±0.07	±1.11	±0.15	±0.9	±0.03

Table IV

Lethality of a Single Intramuscular Injection of Radium-223. The Radium Was Complexed with Sodium Citrate and Administered when the Rats Were 100 Days Old.

	Number of Survivors				
Days after Injection	Controls	0.008μc/gm Ra^{223}	0.017μc/gm Ra^{223}	0.03μc/gm Ra^{223}	0.054μc/gm Ra^{223}
0	10	9	10	9	10
30				7	8
60				7	
90	9			6	
120		8		3	
150				2**	6**
180		7	8		
210		7*	8*		

* Sacrificed 210 days after injection

** Sacrificed 150 days after injection

free from pneumonia, the incidence being of the order of 1% to 2% in the control animals 400 days and older. Apparently in the radium-treated animals there was a depression of resistance to infection, which made them much more susceptible to pneumonia than the control animals.

The effects upon growth of a single dose of radium-223 at the four dose levels employed, namely 0.008 μc/gram, 0.017 μc/gram, 0.03 μc/gram, and 0.054 μc/gram, are shown in Fig. 1. There is considerable overlapping. It may be noted that the curve for the highest-dose-level group terminated at 140 days; animals were sacrificed at this time. The significance of two of the curves is that during a period that extended over 200 days there was no appreciable gain in weight, which is a manifestation of the toxicity of radium-223.

Figure 2 presents the changes of the total leukocyte count employing the same four groups of animals. There may be noted a precipitous drop in all four groups, but there does appear to be a significant difference between the higher and lower dose levels. Only in the lowest dose level, namely 0.008 μc/gram of radium-223, did there appear a tendency towards recovery and an approach to the lower limit of the normal leukocyte count (expressed in terms of thousands of cells per cubic centimeter of blood). Even though the half life of radium-223 is but 11.2 days and there are no long-lived descendants, it is apparent that lack of recovery was due to injury to the cells responsible for production of leukocytes.

Figure 3 presents the effect of radium-223 upon the erythrocyte count of the same group of rats. Here the drop was less precipitous, and an appreciable recovery occurred. This observation suggests that the stem cells for the production of leukocytes and of erythrocytes may be different. Attention should be directed to the fact that these experiments were carried out with two groups of rats for 200 days which is approximately twice the life span of the erythrocyte in the rat.

There may be seen in Fig. 4 the hemoglobin levels from the same group of animals, which in general follow the patterns observed in the erythrocyte count. Likewise in Fig. 5 the hematocrit follows very closely both the erythrocyte counts and the hemoglobin.

Detailed histopathology in the radium-223 rats is either currently being studied or in preparation. This is a most laborious undertaking in preparation and examination, and at present there are approximately several hundred specimens to be examined.

IODINE AND ASTATINE STUDIES

Joseph G. Hamilton, Patricia W. Durbin, and Marshall Parrott

The paper beginning on page 40 was given April 29, 1954 before the American Goiter Society in Boston and has been submitted and accepted for publication by Transactions of the American Goiter Association for the year 1954.

Two additional papers have been accepted for publication by the Proceedings of the Society for Experimental Biology and Medicine. The titles are as follows:

THE EFFECT OF A SINGLE DOSE OF Ra^{223} UPON THE BODY WEIGHT OF THE FEMALE SPRAGUE-DAWLEY RAT.

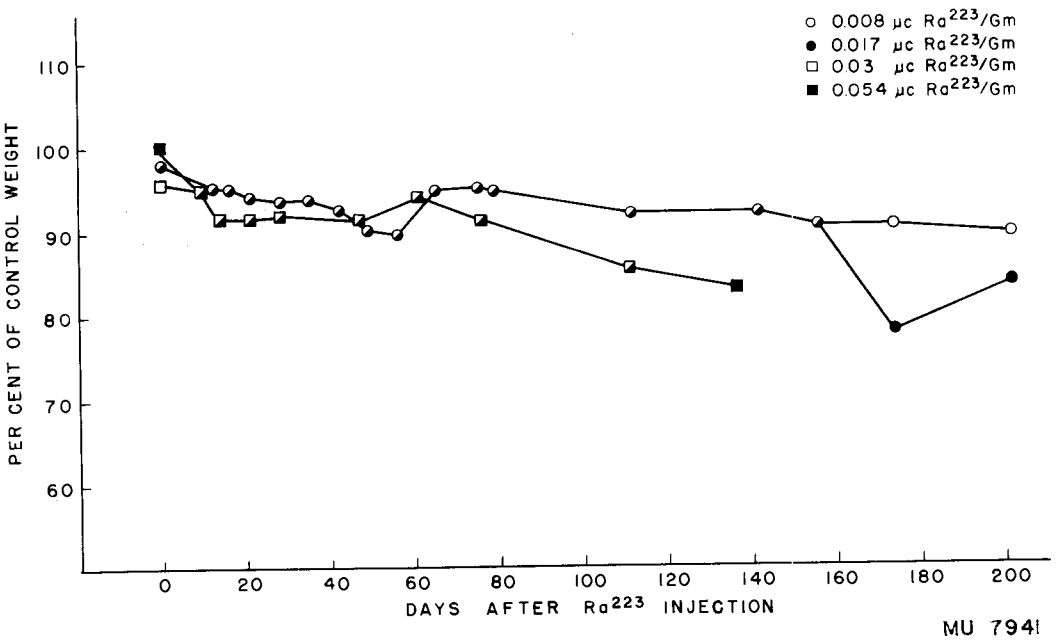


Figure 1

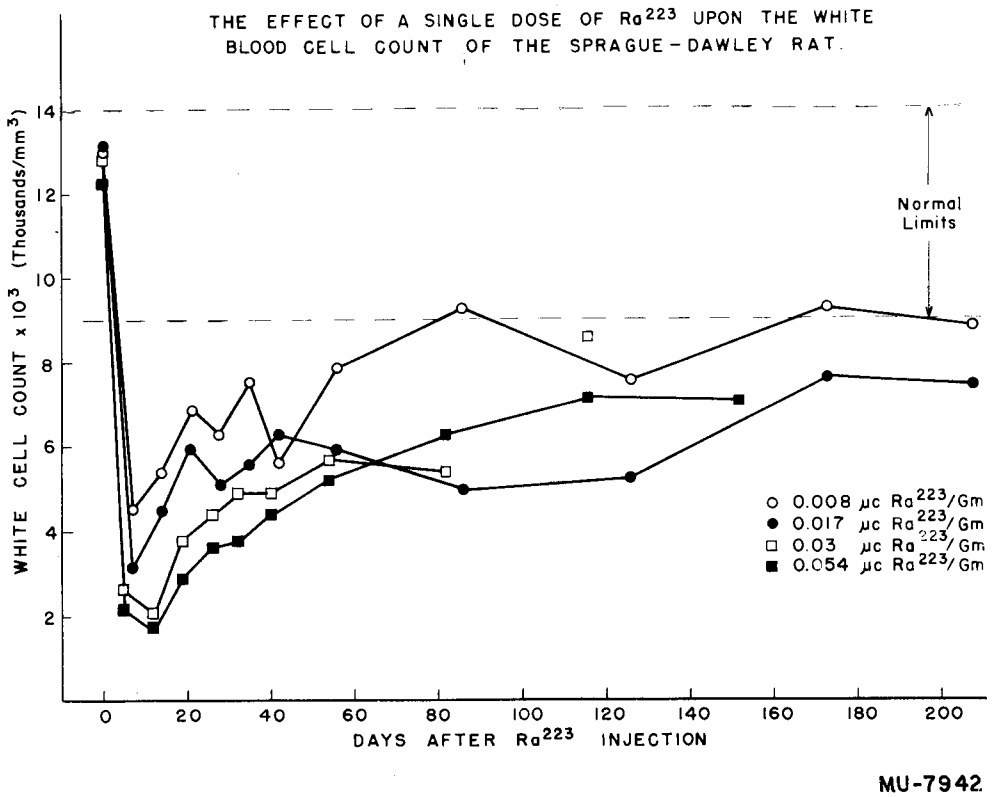

THE EFFECT OF A SINGLE DOSE OF Ra223 UPON THE WHITE BLOOD CELL COUNT OF THE SPRAGUE-DAWLEY RAT.
WHITE CELL COUNT x 10³ (Thousands/mm³)
14
12
10
8
6
4
2
Normal Limits
0.008 μc Ra223/Gm
0.017 μc Ra223/Gm
0.03 μc Ra223/Gm
0.054 μc Ra223/Gm
0
20
40
60
80
100
120
140
160
180
200
DAYS AFTER Ra223 INJECTION
MU-7942

Figure 2

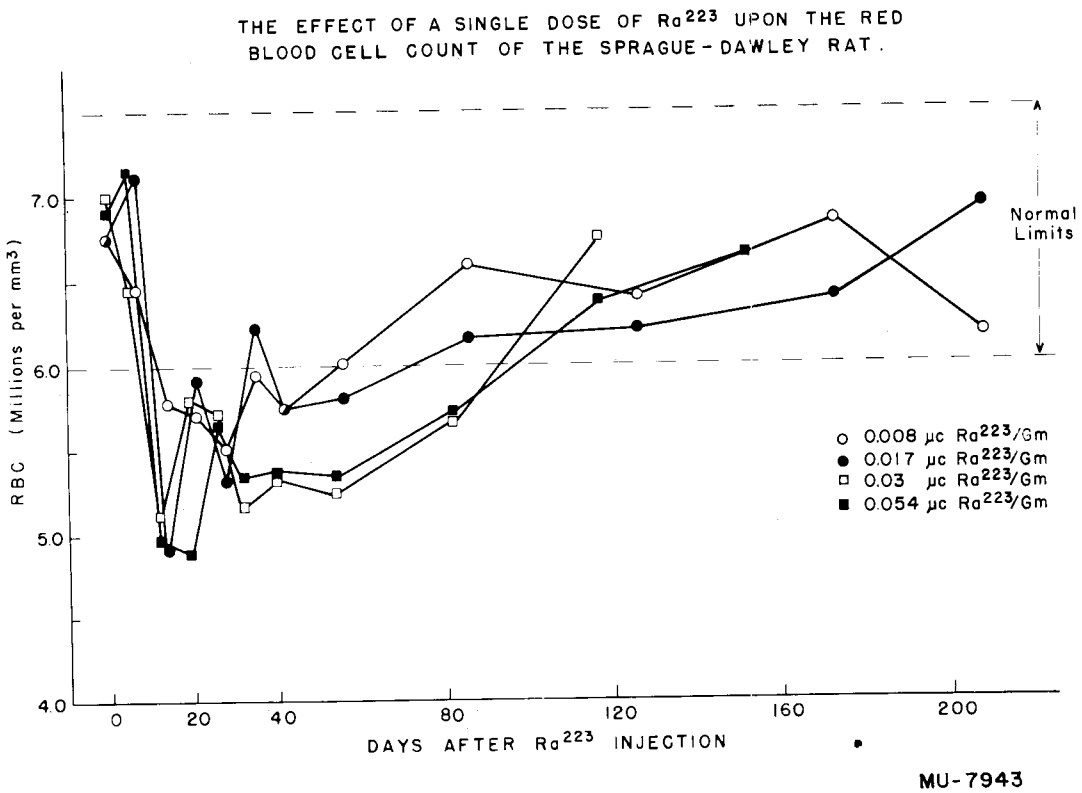

THE EFFECT OF A SINGLE DOSE OF Ra223 UPON THE RED BLOOD CELL COUNT OF THE SPRAGUE-DAWLEY RAT.
RBC (Millions per mm3)
7.0
6.0
5.0
4.0
0
20
40
80
120
160
200
DAYS AFTER Ra223 INJECTION
Normal Limits
0.008 µc Ra223/Gm
0.017 µc Ra223/Gm
0.03 µc Ra223/Gm
0.054 µc Ra223/Gm
MU-7943

Figure 3

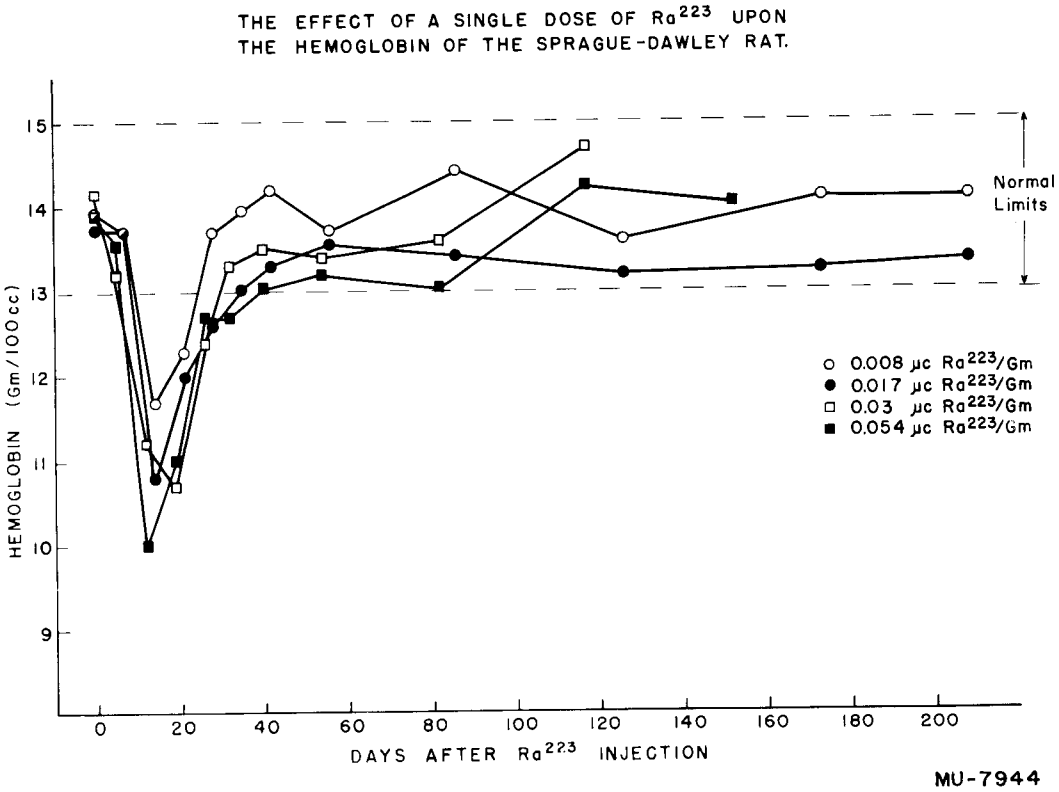

THE EFFECT OF A SINGLE DOSE OF Ra223 UPON
THE HEMOGLOBIN OF THE SPRAGUE-DAWLEY RAT.
HEMOGLOBIN (Gm/100 cc)
15
14
13
12
11
10
9
0
20
40
60
80
100
120
140
160
180
200
DAYS AFTER Ra223 INJECTION
Normal Limits
0.008 µc Ra223/Gm
0.017 µc Ra223/Gm
0.03 µc Ra223/Gm
0.054 µc Ra223/Gm
MU-7944

Figure 4

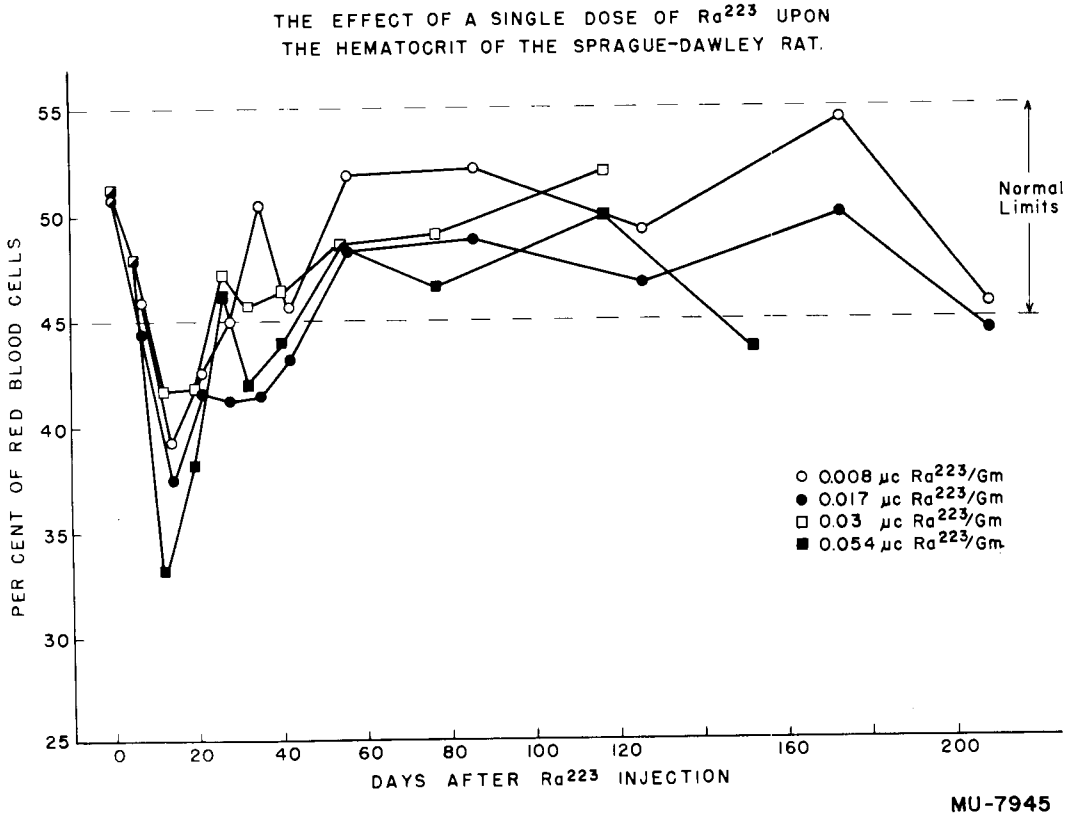

THE EFFECT OF A SINGLE DOSE OF Ra223 UPON
THE HEMATOCRIT OF THE SPRAGUE-DAWLEY RAT.
PER CENT OF RED BLOOD CELLS
55
50
45
40
35
30
25
0
20
40
60
80
100
120
160
200
DAYS AFTER Ra223 INJECTION
Normal Limits
○ 0.008 µc Ra223/Gm
● 0.017 µc Ra223/Gm
□ 0.03 µc Ra223/Gm
■ 0.054 µc Ra223/Gm
MU-7945

Figure 5

"The Effect of Pretreatment with Propyl Thiouracil on the Accumulation of Astatine-211 by the Thyroid Gland of the Rat" by Patricia W. Durbin, Joseph G. Hamilton, and Marshall W. Parrott; and "Accumulation of Astatine-211 by the Thyroid Gland in Man" by Joseph G. Hamilton, Patricia W. Durbin, and Marshall W. Parrott. These two papers are not included in this quarterly report as they will appear as two UCRL reports, which should be available shortly.

The effect of antithyroid drugs upon the accumulation of astatine by the thyroid gland of the rat was independently confirmed by the group at Brookhaven and presented at the American Goiter Society meeting. In this case thiouracil was used and the effect was considerably less than we observed when propyl thiouracil was employed.

Long-Term Studies With Astatine-211

Three groups of ten rats each received 0.83, 0.62 and 0.42 microcuries per gram of At^{211}. This radiohalogen was administered by intravenous injection to 55-day old Sprague-Dawley female rats. The animals were all sacrificed one year after the injection. At the highest dose level there was evidence of endocrine disfunction, which was grossly manifested by loss of hair and a slight retardation of growth. The former effect was less marked, but was still apparent, at the two lower doses.

Table V shows the weights of the spleen, adrenal, and thyroid of the experimental animals and of a group of age controls. The uptake of carrier-free radioiodine given one day prior to sacrifice averaged 7.4% in the controls, 2.6% at the 0.42 μc/gram of astatine dose level, 3.0% for those animals that received 0.62 μc/gram of astatine, and 1.62% uptake at the 0.83 μc/gram astatine dose level. The number of survivors in each group is shown in Table V.

Terminal blood counts including red cell count, white cell count, and hemoglobin were taken from control animals, and from those that had received 0.83 μc/gram at At^{211}. Only the hemoglobin was determined for the other two at At^{211} groups. These data are presented in Table VI. There is a statistically significant difference between the mean weight of the control thyroid glands and the mean weights of thyroids in the three At^{211}-treated groups; p is less than 0.01 in all three cases. The mean weight of the spleen at the highest At^{211} dose level was significantly different from the mean weight of the control spleen; $p = 0.05$. The depression of accumulation of radioiodine by the three groups of At^{211} animals also has a p value of less than 0.01. The p values for the blood counts appear in the table.

Histological specimens were obtained from all the surviving At^{211}-treated animals and the year-old controls. This material is now being processed and will be reported at a later date.

The occurrence of mammary neoplasms in several of the At^{211}-treated animals is discussed in detail in the paper given before the American Goiter Society, which is included in this report.

Table V

The Weights of the Spleen, Thyroid Gland, and Adrenal Gland One Year After the Intravenous Administration of Astatine-211. The Animals Were Female Sprague-Dawley Rats and Were 55 Days of Age When Injected.

Group	Number of rats	Spleen	Adrenal*	Thyroid
Control	9	479 ± 25 mg	35.2 ± 2.0 mg	26 ± 1.4 mg
0.42 μc/g astatine	6	393 ± 28 mg	29.1 ± 2.8 mg	10.6 ± 0.97 mg
0.62 μc/g astatine	5	485 ± 40 mg	30.2 ± 3.7 mg	11.6 ± 0.7 mg
0.83 μc/g astatine	6	344 ± 54 mg	29.3 ± 2.5 mg	9.3 ± 0.9 mg

*Left adrenal only

Table VI

The Terminal Blood Counts of Rats Which Received 0.83, 0.62, and 0.42 Microcuries of Astatine-211 per Gram of Body Weight by Intraperitoneal Injection and Were Sacrificed 1 Year Later. Similar Values Are Included for Control Animals.

Control			Treated				
			0.83 μc/g			0.62 μc/g	0.42 μc/g
RBC million/mm^3	WBC thousands/mm^3	Hemoglobin	RBC million/mm^3	WBC thousands/mm^3	Hemoglobin	Hemoglobin	Hemoglobin
6.99	7.95	11.5	6.23	17.15	10.6	9.8	9.6
8.22	12.20	11.2	7.60	9.775	10.2	10.0	10.0
7.18	9.15	11.8	6.745	7.675	10.6	10.6	10.6
7.86	11.30	11.2	6.05	7.025	9.3	10.0	10.0
6.63	10.05	10.3	6.11	12.3	9.3	10.0	10.0
6.20	9.40	11.2	5.36	11.9	9.8	-	9.0
6.91	9.30	10.0	-	-	-	-	-
7.11	8.10	11.2	-	-	-	-	-
7.075	11.00	10.9	-	-	-	-	-
Average 7.13	9.83	11.0	6.35	11.0	10.0	10.1	9.9
Standard error of the mean: ±0.20	±0.48	±0.2	±0.31	±1.5	±0.2	±0.1	±0.2
			P<0.05	P<0.5	P<0.01	P<0.01	P<0.01

The monkeys that received At^{211} a year ago are showing increasing evidences of presumed simian myxedema as evidenced by apathy, loss of hair, pouchiness under the eyes, and failure to grow. An excellent example of the normal glossy pelt of the control monkey may be seen in Fig. 6. The age of this animal is estimated to be 25 months and it weighs 4.4 kilograms. The astatine-treated animal shown here received 0.8 μc/g 377 days before the photograph was taken. Its age is also estimated to be approximately 25 months, and its weight at the present time is 2.8 kilograms. The loss of hair on the back is most apparent, and may be seen in Fig. 7. (Photographing monkeys is difficult. The control animal was actually further from the camera, and therefore appears smaller.)

Long-Term Studies With Iodine-131

The experiments with radioiodine at the lethal level, which were presented in part in the last quarterly report, are continuing. Dose levels to 90 μc/g have been employed which should be very close to the MLD_{50} at 60 days. A group of eighteen 55-day old Sprague-Dawley rats received 90 μc/g . At about the tenth day there appeared the usual signs of radiation injury from the I^{131} bound by the thyroid gland to the larynx and trachea, as evidenced by respiratory stridor. However, none of the animals of this group has died of suffocation from constriction of the trachea.

It is of interest to note that although at the lower dose levels of iodine, which is roughly twice as lethal as At^{211}, there was no incidence of mammary carcinoma at the end of 200 days. In the case of At^{211}, when the dose level was in the range of from 0.4 to 0.8 μc/g there was a high incidence of mammary neoplasms. The corresponding dose level of I^{131} was apparently from 30 to 50 μc/g. The difference here is as yet not established too conclusively. Consideration should be given to two factors. In the At^{211} experiments where the first mammary neoplasm was seen 150 days after the administration of this alpha emitter, approximately 20% of the astatine was present in a colloidal state (absorbed on colloidal particles, principally aluminum oxide). Despite the increased accumulation of astatine-211 in its colloidal state in the liver, spleen, and lymph nodes, it does not appear reasonable that this colloid could have been responsible for the mammary tumors. A more attractive explanation is that at the lower astatine dose levels endocrine changes may be related to the appearance of the highest incidence of mammary tumors. This is in the dose range in which there remains a small but detectable amount of functional thyroid tissue, which has been demonstrated by the subsequent accumulation of I^{131} one day after the administration of a tracer dose of this radioisotope, both by counting the I^{131} beta particles and by the use of radioautographic techniques. Thus there is a possibility that even in a state of hypothyroidism a small amount of functional thyroid hormone remains and under these conditions is capable of evoking such lesions. A similar situation seen in surgically thyroidectomized rats strengthens this hypothesis (total surgical thyroidectomy is a very difficult thing to achieve). With radioiodine, however, even at the lowest dose level given (10 μc/g) there is complete obliteration of the thyroid gland. This interesting difference deserves further careful study.

Figure 6

Figure 7

In the study of the general chronic endocrine effects of I^{131}, it was considered desirable to obtain information on the state of the reproductive system several months after the administration of a large amount of I^{131} to the female Sprague-Dawley rat.

Examination of stained vaginal smears taken on several consecutive days has been shown to be a fairly reliable index of the functional state of the female reproductive system of the rat , since the changes in the vaginal contents have been correlated with ovarian and uterine changes. Samples of vaginal contents were obtained by the gentle insertion of a small spatula. The material was transferred to a drop of isotonic saline on a microscope slide. After air drying, the slides were stained with Wright's blood stain. Smears were prepared for all the animals tested for ten consecutive days.

Three groups of rats were studied at this time; eight rats 250 days old that had been given 10 μc/g of I^{131} when they were 55 days old; and two control groups of four old rats, 300 days old, and six young rats, 75 days old.

In the young control group five of the six rats were found to be cycling normally with a period of four and one-half to five days. The sixth animal in the group appeared to be cycling erratically. These animals are still quite young, so that the estrous cycle may not yet be firmly established at the accepted interval in all rats tested. Long and Evans found that female rats are sexually mature at from 50 to 60 days of age and that as a rule ovulation occurs at about 72 days (range 45 through 147 days). Further work should establish the age at which the normal estrous cycle occurs in this particular strain under the conditions maintained in this laboratory.

In the old control group three of the animals were cycling at an interval of about seven days. A definite estrous cycle was not determined for the fourth rat in this group.

Six of the animals in the I^{131} group showed no estrous cycle, and the other two animals, if cycling at all, were doing so only erratically. External examination indicated that the vaginas of all the rats in this group had failed to open. Opening of the vagina in the normal rat usually occurs at an age of about 50 to 75 days. Routine vaginal examination prior to the administration of I^{131} with the thyroid glands suitably protected, should enable us to determine whether irradiation or an endocrine disturbance resulting from the ablation of the thyroid gland is the primary cause of the interruption and/or prevention of the normal estrous cycle.

References:

1. J. A. Long and H. M. Evans, The oestrous cycle in the rat and its associated phenomena, Memoirs Univ. Calif. 6, 1922.

2. E. J. Farris and J. G. Griffiths, The Rat in Laboratory Investigation. 2 ed. J. G. Lippincott Co. Philadelphia, 1949, Ch. I, IV.

THE ACCUMULATION AND DESTRUCTIVE ACTION OF ASTATINE-211 (EKA-IODINE) IN THE THYROID GLAND OF RATS AND MONKEYS

Joseph G. Hamilton, Patricia W. Durbin, and Marshall Parrott

Introduction

In 1940, Corson et al. isolated a radioactive element whose chemical, physical, and nuclear properties established it as element 85, the last of the halogen group.[1] This element, now named astatine (from the Greek ἄστατος, "unstable"), was produced by the transmutation of bismuth by alpha particles accelerated to 30 Mev in the 60-inch cyclotron at the Crocker Laboratory, University of California, Berkeley.

Hamilton and Soley compared the matabolism of I^{131} and At^{211} in the guinea pig and demonstrated that At^{211}, like I^{131}, was accumulated by the thyroid gland. This work included the use not only of normal animals but also of guinea pigs in which thyrotoxicosis had been produced by the administration of the thyrotropic hormone.[2] Later a more detailed report was presented by Hamilton et al. which was concerned not only with the metabolism but also with the biological effects of astatine in rats and monkeys. In this work, changes due to the destructive action of astatine in the thyroid gland were investigated at levels that essentially obliterated that organ, and also a rather detailed comparison was made of the metabolism of At^{211} and I^{131} in the rat.[3]

The chemical properties of astatine have been investigated quite thoroughly by Corson et al. and Johnson et al.[1,4] Segre has stated that less is known about the chemical properties of astatine than about any of the other 98 elements.[5] To date, 20 isotopes of astatine have been identified, all of which are radioactive and are primarily formed by transmutation by nuclear reactions employing bismuth and other heavy metals as the target element. The longest-lived radioisotope of astatine, At^{210}, has a half life of 8.3 hours, but unfortunately this decays to form the radiotoxic Po^{210} with a half life of 140 days.[6]

The radioisotope employed in the studies described in this paper and in previously reported experiments is At^{211}, with a half life of 7.3 hours, which decays by emission of alpha particles with an average energy of 6.9 Mev. There is also present the 80-kev x-ray from the 0.52-second Po^{211} daughter.[6]

Methods

The At^{211} was prepared by a modification of the procedure described by Garrison et al.[7] The I^{131} was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tennessee.

The animals employed in these experiments were 55-day-old Sprague-Dawley female rats and six- to eight-month-old rhesus monkeys, both males and females. The rats were maintained on a standard laboratory chow* which is in general use in the University of California Radiation Laboratory,[8] and

* This diet contains added potassium iodide, 300 milliliters of 0.45% solution for every 270 pounds of feed.

were given tap water ad lib. Both At^{211} and I^{131} were given to the rats via the surgically exposed external jugular vein. The solutions administered were isotonic and buffered to pH 7. The amounts of At^{211} administered ranged from 0.4 to 1.8 microcuries per gram of body weight. The I^{131} was administered at dosage levels of 10, 30, 50, and 70 μc/g. During the first two weeks after injection of either At^{211} or I^{131} the animals were examined several times a day, and moribund animals were sacrificed and autopsied. Since the mortality as well as the acute and chronic effects of these two radiohalogens was of interest, no animals were deliberately sacrificed unless it was felt that they would not survive for more than a few hours. At the end of the first week, when it was considered safe to handle the animals, they were weighed individually, and reweighed every week for the first month and every two weeks thereafter.

In order to adequately evaluate the effect of massive doses of At^{211} and I^{131} on the growth of the rat, we have employed the method of Norris and Evans in which the growth trend of normal animals is included in the calculation of the percent of normal weight of the experimental animals.[9] The percent control weight is calculated from the following equations:

$$Q_{(I,t)}(\text{percent control weight}) = \frac{W_{(o,o)}}{W_{(I,o)}} \times \frac{W_{(I,t)}}{W_{(o,t)}} \times 100,$$

where $W_{(o,o)}$ is the weight of the control animals at the start of the experiment, $W_{(I,o)}$ is the weight of the experimental animals when injected, $W_{(o,t)}$ is the weight of the controls at any subsequent time t, and $W_{(I,t)}$ is the weight of the injected animals at time t. The use of the percent control weight as calculated above makes possible the direct comparison of the effects of the toxic agent on weight and growth at different dose levels and their separation from the normal growth pattern. It is justified in this case, because the rats used in these experiments were all the same age and very nearly the same weight when they were injected with either At^{211} or I^{131}.

Rats receiving the lower dose levels of At^{211} have been followed for as long as a year in some cases, not only from the point of view of general appearance and growth, but also for the purpose of detection of mammary tumors. The I^{131} rats are being maintained for the same purpose, but so far the degree of chronicity of this part of the experiment is not as great as in those animals given At^{211}.

The tissues of interest in the acute studies on animals receiving either At^{211} or I^{131} are the thyroid gland and the peritracheal structures. In the chronic studies the following tissues have been taken in addition to those mentioned above: pituitary gland, mammary tumors, and pulmonary metastases when present. With the exception of the pituitary gland, which is fixed in Zenker formol, the tissues are routinely fixed in 10% neutral formalin, dehydrated and cleared with dioxane, and embedded in paraffin. Sections are from five to eight microns in thickness and are stained with hematoxylin and eosin.

The monkeys, four males and two females, were fed "Chim biscuits", reconstituted milk, fresh fruit and vegetables, bananas, and peanuts, and were given tap water ad lib. They were maintained in a thermostatically controlled room at 78° F, at about 70% humidity. The At^{211} was administered intraperitoneally at levels ranging from 0.42 to 0.82 μc/g. One pair of monkeys

has been under observation for more than a year and another pair for nine months. Since the first week after injection thay have been weighed periodically, and detailed notes have been kept regarding the changes in their general condition and appearance.

A previous report has described a reversible simian myxedema one year after the parenteral administration of At^{211}.[3] This animal was given approximately 100 microcuries of At^{211}, or about 0.08 μc/g, injected into the anterior chamber of the eye, the results of which have been described by Shaffer.[10] Two years after the At^{211} injection the animal was given a tracer dose of 100 μc of I^{131} intraperitoneally and was sacrificed the next day. Radioautographs were prepared from sections of tissue taken from the region of the larynx in order to detect the presence of any epithelial cells that might have the ability to accumulate and retain I^{131}. Several other tissues were taken from this animal at autopsy, including the pituitary gland, which was fixed in Zenker formol. Sections of both peritracheal tissue and pituitary gland were stained using Koneff's modification of the Mallory-Azan technique.[11]

Results

The growth curves of the rats receiving I^{131} at levels of 10, 30, 50, and 70 μc/g are presented in Fig. 8. There may be noted an initial drop between the 10th and 25th day after I^{131} administration, which is considerably greater for the 50-μc and 70-μc group than for the two groups receiving 10 μc and 30 μc of I^{131} respectively. After the 30th day, the decrease in percent of control weight tends to be rather similar for all four groups. The At^{211} growth curves are shown in Fig. 9. Here may be seen an initial decrease in percent of control weight, which seemed to occur within approximately the first 10 days. Subsequently the growth curves crossed, and later it would seem that the 1.6-μc and 1.8-μc groups showed a greater depression of growth. The preciseness of these curves of course is subject to some statistical fluctuation, in view of the limited number of animals available.

The body weight, in kilograms, of two male control monkeys and two males receiving 0.42 μc/g of At^{211} and two females receiving 0.80 μc/g of At^{211} may be seen in Fig. 10. In this instance, no definite effect on growth is evident until after the 200th day following the administration of At^{211}. After this time, there does appear to be a difference in the increment of body weight in the control animals as compared to the experimental animals.

Another indication of the disturbance in the pattern of growth in the rat is presented in Fig. 11. On the left is shown a rat that had received 1.8 μc/g of At^{211} and on the right an animal that had been given 70 μc/g of I^{131}. In the center is a control animal. These three animals were all 90 days of age, and 35 days had elapsed since the radiohalogen was given to each of the two experimental animals. The difference in growth is quite evident, and it may be seen that the degree of depression of growth is approximately the same in the two rats receiving radiohalogens.

The effect upon the thyroid gland of a rat receiving 70 μc/g of I^{131} is shown in Fig. 12. Eight days had elapsed between the time of administration of the I^{131} and sacrifice of the animal. There was some hemorrhage, which

is not too evident in this type of photomicrograph. There may be seen what appear to be the remnants of two large follicles, but the degree of injury to this organ is obviously extreme. The over-all impression is that if this animal had not been sacrificed and had survived, the end effect would have been a very marked degree of fibrosis and complete ablation of the thyroid tissue. The chronic effect of the administration of 70 μc/g of I^{131} is shown in Fig. 13. This animal received 70 μc/g of I^{131} and was sacrificed 56 days later. There is no visible evidence of thyroid tissue, and extensive fibrosis may be seen. The degree of injury to the parathyroid gland is shown in the lower right-hand corner of the photomicrograph, and apparent injury was observed in other peritracheal structures adjacent to the site of the thyroid gland.

The acute changes observed in the thyroid gland eight days following administration of 1.8 μc/g of At^{211} may be seen in Fig. 14. In comparison to the acute study employing I^{131}, there may be seen here several follicles that appear to have been less severely injured in this interval of time. This is unlike the I^{131} photomicrograph; here extensive hemorrhage may be seen. Figure 15 shows the chronic destructive action of 0.8 μc/g of At^{211} upon the thyroid gland of the rat at the end of one year. The parathyroid gland on the left shows no visible evidence of injury, whereas there is no recognizable thyroid tissue.

There is presented in Fig. 16 a section of thyroid and parathyroid tissue from a monkey that had received 0.08 μc/g of At^{211} two years previously. The section was mounted on NTB stripping film. Here there is no visible evidence of any follicles. However, dark areas represent regions in which a tracer dose of I^{131} was accumulated by atypical epithelial cells. The parathyroid gland at the top appears normal. For purposes of comparison, a section of the thyroid and parathyroid gland of the normal monkey is shown in Fig. 17. A section of the enterior pituitary gland of the At^{211}-treated monkey is shown in Fig. 18. Representative changes can be seen in the large basophile in the center of the photomicrograph which are characteristic of changes observed following prolonged hypothyroidism.[12]

A high incidence of neoplastic changes in mammary tissue was noted in rats receiving At^{211} at dose levels ranging from 0.4 to 0.8 μc/g. Since there was no statistically significant difference between the incidence of tumors at these high and low dose levels, the results presented are the average for all the animals involved.

Figure 19 presents the incidence of these mammary tumors as a function of age. Frequently these tumors were multiple and ultimately became so large that they weighed as much as 100 grams. Occasionally pulmonary metastases were seen that were presumably from the mammary tumors. There is shown in Fig. 20 a photomicrograph of a primary mammary tumor, and in Fig. 21 (at the same magnification) may be seen a section of a pulmonary metastasis. The pulmonary metastasis shows ductlike structures, which were also noted in the primary tumor. The rat in which this tumor and metastasis were observed had received 0.8 μc/g of At^{211} and was sacrificed 235 days later.

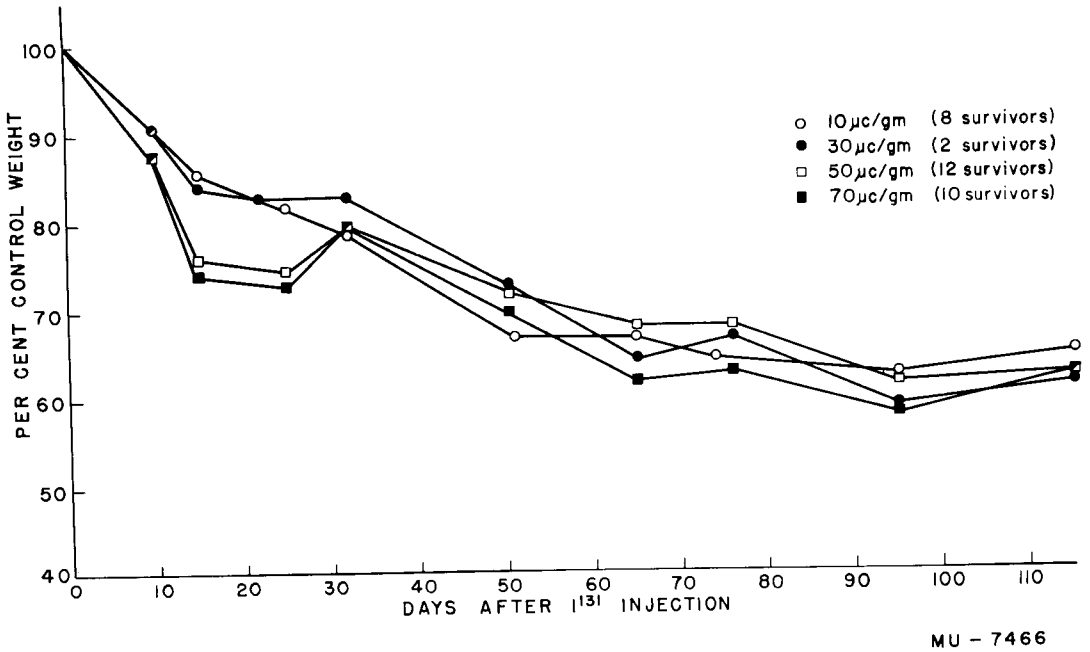


Fig. 8. The effect of a single intravenous injection of I^{131} on the growth of the Sprague-Dawley female rat. The rats were 55 days of age when injected. The weights are expressed as percent of control weight, calculated as indicated on page 41.

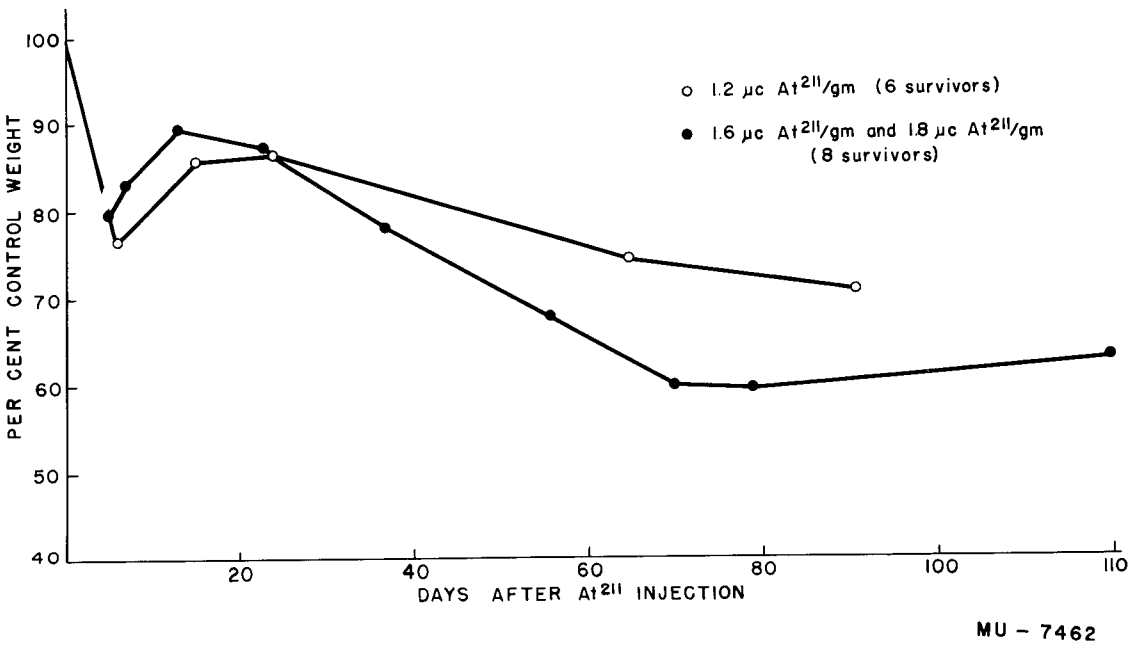


Fig. 9. The effect of a single intravenous injection of At^{211} on the growth of the Sprague-Dawley female rat. The rats were 55 days of age when injected. The weights are expressed as percent of control weight, calculated as indicated on page 41.

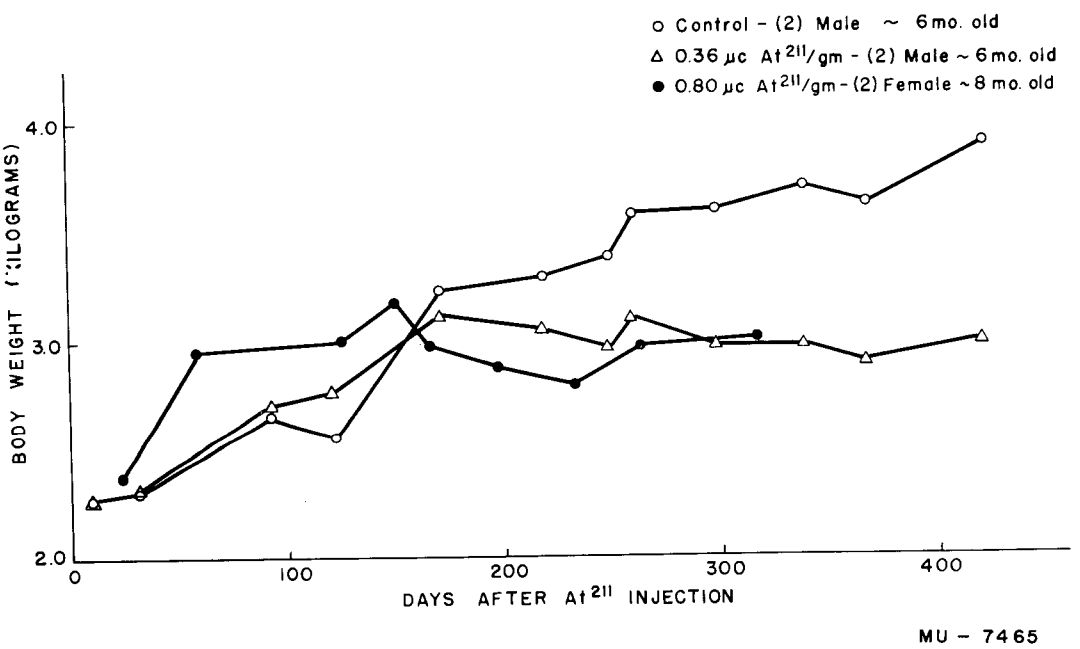
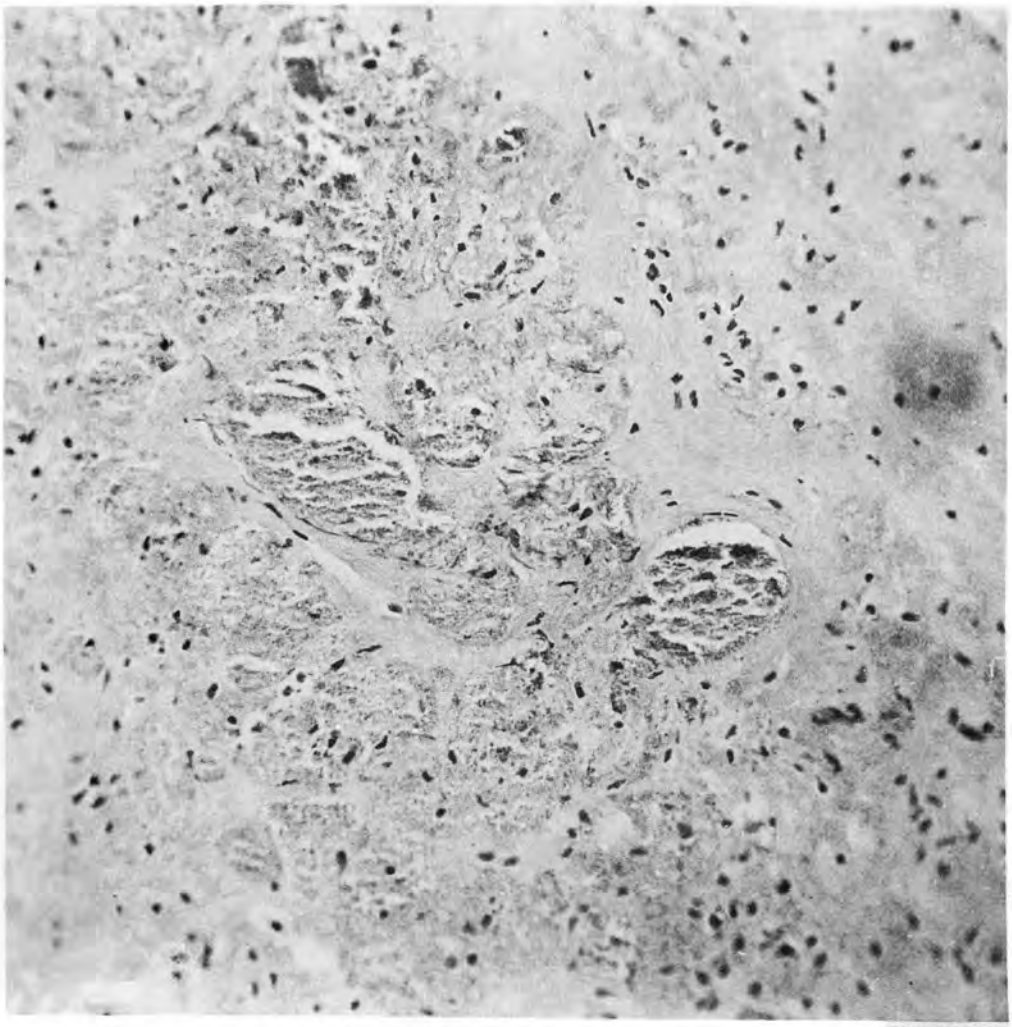


Fig. 10. The effect of a single intraperitoneal injection of At^{211} on the growth of the rhesus monkey. Dose, age, and sex are indicated in the figure.

Fig. 11. The effect of a single intravenous injection of either At^{211} or I^{131} on the growth of the Sprague-Dawley female rat. From left to right are shown: a rat that had received 1.8 μc/g of At^{211}, a normal control rat, and a rat that had received 70 μc/g of I^{131}. The animals were all 90 days of age when the photograph was taken. The radiohalogens were administered when the animals were 55 days old.



ZN-1026

Fig. 12. The histopathological changes in the thyroid gland of a rat eight days after the administration of 70 μc/g of I^{131}. Almost total obliteration of all the follicles may be seen, with a minimal degree of hemorrhage. Magnification x100. Stain: H and E.

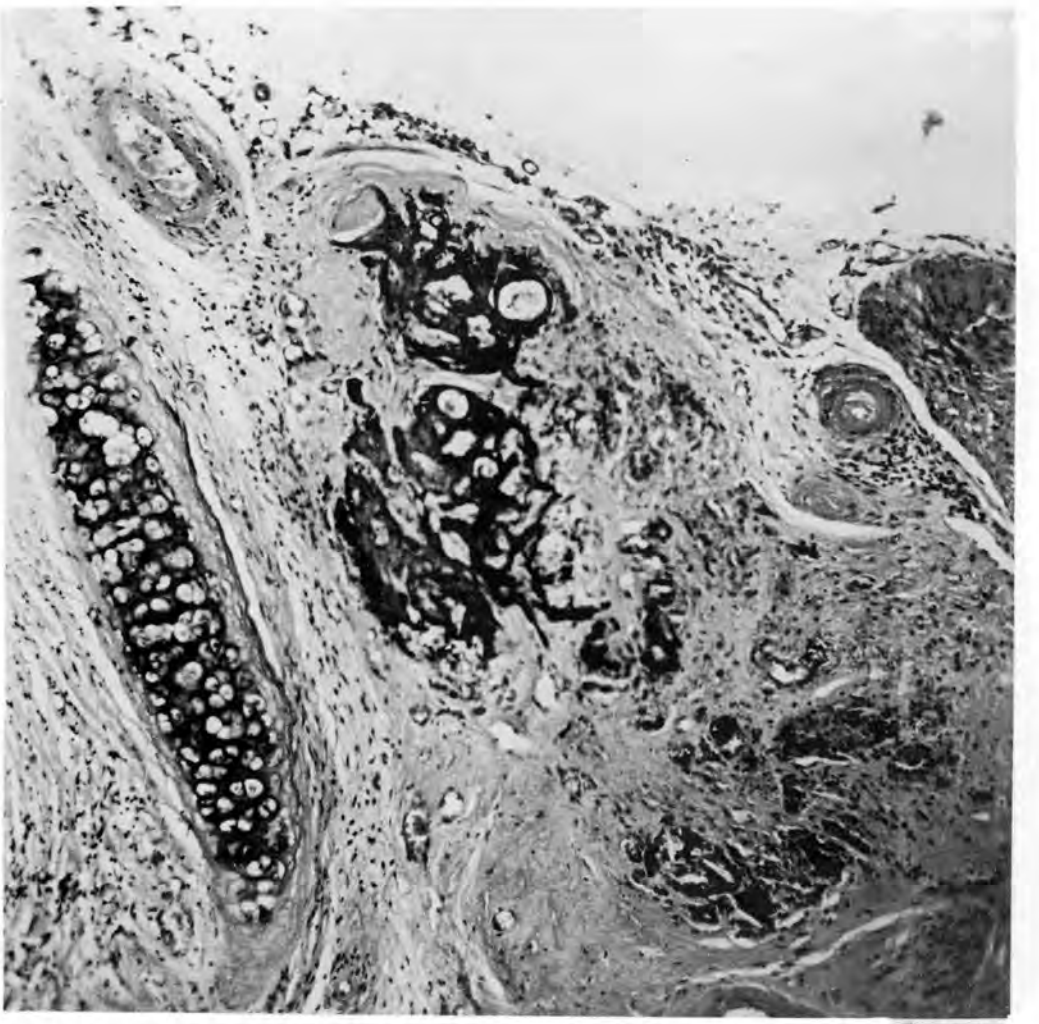


Fig. 13. The chronic histopathological changes in the peritracheal region of a rat that had received 70 μc/g of I^{131} and was sacrificed 56 days later. Complete destruction of the thyroid gland is apparent, as well as an extreme degree of injury to the parathyroid gland, shown at the lower right. Magnification x50. Stain: H and E.

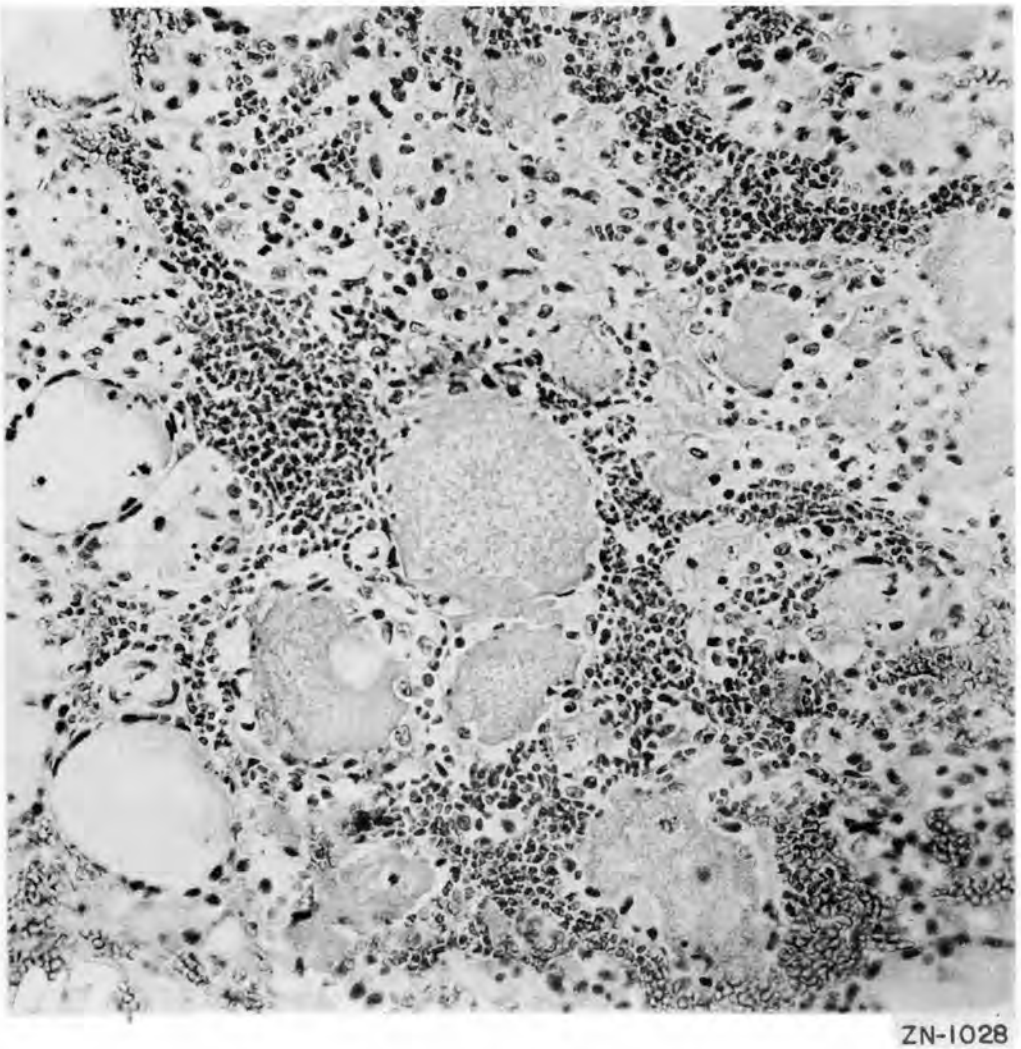
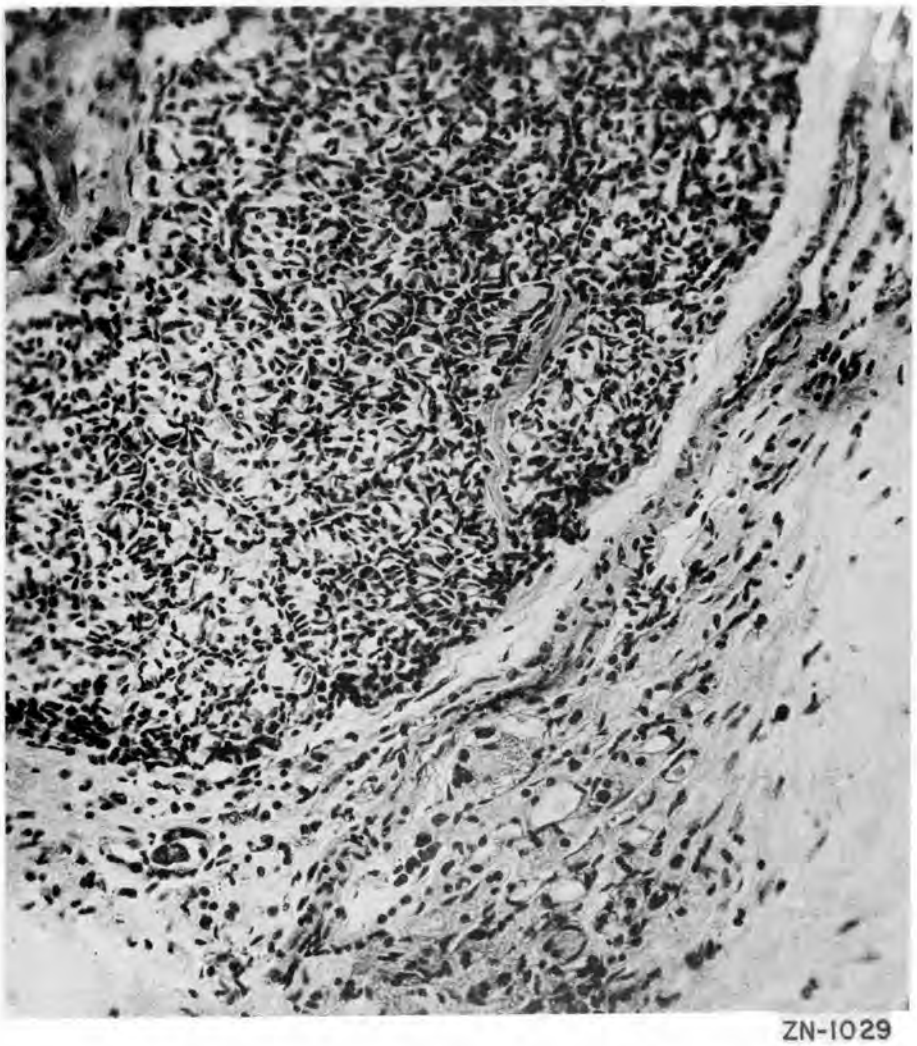


Fig. 14. The histopathological changes in the thyroid gland of a rat eight days after the administration of 1.8 μc/g of At^{211}. Numerous large follicles remain which show some evidence of injury. Marked hemorrhage can be seen in this preparation. Magnification x155. Stain: H and E.



ZN-1029

Fig. 15. A section of the thyroid and parathyroid glands of a rat that had received 0.9 μc/g of At^{211} and was sacrificed at the end of one year. There appears to be no normal thyroid tissue, but the parathyroid gland appears undamaged. Magnification x100. Stain: H and E.

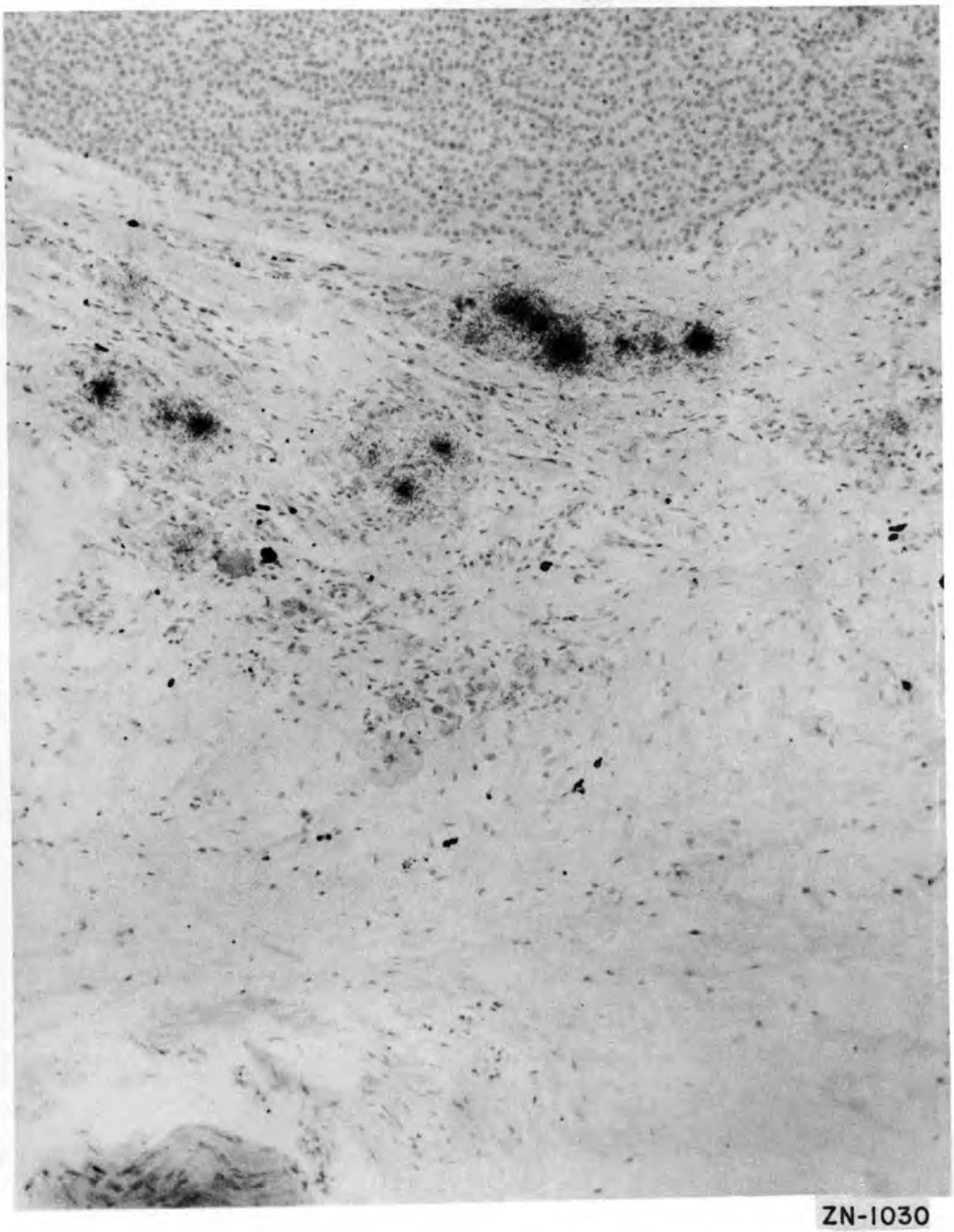


Fig. 16. An NTB stripping film radioautograph of tissue taken from the peritracheal region of a monkey that had received 0.08 μc/g of At^{211} two years previously. A tracer dose of I^{131} was given 24 hours before sacrifice. This preparation demonstrates the accumulation of I^{131} by atypical ephithelial cells. No recognizable follicles can be seen. The parathyroid gland shown in the upper portion of the plate appears completely normal. Magnification x120. Stain: H and E.

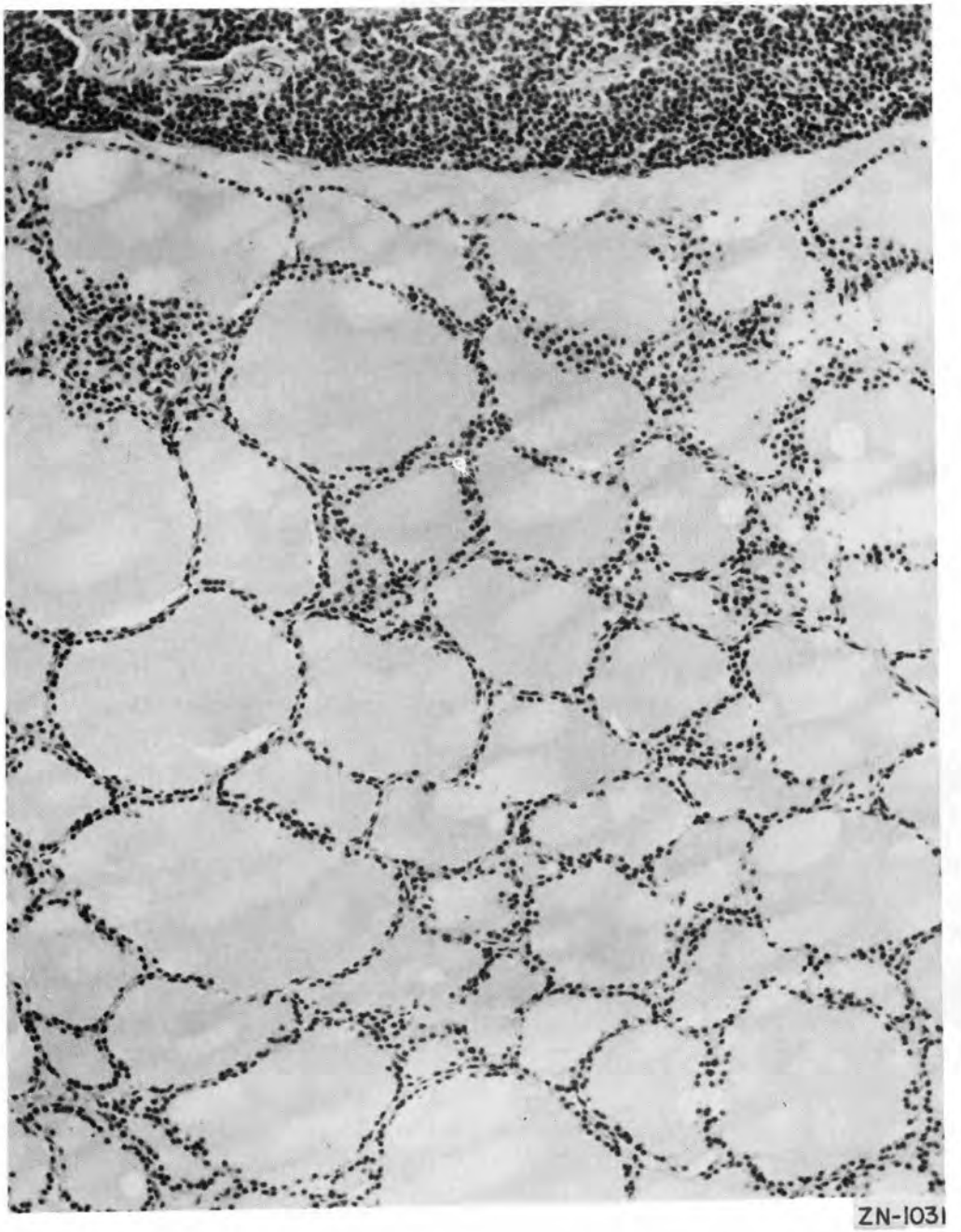


Fig. 17. A section of the thyroid and parathyroid glands from a normal monkey. Magnification x120. Stain: H and E.

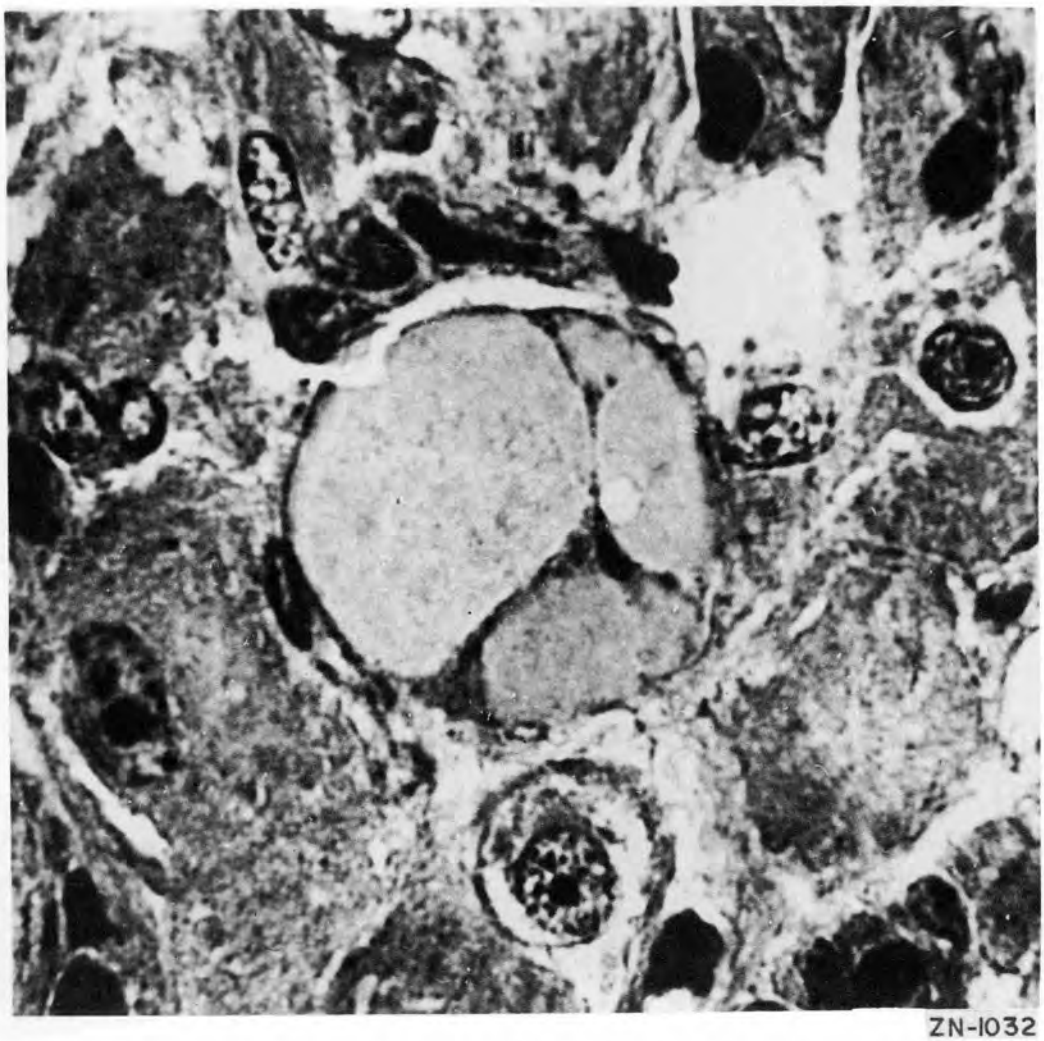


Fig. 18. A section of the anterior lobe of the pituitary gland from the monkey whose thyroid gland is shown in Fig. 14. In the center of the preparation there may be seen a large "thyroidectomy basophile." Magnification x250. Stain: Mallory-Azan.

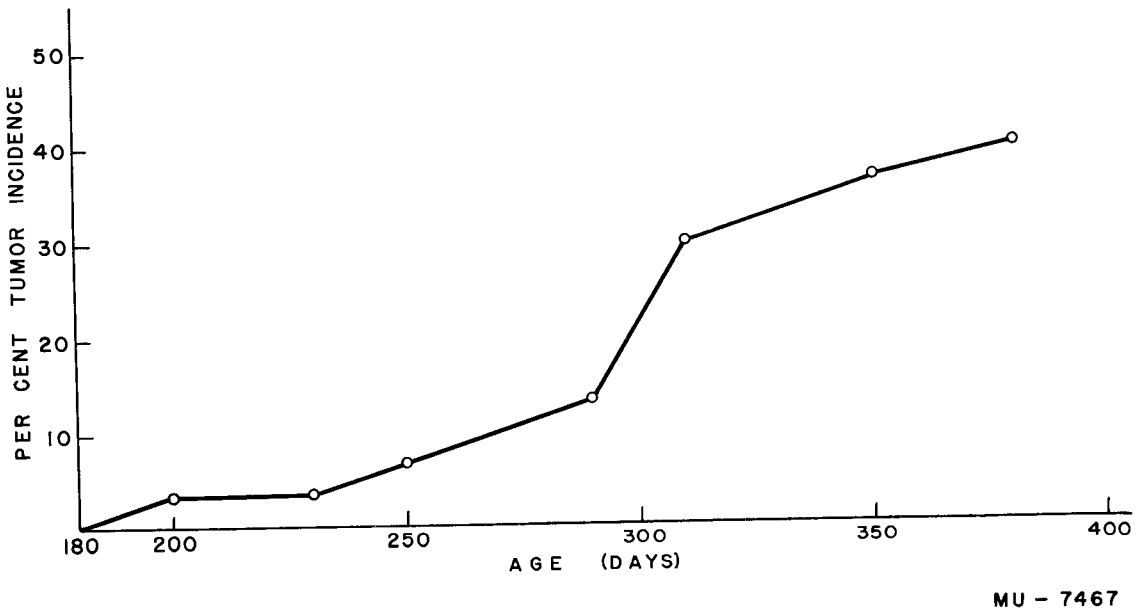


Fig. 19. The incidence of mammary tumors in Sprague-Dawley female rats following a single intravenous injection of At^{211}. The rats were 55 days of age when injected and were given 0.4 to 0.8 μc/g of body weight of At^{211}. No tumors were observed prior to the 135th day after the injection. To date none of the 20 control animals has developed a mammary tumor.

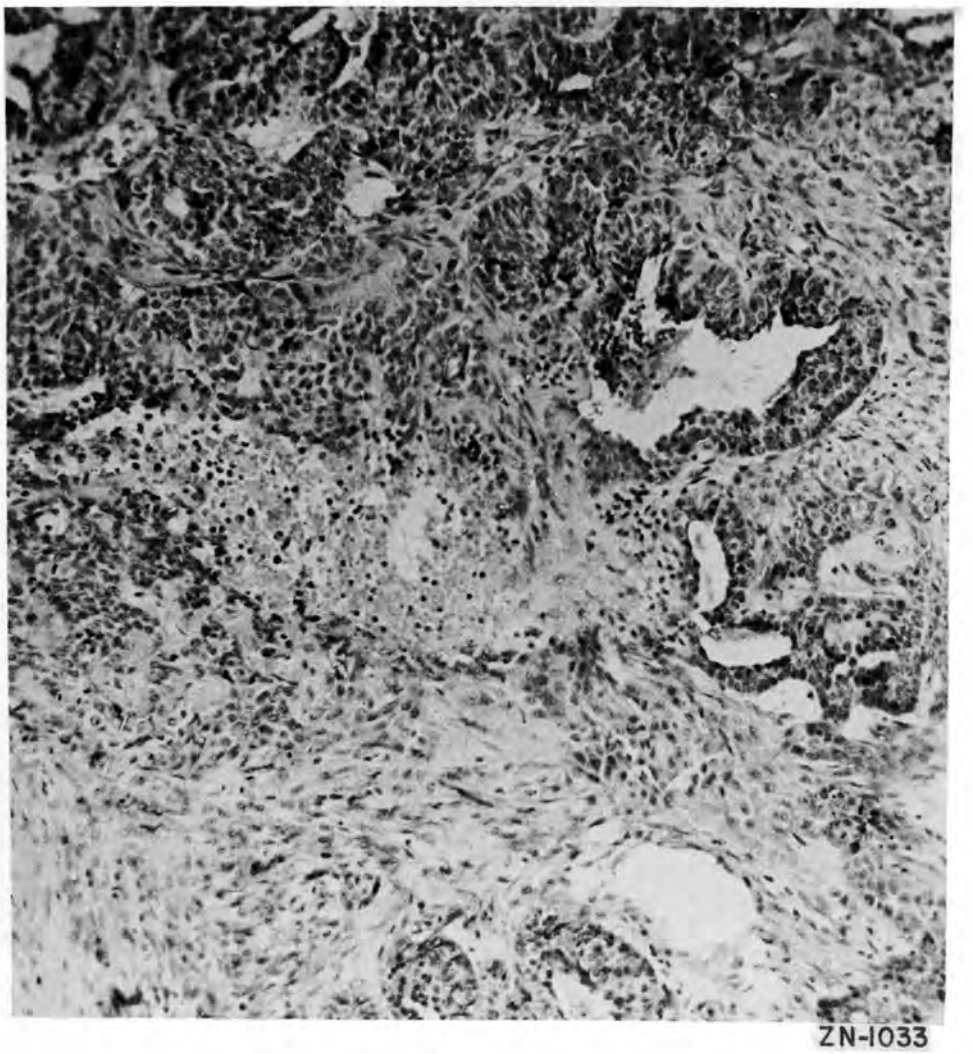


Fig. 20. A section of a mammary tumor taken from a rat that had received 0.8 μc/g of At^{211} 235 days before sacrifice. The fibrous character with ductlike structures is apparent. Magnification x80. Stain: H and E.

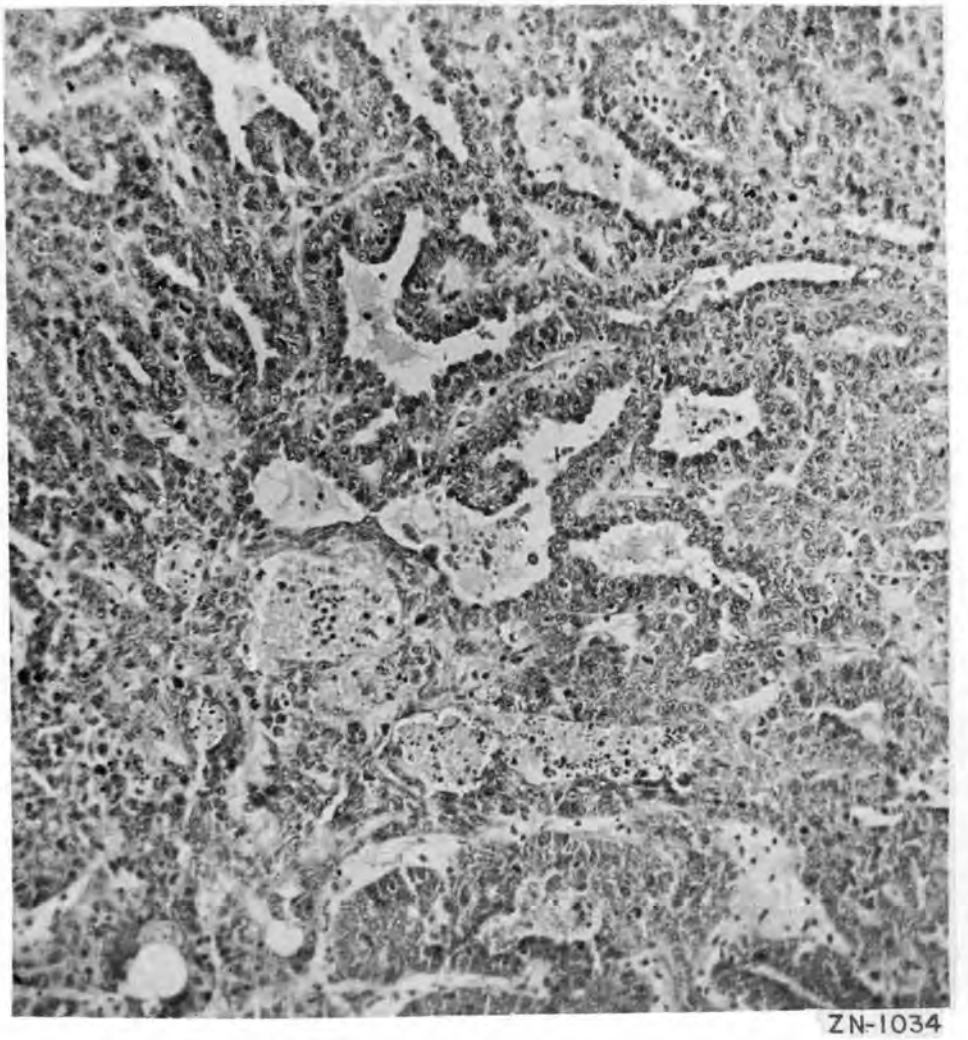


Fig. 21. A section of a pulmonary metastasis removed from the same animal. The general over-all appearance resembles that of the primary tumor. Magnification x80. Stain: H and E.

Discussion

The initial change in the growth pattern observed in rats receiving either At^{211} or I^{131} may be ascribed to radiation injury, and the subsequent deviation from the normal growth rate is presumably related to endocrine disturbances resulting from lack of the thyroid hormone. Similarly, the same interpretation may be applied to the experiments in which monkeys were give At^{211}, except that there does not appear to be a clear-cut radiation effect at the dosage levels employed.

The disassociation of radiation effects from the adverse response of the animals to a prolonged deficiency of the thyroid hormone presents a very real problem. Earlier attempts were made in this laboratory to separate these two processes in rats receiving lethal amounts of At^{211}. This was done by the administration of stable iodine at a level of approximately 10 milligrams per kilo for six days prior to the administration of At^{211}. Even though it was found that the thyroid glands of the animals so treated were completely protected against the destructive action of At^{211}, all these animals died in less than two weeks, while several animals whose thyroid glands were unprotected have survived for nearly eight months. As yet no satisfactory explanation of this phenomenon can be given.[13] Obviously much work remains to be done in order to adequately differentiate between the action of the ionizing radiation from either I^{131} or At^{211} and the subsequent effects of prolonged hypothyroidism resulting from radiothyroidectomy. Attention should be drawn to the fact that in the monkey irreparable radiation injury has been observed in tissues and organs other than the thyroid gland following the administration of At^{211}.[14]

Comparing Figs. 12 and 14, one can see that there is a marked difference between the microscopic appearance of the thyroid glands of the rats given I^{131} and those receiving At^{211}. The more uniform and complete destruction of the thyroid gland by the beta particles from I^{131} is presumably due to their greater range in tissue, which is a maximum of 2000 microns. When At^{211} was given, the injury seemed to be localized in the smaller follicles, especially those in the central portion of the gland, The nonuniform distribution of At^{211} could account for the irregular character of the observed damage. Evidence for this interpretation may be derived from At^{211} radioautographs prepared from the thyroid gland of the rat. An irregular deposition of At^{211} in different follicles was evident, and in general the deposition was greater in those that were centrally located.[3] The explanation for the type of injury to the thyroid gland from At^{211} is reasonable, since the range of its densely ionizing high-energy alpha particles is but 70 microns. Furthermore, it is possible that some of the larger follicles, particularly those at the periphery, may have been less severely damaged. It is also likely that the smaller follicles, which are presumably in a state of greater physiologic activity, accumulated more At^{211} than did the larger ones.

When the chronic injury of the thyroid glands from animals given At^{211} and I^{131} is compared, the effects of the more penetrating beta particles from I^{131} is even more striking. When I^{131} is given, the parathyroid gland, which is quite radioresistant, is almost completely obliterated. In contrast, in the chronic At^{211} experiments with both rats and monkeys, photomicrographs of the thyroid region showed no visible damage to the parathyroid gland, although in both instances the thyroid gland itself was completely destroyed. This is a

further demonstration of the localized action of the energetic short-range At^{211} alpha particles as contrasted to the more sidespread damage resulting from radiation leakage of the more penetrating beta particles from I^{131} and--to a lesser degree--from the associated complex of gamma rays from its decay. The injury seen in these experiments due to the ionizing radiation from the I^{131} accumulated by the thyroid gland is so extensive that not only are the thyroid and parathyroid glands destroyed, but also there is considerable damage to the adjacent peritracheal structures. Goldberg et al. have shown complete obliteration of the thyroid gland in the rat by I^{131}, and some evidence of damage to adjacent peritracheal structures. Injury to the parathyroid gland was minimal. However, the maximum amount of I^{131} given was approximately one-twentith of that used in certain of these studies.[15]

The subsequent accumulation of a tracer dose of I^{131} by atypical epithelial cells in the peritracheal region of a monkey two years after the administration of At^{211} is of interest, since it corroborates Gorbman's experiments on mice that such cells apparently do not attempt any extensive follicular formation even after such an extended period of time.[16]

The complexity of the chemistry of astatine has already been described elsewhere.[1,4] In the body, there is the possibility that astatine may exist in as many as six different chemical states at pH7. It is also likely that these six chemical states may be in rapid equilibrium with one another. The entry of At^{211} into the thyroid gland presumably occurs in the minus-one valence state, which is expressed as At^-. Evidence of this has been derived from a study by Taurog et al. In these experiments the I^- in the thyroid gland was compared to the plasma level of I^-. A selective concentration was found but was relatively small in comparison with the total accumulation of iodine by the thyroid gland. This ratio of I^- in the thyroid gland to I^- in the plasma of the rat was of the order of 20.[17] To a much more limited degree this effect with Br^- was observed by Simon using stable bromine and by Perlman et al, who also demonstrated the selective uptake of Br^- by the thyroid gland employing radioactive Br^- as a tracer.[18,19] Presumably the reason that no further accumulation occurred was that there are no enzyme systems in the thyroid gland capable of oxidizing Br^- to free bromine from which bromine homologues of the iodinated amino acids in the thyroid gland could be produced.

Because of the short half lives of known radioisotopes of astatine and the chemical difficulties in attempting to directly study the biochemistry of astatine in the thyroid gland, all that can be done is to speculate as to the mode of accumulation and retention of astatine in this organ. An attractive hypothesis is that it enters as At^- and that a significant fraction is oxidized to free astatine. The At_2^0 is presumably oxidized from At^- by the epithelial cells and then is in part loosely bound to the thyroglobulin of the colloid. There is concrete evidence that the metabolism of astatine by the thyroid gland is not entirely comparable to that of iodine, since approximately one-half of the At^{211} was leached from the thyroid gland when 80% alcohol was used as a fixative.[13] This is possible evidence of the loose binding of At^{211} as compared to I^{131} in the thyroid gland of the rat.

The increased incidence of mammary tumors following the administration of relatively small amounts of At^{211} was not anticipated. Thirty rats that received from 0.4 to 0.8 μc/g of At^{211} and 20 control rats have now been

observed for 325 days. The first tumor was noted on the 135th day after the injection, or when the animal was six months old, and to date eleven other injected animals have developed tumors, while none has been seen in the control rats. In a lengthy study on a similar strain of albino rat at the Wistar Institute, it was found that spontaneous mammary tumors seldom developed before the age of one year, the average age of incidence for malignant growths was 460 days, and for benign growths 650 days, and in 10% of the cases there were multiple sites of origin.[20] The mammary neoplasms observed in the At^{211} studies appeared to be malignant in character, and the observation of pulmonary metastases showing the same general structure seems to confirm this interpretation. As the incidence and character of the mammary tumors did not seem to be closely related to the amount of At^{211} given, it would appear that they were not the result of the direct action of the ionizing radiation of At^{211}. Therefore, it is reasonable to conclude that the increased incidence of mammary tumors following the administration of At^{211} was due to endocrine disturbances. It has been demonstrated here that At^{211} is capable of destroying the thyroid gland and subsequently producing changes in the anterior pituitary gland in the monkey. Other investigators have observed the occurrence of mammary tumors in the female rat that have been associated with abnormalities of the pituitary gland and ovaries.[21, 22]

Summary

1. (a) Disturbance of the rate of growth in rats and monkeys following the administration of At^{211} has been demonstrated. This phenomenon has also been seen in rats give I^{131}.
   (b) The initial effect upon the growth of the rat following the adiministration of either I^{131} or At^{211} at lethal levels may be ascribed to acute radiation injury. The later disturbance in growth appears to be a result of endocrine changes due to lack of the thyroid hormone.
   (c) The growth pattern in the monkey indicates that the decrease in growth rate of animals receiving At^{211} is presumably the result of endocrine changes.

2. Simian myxedema has been seen in four At^{211}-treated monkeys.

3. A striking difference was seen between the effects at high levels of I^{131} and At^{211} on the thyroid gland in the rat. These differences appear to be quantitative as well as qualitative.

4. The chronic effects of high-level irradiation of the thyroid gland from I^{131} in the rat not only completely obliterated the thyroid gland, but also almost completely destroyed the parathyroid gland and damaged the adjacent peritracheal structures.

5. "Chronic" experiments in the monkey and the rat with At^{211} did not show any injury to the parathyroid gland and adjoining peritracheal structures.

6. (a) Two years after apparently complete destruction by At^{211} of normal thyroid tissue in a monkey, there were atypical cells present which demonstrated the capacity to accumulate and retain a tracer dose of I^{131}.
   (b) Thyroidectomy cells were seen in the anterior lobe of the pituitary gland of this monkey.

7. A high incidence of mammary tumors was observed in the rat following administration of At^{211}. These tumors appeared neoplastic and on occasion there were pulmonary metastases. The incidence of these tumors is ascribed to endocrine changes rather than to direct radiation action from At^{211}.

Acknowledgments

The production of the astatine was the responsibility of Mr. G. Bernard Rossi and the staff of the 60-inch cyclotron. We are indevted to Melvin Calvin, Ph.D., Professor of Chemistry, for the use of certain of his facilities, which were employed for the preparation of the astatine. The authors wish to thank Miss Margaret Gee, Miss Nylan Jeung, Miss Marilyn Hemenway, and Mrs. Ruth Newman for technical assistance in certain phases of the experiments presented here. The preparation and interpretation of the histological specimens from the monkey were the result of collaboration with C. Willet Asling, M.D., Associate Professor of Anatomy, University of California School of Medicine. The histological specimens from the rats were prepared by Miss Muriel Johnston. The assistance in the preparation of the manuscript by Mrs. Barbara Butler and Miss Billie Kelso was invaluable.

The continued interest and encouragement of Robert S. Stone, M.D., Professor of Radiology, and Theodore L. Althausen, M.D., Professor of Medicine, University of California School of Medicine, are gratefully acknowledged.

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Radiation Chemistry

Warren M. Garrison, Winifred Bennett,
Sibyl Cole, H. Ralph Haymond, and Boyd M. Weeks

Recent work has been concerned principally with identification and determination of products of irradiation in solutions of acetic acid, formic acid, and glycine which were irradiated before the shutdown of the 60-inch cyclotron. The following abstract has been submitted for presentation at the forthcoming September meeting of the American Chemical Society.

IRRADIATION OF AQUO-ORGANIC SYSTEMS WITH CYCLOTRON-ACCELERATED PARTICLES

Warren M. Garrison, Boyd M. Weeks,
H. Ralph Haymond, and Joseph G. Hamilton

(Abstract of a paper prepared for presentation as part of a symposium on "Radiation Sterilization of Foods and Pharmaceuticals," at the 126th Annual Meeting of the American Chemical Society, New York, N. Y., September 12-17, 1954)

Radiolysis of aquo-orgnaic systems most generally results in the formation of a mixture of organic product compounds. Identification and quantitative determination of the small amounts of product usually obtained often present a difficult analytical problem. These factors have tended to limit the development of the radiation chemistry of aquo-organic systems. During recent years, however, the application of radiometric methods of analysis and the use of high-intensity radiation sources have made possible the quantitative study of many aquo-organic systems which were not previously amenable to investigation.

The 60-inch cyclotron at the Crocker Laboratory is being used as a high-intensity radiation source in the study of the direct and indirect action of heavy-particle radiation on organic compounds in aqueous solution. Formic acid, acetic acid, glycine, and other two-carbon acids have been investigated. The relatively high radiation levels available make possible a more detailed study of the organic product mixtures. Identification and determination are facilitated by the use of C^{14}-labeled compounds and allied radiometric techniques together with recently developed methods of partition chromatography. The bombardment methods, targets, and analytical procedures have been developed as part of a general investigation of the mechanism of radiation-induced reactions in aquo-organic systems of possible biological interest.

ACETIC ACID (OXYGEN-FREE SYSTEMS)

Winifred Bennett, Sibyl Cole, Herman R. Haymond and Boyd M. Weeks

The following abstract has been submitted for the September meeting of the American Chemical Society.

INDIRECT AND DIRECT ACTION OF HEAVY-PARTICLE RADIATION ON ACETIC ACID IN AQUEOUS SOLUTION

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

Chemical reactions induced by heavy-particle irradiation of acetic acid–water mixtures in the concentration range 0.0625 M to 16 M have been studied. Mechanisms are proposed for the direct and indirect action of radiation on the acetic acid molecule. The 60-inch cyclotron at the Crocker Laboratory was used as the radiation source. Irradiations were made with helium ions and deuterons having energies of 35 Mev and 18 Mev respectively.

The principal products formed in dilute, oxygen-free acetic acid solutions at radiation doses below 5×10^{20} ev/cm^3 are hydrogen, hydrogen peroxide, and succinic acid[1, 2]. Carbon dioxide, methane, ethane, and carbon monoxide are also produced. The radiation yields[3] of all products increase with acetic acid concentration in the range 0.0625 to 1.0 M. With increasing acetic acid concentration above 1.0 M a continuous decrease in G values for hydrogen, hydrogen peroxide, and succinic acid is observed. Values for the latter two products decrease essentially to zero in 16 M acetic acid. Radiation yields of carbon dioxide, methane, ethane, and carbon monoxide, however, increase continuously with acetic acid concentration. With the exception of ethane, G values for these products show a linear dependency on acetic acid concentration. Typical yield data for 35-Mev helium-ion and 18-Mev deutron irradiations at a constant dose of 3.8×10^{20} ev/cm^3 are shown in Fig. 1 and Fig. 2 for the acetic acid concentration range 0.0625 to 16 M.

The production of hydrogen, hydrogen peroxide, and succinic acid in dilute acetic acid solution is discussed in terms of current theory of the mechanism of water decomposition, in which it is customary to attribute the primary chemical effects of radiation to the dissociation, by ionization and excitation, of water molecules to give hydrogen atoms and hydroxyl radicals. The observed chemical changes in irradiated solutions are attributed to subsequent reactions of the H and OH radicals with one another and with solute molecules. The fraction of the radicals that combine to form H_2 and H_2O_2 as compared to the fraction available for reaction with solute increases with increasing specific-ionization density of the effective radiation. These processes have been represented[4, 5] in

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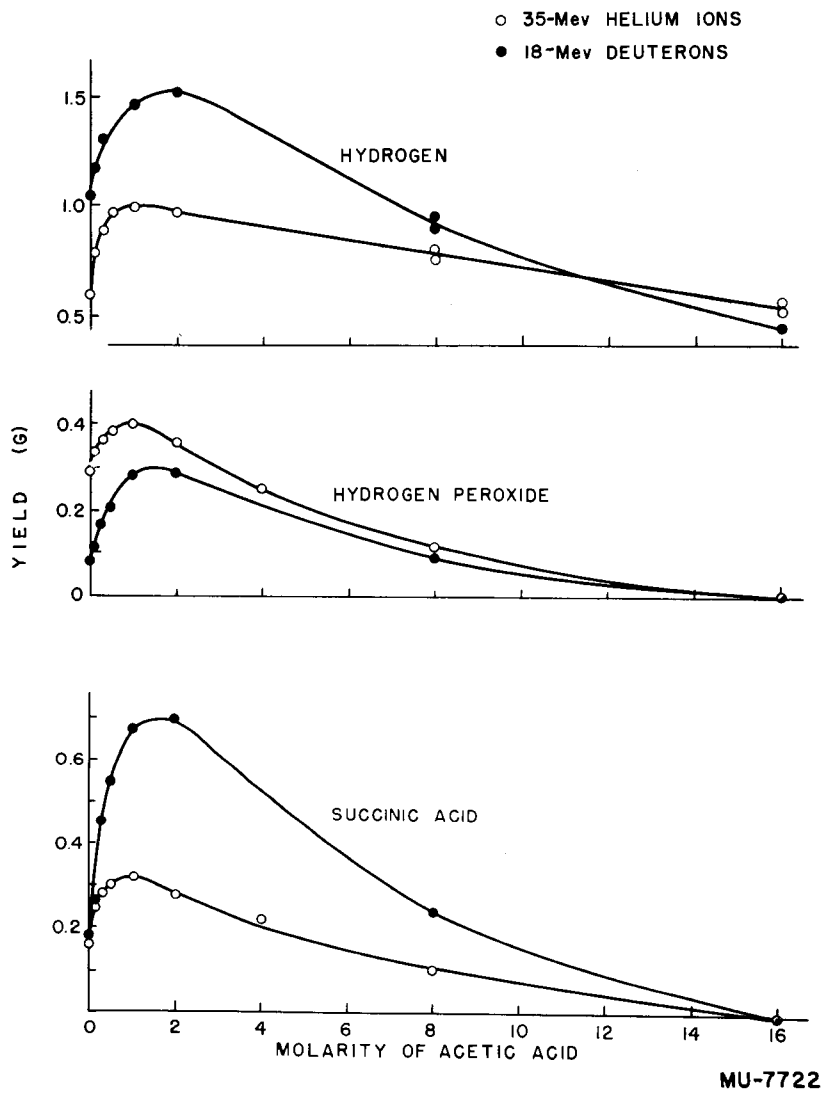
○ 35-Mev HELIUM IONS
● 18-Mev DEUTERONS
HYDROGEN
HYDROGEN PEROXIDE
SUCCINIC ACID
YIELD (G)
1.5
1.0
0.5
0.4
0.2
0
0.6
0.4
0.2
0
2
4
6
8
10
12
14
16
MOLARITY OF ACETIC ACID
MU-7722

Figure 1

terms of the independent primary reactions

$$H_2O \xrightarrow{\sim\sim} H + OH \qquad (1)$$

$$2\,H_2O \xrightarrow{\sim\sim} H_2 + H_2O_2 \qquad (2)$$

Recently,[6] it has been proposed that a third reaction,

$$2\,H_2O \xrightarrow{\sim\sim} 2H + H_2O_2\,,$$

must be added to account for the fact that H and OH may not occur in equal amounts. In the absence of added solute, radicals formed in (1) react with the products of (2) according to the back reactions[4] (3), (4):

$$H_2 + OH \longrightarrow H_2O + H, \qquad (3)$$

$$H + H_2O_2 \longrightarrow H_2O + OH. \qquad (4)$$

In acetic acid solutions both H and OH may react with added solute molecules. The production of succinic acid is most readily accounted for by assuming that reactions (5) and/or (6) occur and that succinic acid is formed by (7).

$$OH + CH_3COOH \longrightarrow CH_2COOH + H_2O\,, \qquad (5)$$

$$H + CH_3COOH \longrightarrow CH_2COOH + H_2\,, \qquad (6)$$

$$2\,CH_2COOH \longrightarrow (COOH)\,CH_2\;CH_2\;COOH. \qquad (7)$$

Evidence that both (5) and (6) contribute to the production of succinic acid through the formation of CH_2COOH has been obtained. The conclusions regarding the occurence of (6) are drawn from data obtained on the isotopic analysis of hydrogen produced by irradiation of solutions of CH_3COOH in D_2O and CD_3COOH in H_2O under conditions that preclude thermal exchange. Evidence for (5) is based on observations of the rate of production of succinic acid in the irradiation of acetic acid solutions containing added hydrogen peroxide at concentrations sufficient to remove all the H radicals by reactions (4) above.

As acetic acid concentration is increased from 0.0625 M, the number of radicals involved in (3) and (4) decreases as the competing reactions (5) and (6) become more important. The radiation yields of hydrogen, hydrogen peroxide,

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6. A. O. Allen, Radiation Research 1, 85 (1954).

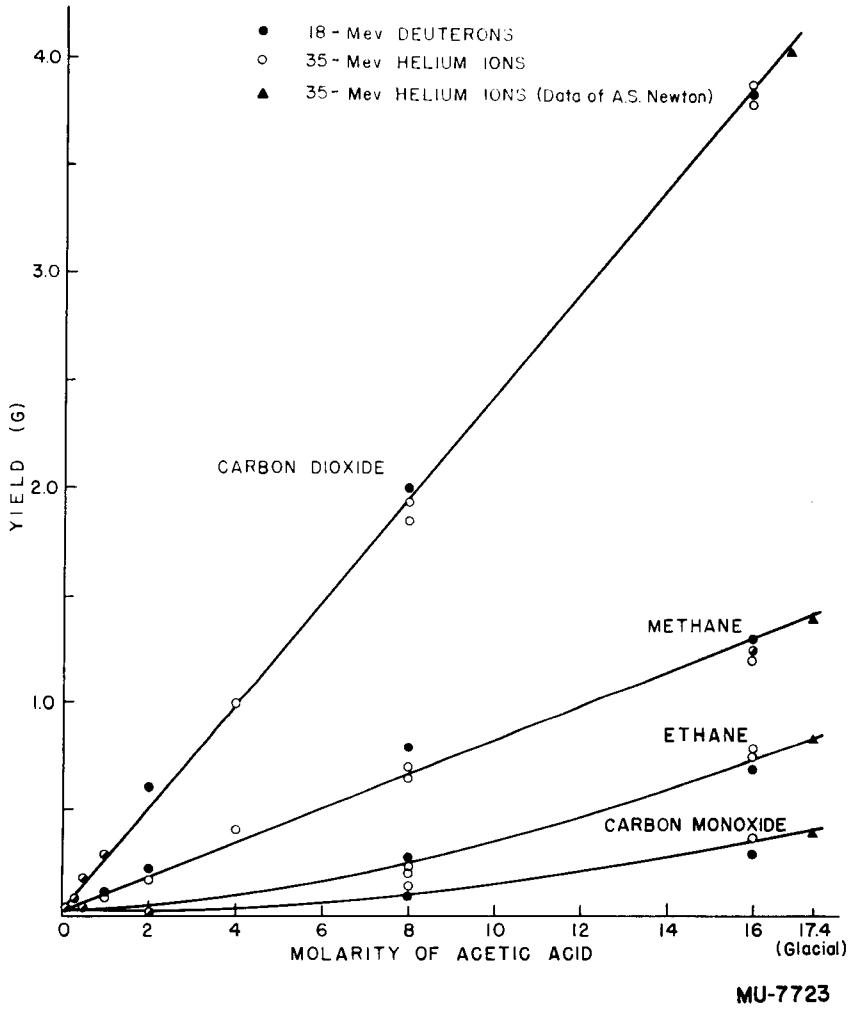
18 - Mev DEUTERONS
35 - Mev HELIUM IONS
35 - Mev HELIUM IONS (Data of A.S. Newton)
CARBON DIOXIDE
METHANE
ETHANE
CARBON MONOXIDE
YIELD (G)
4.0
3.0
2.0
1.0
0
2
4
6
8
10
12
14
16
17.4
(Glacial)
MOLARITY OF ACETIC ACID
MU-7723

Figure 2

and succinic acid therefore increase with acetic acid concentration, as shown in Fig. 1. G values for these products reach a limiting value at an acetic acid concentration of 1.0 to 2.0 M with both helium-ion and deuteron irradiation. This corresponds to the case in which a maximum number of the radicals produced in (1) are utilized in the net production of CH_2COOH. A larger limiting G value for succinic acid is obtained with deuterons in accordance with the observation that (a) the relative number of water molecules decomposed via (1) as compared to (2) decreases with specific ionization of the effective radiation,[5,6] and that (b) the range of a deuteron of energy E is twice that of a helium ion of energy 2E.

Although the reaction sequence (1) to (7) adequately describes the main qualitative features of the observed indirect action of cyclotron-accelerated heavy-particle radiation on acetic acid in aqueous solution, consideration of the absolute product yields in terms of total radical production does not indicate complete removal of H and OH by reactions (5) and (6). It has been shown elsewhere[7] that the total number of water molecules decomposed via (1) and (2) per 100 e.v. corresponds to a GH_2O of 3.1 to 3.8 for γ-rays, β-rays and α-particles. Ionization density determines the relative importance of (1) and (2). Although the measurement of GH_2O, i.e. (1) plus (2), has not been a primary objective of this investigation, similar studies with 35-Mev helium ions gives $GH_2O \simeq 3.1$. Therefore if all available H and OH radicals were removed by (5) and (6) in the formation of hydrogen and succinic acid, higher G values would be expected. Since the activation energy[8] for the H reaction (6) is apparently greater than the corresponding OH reaction, it is proposed that back reactions of the type

$$H + CH_2COOH \longrightarrow CH_3COOH \qquad (8)$$

are important at the high radical concentrations that are obtained in cyclotron irradiations[1].

As the acetic acid concentration is increased, there is a corresponding increase in the fraction of total energy absorbed by direct interaction with acid molecules. The observed decrease in succinic acid and hydrogen peroxide is attributable in part to the fact that a decreasing fraction of the total energy is dissipated via reactions (1) and (2). The relationship between acetic acid concentration and radiation yield for carbon dioxide, methane, ethane, and carbon monoxide seen in Fig. 2 suggests that these products are formed principally by reactions induced by direct interaction of the radiation with acetic acid molecules over the entire range of concentration studied, i.e. from 0.0625 to 16 M. Almost identical G values for these products were obtained with 35-Mev helium ions and with 18-Mev deuterons.

Examination of the data of Fig. 2 shows also that the combined yield of carboxyl carbon as carbon dioxide and carbon monoxide is greater than the yield of methyl carbon as methane and ethane. This difference in 16 M acetic acid corresponds to G of 1.5. It is apparent from this relationship that non-gaseous products are also formed. One of these has been tantatively identified as acetone by chromatographic separation of the 2,4-dinitrophenylhydrazine

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7. E. J. Hart, Radiation Research 1, 79 (1954).

8. N. Uri, Chem. Rev., 50, 378 (1952).

derivative and by mass spectrometric analysis[9] of a volatile sample separated from an irradiated solution by fractional distillation. Carbonyl determinations give a radiation gield of over 1.0 in 16 M acetic acid.

To obtain additional information on the nature of the intermediate processes, irradiations have been made in the presence of added iodine and iodine plus hydrogen iodide. Iodine may affect the observed product yields by reacting with radicals or electrons[10, 11].

$$R + I_2 \longrightarrow RI + I$$

$$e + I_2 \longrightarrow I^- + I$$

The data in Fig. 3 show the effect of the iodine concentration on product yields from 16 M acetic acid. The irradiation conditions were identical with those used in obtaining the helium-ion data of Fig. 2. It is seen that both the methane and ethane yields decrease with increasing iodine concentration and appear to level off in the iodine concentration range 0.02 to 0.05 M. The carbon dioxide and hydrogen yields are relatively unaffected. The presence of 0.25 M hydrogen iodide and iodine up to 0.25 M results in no further decrease in product yield. Figure 4 and Fig. 5 show the effects of hydrogen iodide plus iodine (0.25 M HI, 0.1 M I_2) on the dependancy of the product-gas yields on acetic acid concentration.

Consideration has been given to possible reactions that may be involved in the formation of carbon dioxide, methane, ethane, and carbon monoxide by direct action of radiation on acetic acid molecules in acetic acid-water mixtures. An attempt has been made to draw conclusions regarding the relative importance of ions, free radicals, and excited molecules as intermediates. Both the data reported herein and independent photochemical, spectroscopic, and mass spectrometric evidence have been employed. It is shown that the simplest mechanism in accord with available evidence involves both excited acetic acid molecules and radicals produced from acetic acid as intermediates.

FORMIC ACID (OXYGEN-FREE SYSTEMS)

Sibyl Cole and Winifred Bennett

In a previous report the identification of tartronic acid as a product of the irradiation in vacuo of formic acid solutions was described. This product has been confirmed by another method of coelution of authenic tartronic acid and C^{14}-labeled product from the irradiation of a solution of $HC^{14}OOH$. In the present method column dimensions were as previously described. The aqueous phase was 0.5N H_2SO_4 and eluting agent was 35% isobutanol – 65% chloroform v/v saturated with 0.5N H_2SO_4.

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9. Mass spectrometric analyses of gases and volatile products were obtained through the courtesy of Dr. A. S. Newton.

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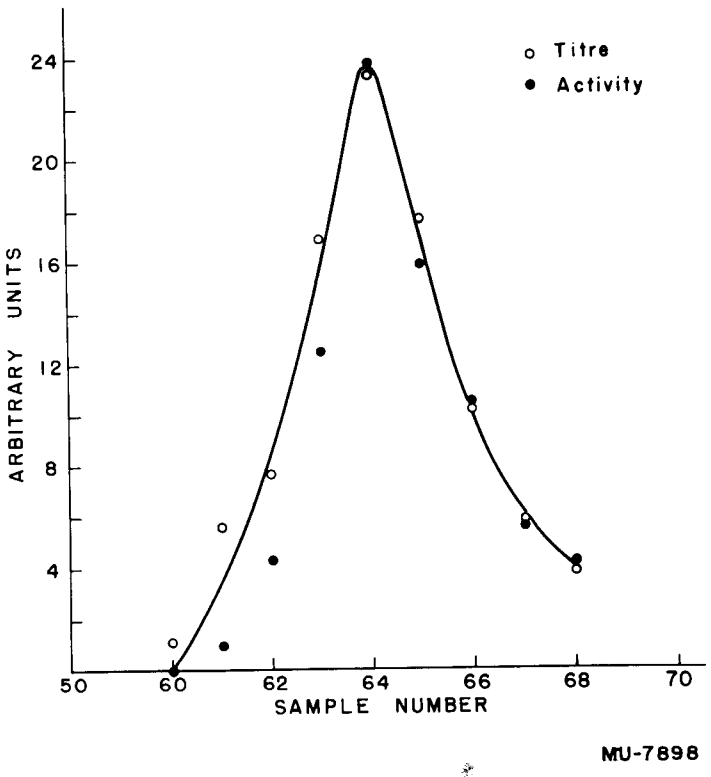
Titre
Activity
ARBITRARY UNITS
24
20
16
12
8
4
50
60
62
64
66
68
70
SAMPLE NUMBER
MU-7898

Figure 3

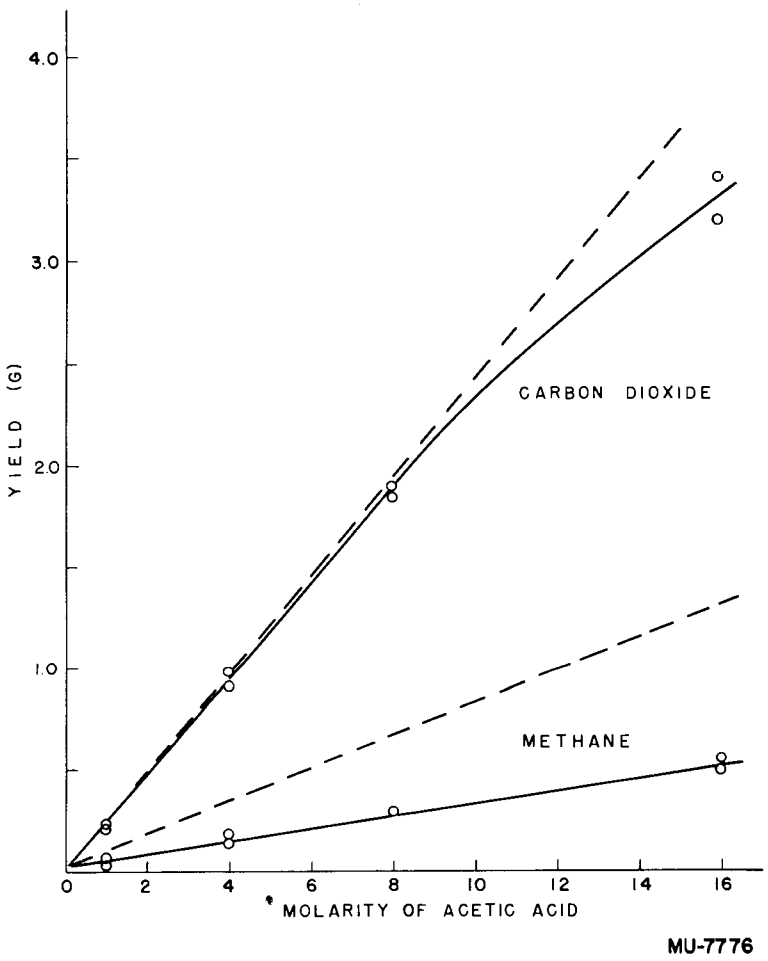
4.0
3.0
2.0
1.0
YIELD (G)
CARBON DIOXIDE
METHANE
0
2
4
6
8
10
12
14
16
MOLARITY OF ACETIC ACID
MU-7776

Figure 4

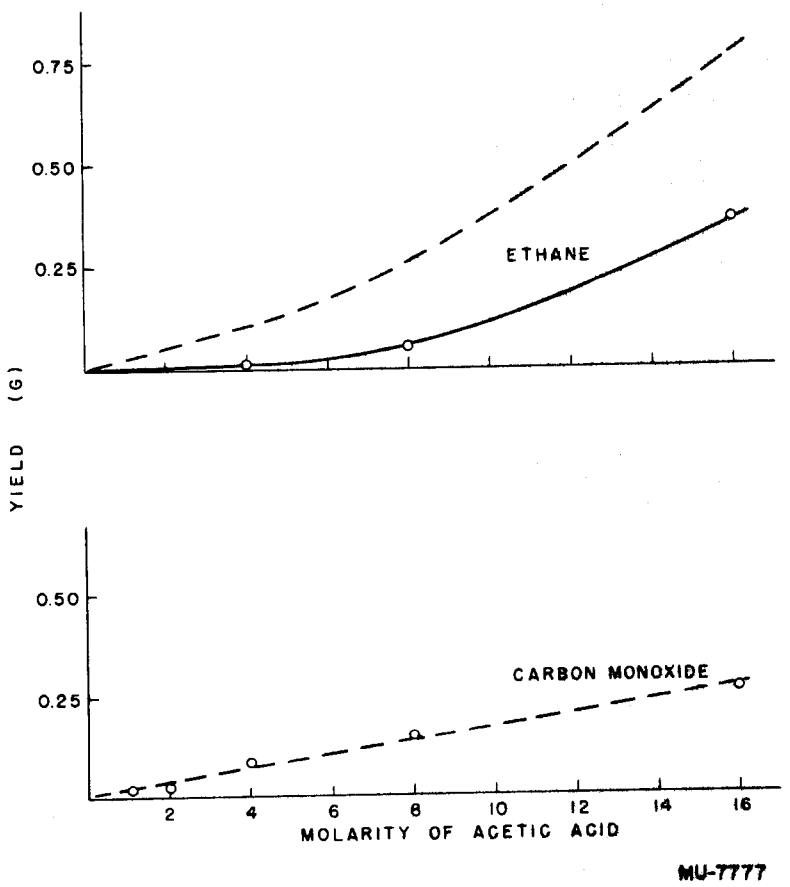
0.75
0.50
0.25
ETHANE
YIELD (G)
0.50
0.25
CARBON MONOXIDE
2
4
6
8
10
12
14
16
MOLARITY OF ACETIC ACID
MU-7777

Figure 5

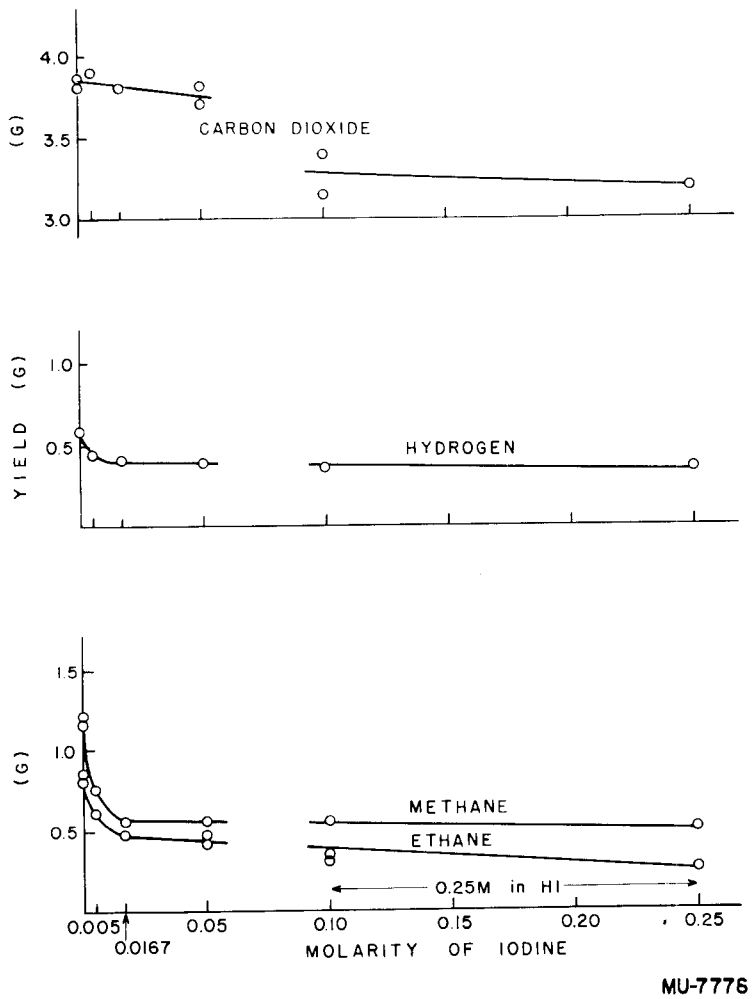

Fig. 6. Correspondence in the eluate from a silica gel column of acid product of irradiation and C^{14} activity from C^{14}-labeled glycolic acid added after irradiation.

EFFECTS OF OXYGEN IN THE IRRADIATION OF AQUEOUS ACETIC ACID

Herman R. Haymond

A previous report described the identification of glycolic acid as a product of irradiation of oxygenated aqueous acetic acid by methods which involved co-elution from silica columns of C^{14}-labeled product glycolic acid and added nonradioactive glycolic acid. Glycolic acid has been further confirmed as an irradiation product by a method which is essentially the reverse of the above.

In order to obtain a titratable amount of glycolic acid in the fraction which is not volatile by distillation in vacuo, eight irradiations, each for 0.05 microampere-hour at 0.20 microampere on 60 ml of 1.0 M acetic acid, were made in a target cell in which the solution could be stirred and oxygenated during irradiation. These target solutions were combined and glycolic acid was determined colorimetrically on a small sample. Then 1.9 mg of glycolic acid containing 2.9 microcuries of 1-C^{14} glycolic acid was added. The nonvolatile products were separated by distillation in vacuo. The mixture was analyzed by a previously described "standard" method of partition chromatography on a silica-gel column. The correspondence of titre from irradiation product and radioactivity from added glycolic acid is shown in Fig. 6. The determinations of glycolic acid by the column method and by the colorimetric method were in agreement.

The carbonyl fraction that results from irradiation of oxygenated dilute aqueous acetic acid has been tentatively identified as formaldehyde. The fraction has been determined by a colorimetric method which gives a measure of total carbonyl in the irradiated solution, as previously described. The identification of formaldehyde is based on paper chromatography methods of separation of 2,4-dinitrophenylhydrazones.[1]

One ml of irradiated solution was added to 1 ml of 0.05% 2,4-dinitrophenylhydrazine in 2N HCl and the mixture was heated at 50°C for 30 minutes. After cooling, one extraction was made with 1 ml CCl_4; 0.1 ml of the CCl_4 phase was dried on a filter-paper strip and then eluted by a descending-solvent method. Figure 7 shows a diagram of a chromatogram obtained when acetic acid solution used for irradiation (A), irradiated solution (B), acetic acid plus formaldehyde (C), and acetic acid plus acetone (D) are carried through the above process and run simultaneously on Whatman No. 1 filter paper with 5% diethyl ether in petroleum ether (bp 65° – 110°C) as the eluting solvent. The chromatogram was sprayed with 10% KOH in water immediately after removal from the solvent trough. This brings out the 2,4-dinitrophenylhydrazones as orange-brown spots, which were outlined with a pencil while the chromatogram was still wet. In Fig. 7 the spots at 2 and 3 which appear in the target solution are due to the presence of H_2O_2 produced during irradiation. This can be shown in two ways. If the irradiated solution is treated with platinum black to decompose the H_2O_2 before it is carried through the above process the blue spots disappear. If a mixture of dilute acetic acid containing H_2O_2 and formaldehyde in concentrations equivalent to the H_2O_2 and carbonyl measured in the irradiated solution is run simultaneously with the irradiated solution the patterns obtained are identical. This is shown in Fig. 8, which is a chromatogram run with 40% $CHCl_3$ – 60% petroleum ether (bp 65°–110°C) v/v as the eluting solvent.

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1. R. G. Rice, G. J. Keller, and J. G. Kirchner, Anal. Chem. 23, 194 (1951).

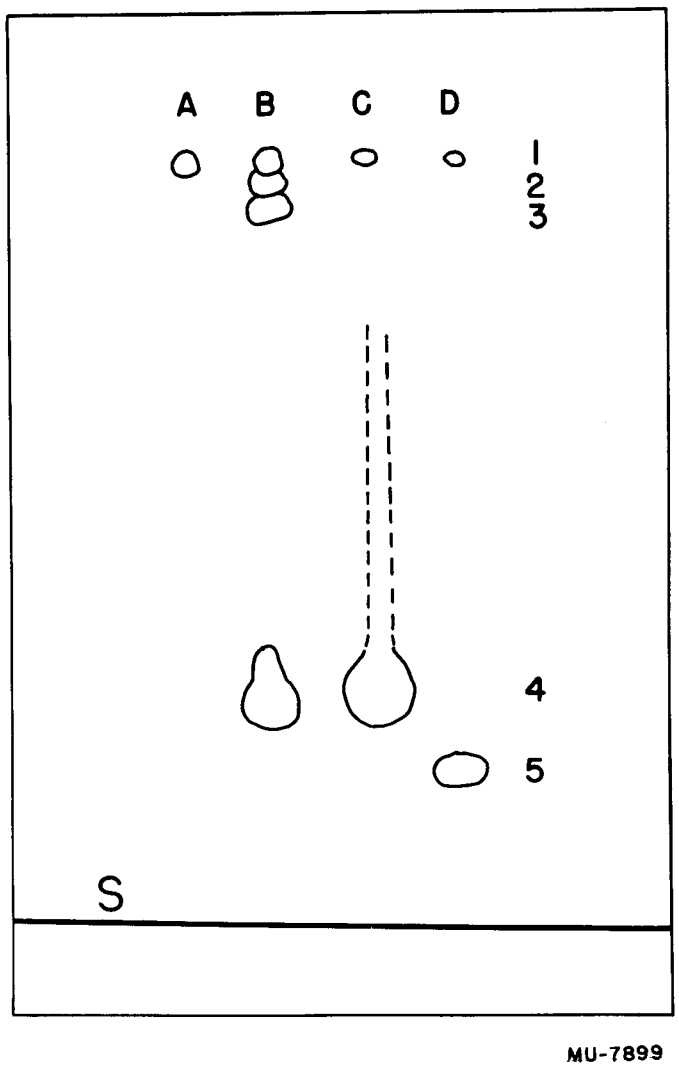

Fig. 7. Diagram of a chromatogram run on Whatman No. 1 filter paper with 5% diethyl ether in petroleum ether (bp 65°–110°C) as the eluting solvent. In each case the CCl_4-extractable 2,4-dinitrophenylhydrazones were dried at 1. A is from acetic acid used for the target solution; B is from the irradiated solution; C is from acetic acid with added formaldehyde; D is from acetic acid with added acetone; 4 and 5 are the 2,4-dinitrophenylhydrazone spots; 2 and 3 (which appear only in the irradiated solution) are due to H_2O_2. (cf. Fig. 8). The dotted lines indicate streaking.

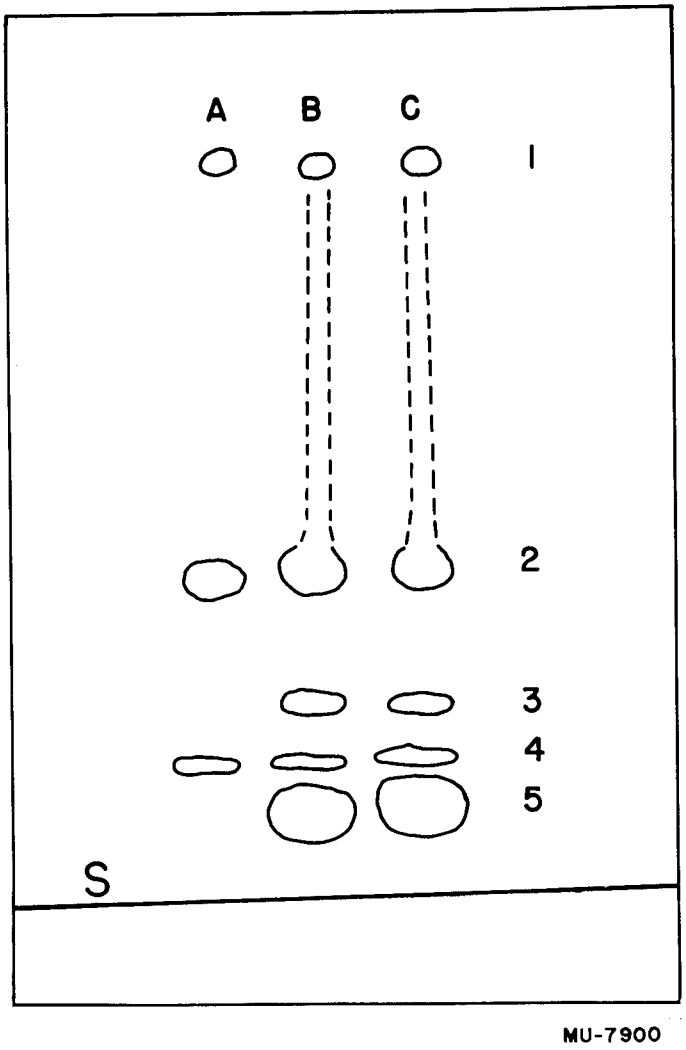

Fig. 8. Diagram of a chromatogram run on Whatman No. 1 filter paper with 40% $CHCl_3$ – 60% petroleum ether (bp 65° – 110° C) as the eluting solvent. CCl_4-extractable 2, 4-dinitrophenylhydrazones were dried at 1. A is from acetic acid solution plus H_2O_2; B is from irradiated acetic acid solution; C is from acetic acid solution with added H_2O_2 and HCHO.

GLYCINE

Boyd Weeks

In the last quarterly report (January, February, March, 1954) details of two series of aqueous glycine irradiations were given and a few of the data presented. In the past quarter, analysis of the gaseous products was completed, and the results are summarized in Figs. 9 and 10. It can be seen that the yield of hydrogen is essentially unaffected by variations of either dose or glycine concentration in the ranges studied (3×10^{21} to 1×10^{23} ev for 100 ml targets and 0.0625 M to 3.3 M). Carbon dioxide and carbon monoxide yields increase with increasing glycine concentration, although there is little significant variation with changing dose.

The two targets employing C^{14}-labeled glycine were analyzed by means of ion-exchange chromatography. The products isolated are being saved to help in the future identification of some of the as yet unidentified products of glycine irradiation.

Considerable effort has been spent in the development of faster techniques of ion exchange chromatography. For the Dowex 50 columns, flow rates up to 1 ml/min/cm^2 column cross section are being used without too much loss in resolution. Columns 0.8 x 100 cm are being used for up to 50 ml of 1M glycine target solution (or its equivalent). Under these conditions about 30% to 40% of the resin bed is saturated with the target glycine. Shorter columns of larger bore give just as good resolution with equivalent glycine load, but a greater volume of elutrient is needed, making the collection and drying of samples more time-consuming. A conductivity cell has been employed together with an Esterline-Angus recording millimmeter and appropriate circuit to give a continuous record of the conductivity of the effluent from the column. Although a quantitative determination of products is not yet possible with this device, it has proved a great value in locating some of the product peaks as they come off the column without having to wait until the collected samples are dry. This allows a more appropriate choice of schedule for elutrient-concentration change, thus reducing the running time of the column and improving resolution. A start has been made in the development of chromatographic techniques employing anion-exchange resins of the strongly basic type (Dowex 1 and 2). A variety of eluting agents and conditions are being tried in an effort to isolate some of the organic acid and carbonyl products in one operation.

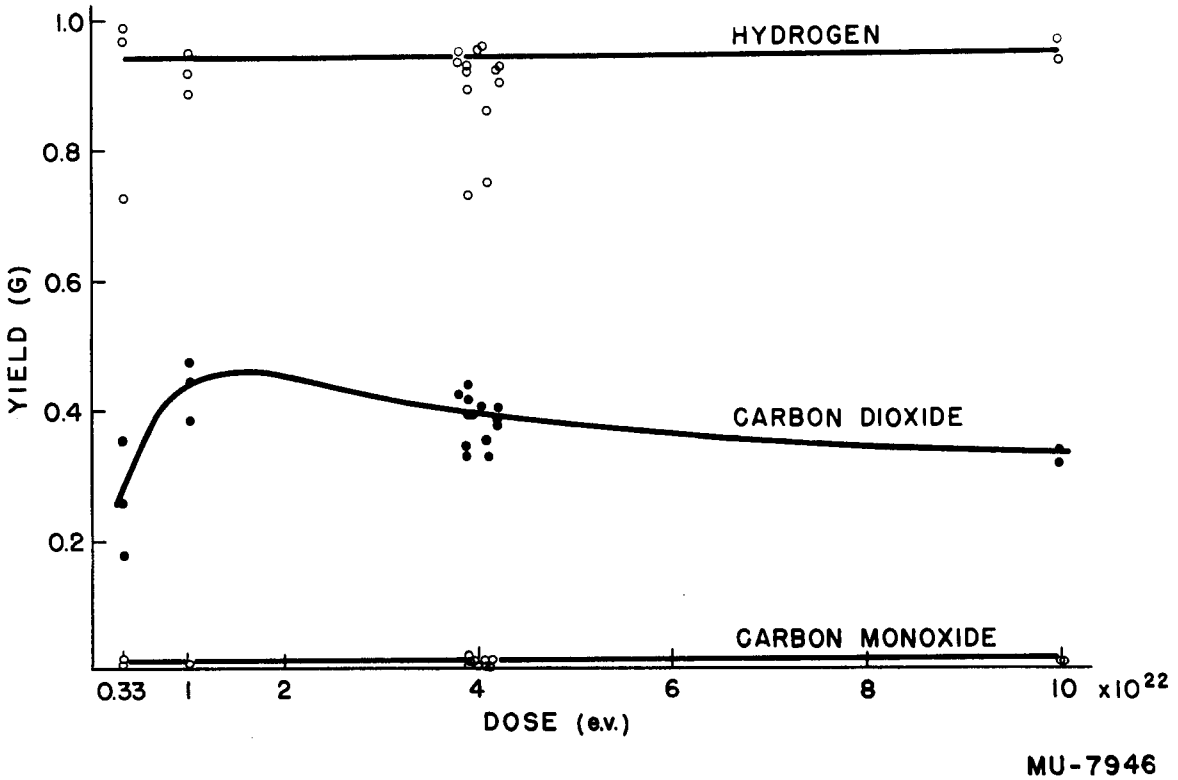

Fig. 9. Yield vs. dose for 0.25 M glycine. 100 to 120-ml evacuated targets.

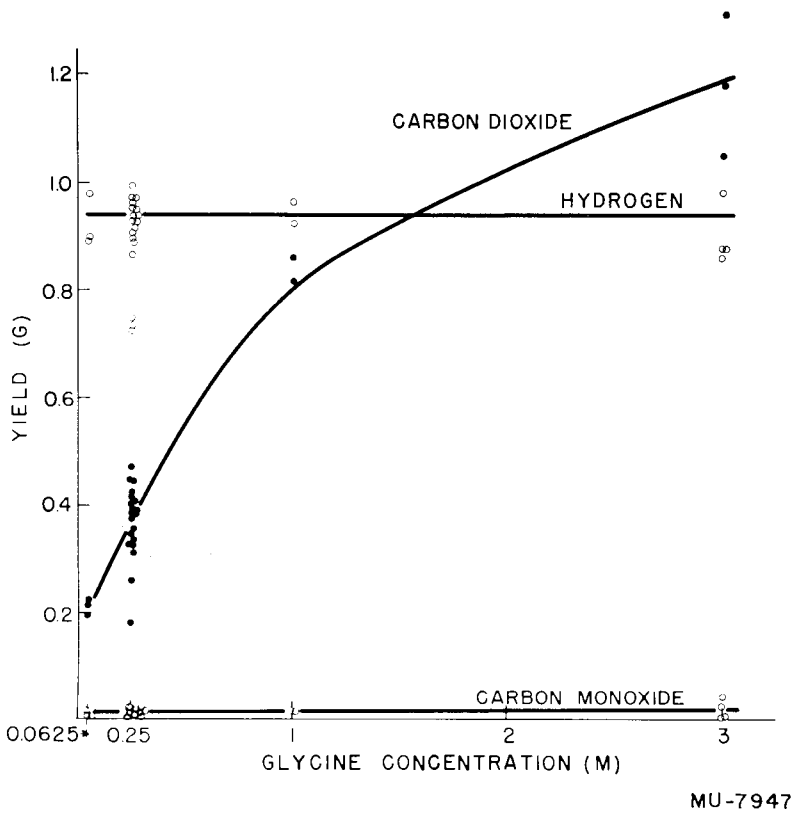

Fig. 10. Yield vs. glycine concentration for 100 to 120-ml evacuated targets. Dose equals 1 x 10^{22} ev.

HEALTH CHEMISTRY

N. B. Garden

EQUIPMENT DEVELOPMENT GROUP

This group has made ready an improved setup for use in the six-inch straight-type lead cave in Room 107, Bldg. 5; this equipment will be used to process another "napkin-ring"-type plutonium irradiation (so called because of the cylindrical features of the capsule, incorporated for the dissipation of hear during irradiation).

Other equipment setups include that for an irradiated depleted uranium sample from Idaho Falls and a large quantity of irradiated neptunium from the same reactor. The need for fuller personal cooperation and contact was revealed in an incident in the processing of the depleted uranium, during which two chemists received considerable but not permanently injurious beta-gamma radiation. Although the equipment and system used at UCRL are not at fault, a series of human factors or misjudgments resulted in this accident, and this aspect of the work has been reviewed and revised in order to arrive at a more foolproof manner of operation. The full details of the incident have been given elsewhere.

MONITORING

An improved device for rupturing quartz capsules containing irradiated material has been introduced, and quartz tubes of as nearly standard dimensions as possible are being put into use.

ACTIVITY HANDLING

The Activity Handling group supervised the preparation of the old 184-inch cyclotron dees for disposal at sea; it was necessary to reduce them to small pieces by sawing and by use of acetylene torches.

Because of the increased number of shipments and the more stringent ICC regulations, the Health Chemistry personnel responsible for outgoing shipments of radioactive material have put into use a new shipping memorandum to be included in each shipment for further assurance to both shipper and receiver that the package has been checked properly with respect to ICC and general safety regulations. Shipping containers for transportation of radioactive materials are being standardized by this group, as an increased number of movements, especially to and from the Idaho Falls Materials Testing Reactor, is taking place.

A minor spill of liquid containing radioactivity from a gloved box being prepared for disposal at sea in the driveway behind Bldg. 5A provided an incident for testing the worth of the paraffin coating put on part of the driveway in that area; it was demonstrated that the surface which has been coated was much more readily decontaminated than the more porous uncoated pavements.

A program is in operation whereby the radium sources held at UCRL are routinely checked for leakage and repackaged or repaired as indicated.

Large quantities of normal uranium and thorium, created over the past several years through machining, etc., were prepared for trucking to a recovery center. A sizable quantity of americium in about two liters of solution was prepared for shipment to the east coast via truck.

A pilot program on the solidification of liquid active waste has been set up and is in operation. This process involves the use of cornstarch on neutralized waste, which is then impregnated with cement in barrels suitable for sea disposal.

GENERAL

For the first time in approximately eight years (the period of existence of the Health Chemistry group) the pressure of work has been reduced to the point where special training can be worked into the schedule and selected men indoctrinated to undertake the responsibilities orginally planned for personnel in contact with the chemists working with radioactive isotopes. These individuals must be able to work with the chemist in planning the experiments and anticipate all requirements--namely, transportation, shielding, equipment, decontamination, waste disposal, etc. The responsibility of this new position (being referred to as Radioisotope Control Chemist or Engineer) is to see that the groups to care for the different phases are alerted and informed as to what will be expected of them. If these positions are properly filled, further improvement in the economy, efficiency, and achieved safety for the Health Chemistry group should result. The training is being given by the leaders of the various groups with specialized knowledge, skills, and functions, and consists of lectures and demonstrations.

Members of the Equipment Development, Airborne Activity Control, Activity Handling, and Decontamination groups have been engaged in examining and evaluating equipment at the former CR&D site in Livermore with the view of adapting the material for Health Chemistry with maximum advantage. Meter equipment has also been reviewed.

The effects on various materials such as plastics, synthetic rubbers, etc., produced by intense gamma fields over long periods has been studied through the use of the Co^{60} source in Bldg. 4A; this source is capable of delivering in excess of 10^5 r/hr.

Bldg. 70, the new chemistry building, is now near completion, and final detailed plans are being crystallized in regard to hoods, ventilation ducting, decontamination features, waste sampling, etc.

HEALTH PHYSICS

Burton J. Moyer

STATISTICAL SUMMARY OF MONITORING PROGRAM

Survey Instruments Maintained

1.	β-γ Ionization Chamber	62
2.	I. D. L. Portable Survey Instruments	20
3.	Cutie Pies	3
4.	Recording γ-Intensity Meters	28
5.	Victoreen Proteximeter	3
6.	Fast-Neutron Proportional Counters	8
7.	Slow-Neutron Proportional Counters	15
8.	Fast-Neutron Proportional Counter (Portable)	11
9.	Slow-Neutron Portable Unit	6
10.	Balanced Chamber--Fast Neutron--Portable	3
11.	Special Tissue Wall Survey Instrument	1

Personnel Meters in Use

1.	Total Personnel Covered with Film Badges	3,088
2.	Total Man Days of Coverage with Pocket Chamber	4,996
3.	Total Man Days of Coverage with Pocket Dosimeters	4,996
4.	Total Man Days of Coverage with Pocket Chambers (S.N.)	2,583

Cases of Weekly Exposure Above 0.3r

Weekly film expos. above	184" Area	60" Area	Lin. Acc.	Chem.	Other	Total
0.3	1	2	3	16	4	26
0.5	0	1	0	10	1	12
1.0	0	1	0	4	0	5
1.5	0	0	0	4	0	4
2.0	0	0	0	4	0	4
2.5	0	0	0	4	0	4
5.0	0	0	0	*4	0	4

* A complete report is being prepared with respect to this exposure and will be submitted to the proper office.

Information Division
8-18-54 og