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THE TOXICITY OF POLONIUM 210 IN MICE. I. THE 30-DAY LD₅₀,
RETENTION, AND DISTRIBUTION

by

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INTRODUCTION

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The first report of the biological behavior of polonium was published in 1913 by Ferneau et al¹, who found that prolonged exposure of human and rabbit skin to large doses of polonium did not result in the local erythema that followed similar exposure to radium. Since 1924 Lacassagne and his associates²⁻⁹ have been studying the excretion, distribution, and pathological effects of polonium injected into rabbits and rats. They have reported that excretion occurs principally via the kidneys and that the retained material is fixed chiefly in the cells of the reticulo-endothelial system. The highest concentration of polonium was found in the spleen, other lymphoid tissues, and renal cortex; the renal medulla and osseous tissue contained only trace amounts. Pathological changes were correlated with the concentration of polonium and cellular sensitivity to irradiation. The injection of lethal amounts was followed in six to twelve days by the destruction of the blood forming organs and a fatal hemorrhagic syndrome, or by acute nephritis. Lower doses resulted in chronic renal or intestinal

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inflammation two to four months later.

In 1950 Fink and his co-workers¹⁰ determined the acutely lethal level, the retention, and the distribution of intravenously administered polonium in rats. They found that 50 $\mu\text{c}/\text{kg}$ killed fifty per cent of the animals in twenty days and that retention (including loss by radioactive decay) was 70 per cent at ten days and 17 per cent at 100 days. Casarette¹¹ described the hematological and histopathological changes that occurred in rats up to 160 days after the intravenous injection of 10 and 20 $\mu\text{c}/\text{kg}$ of polonium.

The establishment of a permissible level of polonium exposure entails investigation of the long-term effects of low doses. This type of study requires a large number of individuals since the latent period for the appearance of pathological changes may approach the life span of the animal. For this purpose the mouse has proved to be a very suitable laboratory mammal. Before long-term studies of the toxicity of polonium in mice could be undertaken, it was necessary to determine certain facts for this species that already have been established for the rat, e.g., the acutely lethal dose of polonium, the rate at which the injected material disappears from the body, and the selective uptake by the various organs. The results of these investigations are reported in this paper.

EXPERIMENTAL PROCEDURE

Polonium 210 (radium F) has a half-life of 138 days and decays by the emission of an alpha particle and a gamma ray with respective energies of 5.3 and .77 mev. It was injected into a tail vein in 0.3 ml of hydrochloric acid solution in isotonic saline at pH 2.

Seventy-four CF#1 female mice, which were 62-68 days old and whose average weight was 22 grams, were randomly distributed into the groups shown in Table 1. It was estimated from the results of other workers that the dose that would kill fifty per cent of the animals in thirty days (30-day LD₅₀) would fall between 50 and 100 µc/kg. Therefore, animals of groups 1 and 2 were permitted to live until death occurred. Group 3 was selected for the separate analyses of the following: liver, spleen, kidneys, ovaries, mesenteric lymph node, lungs, femur including marrow, gastrocnemius muscle, and remaining carcass. Groups 4 and 5 were used to study retention over a longer period of time and to compare retention at different doses. The animals were killed with intravenous Nembutal.[®]

Table 1

Mouse Groups

Group	Dose level (µc/kg)	Number of animals	Disposition
1	100	15	Total body analysis at natural death
2	50	15	Total body analysis at natural death
3	25	15	Organ analysis at timed intervals
4	10	15	Total body analysis at timed intervals
5	5	14	Total body analysis at timed intervals

ANALYTICAL PROCEDURE

Polonium determinations are complicated by the adsorption of the material on glass, colloid formation, and the volatility of many of its compounds above 300°C. The analytical procedure developed for the present study was designed to reduce these complicating factors to a minimum.

Since, under proper conditions, polonium spontaneously plates from acid solution on metals such as copper, iron, and silver, it usually is assayed by measuring the alpha-ray activity of a plated disc. Silver was used in these studies because of its relative inertness to hydrochloric acid and because of its availability in thin foils.

The animals or organs to be assayed were placed in Kjeldahl flasks. A minimum of 2.⁵/₄ ml of concentrated perchloric acid, or 2.5 ml for each gram of tissue, was added. Superoxol (H_2O_2 --30 per cent Merck) equal to one-half the volume of perchloric acid and water equal to one-fifth the volume of perchloric acid were then added, and the flasks were shaken briefly. After 48 hours, during which time preliminary decomposition occurred, the flasks were placed on a digestion rack and maintained at 200°C. In two to twenty minutes the solutions became clear except for an oily layer on the surface. After further heating they became black and started to fume. One to two ml of Superoxol were added immediately whenever this occurred; digestion was considered to be complete when a clear white to yellow solution remained that did not blacken on further heating. Total digestion required fifteen minutes to ten hours, the time depending upon the size and fat content of the sample. The cooled solutions were transferred to volumetric flasks and brought to 1N with hydrochloric acid.

An aliquot that was estimated to contain approximately 0.01 μ c of polonium was placed in a 100 ml round-bottom centrifuge tube, and sufficient 1N hydrochloric acid was added to bring the volume to 50 ml. A clean, bright, silver disc, one inch in diameter and 0.002 inches thick, was placed in the vessel for the plating of the polonium. The solution was

stirred at such a rate that the disc rotated on edge while the tube was heated in a water bath at 90-100°C. After two and one-half to three hours the disc was removed, washed with water, and allowed to dry. Both sides were counted in a standard parallel plate alpha chamber at fifty-two per cent geometry¹²; the sum of the counts of the two sides was taken as the total count. Similar discs that had been plated from aliquots of the injection solutions were counted at the same time and used as standards.

Sixteen mice were processed by the analytical procedure described above, and mean recovery was found to be 100 ± 2.8 per cent (S.D.) Ashing in sulphuric acid proved to be unsatisfactory because it required temperatures in excess of 200°C, which resulted in loss of polonium by volatilization. Plating was found to be equally good from hydrochloric, sulphuric, and phosphoric acids, but nitric and perchloric acids were inferior, probably because they tended to dissolve the silver disc. The concentration of acid in the plating bath was not critical from 0.01N to 6N. The plating time required for 100 per cent recovery from 50 ml of solution was determined by removing the silver disc from the solution and counting it at intervals during a four-hour plating period. Complete recovery was attained in less than two and one-half hours (Figure 1). Larger volumes of solution required longer plating times so that complete recovery from 500 ml of solution required about four days.

EXPERIMENTAL RESULTS

Survival: The survival data plotted on probit paper were found to approximate closely the normal probability function. The curves that were derived from the probit graphs and the actual survival points are presented

in Figure 2. All the animals that had received 5 or 10 $\mu\text{c}/\text{kg}$ lived until their specified sacrifice dates. In Figure 3 the logarithm of the number of days to fifty per cent mortality is plotted against the logarithm of the dose. This curve places the 30-day LD_{50} of intravenously administered polonium 210 between 30 and 40 $\mu\text{c}/\text{kg}$ for CF#1 female mice.

Effect on body weight: At the time the mice were injected with polonium they had not attained full size and were growing rapidly. Those groups that received more than the 30-day LD_{50} (50 and 100 $\mu\text{c}/\text{kg}$) lost weight precipitously after the first week; those that received 10 or 25 $\mu\text{c}/\text{kg}$ progressed almost as well as 120 control animals that had received intravenous injections of a comparable volume of isotonic sodium chloride solution brought to a pH of 2 with hydrochloric acid.

Retention: The percentage of the injected dose that was retained appeared to be independent of the dose level over the range studied. A representative sample of the data is given in Table 2. It was therefore possible to combine the various doses for the establishment of the retention curve presented in Figure 4, where the individual values plotted semilogarithmically are adequately represented by a straight line after an initial rapid drop of approximately twenty per cent during the first three days. After this initial drop the biological half-period, when reduction by both excretion and radioactive decay are considered, was 30 days.

failed to grow during the period of observation; those that received 5 $\mu\text{c}/\text{kg}$

Table 2
Per Cent of Various Injected Doses Retained

Dose ($\mu\text{c/kg}$)	Days after injection			
	3	10	30	120
100		65.9 62.3		
25	83.4 81.9	66.8 63.5	45.4 43.7	7.9
10	89.6 88.6	85.2 62.9	55.8 51.5	17.3 11.8
5	85.5 70.7	66.5 63.6	42.5 41.8	12.9 12.5

Distribution and concentration: The fifteen animals that received 25 $\mu\text{c/kg}$ of polonium were used for this portion of the study. This dose proved to lie so close to the 30-day LD_{50} that in several cases death occurred before the scheduled sacrifice time. As a result the series includes two animals each at 1, 3, 10, 30, and 38 days and only one animal at 45, 51, 66, and 120 days. This number, unfortunately, is small, but it does permit the establishment of orders of magnitude for the distribution and concentration of polonium in various organs.

The percentages of the retained dose found in the selected organs are presented in Figure 5. This quantity was selected for illustration rather than the percentage of the injected dose in order to facilitate comparison between the organs and the body as a whole, and so that changes in distribution would not be masked by the decline in over-all retention. During the

entire 120-day period relatively constant percentages of the retained polonium were found in the liver, skeleton (3), and the mesenteric lymph node. The values for both the kidneys and the lungs decreased by a factor of 6, while for the spleen the value increased by a factor of 3. There was considerable variation in the polonium content of the ovaries sampled during the first ten days. The highest value was 1.3 per cent and the lowest 0.23 per cent; at 120 days no polonium could be detected in them.

The concentration of polonium in the various organs ($\mu\text{c/g}$) at from one to sixty-six days expressed as the per cent of the injected dose in $\mu\text{c/g}$ is illustrated in Figure 6. This method of presentation demonstrates the relative concentrations in the different organs and, in addition, it permits the calculation of the radiation dosage to an organ that will result from the administration of any amount of polonium. It is assumed that, since per cent retention proved to be independent of dose, distribution and concentration likewise are independent of dose. In fact, this situation was found to be the case for rats by Fink.¹⁰ The curve that describes the total body concentration of polonium, or the concentration if deposition were uniform throughout the body, is actually the retention curve in terms of $\mu\text{c/g}$ for this group of animals. At 120 days significant concentrations of polonium were found only in the spleen, the mesenteric lymph node, liver, and femur (Table 3).

3. Based on the approximation that a femur comprises 1/20 of the total skeleton.

Table 3

The Concentration of Polonium 120 days after Injection

$\frac{\mu\text{c/g in tissue} \times 100}{\mu\text{c/g injected}}$	
Organ	Per cent
spleen.....	97
mesenteric lymph node.....	25
liver.....	9
femur.....	9
kidneys.....	4
total animal.....	4
gastrocnemius muscle.....	2
ovaries.....	0
lungs.....	0

Since the weight of an organ is involved in the calculation of its polonium concentration, and since this weight may be influenced by irradiation from the contained polonium, attention should be given to organ weights. In Figure 7 these data are presented as the percentage of the weight found the day after injection. During a comparable 120-day period both the body weight and the spleen weight of control CF#1 female mice have been found to increase forty per cent. As was noted previously, the animals that were used for this portion of the study (the 25 $\mu\text{c/kg}$ group) failed to gain weight. However, the lungs and gastrocnemius muscle showed a forty per cent gain, but all of this increase occurred after the sixty-sixth day. These organs were subjected to less irradiation than any of the others with the exception of the femur, which failed to grow even after the sixty-sixth day. The explanation ^{for the lack of femur growth} may lie in the fact that whereas the concentration of polonium

in the muscle and lungs was negligible at 120 days, that in the femur was still nine per cent of the injected concentration (Table 3). The kidneys received almost ten times as much irradiation as the femur during the first sixty-six days, and they responded with a significant decrease in weight. However, at 120 days the concentration of polonium in them was only four per cent of the injected concentration, and recovery is evidenced by the fact that they weighed seventeen per cent more than they had at one day. Although the liver received only about one-third as much irradiation as the kidneys on a weight basis during the first sixty-six days, it showed a greater loss of weight. In contrast to the kidneys, the liver did not exceed its initial weight at 120 days, and this again may be explained on the basis of the relative concentrations of polonium in these two organs at 120 days, viz., nine per cent for the liver and four per cent for the kidneys. From the sixty-sixth to the 120th day the concentration of polonium decreased in the kidneys by a factor of 50 and in the liver by a factor of 7. It appears that the ovaries were so severely damaged that, in spite of the fact that no polonium could be detected in them at 120 days, weight recovery did not occur. The spleen and the mesenteric lymph node showed the greatest proportional loss of weight and, with the exception of the ovaries, the least recovery. This is in accord with the fact that at 120 days their concentration of polonium was 97 per cent and 25 per cent, respectively, of the injected concentration.

DISCUSSION

There appears to be little difference between the response of rats and that of mice to acutely lethal doses of polonium administered intravenously. A comparison of the data reported by Fink and his associates for the rat¹⁰ and those reported here for the mouse shows only minor differences, which may well be the result of the small numbers of animals used rather than an actual species difference. For example, the percentage of the retained dose that was present in the rat spleen decreased from the tenth to the fiftieth day while in the mouse spleen it increased. Another discrepancy ~~is~~ ^{appears} between the values for the concentration in the lung. The rat lung contained approximately 2 1/2 times as much polonium per gram of tissue as the average body tissue whereas the concentration in the mouse lung remained very close to that of the body as a whole.

The liver, spleen, kidneys, and skeleton of the mouse contained between thirty and sixty per cent of the retained polonium during the first sixty-six days after injection. At 120 days thirty per cent of the retained material was present in the liver, spleen, and skeleton. No attempt was made to analyze separately the bone and the bone marrow in this small animal, but Fink¹⁰ has reported for the rat that on a weight basis the marrow contained five times as much polonium as the bone. Therefore, if "bone marrow" may be substituted for "skeleton," the distribution of polonium in the mouse is found to coincide closely with the principal locations of the reticulo-endothelial cells. This distribution may be a result of the injected polonium becoming colloidal immediately upon injection into

the blood stream. This hypothesis is substantiated by the work of Boyd¹⁴, who found polonium aggregates in the cardiac blood of the rat 1 1/2 minutes after intravenous administration *of a noncolloidal solution.* Within a few minutes these colloids disappeared from the blood and appreciable amounts of polonium appeared in the liver, spleen, and bone marrow.

The concentration of polonium in particular organs and its uneven distribution within these organs complicates the calculation of radiation dosage. This situation is true for all radioisotopes that are not distributed uniformly. In spite of this difficulty there is, perhaps, some justification for translating the energy resulting from radioactive decay into a unit such as the roentgen-equivalent-physical (rep) so that the effects of one type of irradiation may be compared with those of another for purposes of determining their relative biological effectiveness. If the assumption is made that one microcurie of polonium is evenly distributed within one gram of tissue, the dosage therein is equivalent to 294 rep/day. Calculation of the dosage to the various organs of those mice that received 25 μ c/kg reveals that, if the material had been evenly distributed within them, the spleen and the mesenteric lymph node received 170 and 48 rep/day, respectively, at the time that they had their highest polonium concentration. These values were reduced to 7 and 1.7 rep/day on the 120th day. Therefore, it is not surprising that these organs did not recover their pre-injection weight. Similarly, the ovaries were unable to recover after their initial maximum dose of 73 rep/day.

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The uneven distribution of polonium among and within the organs indicates that at low levels the major pathological changes will be found in the lymphoid and myeloid tissues, renal cortex, ovaries, and perhaps in the liver. Casarette¹¹, in his studies of rats that had received 10 or 20 $\mu\text{C}/\text{kg}$ one-half to 160 days previously, found changes in the peripheral blood, the lymphatic tissues, kidneys, and testes. Although polonium is not a bone seeking isotope, he found alterations in the zone of endochondral ossification in the femur, primarily in the higher dosage group and at the later intervals. Therefore, it is not improbable that pathological changes will occur in the bones of those animals that receive above tolerance levels of polonium.

The ovary may prove to be the critical organ in determining the lowest injurious dose since it is one of the more radiosensitive organs and its concentration of polonium during the first ten days after injection was exceeded only by that of the spleen. Lorenz¹³ found an increase in the incidence and a decrease in the latent period of ovarian tumors in LAF₁ mice that were subjected to gamma irradiation from radium at the rate of 0.11 r/day given in eight hours per day. The first tumor appeared when the total accumulated dose was approximately 90 r. The ovarian tumor incidence was the same as that following a single dose of 50 r X-ray delivered in four and one-half hours. It can be calculated from the present study that at the lowest proposed dose for the long-term experiment (1/100 of the 30-day LD₅₀, or 0.4 $\mu\text{C}/\text{kg}$) the ovaries will receive approximately 35 rep during the first sixty-six days, or an average of 0.5 rep/day. From the third through the

tenth day after injection the dose rate will be of the order of one rep/day.

SUMMARY AND CONCLUSIONS

1. A reliable method for the quantitative determination of polonium 210 in animal tissues is presented.
2. The 30-day LD_{50} of polonium given intravenously in hydrochloric acid solution to young adult CF#1 female mice lies between 30 and 40 $\mu\text{c}/\text{kg}$. The deleterious effects of 10 $\mu\text{c}/\text{kg}$ were manifest as early as two weeks after injection by the failure of the mice to grow.
3. The per cent retention of intravenously injected polonium was independent of dose level over the range studied (5 to 100 $\mu\text{c}/\text{kg}$). Excretion alone reduced the body content to 71 per cent at ten days, 50 per cent at thirty days, 14.5 per cent at one hundred days, and 6 per cent at one hundred and fifty days. The biological half-period, including loss by radioactive decay, was thirty days.
4. During the first sixty-six days after the injection, the greatest portion of the retained polonium was located in the liver, kidneys, and skeleton. The major change at 120 days was the decrease in the polonium content of the kidneys.
5. During the first sixty-six days the concentration of polonium ($\mu\text{c}/\text{g}$) was greatest in the spleen, kidney, mesenteric lymph node, ovary, and liver. At 120 days the microcuries of polonium per gram of spleen were 97 per cent of the microcuries that had been injected per gram of animal. The value for the mesenteric lymph node was 25 per cent. At this time the concentrations in both the liver and the femur were nine per cent of the initial concentration in the total animal.

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6. The localization of polonium indicates the probable sites of pathological change after the administration of low doses of the isotope. It is suggested that the ovary may prove to be the critical organ in determining the lowest injurious dose.

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LEGENDS FOR FIGURES

- Fig. 1. Recovery of polonium from hydrochloric acid solution as a function of time.
- Fig. 2. Survival of CF#1 female mice following the intravenous administration of polonium 210. The percentages were corrected for those animals taken for time-serial sacrifice at the 25 $\mu\text{c/kg}$ level.
- Fig. 3. Dose and interval to 50 per cent mortality.
- Fig. 4. Retention of polonium following intravenous administration.
- Fig. 5. Distribution of retained polonium: $\frac{\mu\text{c in organ} \times 100}{\mu\text{c in total animal}}$
- Fig. 6. Concentration of polonium: $\frac{\mu\text{c/g in organ} \times 100}{\mu\text{c/g injected}}$
- Fig. 7. Weight change of organs following intravenous administration of polonium 210. The weight on the day after injection establishes the base line of 100 per cent.

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PER CENT OF RETAINED DOSE

2-1-23

PER CENT OF WEIGHT AT ONE DAY

DAYS AFTER INJECTION