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REPORT FOR BIOLOGICAL RESEARCH

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Date: April 23, 1951

Approved By: for D. S. Anthony
M. N. Haring
Laboratory Director

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GENERAL SUMMARY

STUDIES OF THE PHYSIOLOGICAL EFFECTS OF POLONIUM UPON THE METABOLISM OF LABORATORY ANIMALS

Study of the Recovery of Polonium from the Blood of Rats

The level of polonium in blood of rats injected with 7.4 microcuries of polonium per kilogram of body weight was followed for more than eight months. There is an initial drop in polonium concentration with time, followed by a period of several weeks during which there is actually a slight increase in concentration. At late time intervals there is again a drop in concentration with time. Polonium was detectable in the blood of these rats for at least 31 weeks.

A Study of the Variations in the Amount of Reducing Sugars Present in the Blood of Polonium-Injected Rats

A statistical analysis of the data showed that there is a significant increase in the blood-sugar level of polonium-injected rats. However, these studies showed that the determination of blood-sugar levels has no reliable diagnostic value for polonium intoxication.

Twenty-Day LD_{50} Determinations for Different Species of Laboratory Animals Part I. Studies on Dogs

This experiment gave a rough working figure of about 70 microcuries of polonium per kilogram of body weight as the twenty-day LD_{50} in dogs.

Twenty-Day LD_{50} Determinations Upon Laboratory Animals Part II. Survival Studies on Rabbits

In the dosage range from 85.1 to 23.1 microcuries of polonium per kilogram of body weight, the survival time ranged from 8 to 160 days. Over this range the survival time was roughly proportional to the dose given.

Effect of Repeated Injections of Polonium Upon Laboratory Animals. Pilot Study

The data indicate successively diminishing fecal excretion of polonium per unit time with each new injection. Urinary excretion was more in harmony with the increasing body burden. The experiment provided a basis of fact and experience for subsequent multiple-dose studies.

Effect of Repeated Injections of Polonium Upon Laboratory Animals. Main Study

Since this experiment is in the very earliest stages only the plan of experiment is given here. Rats will receive 10 biweekly polonium injections totaling 7.2 microcuries of polonium per kilogram of body weight. Tissue samples and polonium assay samples are being taken. Hematological data are also being obtained.

Clinical Tests of the Kidney Function of Polonium-Injected Sprague-Dawley Rats

This experiment is in the very early stage, hence no data are presented. Two injection levels, 9.8 and 4.0 microcuries of polonium per kilogram of body weight were used. The study will be divided into three main sections, (1) clinical pathology, (2) histopathology, and (3) hematology.

An Adaptation of the Phenolsulfonphthalein Test for Estimating Renal Function for Use on Rats

The technique of Geraghty and Rowntree for the clinical determinations of the function of the collecting tubules of the kidney has been modified for use on rats. The modified test has been used successfully in this laboratory yielding reproducible results.

A Modification of Ohlsson's Micromethod for the Determination of Blood Urea Nitrogen in Rats

The modification involves alterations in the blood sample size, preparation of reagents, and a change in the technique for spectrophotometric analysis.

THE EFFECTS OF POLONIUM UPON CELL METABOLISM

Growth and Cell Division Changes

The observation that polonium exerts a greater effect upon yeast cell divisions than upon yeast cell growth, resulting in the formation of large cells, has been extended to the bacterium E. coli. Within the range of polonium concentrations used it appears that the growth of this organism is affected even less than is the growth of yeast. However, cell division is again inhibited, thus, large cells are formed.

Oxygen Effect on Polonium Injury

The effect of polonium upon yeast cell growth is markedly reduced when oxyge is excluded from the cultures. However, there is little difference in the effect of polonium on cell division; large cells are still produced.

Nitrogen Compound Studies

The data show that large individual cells contain increased amounts of various nitrogenous constituents. Thus, the increased cell size mentioned above is not caused by mere water swelling. On a total mass basis, small increases in total nitrogen and protein nitrogen appear in large cells in each run. Acid-soluble nitrogen levels, however, are lower than in control cells. Several constituents of the acid-soluble nitrogen fraction are low also. Amide-nitrogen and ammonia-nitrogen levels are lowest in comparison with controls. Although no actual reduction in the amount of any of these constituents occurs, amide-nitrogen is at the point where little or no increase may occur after exposure of the cells to polonium. The low amide levels found in large cells are in direct contrast to previous measurements. No complete explanation is yet evident.

HEMATOLOGICAL AND PATHOLOGICAL EFFECTS OF POLONIUM ON LABORATORY ANIMALS

Hematological and Pathological Studies on Sprague-Dawley Rats Injected Intravenously with Varying Amounts of Polonium

This report presents the complete histopathological examination of skin, brain, eye, lymph nodes, thymus, thyroid, pancreas, adrenals, and lungs of rats which have been injected with 23 or 8 microcuries of polonium per kilogram of body weight.

No abnormalities were seen in either the epidermis or dermis at either injection level.

Lymph nodes of rats injected with 23 microcuries of polonium per kilogram of body weight show degenerative changes as early as three days post-injection. There was a loss in the medium sized and small lymphocytes and an increase in nuclear debris. Progressive atrophy and degeneration of the lymphoid tissue occurs with time, and there was no regeneration of the tissue in these rats. At the 8 microcuries per kilogram of body weight dose level, lymphoid changes did not show up until about seven days post-injection. The changes observed were less severe and were variable from animal to animal and even from node to node in the same animal. Regenerative changes were seen in many animals beginning at about 56 days. All rats sacrificed from 70 through 305 days post-injection revealed some type of regenerative changes with restoration of some of the nodes to almost normal lymphoid activity.

The adrenals showed collagenous degeneration of the zona reticularis. Degeneration was slight at the 8-microcurie level and moderate to marked at the 23-microcurie level.

The thymus of rats from both injection levels underwent progressive atrophy with time. From 244 days post-injection onward in the 23-microcurie animals, and from 305 days onward in the 8-microcurie level animals, only connective tissue was found in the area of the thymus. At the 8-microcurie level there was a transient partial regeneration in the period from 70 to 98 days post-injection.

No histopathological changes were found in the eye sections at either dose level.

No histopathological changes were found in the brain sections of rats at either level. Only Azure II-eosin, hematoxylin-eosin, and Masson's Trichrome stains were used.

No histopathological changes were seen in the pancreas of rats at either dose level.

There was no consistent pathological change in sections of thyroid gland that could be attributed to the action of polonium. Changes in thyroid architecture caused by the pressure of forceps in handling the tissues are discussed.

The lungs of the controls and both injected series of rats showed small multiple cystic areas or punctate nodules in the visceral pleura. In later sacrifices the lungs of polonium-injected rats showed less cellular infiltration into the lesions, and there was a more consistent finding of large vacuolated lipoid-like cells than in the lungs of the control rats. Otherwise the lesions in the lungs of control and polonium-injected rats showed the same histopathological picture.

The Minimum Effective Dose of Polonium Causing Hematological and Pathological Changes in Rats

Only the experimental plan is given. Two injection levels were used, 0.9 and 3.5 microcuries of polonium per kilogram of body weight. The rats in each injection level were divided into a pathological and hematological group. Serial sacrifices and frequent hematological examinations will be carried on for at least 52 weeks.

A Study of Spontaneous Lung Lesions in Sprague-Dawley Rats

It was concluded that interstitial pneumonitis, probably of a viral origin, is endemic in rats of the Sprague-Dawley strain. Lung lesions seen in injected rats may vary from mild infection with but a few sparse lesions, to extensive lesions involving a large portion of the lung tissue. The etiology of the pneumonitis has not yet been determined. Sprague-Dawley rats with a nonspecific pneumonitis that are injected with polonium revealed only a loss in the cellular content of the lesion. The lung lesions previously described in MLM-381 as characteristic for that dosage of polonium were incidental to the polonium and secondary to a previous lung infection.

A Kidney Function Study on Polonium-Injected Rats II. Histopathological Study of Animals from a Pilot Experiment

There is a relationship between the amount of hydronephrosis present, the amount of abnormal fat in the glomeruli and proximal convoluted tubules, and the number of fat casts in the collecting tubules of the kidney with the percentage excretion of phenolsulfonphthalein dye by that kidney. The liver and spleen were also examined in these rats.

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STUDIES OF THE PHYSIOLOGICAL EFFECTS OF POLONIUM UPON THE METABOLISM OF LABORATORY ANIMALS

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Problem Title **A Study of the Recovery of Polonium from the Blood of Rats**

Report By **D S Anthony, R K Davis, W P Jolley, and L H Talley**

Work Done By **D E Etter, W P Jolley, and L H Talley**

INTRODUCTION

This pilot study was undertaken to further the understanding of the polonium distribution in the organs of Sprague-Dawley rats. The experiment is also considered important as a prerequisite for skin adsorption experiments, where the amounts of polonium absorbed through the skin into the blood is expected to be small. It was found previously[1] that the polonium recovery from rat urine increased sharply to a peak at five days post-injection and then declined very sharply until a plateau was reached.

DETAILED REPORT

Twelve rats, six males and six females, were used in this experiment. One rat of each sex was designated as a carrier control and was injected with the phosphate-buffered saline carrier solution. The other five rats of each sex were injected via the caudal vein with 7.4 microcuries of polonium per kilogram of body weight, as measured in dummy injection solutions. Blood samples of 0.4 milliliter were taken from the rats by cutting the tip of their tails. Samples were collected at intervals of 1, 4, 6, 24, and 48 hours post-injection, at weekly intervals for the next 13 weeks, then semimonthly until the conclusion of the experiment at 35 weeks. The blood samples were heparinized, and two 0.1-milliliter aliquots were chemically digested and the polonium content measured.

A graphic representation of the average polonium concentration in the blood of 10 rats (five male and five female) is given in Figure 1 where time in hours is plotted semilogarithmically against polonium concentration expressed in 10^{-9} microcuries per milliliter of whole blood. The lower curve represents an average of one male and one female control animal and thus the curve is the equivalent of a blank in other types of analyses.

It will be noted from Figure 1 that there is a very rapid straight-line decrease in the blood polonium concentration from 4 hours to 168 hours (one week). During this period the concentration dropped from approximately 79 to 8.3 x 10^{-9} microcurie per milliliter of whole blood. From this point there is a slight increase in the blood polonium concentration to a maximum at 1,848 hours (11 weeks) post-injection of 11.5 x 10^{-9} microcurie of polonium per milliliter of whole blood. The 1,680 hours (10 weeks) of slowly increasing polonium concentration in the blood is very different from the usual behavior of radioisotopes present in subacute toxic amounts in the rodent body. The concentration of such materials in the blood generally drops continuously and in a more or less regular manner with time. In fact, in the case of Sr 89 the blood level drops in such a regular fashion that it is an unbroken straight line on a logarithmic plot.[2] This is true for both rats and rabbits containing radioactive strontium.

After the 11-week point there is again a more or less straight-line decrease in polonium concentration to about 7.5 x 10^{-9} microcurie of polonium per milliliter of whole blood at 3,864 hours post-injection. After this point there is a downward curvature in the line to the end of the experiment at 5,880 hours (35 weeks) post-injection. At this time the blood contained 0.8 x 10^{-9} microcurie per 1.0 milliliter. This is essentially the same as the control value of 0.2 x 10^{-9} microcurie per 1.0 milliliter of whole blood.

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The last two points on the experimental curve in Figure 1 represent an average of three males and one female as the rest of the experimental animals had died by that time. The final point was determined on a 1.0-milliliter aliquot rather than the usual 0.1-milliliter, giving a much larger sample, and a more accurate analysis. At the conclusion of the experiment the remaining animals were sacrificed and tissue samples were taken for histopathological study.

It is interesting to speculate concerning the cause of the peculiar shape of the blood-polonium concentration curve of Figure 1. One attractive explanation is that there is an excretory disfunction, at least for polonium, during the 168 to 1,848-hour post-injection period. Thus, polonium mobilized from body depots would tend to pile up in the blood. One piece of evidence recently obtained supports this view. Tests on animals receiving comparable doses of polonium show some evidence of reduced kidney function at the earliest test period, two weeks (336 hours) post-injection.[3] Additional evidence may be obtained from a study of the correlation of polonium concentrations in blood and urine.

The scatter of individuals making up the average points recorded in Figure 1 is shown in Figure 2. In the interest of brevity, only four arbitrarily selected times were graphed.

As another test of the consistency of the data, all ten individuals were graphed in the manner of Figure 1. The graphs are not presented in this report because they can be accurately summarized in the following single sentence. Every rat showed the same shape of curve with minor variations in slopes.

In conclusion, this experiment has demonstrated that in subacute-dose experiments it is possible to take repeated blood samplings over a span of more than eight months until the levels of polonium in the blood approach or reach control values. Further, it has been learned that the technique of digestion, plating, and counting small samples of blood is practical. However, this method has recently been supplanted by an equally practical but simpler method of direct blood-smear assay.[4]

From Figures 1 and 2 it can be seen that in this experiment the biological life of polonium in the blood of rats injected with 7.4 microcuries of polonium per kilogram of body weight was approximately 8,900 hours (35 weeks).

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3. Unpublished data, this Laboratory.

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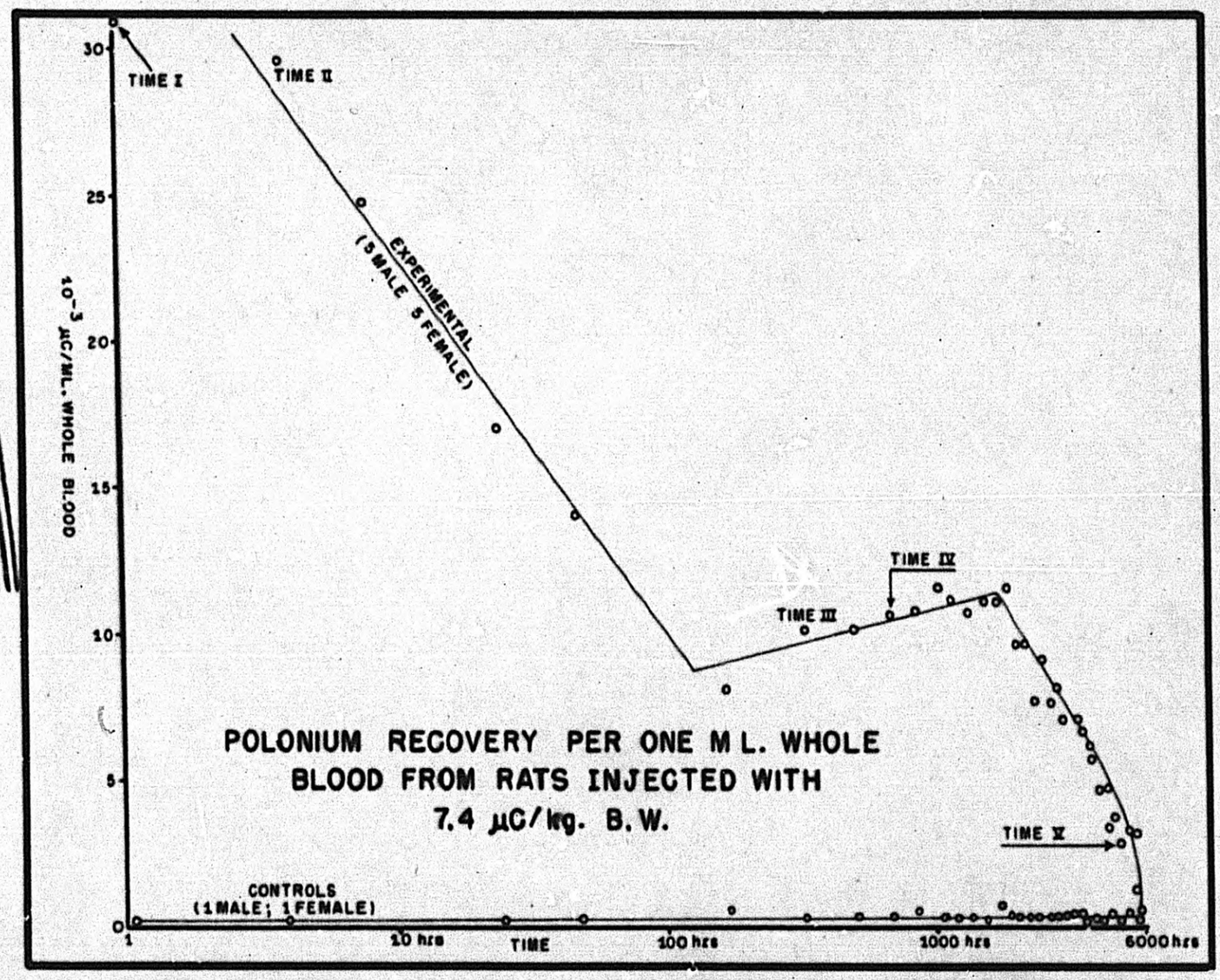
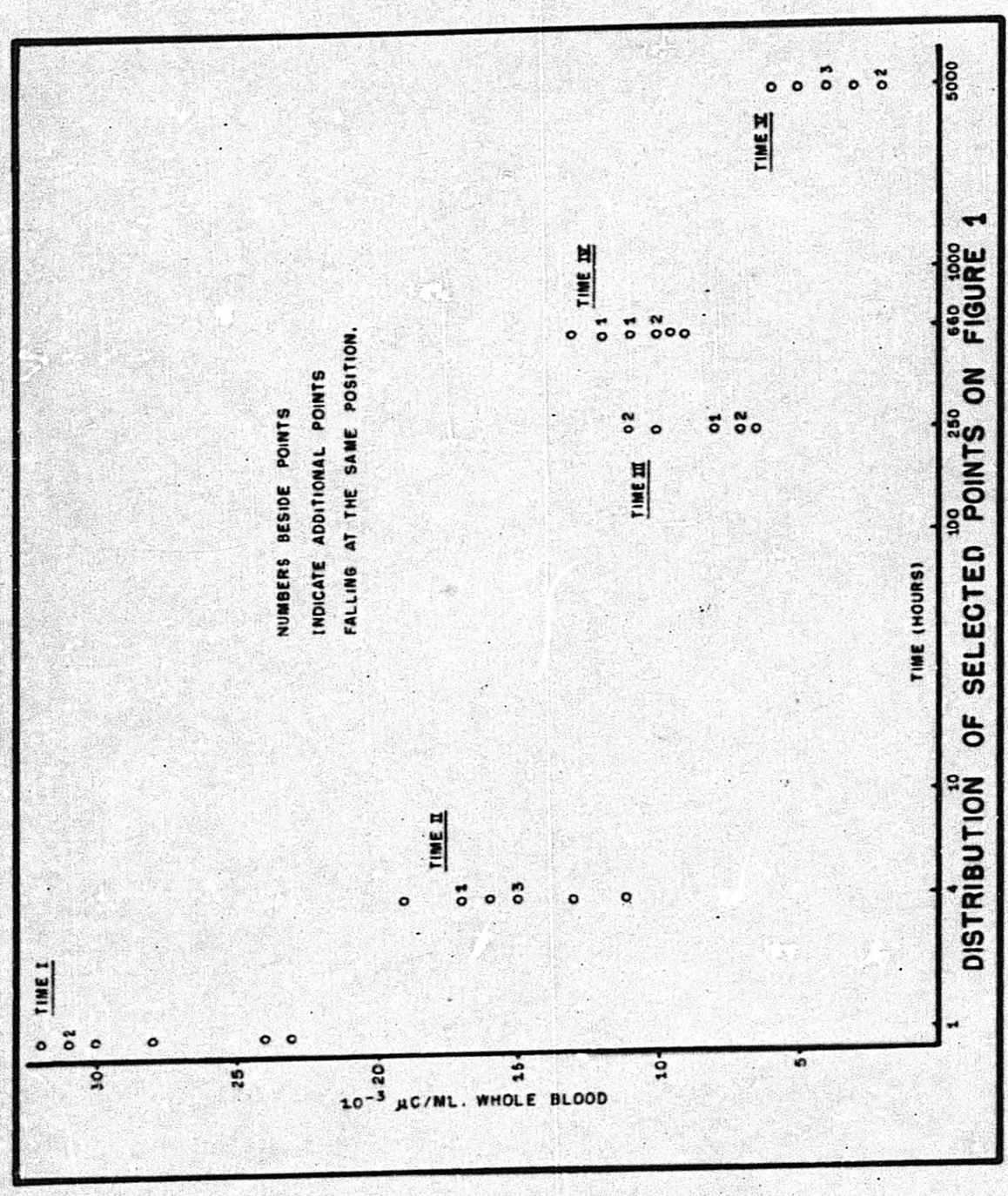

TIME I
TIME II
TIME III
TIME IV
TIME V
EXPERIMENTAL
(5 MALE 5 FEMALE)
CONTROLS
(1 MALE; 1 FEMALE)
30
25
20
15
10
5
0
1
10 hrs
100 hrs
1000 hrs
6000 hrs
TIME
10⁻³ µC/ML. WHOLE BLOOD
POLONIUM RECOVERY PER ONE M L. WHOLE
BLOOD FROM RATS INJECTED WITH
7.4 µC/kg. B. W.

FIGURE 1

FIGURE 2


DISTRIBUTION OF SELECTED POINTS ON FIGURE 1
TIME (HOURS)
1
4
10
100
250
660
1000
5000
10^{-3} µC/ML. WHOLE BLOOD
30
25
20
15
10
5
TIME I
TIME II
TIME III
TIME IV
TIME V
NUMBERS BESIDE POINTS
INDICATE ADDITIONAL POINTS
FALLING AT THE SAME POSITION.

[redacted]

Problem Title – **A Study of the Variations in the Amount of Reducing Sugars Present in the Blood of Polonium-Injected Rats**

Report By – **R. K. Davis, W. P. Jolley, and L. H. Talley**

Work Done By – **D. E. Etter, W. P. Jolley, and L. H. Talley**

INTRODUCTION

Early experiments in this laboratory consistently showed restricted nutritional effects following polonium administration to Sprague-Dawley rats. Subsequently an inanition experiment[1] was conducted where it was found that starvation produced histological and hematological effects very similar to those found in polonium-treated rats. In an attempt to further evaluate this nutritional syndrome, attention was focused upon the blood-sugar levels of the laboratory animals. Kohn[2] noted a 40 per cent increase in the blood-sugar level of guinea pigs after total body irradiation with X-rays. In view of these findings the present experiment was designed to study the variations in the amounts of reducing sugars in the blood streams of polonium-injected rats and to determine if these variations would have diagnostic significance.

DETAILED REPORT

A total of 36 rats, divided into three groups of six males and six females each, were used in this experiment. Three rats of each sex in each group were designated as carrier controls and received an injection of phosphate-buffered, saline, carrier solution. The remaining rats of each group (three males and three females) were injected via the caudal vein with 7.4, 21.4, and 33 microcuries of polonium per kilogram of body weight, respectively. The rats were fasted 24 hours prior to injection, and the fasted weights were used to determine the injection volumes.

Tests were run on three normal male and three normal female rats to establish a normal range for the blood-sugar levels in this experiment. These tests showed the mean fasted, blood-sugar level to be 86 milligrams of glucose per 100 millliliters of blood. These values agree with those of Mann[3] for the Wistar strain of rats.

The modified Somogyi method[4] was used to determine the amount of reducing sugars present in the blood samples. A standard glucose curve was plotted from the analyses of the standard stock solutions. The method of analysis used on these standard solutions was the same as that used for the analyses of the unknowns.

All experimental animals were fasted for 16 hours prior to testing, and then venous blood samples were obtained by slicing off the extreme tip of the rat's tail and drawing 0.1 milliliter of whole blood into a standard blood micropipette. The samples were transferred to a precipitating solution, and the pipette was rinsed several times with the precipitating solution. The samples were then immediately analysed for the sugar content. Analyses were run one day post-injection, then weekly for the first 30 days, and then semimonthly until the conclusion of the experiment.

The blood-sugar curves shown in Figure 1 for the three groups of polonium-injected animals tend to follow the general pattern of the control blood-sugar curves. Each curve plotted represents the average values for all the rats in that particular group. The blood-sugar levels of the injected rats were slightly higher than the control rats. This is particularly true for Group II (21.4 microcuries per kilogram of body

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weight) and Group III (33 microcuries per kilogram of body weight). The rats of Group I (7.4 microcuries per kilogram of body weight), however, had slightly lower blood-sugar levels than the controls, although they followed the same general trend as the controls.

Groups II and III were not carried to the termination of the experiment because of the death of the polonium-injected rats. As each of the injected rats died, in any group, its corresponding control rat was sacrificed. The experiment ran for a total of 285 days. During the last 125 days of the experiment the blood-sugar determinations were run on only three polonium-injected and three control rats. Additional blood-sugar determinations made since the last report[5] have shown no further variation.

It has been concluded from these data that the determination of blood-sugar levels at various intervals following polonium injection has no reliable diagnostic value. However, a statistical analysis of the data shows that there is a significant increase in the blood-sugar level of polonium-injected Sprague-Dawley rats.

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4. Nelson, N. A., J. Biol. Chem., *153*, 375, 1944.
5. Davis, R. K., and Jolley, W. P., Quart. Rpt. Biol. Res., MLM-493, p. 29, October 9, 1950.

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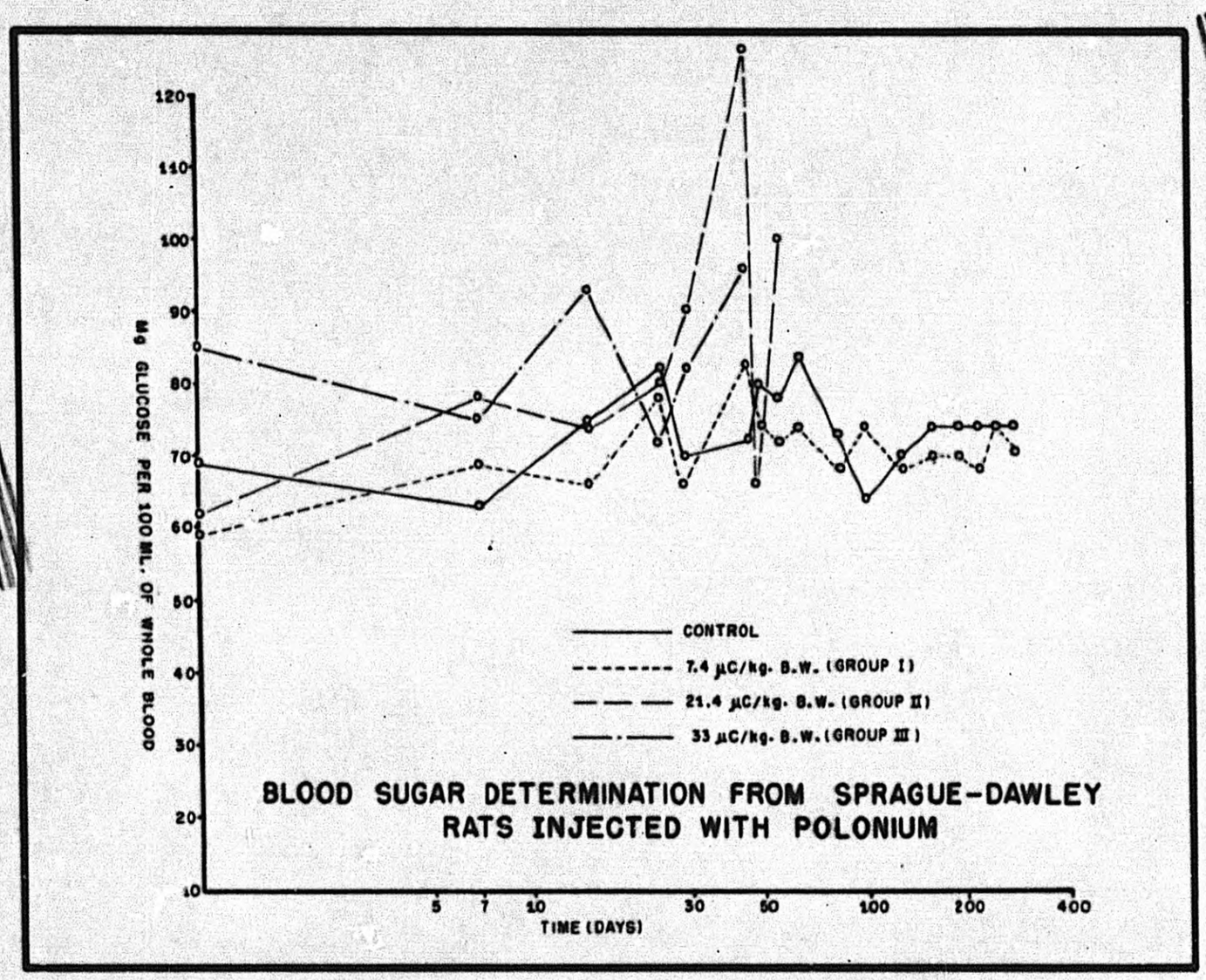
120
110
100
90
80
70
60
50
40
30
20
10
MG GLUCOSE PER 100 ML. OF WHOLE BLOOD
CONTROL
7.4 µC/kg. B.W. (GROUP I)
21.4 µC/kg. B.W. (GROUP II)
33 µC/kg. B.W. (GROUP III)
BLOOD SUGAR DETERMINATION FROM SPRAGUE-DAWLEY RATS INJECTED WITH POLONIUM
5
7
10
30
50
100
200
400
TIME (DAYS)

FIGURE 1

Problem Title - **Twenty-Day LD_{50} Determinations for Different Species of Laboratory Animals I. - Studies on Dogs**

Report By - **R. K. Davis and W. P. Jolley**

Work Done By - **R. K. Davis and W. P. Jolley**

INTRODUCTION

Animal biological research is concerned with the over-all understanding of the effects of polonium upon the physiology of laboratory animals, and the extrapolation of these findings to human data. In accordance with this plan, research work is progressing from work on mice and rats through rabbits and cats to dogs.

As in the past, one of the first problems of importance to be solved is that of what constitutes a lethal dose of polonium for the animal. Accordingly, a small dosage experiment on dogs was undertaken. From this experimental survey[1] a dosage range of 40 to 60 microcuries of polonium per kilogram of body weight was established.

The present 20-day polonium LD_{50} experiment on dogs was completed as the culminating experiment for this series of dog studies.

DETAILED REPORT

Twelve dogs of mongrel ancestry and of both sexes were selected from the laboratory colony. These dogs were selected for their generally excellent health and the length of time they had been in the stock colony. Their body weights ranged from 8,700 to 14,750 grams and the 12 animals were divided equally into four general weight groups. One dog from each of these four weight groups was used to make up the membership of each of three injection levels of 64.5, 77.1, and 89.8 microcuries of polonium per kilogram body weight.

The dogs were injected via the cephalic vein in the right fore-leg with polonium in buffered physiological saline. The injection volumes ranged from 0.50 to 0.94 milliliter of solution depending upon the dog's weight and its injection level.

The dogs were caged in individual cages, their food and water consumption was measured, and their body weights were recorded. The curves derived from plotting the average food and water consumptions of the dogs in each of the three dosage levels had essentially the same slopes for the first two weeks. A composite food consumption curve representing all three injection levels was parabolic, starting at an average daily food consumption of a little more than 600 grams per dog per day and dropping to zero consumption at 14 days. The water intake composite curve indicates that an average of 1,160 milliliters of water per dog per day was taken in. This consumption fell to about 140 milliliters after two weeks post-injection. At this time, the water consumption curve showed a small abrupt rise to 225 milliliters per dog per day. This is probably caused by two factors, first, by this time the three sickest dogs had died and secondly, since all of the remaining dogs had stopped eating, there resulted a slight compensatory increase in water intake. Perhaps the most striking feature of the food and water consumption data was the fact that the dogs in all injection levels behaved the same. That is, regardless of the dosage the dogs decreased their intake and finally quit eating at about the same rate and time. Similarly their consumption of water paralleled one another. Too few points

were obtained on body weights to enable a body weight curve to be plotted but data on the per cent of the original or injection weight lost by the dogs during the course of the experiment are given in Table I. Also included in this table are data on the individual dosages of polonium administered to the dogs and the number of days they survived.

In all cases, the deaths of the dogs were preceded by a passage of a stool of clotted blood sometime within the final 12 hours. Complete blood counts were taken at weekly intervals starting with the day of injection. The hemoglobin, hematocrit, and red blood cell counts showed no marked changes. The white blood cell counts tended to decrease as the survival time lengthened. However, in all 12 dogs the white blood cell counts decreased tremendously. Table I gives the per cent decrease in these cell counts for the 12 dogs.

The general appearances of the dogs just prior to their death were very similar. There was marked loss of hair, loss of body weight, and muscular asthenia, lethargy, and lack of appetite. Cutaneous ulcers developed and refused to heal. All of these symptoms were the same as have been observed in other animals that had been given polonium.

The data from this experiment indicate that the polonium 20-day LD_{50} for dogs lies somewhere between the low and middle levels of injection. The data do not lend themselves to statistical proof because of the deaths of all of the high-level dogs before there were any deaths in the low level. Essentially this left only two dosage levels that could be used in the calculations, and this did not meet the criteria used in the statistical analyses. However, a working figure for the LD_{50} value is taken as the midpoint between the low and middle injection levels, and is found to be about 70 microcuries of polonium per kilogram of body weight.

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1. Davis, R.K. and Jolley, W.P., Quart. Rpt. Biol. Res., MLM-527, p. 16, January 2, 1951.

TABLE I

SUMMARY OF DATA ON THE LD_{100} FOR POLONIUM INJECTED INTO DOGS

Dog No	Individual Dosage (μC /kg B.W.)	Ave Dosage By Group (μC /kg B.W.)	Weight Loss (Per Cent)	Average Weight Loss By Group (Per Cent)	Survival Time (Days)	Average Survival Time By Group (Days)	Decrease in W.B.C. (Per Cent)
2	64.7		28.0		18		99.6
3	64.4		29.0		19		98.5
9	64.5	64.5	37.0	33.0	21	21	98.5
10	64.5		38.0		25		97.5
1	77.6		30.0		18		99.2
4	77.0		19.0		13		99.0
6	77.2	77.1	19.0	27.0	21	19	99.6
7	76.6		39.0		24		99.5
5	90.0		20.0		11		86.9
8	90.1		18.0		12		85.5
11	89.6	89.8	28.0	24.0	16	14	99.1
12	89.6		28.0		16		99.7

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Problem Title — **Twenty-Day LD_{50} Determinations Upon Laboratory Animals II - Survival Studies on Rabbits**

Report By — **R. K. Davis, W. P. Jolley, and W. T. Hockhold**

Work Done By — **R. N. Cowden, R. K. Davis, W. P. Jolley, and C. Lizardi**

INTRODUCTION

Dosages of toxic substances in laboratory investigations are frequently based on LD_{50} values. The establishment of a polonium LD_{50} in several species is important to the undertaking of the research program of this laboratory. Not only does this value furnish a concrete dosage limit but also enables estimation of acute and sub-acute doses.

Preliminary studies on the polonium 20-day LD_{50} for rabbits were undertaken [1,2] to establish a dosage range within which further extensive determinations could be made. After observations for the experimental 20-day period were completed, it was deemed advisable to continue observing the remaining rabbits. In this manner, survival time could be obtained.

DETAILED REPORT

Eleven, young, adult, male, New Zealand white rabbits were injected via the marginal ear vein with polonium doses ranging from 23.1 to 85.1 microcuries per kilogram of body weight. The animals were then caged separately and observed for a 20-day period. Food (Vitality All Purpose rabbit pellets) and water were allowed ad libitum. By 20 days three rabbits had died. These rabbits had received 85.1, 61.4, and 51.3 microcuries of polonium per kilogram of body weight and had died on the 10th, 15th, and 11th post-injection days, respectively. On the basis of these findings the dosage range for an extensive 20-day LD_{50} study will be centered around the higher dosage levels.

The surviving rabbits were then maintained in the same surroundings and on the same diet for the purpose of collecting data for a survival study. The dosage level and the number of days post-injection that the animals lived is shown in Table I. When the injection levels were plotted against survival time, beyond 70 days, a nearly straight-line relationship was found to exist within the limits of dosages employed (Figure 1).

These animals exhibited the same symptoms of radiation sickness as have been observed in other laboratory animals (rats, dogs) that succumbed because of polonium administration. There was marked anorexia, loss of weight, lack of interest in surroundings, and in the terminal stages they had a bloody stool and inflammation of the mucous membranes.

No conclusions are drawn from these data, but they are presented here for academic interest to show that the toxic effects of polonium upon rabbits are very similar to those observed for rats, cats, and dogs.

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1. Davis, R. K., Quart. Rpt. Biol. Res., MLM-493, p. 47, October 9, 1950.
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TABLE I

RELATIONSHIP BETWEEN SURVIVAL AND DOSAGE LEVEL
OF POLONIUM-INJECTED MALE NEW ZEALAND RABBITS

Animal No	Microcuries of Polonium Per Kilogram Body Weight	Days Survival
1	23.1	160
2	26.9	147
3	32.8	150
4	36.6	139
5	45.9	95
6	50.8	124
7	51.3	10
8	58.2	70
10	61.4	10
9	64.5	86
11	85.1	8

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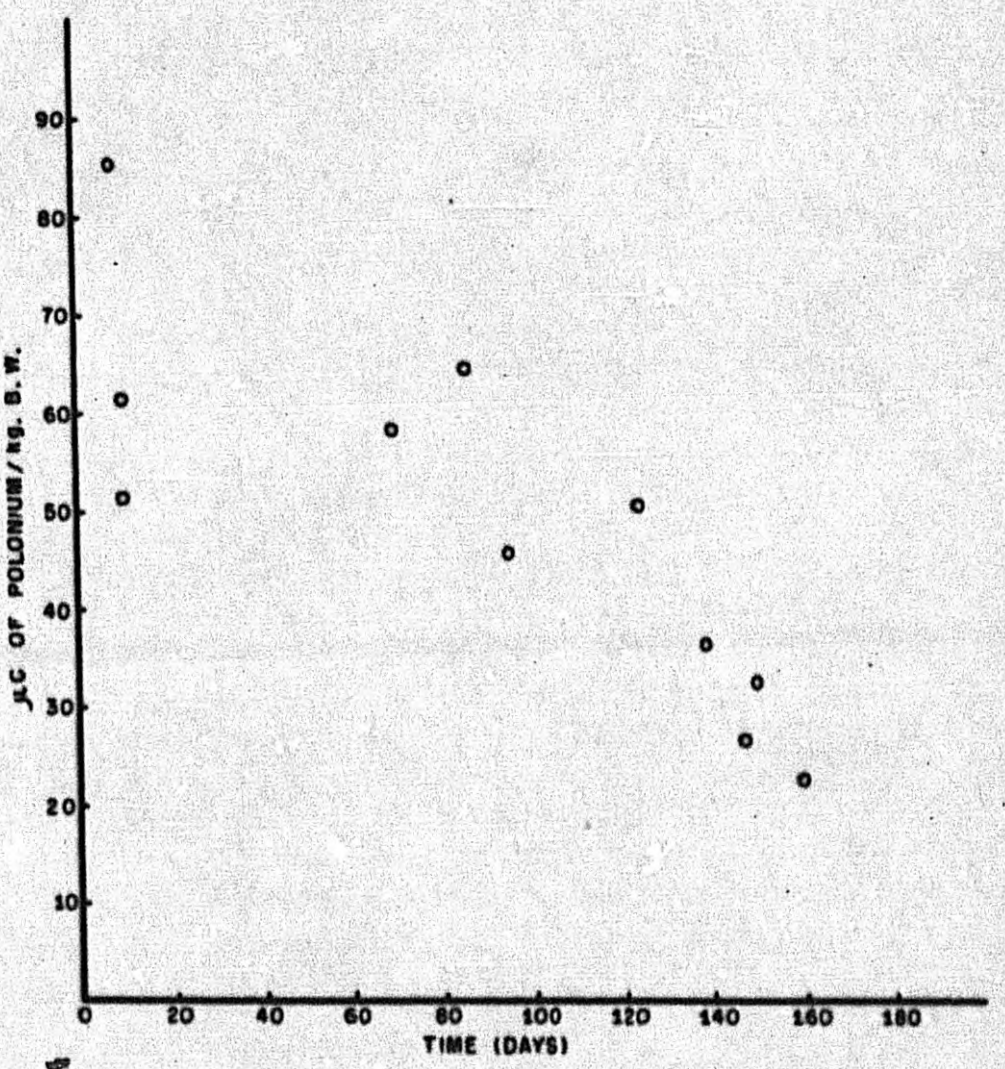
FIGURE 1



RELATIONSHIP OF POLONIUM DOSAGE TO THE SURVIVAL TIME OF ELEVEN MALE RABBITS.

Problem Title — **Effects of Repeated Injections of Polonium Upon Laboratory Animals. Pilot Study.**

Report By — **R. K. Davis, and W. T. Rockhold**

Work Done By — **D. H Couden, J L. Crom, R.K. Davis, D.E.Etter, P.K.Glass, W.P.Jolley, J. Mendicino, W.T.Rockhold, and L.H. Talley**

INTRODUCTION

All the information on the toxic effects of polonium upon animal tissues that have been collected to date were obtained from experiments employing single doses. These studies were obviously necessary for many reasons. However, if polonium tolerance levels based on actual experience are to be set up, studies of multiple exposures to low levels of polonium constitute the next forward step in the biological research program.

A pilot experiment was set up that involved four successive injections of polonium into albino rats, and a preliminary description was given in a previous report.[1] The pilot experiment was designed to give an approximate idea of the trends of urinary and fecal excretion of polonium, its various levels in the blood relative to the time of injection, and other pertinent data necessary for the successful operation of a full-scale multiple-injection experiment.

DETAILED REPORT

Twenty, female, Sprague-Dawley rats of uniform body weight (about 250 grams) were paired according to their body weight, and the pairs then were separated into two groups of ten rats each. The pairs were so matched in weight that the two members received identical injection volumes. An exception occurred in the case of three rats where their weight differences caused a difference of 0.01 milliliter in the injection volumes. Four biweekly dosages of 4.88, 4.36, 4.48 and 4.69, microcuries of polonium per kilogram of body weight were injected via the caudal vein into both series of animals. This dosage rate produced actual dosages per rat that averaged 1.16, 1.13, 1.17, and 1.21 microcuries of polonium for each successive injection, respectively. Immediately after receiving the first injection the rats were placed in individual metabolism cages so that separate urine and feces collections could be made. To prevent food particles spilling in the urine collection beakers, and to produce a hard, dry feces, the rats were fed a specially-prepared wet-mash diet. This diet contained all the necessary dietary requirements plus Cellu-flour to give the necessary bulk.[2]

Daily urine and feces collections were recorded, chemically digested, and analyzed for their polonium content. Blood samples for polonium assay were taken one hour before and one hour after injection from all rats, and daily thereafter, from one matched pair of rats. These blood samples were analyzed for polonium concentrations by the direct blood-smear assay technique.[3]

The body burden of polonium was calculated by subtracting the amount of polonium recovered in the excreta from the amount injected into the rat. The amount of polonium remaining in the body of the rat at the end of the two-week period was corrected for decay and this residual amount plus the amount given in the new injection gave an estimate of the amount of polonium in the animal, (body burden) following each injection. One member from

each of two matched pairs was sacrificed just prior to each injection period. Since the animals were matched, and the polonium recovery from the excreta and blood was comparable, the polonium content of the carcass of the sacrificed animal was assumed to be referable to that remaining in the body of its unsacrificed mate. The disagreement between the analyzed carcass content and the estimated carcass content varied from 1 per cent to 18 per cent.

The polonium recovery from the urine, feces, and blood of the experimental rats is graphically represented in Figure 1. The graph shows that a much greater quantity of the injected polonium is excreted in the feces than in the urine. The polonium recovery from the feces during the two-week intervals represents approximately 20 per cent of the injected dose with a trend toward a decrease in the percentage of recovery as the body burden increases. The fecal polonium excretion reached a peak on the third day post-injection for the third and fourth injection periods. Whether this phenomenon indicates a decreased detoxifying action in the liver or not is unknown at present. However, the per cent of the body burden of polonium excreted in the feces during each of the succeeding post-injection periods diminished according to the following schedule: 22.6 per cent, 18.2 per cent, 17.6 per cent, and 9.4 per cent. The corresponding figures for polonium excretion in the urine were: 1.25 per cent, 1.47 per cent, 1.82 per cent, and 0.96 per cent. Here the trend appears more in harmony with the increased body burden. Since the fourth post-injection period was only one week in duration, the polonium excretion figures for this period are low.

From these excretion data it seems that the mechanism for excreting polonium by way of the feces is more adversely affected by repeated polonium insults than the mechanism for its excretion via the urine. Due to the relatively short duration of this experiment and its necessarily modest scope, the relationships of these excretory mechanisms to time and the extent of the body insult are unknown.

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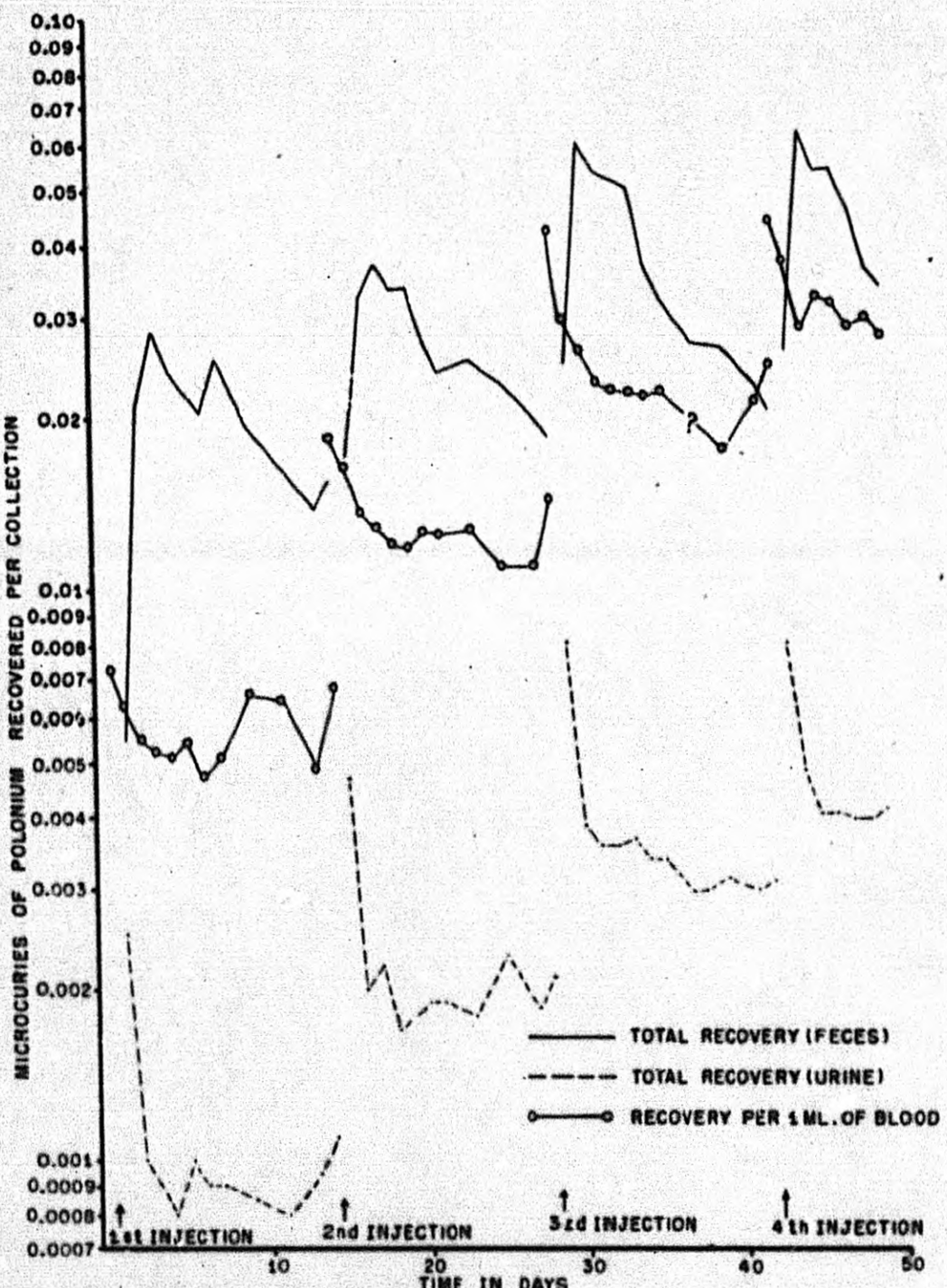
FIGURE 1



RECOVERY OF POLONIUM FROM URINE, FECES AND BLOOD OF RATS FOLLOWING MULTIPLE INJECTIONS TOTALING 18.4 μC/kg. B.W.

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Problem Title - **Effects of Repeated Injections of Polonium Upon Laboratory Animals. Main Study.**

Report By - **H. K. Davis, and W. T. Rockhold**

Work Done By - **D. H. Cowden, J. L. Cron, H. K. Davis, D. E. Etter, P. K. Glass, W. P. Jolley, J. Mendicino, W. T. Rockhold, and L. H. Talley**

INTRODUCTION

The exposure of personnel in this laboratory to polonium often is intermittent in nature. Repeated injections of polonium into laboratory animals offers a method to study such exposures. Further, information regarding the effects of repeated exposures of laboratory animals to polonium via the injection routes is necessary before multiple skin exposure to polonium and continuous polonium inhalation studies can be adequately evaluated. A previous experiment in the form of a pilot study has been reported.[1] The present experiment has been designed from the data gathered from the pilot study.

The methods of calculating polonium decay with respect to dosage and the general plan of this experiment are the same as those used in the earlier pilot experiment. However, a much larger number of animals is being used in this main study.

DETAILED REPORT

Young, adult, Sprague-Dawley rats, (60 males and 60 females) were divided into five body weight groups per sex. One rat from each of these groups was placed in a metabolism cage so that 12 cages of rats per sex were set up; body weights per cage were almost identical. The average total weight for each of the 12 cages of male rats was 1507 ± 4 grams and for each of the cages of females was 1007 ± 3 grams. This similarity in the total body weights within the sexes made the polonium injections the same for each cageful of rats.

The rats were housed in groups of five in the colony-type cages. Specially constructed Lucite trays [2] permits separate collections of urine and feces. A specially prepared wet mash diet [2] is fed the rats to prevent them from spilling food into the collection trays and to produce a more compact feces. Food and water are given ad libitum, and their consumption measured.

The experiment is designed to run for 20 weeks with each rat receiving a biweekly intravenous injection of a theoretical two microcuries of polonium per kilogram of body weight. Urine and feces collections are being made on the first day post-injection and every other day thereafter during each injection period. The excreta collections are measured, chemically digested, and analyzed for polonium. Blood samples for hematological studies and for polonium analysis are being collected three times weekly during each collection period. The polonium concentration of the peripheral blood is assayed by the direct blood-smear technique[3].

One cage of male and one cage of female rats are being sacrificed just prior to each injection. Tissue samples for histopathological examination are taken from the organs of the animals at that time. The remainder of the organs of the rats from each cage are pooled and weighed. After weighing, the organs, carcass, and debris are digested separately and analyzed for their polonium content.

Two cages each of male and female rats will remain at the end of the 20-week injection period. By comparison of the polonium recovery data from these cages of rats with that of the previously sacrificed cages of rats, the body burden of polonium can be calculated for each injection period.

To date the rats have received three of the ten biweekly polonium injections totaling 7.18 microcuries per kilogram of body weight for the males and 7.25 microcuries per kilogram of body weight for the females. Four cages of rats (10 males and 10 females) have been sacrificed. Tissue samples have been taken and prepared for study, and the remainder of the organs have been stored for digestion. No polonium recovery figures, hematological data, or physiological data are complete enough for presentation at this time.

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SECRET

Problem Title – **Clinical Tests of the Kidney Function of Polonium-Injected Sprague-Dawley Rats**

Report By – **R. N. Cowden and L. H. Talley**

Work Done By **R N Cowden W P Jolley and L H Talley**

INTRODUCTION

The interrelationship between the histopathological damage and the physiological function of the kidney of the albino rat, which has been subjected to polonium poisoning, has been under investigation by this laboratory. Previous reports [1,2] have shown that the time of the appearance of histopathological changes is relatively constant and has little relationship to polonium dosage, but the extent and severity of kidney damage is proportional to the dosage. It seemed that some correlation might exist between the ability of the kidney to eliminate nitrogenous wastes and the survival time of the rat following varying degrees of body insult by polonium. Accordingly, investigations were undertaken to test the level of the blood urea nitrogen in polonium-injected rats as a measure of the extent of the damage to the glomerular filtration system. A phenolsulfonphthalein excretion test will measure the extent of the damage to the tubular cells of the proximal convoluted tubules.

DETAILED REPORT

Two injection levels, consisting of 9.79 and 4.04 microcuries of polonium per kilogram of body weight, were used. A total of 114 rats were used, with each injection level consisting of the following:

1. Sixteen rats (8 males and 8 females) made up the Clinical Pathology Group.
2. Twenty rats (10 males and 10 females) made up the Histopathology Group.
3. Fourteen rats (7 males and 7 females) made up the Hematology Group.

The hematological carrier-control rats will consist of the same seven males and seven females for the two hematological injection levels.

Pre-injection red blood cell counts, white blood cell counts, and hemoglobin determinations were taken on the hematology group, as well as the percentage excretion of phenolsulfonphthalein dye and blood urea nitrogen determinations on the clinical pathological group. These latter tests on the clinical pathology group of rats are being done at biweekly intervals until a change from normal limits are found, and then at weekly intervals until the death of the animal or termination of the experiment.

The red blood count, white blood count, and the hemoglobin determinations are being done at monthly intervals on the hematological groups of rats until the termination of the experiment.

The serial sacrificing of rats of the histopathological group will depend somewhat on the clinical pathological picture of the clinical pathology groups of rats and on the peripheral blood picture of the hematological groups of rats. An arbitrary schedule has been set up calling for a serial sacrifice of two injected rats at 60 days post-injection and then two rats (one male and one female) at monthly intervals until 330 days post-injection.

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The kidneys of rats of the hematological and clinical pathological groups that die during the course of the experiment as well as the kidneys of the rats of these two groups that survive until the termination of the experiment will be used for histopathological study.

The kidneys of all rats of the eight-microcurie level that die or are serially sacrificed will be saved and stained with hemotoxylin and eosin, glycogen, and fat stains. Only kidneys will be saved from rats of the eight-microcurie level.

Various organs will be saved from the rats of the three-microcurie level as follows: heart, lungs, thyroid, liver, spleen, lymph node, stomach, and intestine (duodenum and ileum), gonads, adrenals, and kidneys. Only the kidneys will be routinely embedded and sectioned for staining with hemotoxylin and eosin, glycogen, and fat stains. The other organs will be saved for future reference and study.

Bone marrow sections will be taken at every other sacrifice period of rats of both the eight-microcurie and three-microcurie levels.

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Problem Title - **An Adaptation of the Phenolsulfonphthalein Test for Estimating Renal Function for use on Rats**

Report By - **W. T. Beckhold and L. H. Talley**

Work Done By - **L. H. Talley**

INTRODUCTION

The technique of Geraghty and Rowntree[1] for the clinical determinations of the function of the collecting tubules of the kidney has been modified for use on albino rats. The modified test has been used successfully in this laboratory yielding reproducible results.

DETAILED REPORT

Because of the small size of the rat relative to the human, it was first necessary to modify the clinical technique to reduce the quantity of phenolsulfonphthalein dye that had to be injected into a rat to run the test. By experimentation it was found that 0.5 milliliter of a solution containing three milligrams of phenolsulfonphthalein per milliliter of solution was most satisfactory. This dye solution was prepared by dissolving three grams of phenolsulfonphthalein in 700 milliliters of distilled water. The maximum color intensity was produced by adding two normal sodium hydroxide dropwise until a Bordeaux wine color is developed.

To insure adequate clearance of the dye during the collection period, it was necessary to induce diuresis. This was accomplished by bilateral subcutaneous injections of a total of 20 milliliters of a normal physiological saline solution. This treatment yielded excellent renal flow.

In a normal excretion test the rats are injected with the physiological saline solution and then receive an intramuscular injection of 0.5 milliliter of the phenolsulfonphthalein dye solution. The rats are then placed in individual immobilization metabolism cages for two hours, and the excreted urine is collected. At the end of the collection period complete emptying of the bladder was brought about by light anesthetization with ether. To insure collection of all of the excreted dye the abdomen and pelvic regions of the rats, and the collection cages, are washed with a stream of distilled water.

The urine samples are quantitatively transferred to 250-milliliter volumetric flasks. Two drops of two normal sodium hydroxide are added to produce maximum color, and the solution is diluted to volume. Aliquots of the solution are then transferred to standard cuvettes and the optical density read in a spectrophotometer at a wavelength of 520 millimicrons.

A standard solution was prepared by placing one-half the amount of injected dye (0.25 milliliter) in a 250-milliliter volmetric flask following the addition of two drops of two normal sodium hydroxide, the standard was diluted to volume and the optical density read at the same wavelength as the unknown.

The per cent of the phenolsulfonphthalein dye excreted in the two-hour collection period was calculated according to the formula:

$$\frac{\text{Optical density of the unknown}}{\text{Optical density of the standard}} \times 0.50 = \text{per cent of dye excreted}$$

Twenty Sprague-Dawley rats were used to determine a normal distribution curve for the recovery of phenolsulfonphthalein dye from rat urine by this technique. These data showed an average recovery of 60.5 per cent of the dye in a two-hour collection period. The range was from 45.8 per cent to 69.5 per cent. This technique has been used and reported previously[2].

This modified kidney function test has proven satisfactory in this laboratory for use on rats. The main modifications of the original test involves: a change in the amount of phenolsulfonphthalein dye injected, a method for inducing diuresis, and application of the urine collection method devised in this laboratory.

REFERENCES

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2. Cowden, R. N., Davis, R. K., and Talley, L. H., Quart. Rpt. Biol. Res., MLM-527, p. 9, January 2, 1951.

Problem Title - **A Modification of Ohlsson's Micromethod for the Determination of Blood Urea Nitrogen in Rats**

Report By - **V. T. Rockhold and L. H. Talley**

Work Done By - **L. H. Talley**

INTRODUCTION

The technique of Ohlsson[1] as devised for clinical determination of blood urea nitrogen has been modified for use in like tests on albino rats. This modification as herein reported involves alterations in the blood sample size, preparation of reagents and a change in the technique for spectrophotometric analyses.

DETAILED REPORT

Blood samples were taken from the tails of rats by slicing off the extreme tip of the tail. One-tenth of a milliliter of whole blood was drawn into a standard blood pipette. The blood sample was placed in a 15-milliliter centrifuge tube containing four milliliters of a molybdate reagent, and the pipette was rinsed several times. The molybdate reagent was prepared by mixing 10 milliliters of 10 per cent Na_2MoO_4 and 6.5 grams of potassium sulfate and diluting to 500 milliliters with distilled water. One drop of a urease extract was then added to the blood-molybdate mixture, mixed thoroughly, and allowed to stand at room temperature (25° ± 0.5) for 30 minutes. After standing, one milliliter of precipitating solution, (40 milliliters in H_2SO_4 diluted to 300 milliliters) was added to remove the excess urease. The solution was then mixed and centrifuged at 2,500 revolutions per minute for 10 minutes, and the supernatant decanted. One milliliter of one per cent gum acacia (freshly prepared weekly) and 2.5 milliliters of Nessler's solution were then added to four milliliters of the supernatant, and the optical density was read immediately on the spectrophotometer at a wavelength of 440 millimicrons. A blank solution was run with each determination.

The volume per cent of urea nitrogen was then calculated from:

$$\frac{\text{Optical density of unknown}}{\text{Optical density of known}} \times 20 = \text{Urea nitrogen (mg./100 ml.)}$$

where the known is a standard solution of urea which contains 20 milligrams of urea nitrogen per 100 milliliter of solution.

DISCUSSION

This modified technique for blood urea nitrogen determinations in rats has proven very satisfactory in this laboratory. Experimental findings employing the technique have been reported.[2] The major modifications in the technique were those which would render the determinations suitable for use on laboratory animals.

Of necessity, and to allow repeated samplings, the blood volume required for the determination has been reduced. We have found that repeated blood samples are easily obtained by slicing a very thin piece from the tip of a rat's tail and drawing the blood sample from the wound. This method of sampling was used for weekly blood samples for a period of 35 weeks on one group of rats.

We have also made slight modifications in the preparation of the reagents. Ohlsson's technique used urease tablets for the preparation of the urease extract which

is added to break down the urea. For both economy and simplicity we prepared our own urease extract after the method of Sobel et. al.[3] We also prefer the method of Vanselow[4] for the preparation of the Nessler's solution.

In the original technique a time period of 15 minutes was allowed for Nesslerization. We have found that in our modification the optical density can be read immediately after adding the Nessler's solution.

These changes have increased the speed with which these tests may be run and the number of the animals that may be tested. It has been found very useful as a check for kidney damage in laboratory rats.

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EFFECTS OF POLONIUM UPON CELL METABOLISM

[redacted]

428-33

[redacted]

Problem Title - **Effects of Polonium Upon Cell Metabolism**

Report By - **H. B. Speerl**

Work Done By - **B. Baisden, B. Carleton, E. Greve, H. Smith, H. Speerl, and I. Sorensen**

INTRODUCTION

The formation of large cells provide a ready index for the study of the effects of polonium on yeast cells. Because large cells result from an inhibition of cell division, these cells have provided excellent material for studying the mechanism of division. Measurements aimed at determining the metabolism which is affected by the polonium and the manner in which it is affected are also underway in these large cells.

This work, heretofore limited to yeast, has been extended to other organisms. A description of studies with E. coli is included in this report.

DETAILED REPORT

Growth and Cell Division Changes

Polonium has been shown to exert a greater effect upon yeast cell division than upon yeast cell growth.[1] The formation of large cells results. Comparable experiments have recently been performed with the bacterium E. coli. Within the range of polonium concentration used, it appears that the growth of this organism is affected even less than is the growth of yeast. However, cell division is again inhibited. Thus, large cells are formed. Details of these experiments are given below.

E. Coli, Strain B, was used in these experiments. The growth medium was composed as follows: 3 grams KH_2PO_4, 1 gram NH_4Cl, 6.7 grams Na_2HPO_4, 0.5 gram NaCl, 0.2 gram $MgSO_4 \cdot 7H_2O$, and 4 grams of glucose in one liter of water. Three hours after inoculation, cells in this medium have reached the steady growth stage, and at about five hours, growth of the culture ceases. In these experiments inoculum was grown overnight. Growth media were then seeded, the culture was grown at 37° with shaking, and polonium was added after three hours of growth. Cell counts were made (Petroff-Hauser counter) and dry weights per milliliter were determined at one and two hours after polonium addition.

The results obtained in some of these experiments are recorded in Table I. The differences in dry weight cannot be considered significant. It appears, therefore, that no inhibition of weight increase occurs in coli cultures containing as much as 250 microcuries per milliliter of polonium. On the other hand, differences do occur in individual cell weight. An increased average weight per cell occurs with an increased polonium concentration. Cell division is inhibited more severely by greater amounts of polonium. As a result of continued growth but inhibited division, individual cells grow larger.

It is seen, also, when considering the average cell-weight figures, that differences appear between cultures started with different cell concentrations. Likewise, there are differences between results obtained at the first and second hours. The percentage figures may vary quite widely before significance can be attached to differences between them. This is true because variations which come from the not-very-precise cell count and dry weight determinations are included. Though further substantiation is needed, real differences do appear to be present in these data. The growth of control cultures used in these experiments is described in Table II.

[redacted]

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Oxygen Effect on Poisoning Injury

The presence of oxygen has been demonstrated to be a factor leading to increase radiation injury to living organisms. Oxygen can increase the formation of oxidizing agents produced by irradiating water. In this way it may be responsible for greater cell injury. With microorganism cultures, available oxygen can be reduced or increased. However, such changes may themselves affect cellular metabolism in several ways. It is not clear whether the effect of oxygen on poisoning injury is the result of an altered metabolism or is a specific product of radiation on oxygen or oxygen-containing materials.

Table III records the results of experiments in which oxygen levels were reduced by aerating tube cultures of yeast with purified nitrogen. Cells were grown in large cultures. Aliquots of these cultures were then transferred to tubes. Half of the tube cultures were stirred by aeration with purified nitrogen and half by aeration with compressed air. After two hours of such growth, poisoning was added, and the cells were grown for a further period of four hours. Cell counts were made, and culture weights were then determined.

It is quite evident from the per cent figures of Table III that the effect of poisoning upon yeast cell growth is markedly reduced when oxygen is excluded from the cultures. It appears, however, that little difference is found in the effect of poisoning upon cell division. Large cells are still produced.

The manner in which this reduced effect upon growth is brought about is not immediately evident. In control cultures there is a considerable difference in growth rate between the cells aerated with nitrogen and those aerated with air. During the four hour growth period the increase in culture weight may be three times greater with air than with nitrogen aeration. Such a difference indicates that a variety of cell functions may be altered by oxygen level changes alone. It is not yet clear whether growth rate changes or irradiation-product formation in water is most important in the observed cell response. Most probably both factors are involved.

Nitrogen Compound Studies

One means for establishing [illegible] changes correlated with the inhibiting effect of poisoning upon cell division is a study of cell nitrogen compounds. Accordingly analyses for major nitrogen constituents of normal and division inhibited yeast cells have been made.

Several modifications in nitrogen analytical methods[2] have been introduced since data on yeast-cell nitrogen fractions were previously reported.[3] In addition several cell analyses have been made on cells grown in a synthetic medium in contrast to the complex organic medium used in the previous work. The data in Tables IV and V summarize the results which have been obtained. The nitrogen measurements made and the proportions of each constituent present in large and normal cells for one experiment are listed in Table IV (Run No. 3). The changes which have been found to occur in the several nitrogen fractions measured in each of three experiments are summarized in Table V.

The data show that large individual cells contain increased amounts of the various constituents. Thus, increased size is not caused by more water swelling. On a total mass basis, small increases in total nitrogen and protein nitrogen (TCA precipitate) appear in large cells in each run. Acid soluble nitrogen levels, however, are lower than in control

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[illegible]

[illegible]

1. [illegible]
2. [illegible]
3. [illegible]

[illegible]

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TABLE I

POLONIUM EFFECTS UPON GROWTH AND CELL DIVISION IN E.COLI

Starting Cell Count	[illegible]	Per Cent of Control: Dry Weight				Per Cell Weight			
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.5 x 10[illegible]	1	100	100	97	103	115	123	105	120
1.5 x 10[illegible]	2	100	95	93	98	104	105	141	231
3.6 x 10[illegible]	1	98	93	99	97	108	148	138	178
3.6 x 10[illegible]	2	100	100	102	102	123	116	175	200
7.7 x 10[illegible]	1	103	99	98	101	98	130	162	208
7.7 x 10[illegible]	2	100	109	103	101	115	141	136	161

* Polonium concentration in microcuries per milliliter

TABLE II

GROWTH CHARACTERISTICS OF E. COLI CONTROL CULTURES

Starting Cell Count	Starting Dry Weight*	Weight Increase During (Per Cent x 10[illegible])		Cell Number Increase During (Per Cent x 10[illegible])		Average Cell Weight (x 10[illegible] G.)		
		1st Hr.	2nd Hr.	1st Hr.	2nd Hr.	0 Hr.	1st Hr.	2nd Hr.
1.5 x 10[illegible]	0.0011	2.5	1.4	3.3	2.5	7.1	6.2	5.9
3.6 x 10[illegible]	0.0032	3.0	1.6	3.7	1.8	6.1	4.9	4.3
7.7 x 10[illegible]	0.0040	1.8	1.3	2.9	1.3	5.2	3.5	3.5

* Grams per 10 milliliter

TABLE III

POLONIUM EFFECTS UPON GROWTH AND DIVISION OF YEAST CELLS GROWN AT DIFFERENT OXYGEN LEVELS

Starting Cell Concentration	Polonium Concentration (μC./ml.)	Per Cent of Control: Culture Weight		Per Cell Weight	
		Nitrogen	Air	Nitrogen	Air
1×10^7	8.4	96.5	101.4	133.7	118.1
1×10^7	26.1	104.4	94.5	162.2	205.2
1×10^7	80.9	97.6	81.5	143.2	196.5
1×10^7	240.7	90.4	65.3	176.0	175.2

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TABLE IV

EFFECTS OF POLONIUM UPON YEAST CELL NITROGEN

Nitrogen Fraction	Hours Treatment	gN./Cell* ($\times 10^{-10}$) Control	gN./Cell* ($\times 10^{-10}$) Treated	Per Cent Change	µg./Mg. of Dry Weight Control	µg./Mg. of Dry Weight Treated	Per Cent Change
Total N	0	54.5	54.5		121.1	121.1	
	2	41.2	90.0	+ 94	110.7	112.8	+ 2.0
	4	38.1	93.0	+144	102.9	104.6	+ 1.6
Protein N	0	43.5	43.8		97.3	97.3	
	2	33.2	65.0	+ 96	89.2	91.6	+ 2.6
	4	30.0	77.5	+159	81.0	87.4	+ 7.9
Acid	0	10.0	10.0		22.0	22.2	
Soluble N	2	8.0	14.1	+ 76	21.5	19.9	- 7.2
	4	7.8	16.3	+109	21.2	18.3	-13.7
Alpha	0	6.9	6.9		15.3	15.3	
Amino N	2	5.8	10.1	+ 74	15.6	14.2	- 8.7
	4	5.6	10.9	+ 95	15.1	12.3	-18.3
Ammonia N	0	0.24	0.24		0.52	0.52	
	2	0.22	0.31	+ 41	0.60	0.44	-27.0
	4	0.19	0.31	+ 63	0.51	0.35	-30.6
Amide N	0	0.77	0.77		1.71	1.71	
	2	0.62	1.19	+ 45	2.19	1.68	-23.5
	4	0.77	0.96	+ 25	2.09	1.08	-48.5

* Average weight of cell ($\times 10^{-10}$ g.): Control, 6.3 at two hours, 6.2 at four hours. Treated, 11.5 at two hours, 14.9 at four hours.

TABLE V

CHANGES IN YEAST CELL NITROGEN FOLLOWING POLONIUM TREATMENT

Nitrogen Fraction	Hours Treatment	Per Cent Change (on µg. N/Mg. Cell Basis) Run No. 1	Run No. 2	Run No. 3
Total N	2	+ 0.5	+10.5	+ 2.0
	4	+ 7.5	+10.9	+ 1.6
Protein N	2	+ 4.7	+11.0	+ 2.6
	4	+16.0	+11.2	+ 7.9
Acid Soluble N	2	- 4.9	- 1.0	- 7.2
	4	-19.2	- 9.4	-13.7
Alpha Amino N	2	-16.7	-15.7	- 8.7
	4	-24.8	-10.8	-18.3
Ammonia N	2	-22.9	- 2.8	-27.0
	4	-43.6	-21.8	-30.4
Amide N	2	-26.4	-13.3	-23.5
	4	-40.2	-55.7	-48.5

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HEMATOLOGICAL AND PATHOLOGICAL EFFECTS OF POLONIUM ON LABORATORY ANIMALS

[redacted]

Problem Title - **Hematological and Pathological Studies on Sprague-Dawley Rats Injected Intravenously with Varying Amounts of Polonium**

Report By - **R. A. Conden and R. A. Zigel**

Work Done By - **R. A. Conden, R. R. Conden, F. A. Olson, R. F. Bailey, C. Lisowski, J. Bedirian, G. L. Norris, and R. H. Zigel**

INTRODUCTION

Histopathological studies have been completed on two groups of Sprague-Dawley rats which had been injected with 23 (Level II) and 5 (Level I) microcuries of polonium per kilogram of body weight, respectively. Previous reports on this experiment may be found in the Quarterly Reports for Biological Research, MLR-270, MLR-427, MLR-442, MLR-471-2, MLR-501, MLR-630, and MLR-127. Histopathological examinations of the skin, brain, eye, lymph nodes, thymus, thyroid, pancreas, adrenals, and lung of rats of Level I and Level II are presented in this report.

DETAILED REPORT

Skin (5 and 23 μc /kg. R.B.)

Gross Description - After a variable period of time post-injection, the hair appears lusterless and often times appears grayish-yellow in color. These skin sections were always taken from the abdomen in the region overlapping the xiphoid process.

Histopathological Description - No abnormalities were seen in either the epidermis or dermis.

Lymph Nodes (23 μc /kg. R. B.)

Gross Description - There is a moderate to marked atrophy of the lymph nodes by the seventh post-injection day, and only markedly atrophic nodes are seen in subsequent sacrifices.

Histopathological Description - No consistent histopathological change was noted in the lymph nodes of rats sacrificed one day post-injection. On the third post-injection day there is a slight increase in nuclear debris, some of which is in histiocytes. There is a slight atrophy of the lymph nodules and lymph cords with a slight to moderate reduction in the size of their germinal centers and in the number of small lymphocytes at the periphery of the nodules.

The lymph nodes of rats sacrificed 7 days post-injection show a marked atrophy of many of the primary nodules while the remaining nodules show moderate to marked reduction in the number of medium and large lymphocytes in the smaller germinal centers with a corresponding loss of small lymphocytes around the periphery. There is a marked increase in nuclear debris found in the histiocytes and only an occasional mitotic figure is seen.

By the 11th post-injection day there is marked atrophy of the lymph nodes with a complete loss of the germinal centers of the nodules. Only a diffuse scattering of lymphocytes is seen throughout the nodes, and many of these are undergoing karyorrhexis and karyolysis. There is a marked increase of nuclear debris, most of which has been engulfed by histiocytes. There is moderate hemorrhage into the lymph sinuses.

[redacted]

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By the 14th post-injection day there is progressive atrophy of the lymph nodes with increased hemorrhage into the lymph sinuses. There is proliferation of fibroblastic connective tissue into the trabeculae and the reticular stroma. The histiocytes contain a marked amount of nuclear debris.

By the 21st post-injection day there is edematous thickening and collagenous degeneration of the reticulum with marked atrophy and marked hemorrhage into all the lymph sinuses. There is a relative as well as an actual increase in fibroblastic connective tissue with many histiocytes containing nuclear debris.

By the 28th post-injection day only a few scattered lymphocytes remain. There are many histiocytes loaded with hemosiderin. There was one small lymph node showing a marked increase in fibroblastic connective tissue proliferation and collagenous degeneration especially marked in the cortical zone of the lymph node.

Rats sacrificed 42, 56, and 70 days post-injection show almost complete atrophy of the lymph nodes with marked hemorrhage into the lymph sinuses. One of the rats sacrificed 56 days post-injection shows a lymph node with a few primary nodules remaining and in which a rare mitotic figure was seen.

One rat was sacrificed 88 days post-injection and the lymph nodes revealed a very marked atrophy and marked collagenous degeneration with a complete loss of the reticular structure.

The rat sacrificed at 98 days post-injection shows a proliferation of small mature and large lymphocytes in several of the lymph nodes studied, while other lymph nodes remain quiescent and atrophic. The proliferation is in the form of lymph cords without nodule formation. Some mitotic figures are seen in the lymph nodes showing proliferative changes.

One rat was sacrificed at 154 days and one rat at 231 days post-injection. These rats were sacrificed at 364 days post-injection. The lymph nodes of these rats show a somewhat variable picture. A few lymph nodes show some aggregates of lymphocytes but for the most part there is a marked atrophy with a destruction of the normal architecture of the lymph nodes. There is marked collagenous degeneration with proliferation of fibroblastic connective tissue and infiltration of many macrophages which contain hemosiderin and nuclear debris. There are many plasma cells and scattered lymphocytes throughout the stroma.

Summary of Observations

1. Tissue sections from lymph nodes of rats injected with 20 microcuries per kilogram of body weight and sacrificed after three days show early degenerative changes with a loss in the medium sized and small lymphocytes and increase in nuclear debris.
2. Lymph nodes from rats injected with 20 microcuries per kilogram of body weight show progressive atrophy and degeneration of the lymphoid tissue.
3. There is no regeneration of lymphoid tissue in these rats.

Lymph Nodes (5 μC./kg. B.W.)

Gross Description - There is a slight atrophy of the lymph nodes by the 7th day which becomes moderate to marked by the 21st day post-injection. The lymph nodes appear somewhat larger in those rats sacrificed from the 56th through the 84th post-injection days.

[redacted]

From the [illegible] to the [illegible] post-injection days the lymph nodes are variable in size with some showing marked atrophy and hemorrhage, while others appear much less atrophic and show little or no hemorrhage. From the [illegible] post-injection day to the termination of the experiment at [illegible] days post-injection, the lymph nodes are markedly atrophic and hemorrhagic.

Histopathological Description. - There was no consistent histopathological change noted in the lymph nodes of rats sacrificed at 1 day post-injection. On the third post-injection day there is a slight decrease in the size of the germinal centers with a slight to moderate decrease in the number of small lymphocytes at the periphery of the germinal centers.

By the seventh post-injection day there is a moderate atrophy of the lymph nodes with a decrease in the number of medium and large lymphocytes within the germinal centers and a moderate to marked decrease in the number of small lymphocytes at the periphery of the germinal centers. There is an increase in nuclear debris some of which is in macrophages. A few of the lymph nodes show complete loss of nodule formation.

On the 11th post-injection day the lymph nodes of one rat show a marked number of megakaryocytes and almost normal lymphoid activity. The other three rats sacrificed show moderate to marked atrophy with loss of size of the germinal centers and moderate reduction of medium, large, and small lymphocytes. There is a slight hemorrhage into the lymph sinuses, and an increase of nuclear debris within the germinal centers.

One of the rats sacrificed on the 14th post-injection day shows almost normal lymphoid activity while the other three rats show moderate to marked atrophy, slight hemorrhage, and loss of some of the germinal centers of the lymph nodules. For the most part there are some small germinal centers containing a marked amount of nuclear debris. There is some fresh hemorrhage into the lymph sinuses with many macrophages containing erythrocytes, altered blood pigment, and some nuclear debris. Few mitotic figures are seen. There is an edematous thickening of the stroma and trabeculae in a few of the nodes.

On the 21st post-injection day only small lymph nodules with small germinal centers are present. Mitotic figures are rare, and there is karyorrhexis and karyolysis of many of the large and medium sized lymphocytes. There is marked edematous thickening of the reticular stroma, and diffuse collagenous degeneration. There is a marked increase in nuclear debris and a moderate increase in altered blood pigment within the macrophages.

On the 28th post-injection day the conditions described for the lymph nodes of the 21st post-injection day are more marked. On the 42nd post-injection day there is a marked edematous thickening of the reticular stroma with small areas of edematous fluid and small hemorrhages into the lymph sinuses. There is a marked amount of nuclear debris. The lymphoid tissue is diffuse, and there is karyorrhexis and karyolysis of many of the lymphocytes. There is a diffuse collagenous degeneration of the reticular stroma, and a few of the nodes show marked fibrosis.

On the 56th post-injection day a few of the nodes are similar to those described for the 42nd post-injection day, but most of the lymph nodes show moderate regenerative changes. These changes are characterized by a moderate increase in small, medium, and large lymphocytes in the peripheral lymph cords and the formation of lymph nodules with active germinal centers. Mitotic figures are fairly common.

[redacted]

On the 70th post-injection day all nodes show regenerative changes with increases in number of all sizes of lymphocytes. In some nodes the regeneration is one of diffuse increase of lymphocytes in the cords, while in others the lymphocytes show nodular formations with active germinal centers.

On the 84th post-injection day further regenerative changes are seen with the formation of some nodules containing germinal centers in the medullary portion of the nodes. There is a moderate amount of altered blood pigment within macrophages. Some mitotic figures are seen.

On the 98th post-injection day one of the four rats sacrificed shows well-formed lymph nodules with some mitotic activity. The lymph nodes of the other three rats show variable reactions. For the most part there is loss of nodule formation with massive fresh hemorrhage into the lymph sinuses. There are many macrophages containing altered blood pigment and nuclear debris. Some nodes show collagenous thickening of the capsule and stroma. A few of the lymph nodes show primary nodule formation with an occasional mitotic figure and little or no hemorrhage. A few of the lymph nodes show only cords of lymphoid cells in the medullary zone of the lymph node. The lymph nodes of one rat show this variable degree of hypoplasia in the extreme.

The histopathological picture of the lymph nodes of rats sacrificed 112, 126, and 140 days post-injection is similar to the rats sacrificed at 98 days post-injection. The lymph nodes of one of these rats show localized areas of regenerative change. The other three rats show an extremely variable degree of atrophy and hypoplasia of the lymph nodes.

Rats sacrificed 161 days post-injection show an extremely variable histopathological picture of the lymph nodes. There are localized areas of marked atrophy with a few of the nodes showing some lymph nodules containing small germinal centers. Little or no mitotic activity is seen. There are many macrophages loaded with altered blood pigment and nuclear debris. The capsule and reticular stroma show more pronounced collagenous degeneration in some of the lymph nodes and moderate to marked increase in fibroblastic connective tissue in many of the lymph nodes.

Rats sacrificed at 182 and 238 days post-injection show a few primary nodules in the lymph nodes, but little or no mitosis is seen. The lymph nodes are markedly atrophic and the remaining lymphocytes are in cord formation.

The lymph nodes of rats sacrificed 305 days post-injection show marked atrophy, but there is a marked variation in the histopathology of the lymph nodes from the different rats sacrificed as well as from different lymph nodes of the same chain in a given rat. A few of the lymph nodes show small primary nodules with a rare mitotic figure, or lymph cords containing small, medium, and large lymphocytes. However, for the most part, the lymph nodes show capsules thickened with fibroblastic and collagenous connective tissue with trabeculae dipping into the medullary portion of the gland. The reticular stroma is thickened by collagenous degeneration and shows marked fibroblastic proliferation in many instances. Many of the lymph nodes show fresh hemorrhage with fine fibrin threads throughout the lymph sinuses. There are many macrophages filled with erythrocytes, altered blood pigment, and nuclear debris. In one instance a lymph node shows lobule formation with collagenous and fibroblastic connective tissue separating the lymphoid tissue into several isolated entities.

Summary of Observation

1. The degree of lymph node atrophy from the injection of 8 microcuries per kilogram of body weight varies with time in all rats. At a given time there is also considerable variation from animal to animal and even from node to node in the same animal.

2. There is little microscopic change in the lymph nodes of rats sacrificed from 1 through 4 days post-injection.

3. The lymph nodes of those animals sacrificed 7 through 56 days post-injection show variable degrees of lymph node destruction as manifested by a loss in the medium and small lymphocytes, reduction in the size and activity of the germinal center, increased altered blood pigment in the macrophages, increased connective tissue proliferation, edema, and collagenous degeneration.

4. From 56 days through 305 days post-injection the lymph nodes show variable degrees of regeneration, sometimes more marked in one rat than in another.

5. Regenerative changes may be entirely localized within a lymph node. One area may show marked atrophy and complete absence of a lymphoid stroma along with hemorrhage, and other localized areas may show almost complete regeneration to a normal appearing lymphoid architecture.

6. All rats sacrificed from 70 through 305 days post-injection reveal some type of regenerative changes with restoration of some of the nodes to almost normal lymphoid activity.

Adrenal (8 μC /kg. B.W.)

The only specific change seen in the adrenals through 305 days is a slight collagenous degeneration of the stroma of the zona reticularis.

Adrenals (23 μC./kg. B.W.)

From the 7th through the 28th post-injection day there is a moderate congestion of the adrenals with slight to moderate dilatation of the sinusoidal capillaries and of the veins of the medullary portion of the gland. On the 42nd post-injection day the adrenals of one of the sacrificed rats show marked collagenous degeneration in the inner aspect of the zona reticularis.

One of the two rats sacrificed at 70 days post-injection shows moderate collagenous degeneration of the reticular stroma of the zona reticularis.

The adrenals of both the rats sacrificed at 84 days post-injection show moderate collagenous degeneration of the reticular stroma of the inner part of the zona reticularis with slight collagenous degeneration of the stroma of the medullary portion of the gland. There is a slight proliferation of connective tissue fibroblasts.

On the 124th and 244th post-injection days there is marked collagenous degeneration of the zona reticularis. No other changes are noted with hematoxylin-eosin, azure II-eosin, or Masson's trichrome stains.

Thymus (Control Rats)

Normal histological changes were studied on a control group of rats to determine the involutionary changes that might be found over the period covered by this experiment.

The rats were 90 days of age at the beginning of the experiment, which lasted for 305 days, making the controls and injected rats of the 8-microcuries-per-kilogram-of-body-weight level 395 days of age at the time of the final sacrifices. There were non-injected controls as well as controls injected with the carrier solution. The thymuses from both these control groups show a very gradual atrophy, so that at 305 days post-injection (395 days of age) there is a considerable loss of small lymphocytes from the cortex. However, mitosis is still evident, and the cortex and medulla show normal structural relationships in most instances. A few lobules do show loss of lymphocytes and increased collagenous degeneration.

Thymus (8 μC./kg. B.W.)

Tissue sections of the thymus from rats sacrificed 1 day post-injection revealed no consistent histopathological change Rats sacrificed on the third post-injection day show an abundance of mast cells in the capsule and interlobular septa of the thymus. A few lobules show hemorrhage into the glandular tissue, and there is a slight increase in nuclear debris in the medulla. The thymus from one of the four rats sacrificed shows these changes much more markedly than do the thymuses of the other three sacrificed rats.

On the seventh post-injection day two of the four rats sacrificed show a moderate atrophy with a gradient of change in the lobules from near normal to marked depletion of the lymphocytes. The more atrophic areas also show more nuclear debris, hemorrhage, and proliferation of connective tissue fibroblasts. The other two rats sacrificed show almost normal thymic tissue.

On the 11th post-injection day there is some thinning of the cortex, and it appears "pitted" under low power magnification. This pitting is caused by the large vacuolated macrophages which can be seen in the reticular stroma. The medullary portion of the gland is relatively wider, and the nuclear debris is still extracellular in position.

Rats sacrificed on the 14th post-injection day show the same histopathological changes as the 11-day sacrifices, except that one of the rats shows an almost complete loss of cortical lymphocytes in one of the thymic lobules with much more pronounced atrophy of the entire thymus.

By the 21st and 28th post-injection days most of the nuclear debris is within macrophages.

From the 42nd through the 56th post-injection days there is a gradual atrophy with thinning of the cortex. There is an increase in nuclear debris with a concomitant increase in the karyorrhexis and karyolysis of lymphocytes, and no mitotic figures are discernible. Small foci of hemorrhage are scattered through the glandular tissue. These changes are more pronounced in some sacrifices than in others, and in the same thymus there is a gradient in the ratio of damage between adjoining lobules.

On the 70th and 84th post-injection days there is a moderate regeneration of the thymus in all sacrifices. There are some mitotic figures with an increase of small lymphocytes in the cortical areas and in the medulla, so that the underlying stroma is more difficult to see. Most of the nuclear debris is within the macrophages.

Two of the rats sacrificed at 98 days post-injection show regenerative changes as seen in the thymuses of rats sacrificed at 84 days post-injection. The thymuses of the other two rats sacrificed at this time show only atrophic changes.

In subsequent sacrifices on the 112th, 126th, 140th, 161st, and 182nd post-injection days, the thymus shows a gradual atrophy with loss of small lymphocytes from the cortex and of medium and large lymphocytes from the medulla. There is a gradual increase in fibroblastic connective tissue and collagenous fibers, and this is probably in part a relative increase caused by the marked contraction of the reticular stroma attendant to atrophy.

On the 238th post-injection day there is a very marked atrophy of the thymus with inversion of the cortex and medulla in some lobules. Some thymus tissue is much more atrophic than others at the same period of sacrifice, and the lobules of a few show gradients from inversion of the cortex and medulla to almost normal appearing lobules in the same thymic tissue (Figure 1). Other lobules show a complete loss of lymphocytes with the stroma contracted to form solid or thin sheets of lymphocytes. Some lobules show thin sheets of lymphocytes. There is an increase in connective tissue fibroblasts and collagenous fibers, although again this is in part relative to the marked atrophy of the thymus. Many of the blood vessels show a moderate hyalinization of their walls. Many mast cells are present in the connective tissue of the capsule and stroma of the gland.

No recognizable thymic tissue was found in the injected rats at 305 days post-injection.

Thymus (23 μC/kg B W.)

No consistent histopathological changes were noted on the first post-injection day. On the third post-injection day there are a few scattered areas of hemorrhage. There is a slight loss of small lymphocytes from the cortex of a few of the lobules which exposes some of the underlying reticular stroma. There is a slight amount of extracellular nuclear debris in the medulla. The medullary blood vessels show moderate congestion.

Rats sacrificed on the 7th and 11th post-injection days show a moderate atrophy with narrowing of the cortex and a loss of small lymphocytes. Many macrophages are present, especially in the cortical portions. There is karyorrhexis and karyolysis of some of the lymphocytes, and many show peripheral clumping of the nuclear chromatin. There is a moderate amount of nuclear debris, most of which is extracellular in position. Azure II-eosin stains show a marked increased in the number of mast cells in the capsule and interlobular connective tissue. Mitotic figures are rare, and Hassall's corpuscles are prominent.

On the 14th day the lymphocytes show marked peripheral clumping of their nuclear chromatin, and no mitotic figures are seen. The stroma of the gland is much more prominent, especially at the inner aspect of the cortex where the small lymphocytes are almost entirely absent. There are some large lymphocytes in the medulla, but the medium sized lymphocytes are exceedingly rare. The nuclear debris are less conspicuous at this time because they have been phagocytized by the large macrophages.

On the 28th day post-injection the thymus of one of the sacrificed rats shows the so-called inversion of the thymus. There is a solid sheet of epithelial cells making up the cortex, and phagocytic cells can be distinguished by their vacuolated cytoplasm and darker, wrinkled nuclei. There are a few thin sheets of lymphocytes in the medullary area (Figure 2), but no mitotic figures are seen. There is a marked collagenous degeneration of the capsule and of the connective tissue surrounding the blood vessels as well as a diffuse collagenous degeneration of the reticular stroma of the cortex and medulla.

Other sacrifices do not show this inversion of the cortex and medulla, but a few of their lobules show this tendency. The thymus of these later-sacrificed rats is essentially like those of the 14-day sacrifices.

The thymus of one of the rats sacrificed 42 days post-injection shows solid sheets of stromal cells with marked collagenous degeneration and the presence of numerous mast cells. There are many fat cells as well as an abundance of fibrin threads surrounding and separating the atrophic lobules. Some plasma cells are enmeshed in the fibrin threads and collagenous connective tissue.

By the 56th and 84th post-injection days there are solid sheets of stromal cells along with pronounced collagenous degeneration. Fat cells surround and separate the atrophic lobules. The macrophages are very prominent at this time and consist of large cells with a vacuolated, frothy cytoplasm and a darker nucleus than that of the stromal cells. The nucleolus is prominent, and some of these cells are grouped together in an acinar arrangement. Some of the macrophages appear to be undergoing changes similar to the formation of Hassall's corpuscles. There are refractile granules and debris in some of the macrophages, while others show engulfed nuclei. (Figure 3.)

By the 99th and 124th post-injection days the histopathological picture is the same as on the previous sacrifices except that Hassall's corpuscles show slightly more hyalinization. Some blood vessels as well as the cell membrane of a few of the macrophages show slight hyalinization.

In all subsequent sacrifices through 244 days post-injection, only connective tissue was found in the area of the thymus.

Eye (8 and 23 μC./kg. B.W.)

No histopathological changes were found in the eye sections studied on rats at the injection levels of 8 microcuries per kilogram of body weight and 23 microcuries per kilogram of body weight.

Brain (8 and 23 μC./kg. B.W.)

No histopathological changes were found in the brain sections of rats from either the 8-microcuries-per-kilogram-of-body-weight injection level or the 23-microcuries-per-kilogram-of-body-weight injection level. Only Azure II-eosin, hematoxylin-eosin, and Masson's trichrome stains were used. Special stains for the staining of nerve cells and processes should be used for conclusive proof of the resistance of neurological tissue to the alpha radiations of polonium.

Pancreas (23 μC./kg. B.W.)

No histopathological changes are seen in the pancreas of rats through 244 days post-injection.

Pancreas (8 μC./kg. B.W.)

Gross Pathological Description - No gross changes of the pancreas were noted through 305 days post-injection.

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Histopathological Description - No histopathological changes were noted through 305 days post-injection with one exception. Rat number 15A, which has also shown pronounced degenerative changes of the heart, lymph nodes, etc., shows a marked collagenous degeneration of many pancreatic lobules. The interlobular tissue and the connective tissue surrounding the blood vessels show marked collagenous degeneration. The acinar cells in many areas are pale and vesicular with a loss in the acinar arrangement of the cells. The basement membranes of the acinar cells show marked collagenous degeneration. No zymogen granules are seen in the cells, and the basophilic substance usually seen in the basal zone of the acinar cells with hematoxylin-eosin stain is absent. The nucleoli of the basal cells are faint or entirely absent, and many of these cells show peripheral clumping of the nuclear chromatin. The intercalated ducts are dilated. A few of the small, interlobular arteries show marked hyaline degeneration. This degenerative change occurs throughout some of the secondary lobules and is of a patchy nature in most other lobules of the pancreatic section. The islands of Langerhans show no abnormalities. There are localized areas of inflammatory cell infiltration which gives the appearance of a chronic active pancreatitis.

Thyroid (8 and 23 μC./kg. B.W.)

Gross Description - There are no gross pathological changes noted in the thyroid gland through 346 days in one group and through 345 days post-injection in another group of rats.

Histopathological Description - There was no consistent histopathological change in sections of thyroid gland that could be attributed to the action of polonium. There is one consistent change noted in the thyroid gland of both control and injected animals that is caused by pressure from the application of tissue forceps in the handling of tissue during the preparation of the tissue for sectioning. Compression of the thyroid gland results in distortion of the glandular elements to such an extent that ante-mortem pathological changes may be simulated.

There is a marked distortion of the normal thyroid architecture in most of the sacrificed rats. For the most part the lobes show marked atrophy with a loss of the acinar structure of the gland in many areas. Some of the acini contain colloid with a large number of pyknotic nuclei in the lumen. There is desquamation of the epithelial lining cells; with karyorrhexis of some of the cells. Some areas appear to be undergoing necrotic changes. These changes appear to involve a large number of acini or a solitary acinus in various lobes, and appear to be more extensive in the animals sacrificed later in the experiment. Azure II-eosin stains showed the presence of numerous mast cells in the interfollicular connective tissue. Vacuolization and atrophy appears more marked in the thyroid of the rats injected with polonium, but so many sections from control rats (Figure 4) show almost the same degree of atrophic distortion and degeneration that these changes can be attributed for the most part to pressure. Some of the acini from both control and polonium injected rats show hyperplasia of the epithelial lining cells so that there is little or no colloid in the lumina and the epithelium appears to be stratified. The inner part of the lobe shows degenerative change with loss of acini more than does the periphery where the acini are often large and filled with colloid. Small localized areas of degeneration within large sections of tissue (Figure 5) is often difficult to explain, but the appearance is similar to those changes seen in thyroid gland tissue following pressure.

428-48

[redacted]

[redacted]

Lungs (8 and 23 μC./Levels)

Gross Description - The gross examination of the lungs of both control and injected rats revealed small, multiple cystic areas or discrete, punctate nodules in the visceral pleura. These lesions were present in the majority of the sacrificed rats, but the degree of injury was variable. The cystic areas were probably a resolution of the nodular areas, and the lesion appears to be a chronic endemic infection in the rat colony. Serial sacrifices of the 23-microcurie level of rats through 240 days post-injection and of the 8-microcurie level of rats through 305 days post-injection shows the chronic nature of the lung lesion. The lung sections from the rats of various serial sacrifices were stained with Wright's Stain and Gram's Stain, but no etiological organisms could be found.

Histopathological Description - The lesions were present microscopically in almost every instance. Many of the lesions were cystic and consisted of a peripheral, vacuolated area with atelectatic lung tissue at the margin of the cyst. Other lesions showed consolidation with small, round cell infiltration, various sized lymphocytes, an occasional eosinophil, and large cells with a clear nucleus and a frothy, granular cytoplasm that gives it the appearance of a macrophage. There are numerous extravasated erythrocytes, fibrin threads, and edematous fluid. The surrounding alveolar sacs are atelectatic. These lesions appear to undergo organization with collagenous degeneration of the alveolar stroma, and the cystic phase is probably a resolution of this lesion.

In later sacrifices the lungs of polonium-injected rats show less cellular infiltration into the lesions and there is a more consistent finding of the large vacuolated lipoid-like cells than in the lungs of the control rats. Otherwise, the lesions in the lungs following serial sacrifice of both control and polonium-injected rats show the same histopathological picture. This loss in the cellular infiltrate in the lung lesions of polonium-injected rats is probably caused by the action of polonium on the cells in the same manner in which the polonium destroys the lymphocytes of the peribronchial lymph nodules.

No other histopathological changes are noted in the lungs of rats from either the 8 or 23 microcurie injection levels

[redacted]

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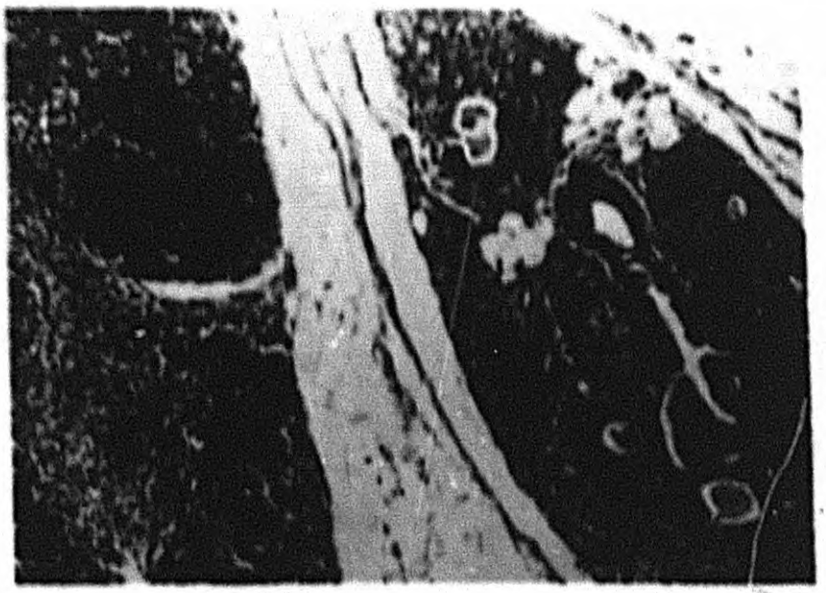
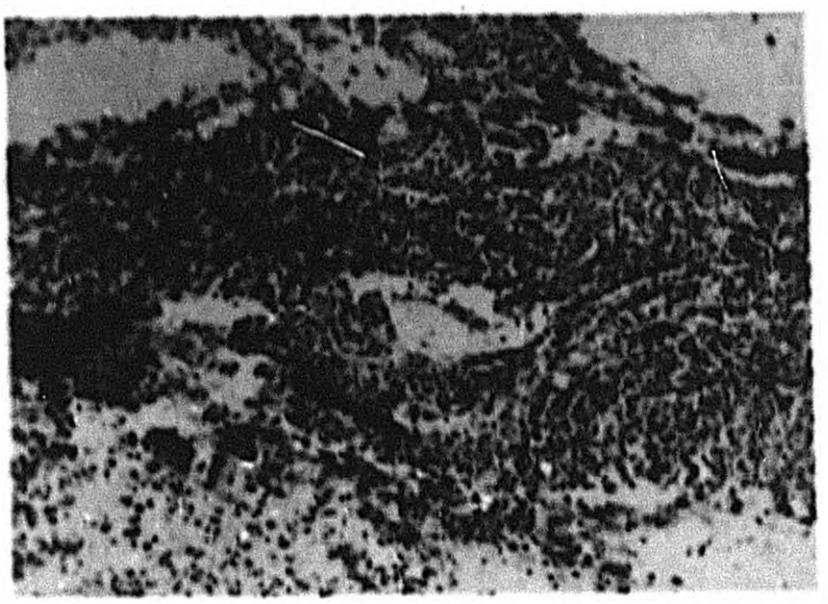
FIGURE 1



THYMUS OF POLONIUM INJECTED RAT (88A) SACRIFICED 238 DAYS POST-INJECTION.

NOTE: ADJACENT THYMIC LOBULES SHOWING GRADIENT OF DEGENERATIVE CHANGE. (100X)
(THIS PHOTOGRAPH IS UNCLASSIFIED.)

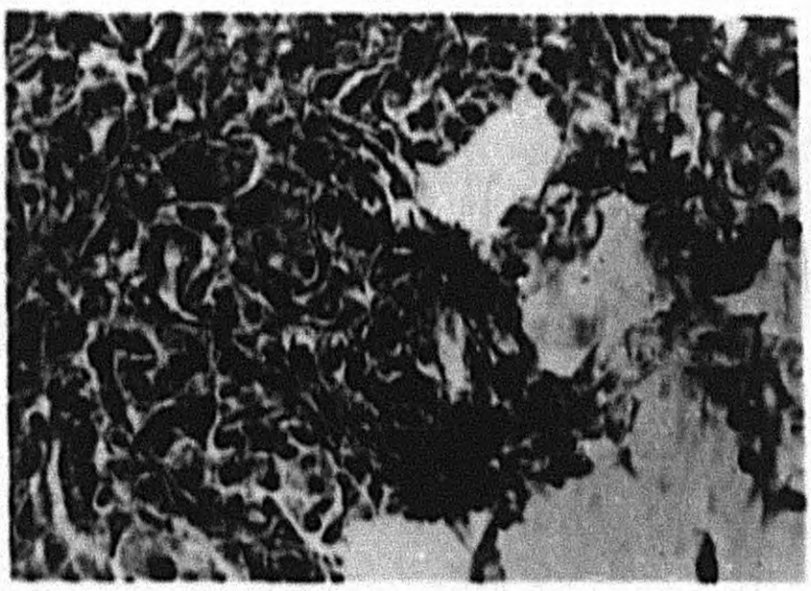
FIGURE 2



THYMUS OF POLONIUM INJECTED RAT (45B) SACRIFICED 28 DAYS POST-INJECTION.

NOTE: ALMOST COMPLETE ATROPHY OF GLAND.
(THIS PHOTOGRAPH IS UNCLASSIFIED.)

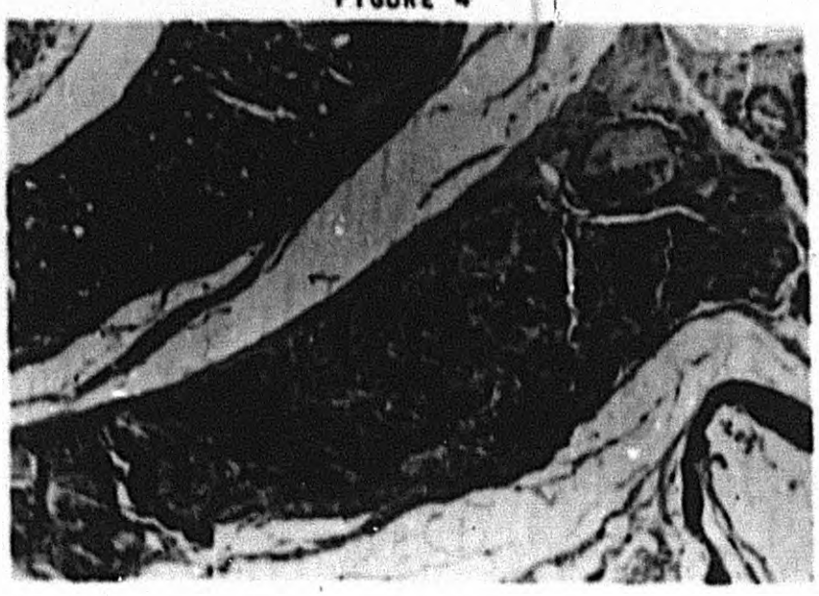
FIGURE 3



THYMUS OF POLONIUM INJECTED RAT (76B) SACRIFICED 56 DAYS POST-INJECTION.

NOTE: LOSS OF LYMPHOCITES, LARGE MACROPHAGES. HASSALL'S BODY. (400X)
(THIS PHOTOGRAPH IS UNCLASSIFIED.)

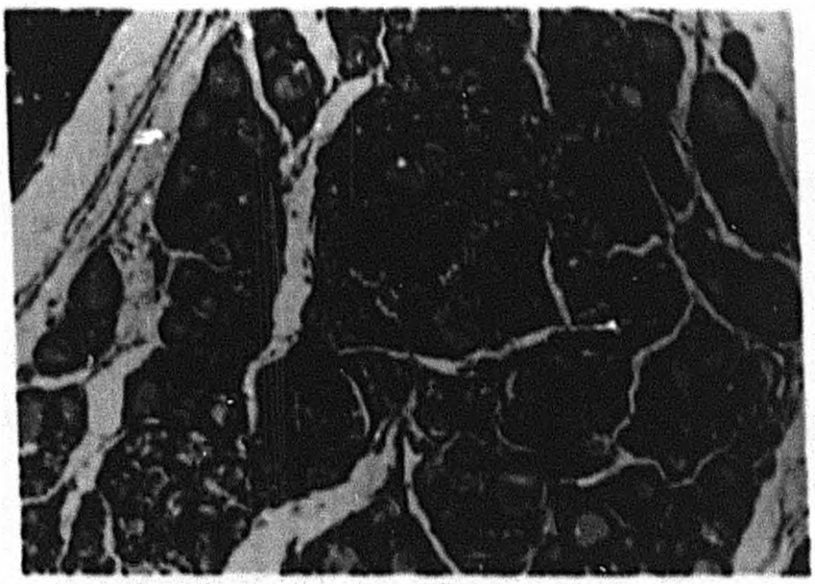
FIGURE 4



THYROID OF CONTROL RAT (118B)

NOTE: DEGENERATIVE CHANGES OF ISTHMUS OF THYROID GLAND. (100X)
(THIS PHOTOGRAPH IS UNCLASSIFIED.)

FIGURE 5



THYROID OF POLONIUM INJECTED RAT (62B) SACRIFICED 244 DAYS POST-INJECTION.

NOTE: SMALL DEGENERATED ACINUS NEAR CENTER OF LATERAL LOBE OF GLAND. (100X)
(THIS PHOTOGRAPH IS UNCLASSIFIED.)

[redacted]

Problem Title – **The Minimum Effective Dose of Polonium Causing Hematological and Pathological Changes in Rats**

Report By – **E.K.Davis, P.E.Glass, and E.T.Bechhold**

Work Done By – **D.L.Cowden, B.N.Cowden, P.E.Glass, C.Linardi, J.Bendicsen, G.L.Norris, and E.E.Zipf**

INTRODUCTION

Extensive studies have been completed on the hematological and pathological changes in rats caused by intravenous polonium injections of 8.23, and 35 microcuries of polonium per kilogram of body weight.[1-6] The concluding studies are presented in this report. To supplement these data, experiments were initiated to find the minimal injected dose of polonium that will cause detectable hematological and pathological changes. This information is expected to be very useful in evaluating long-term single and multiple dosage experiments and as an aid in establishing valid personnel tolerances by the Health-Physics Division.

DETAILED REPORT

A total of 256 young, adult, Spr-cue-Dawley rats comprised both of the polonium injection levels and their requisite controls. The rats in each injection level were divided into a pathological and a hematological group. The two injection levels were 0.9 and 3.5 microcuries of polonium per kilogram of body weight.

The pathological group consisted of 30 male and 30 female rats per injection level. The control rats for this group were made up of 12 male and 12 female non-injected control rats and six male and six female rats injected with carrier solution only. The polonium-injected rats were sacrificed on a schedule of three male and three female rats at 4 days post-injection and then at 6-week intervals thereafter for 52 weeks. Control rats will be sacrificed on the same schedule as the polonium-injected rats. At the time of sacrifice, tissue samples of thyroid, liver, spleen, lymph nodes, kidney, adrenal, and gonad will be saved and processed for histopathological study. To check for bone tumor incidence, whole body, dorso-ventral X-ray photographs of the polonium-injected rats are being taken prior to their sacrifice. Bone marrow smears and sections from the tibia of all polonium-injected and two control rats are taken at each sacrifice period.

The hematological group consisted of eight male and eight female polonium-injected rats for each injection level and the same number of control rats injected with the carrier solution. Complete pre-injection hematology, consisting of erythrocyte, leukocyte, and differential counts, hematocrit and hemoglobin determination, were taken on all rats. The post-injection hematology consisted of leukocyte and differential counts at 1,4,7,14,21, and 28 days post-injection. Complete blood counts will be taken at 10 weeks post-injection and then at 6-week intervals thereafter for 52 weeks. At the termination of the experiment, the surviving rats in the hematology group will be sacrificed and used for histopathological study.

Currently, the experiment is in the 9th post-injection week. No data are available for presentation. Interim reports will be published as the data become available.

[redacted]

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SECRET

Problem Title - **A Study of Spontaneous Lung Lesions in Sprague-Dawley Rats**

Report By - **R. N. Cowden, W. T. Rockhold, and R. E. Zipf**

Work Done By - **D. L. Cowden, R. N. Cowden, P. K. Glass, J. Hendricino, G. L. Norris, and R. E. Zipf**

INTRODUCTION

Lung lesions had been seen and previously reported[1] in rats injected with 35 microcuries of polonium per kilogram of body weight. These lesions were first attributed to polonium damage alone, but later investigations revealed lesions of the same type in the lungs of control rats.[2] Further investigations of the laboratory rats revealed gross contamination of one group of experimental animals[3] with this same type of lung lesion, necessitating the termination of the experiment. This investigation was instituted to study the etiology of the lung lesions and to investigate whether the lesions were endemic in the Sprague-Dawley strain of rats used in this laboratory.

DETAILED REPORT

Twenty-four rats were selected from the laboratory colony and divided into two groups of 12 rats each. One group received an intravenous injection of 33.1 microcuries of polonium per kilogram body weight, and the other group served as control animals. Two control and two polonium-injected rats were sacrificed and autopsied at 1, 3, 7, 11, 14, and 15 days post-injection. Gross examination of the organs was made, and tissue sections of lung, liver, and spleen were taken for histopathological study.

In addition, tissues from 12 rats used in an earlier experiment,[4,5] where they had been sacrificed at four-hour intervals, were also examined further to investigate the cause of these spontaneous lung lesions. These rats had received an intravenous injection of 31.0 microcuries of polonium per kilogram of body weight, and tissues from these rats had been preserved. The lung tissues of the rats that were sacrificed at four-hour intervals, through a 48-hour period, were sectioned and examined histopathologically for the presence of lung lesions and for signs of the development of the lesions.

Gross Examination - Gross examination of the lungs of the rats in the 33.1-microcurie series revealed either nodules the size of a pin head or cystic areas in both the control and the polonium-injected rats. These lesions had the same gross appearance as those reported previously.[1] Six of the control rats and ten of the polonium-injected rats were found to have these lung lesions. The lungs of one control rat exhibited evidence of a more advanced disease than did the lungs of any of the injected rats.

Histopathological Examination - Examination of the tissues, stained with hematoxylin-eosin, from the rats injected with 31.0 microcuries of polonium per kilogram of body weight revealed lesions in all of the lung tissues from the 12 rats studied. These lesions were located on the periphery of the lung and consisted of a filtrate containing both lymphocytic and an occasional eosinophilic cell and a few of the lesions contained lipoid cells. There was little or no progressive change in the lesions through the 48-hour post-injection period covered by this portion of the study. Many of the lesions were cystic, and others showed a round cell infiltration. The lungs from one rat showed this change to be rather extensive, involving a great portion of the lung, whereas the lesions in the

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other 11 rats showed only minimal changes. The examination of sections stained with Masson's trichrome stain gave no additional information. No alpha tracks were seen in autoradiographs of these lung sections.

Tissue sections similarly prepared from the lungs of the control rats and the rats injected with 33.1 microcuries of polonium per kilogram of body weight revealed the same type of lesions. The tissues from the rats sacrificed in the latter part of the experiment showed that the lesions contained less cellular infiltrate and had permitted the fibroblastic proliferation and connective tissue spaces to be more prominent.

Summary of Observation

1. Interstitial pneumonitis probably of viral origin is endemic in rats of the Sprague-Dawley strain.

2. Lung lesions seen in injected rats may vary from mild infection, with but a few sparse lesions, to extensive lesions involving a large portion of the lung tissue.

3. The etiology for the pneumonitis is as yet undetermined.

4. Sprague-Dawley rats with a non-specific pneumonitis that are injected with polonium reveal only a loss in the cellular content of the lesion.

5. The lung lesions previously described in MLM-381 as characteristic for that dosage of polonium are incidental to the polonium and secondary to a previous lung infection.

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Problem Title – **A Kidney-Function Study on Polonium-Injected Rats II Histopathological Study of Animals from a Pilot Experiment**

Report By – **R. N. Cowden, R. E. Zipf**

Work Done By – **D. L. Cowden, R. N. Cowden, L. H. Talley, J. Mendicino, and R. E. Zipf**

INTRODUCTION

Histopathological changes in the kidney of the albino rats caused by intravenous polonium injections have been studied and reported [1,2] by this laboratory. To evaluate and correlate the observed histopathological changes and physiological functions of the kidney of polonium-injected rats, an experiment was instituted to study the functions of the rat kidney by means of clinical tests. The data collected from this first, small, kidney-function study has been reported and is Part I of the present report. The present report covers the histopathological examination of various organs taken from the rats sacrificed during the course of this experiment.

DETAILED REPORT

Methods

Thirty-four Sprague-Dawley rats remaining from a previous experiment[4] were used for these studies. The rats were three months old at the time of injection of 8.9, 19.7, and 29.7 microcuries of polonium per kilogram of body weight, respectively. The phenolsulfonphthalein kidney-function tests were started 175 days post-injection. At about 225 days post-injection the polonium-injected and control animals were sacrificed and autopsied. Tissue sections from the kidney, liver, and spleen were taken and routinely stained with hemotoxylin-eosin and Masson's trichrome stains. Frozen sections from kidney and liver were stained with Oil Red "O" for demonstration of fat.

Histopathological Description - Tissue sections of the kidney stained with hematoxylin-eosin revealed a moderate destruction of the most peripheral aspects of the cortical zone of the kidney. The cortical zone of the kidney consists of the malpighian corpuscles, proximal convoluted tubules, distal convoluted tubules, thick segments of the ascending limb of Henle's loop, and the arched collecting tubules. The proximal convoluted tubule is the longest and most convoluted segment of the nephron, and it forms a major part of the cortical area. The proximal convoluted tubule is lined by a single layer of low columnar or pyramidal cells which contain round basally located nuclei and granular, eosinophilic cytoplasm. The basal portion of the cell has a distinct striated appearance caused by the arrangement of the basal cytoplasmic granules in rows. These epithelial lining cells of the proximal convoluted tubules are also characterized by a brush border which is a narrow, finely striated zone which lines the free surface of the cell. This brush border undergoes rapid post-mortem change.

The greatest change seen in the kidneys of the rats injected with 8.9 microcuries of polonium per kilogram of body weight and sacrificed 225 days post-injection is confined to the proximal convoluted tubules. The epithelial lining cells of the proximal tubules first appear to lose their brush borders. This condition is apparent in many of the proximal tubules. Any specific proximal convoluted tubule may show a section of epithelial lining cells in which the brush border is absent, whereas the remainder of the cells may have the brush border intact. There is also an increased granularity of the cytoplasm

which is most marked in the section of the cytoplasm nearest the lumen of the tubule. Some of the epithelial lining cells show edematous changes with projections of the cytoplasm into the lumina of the proximal convoluted tubules. Many of these lining cells also show large, bizarre nuclei. The nuclei are irregular in shape and may be two to five times the size of the nuclei of a normal epithelial lining cell. Many of the tubules, especially those adjacent to the capsule, may be lined by one or two of these large atypical cells in which the cytoplasm is markedly thinned out as if in an apparent attempt to line completely the wall of the entire proximal tubule.

Some of the kidneys show moderate destruction of the proximal convoluted tubules while others show a more marked destruction. The proximal convoluted tubules, the distal convoluted tubules, and the collecting tubules all show dilatation which vary in the degree and in the number of tubules involved. Many of these dilated tubules contain an acidophilic staining material. There is apparently an inverse relation between the degree of hydronephrosis present in a given kidney and the per cent excretion of phenolsulfonphthalein dye previously observed with the same kidney and its pair.

The malpighian corpuscles show little histopathological change at 225 days post-injection. Many of the glomerular tufts are distended with blood. There is some enlargement of Bowman's space which may be caused by a shrinkage of the glomerular tuft. A few glomerular tufts show hyalinization, but this is not a consistent finding in the routine section.

The blood vessels, especially the arteries, show little change in their walls. There is evidence of a slight degree of intimal proliferation in the smaller arterioles and in the afferent glomerular arteries. This proliferation does not appear to encroach a great deal on the size of the lumen. The afferent arteries of these kidneys do not show the degree of intimal thickening of their walls, as described by other investigators [5].

The tissue sections of kidneys prepared by the frozen-section technique and stained with Oil Red "O" show fat deposits in the epithelial lining cells of the proximal convoluted tubules. These fat deposits in the proximal tubules are first noted in the peripheral aspects of the cortex. Fine droplets of fat appear in the basal portions of the cells. In the proximal tubules the cells that show the most severe destruction, the fat droplets appear as globules. The kidneys from the rats that show the lowest per cent output of phenolsulfonphthalein dye also show the greatest amount of fat in the proximal convoluted tubules. The large, bizarre, epithelial cells show little or no fat.

A plausible hypothesis may be that the sequence of events in the histopathological change of the epithelial lining cells of the proximal convoluted tubules is an initial cloudy swelling of the cell cytoplasm, followed by fatty degeneration of the cytoplasm. The cell swells, and fat appears in the lumina of the tubules. The cell nuclei show changes in morphology which appear to be attempts at regeneration. The cell nuclei are elongated, and the cytoplasm spreads over a greater surface of the wall of the proximal convoluted tubules.

There is an occasional glomerulus which contained fat, and again the rat kidney that showed the largest amount of fat in the glomeruli also showed the lowest percentage excretion of phenolsulfonphthalein dye. Fat casts were present in the collecting tubules and consisted of columns of fat filling the lumina. The fat casts were also more prevalent in the collecting tubules of the kidneys from the rats that showed the lowest per cent excretion of phenolsulfonphthalein dye.

The tissue sections of the kidneys of rats injected with 19 7 microcuries of polonium per kilogram of body weight show more pronounced changes than do the kidneys of the rats of the 8 9-microcurie level. In most instances the brush borders are completely lost, the basal striations of the epithelial lining cells of the proximal convoluted tubules are absent, and the convoluted tubules and collecting tubules show more dilatation. Numerous acidophilic casts are present in the collecting tubules. There are more bizarre giant nuclei in the epithelial lining cells of the proximal tubules, and many of these tubules show complete loss of their lining epithelium. More of the glomeruli show hyalinization, and many of the glomerular tufts show vacuolization of their endothelial cells. The arteries show a slightly more marked intimal proliferation than is seen in the arteries of the kidneys from rats injected with 8.9 microcuries per kilogram of body weight.

The fat found in the lining cells of the proximal convoluted tubules, the fat in the glomeruli, and the fat casts in the collecting tubules show the same relationship to the per cent excretion of phenolsulfonphthalein dye as was found in the rats of the 8 9-microcurie injection level, namely the lower the per cent dye excretion in a given kidney, the greater is the abnormal content of fat in that kidney.

The two rats that were sacrificed and autopsied from the 29 7-microcuries-per-kilogram-of-body-weight injection level showed less pronounced changes in the kidneys than those found in the 19 7-microcuries-per-kilogram-of-body-weight level. The hydronephrosis was not as marked and the abnormal fat content of the glomeruli and proximal convoluted tubules was not as pronounced. The percentage excretion of phenolsulfonphthalein dye was also higher, which, therefore shows the same relationship of percentage of dye excretion to kidrey damage as was found on the other two injection levels.

Summary of Kidney Observations

1. There is a relationship between the amount of hydronephrosis present, the amount of abnormal fat in the glomeruli and proximal convoluted tubules, and the number of fat casts in the collecting tubules of the kidney with the percentage excretion of phenol sulfonphthalein dye by that kidney.

2. The large bizarre epithelial lining cells of the proximal convoluted tubules show little or no fat.

Liver

Both hematoxylin-eosin and Oil Red "O" stains were used on the liver sections from the 15 rats injected with 8 9 microcuries of polonium per kilogram of body weight. The liver sections from 20 per cent of these rats show many large bizarre liver-cord cell nuclei. These cells show peripheral clumping of the nuclear chromatin and appear to be cells undergoing attempted regenerative changes. Another 20 per cent of the liver sections show no large liver-cord cell nuclei. The other 60 per cent show a rare or occasional large bizarre liver-cord cell nucleus.

Liver sections stained with Oil Red "O" for the identification of fat show a variable picture. The liver sections from only one rat showed marked amounts of fat within the liver cord cells, and this was mainly in the cells at the periphery of the liver lobules. Liver sections from three of the sacrificed rats showed a moderate increase in fat content in the cord cells adjacent to the central vein. The liver sections from the

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rest of the sacrificed rats showed only slight accumulations of fat in the liver-cord cells. These accumulations were at or only slightly above the amount found in the livers of control rats of the same age.

The liver sections of 50 per cent of the rats from the 19.7-microcuries-of-polonium-per-kilogram-of-body-weight injection level show many large, bizarre, liver-cord cell nuclei. These nuclei show peripheral clumping of the nuclear chromatin and appear to be undergoing attempted regeneration. Twenty-five per cent of the liver sections from the sacrificed rats show only a rare, large, liver-cord cell nucleus, and 25 per cent of the liver sections show no large, liver-cord nuclei.

Liver sections stained with Oil Red "O" showed a marked amount of fat in only one of the eight rats studied. The other seven livers from the remaining rats show moderate to slight amount of fat in the liver-cord cells. The large, bizarre, liver-cord cells show little or no fat.

The liver sections from both rats that had been injected with 29.7 microcuries of polonium per kilogram of body weight show many large, bizarre, liver-cord cell nuclei. There is also a moderate amount of fat present in the liver-cord cells. The fat deposition is mainly in the cord cells abutting on the central vein of the liver lobule.

Spleen

The spleens from the rats of the 8.9-microcuries-per-kilogram-of-body-weight injection level show some lymphoid activity. The histopathological changes are the same as those observed in rats injected with eight microcuries of polonium per kilogram of body weight.

The spleens from the rats injected with 19.5 and 29.7 microcuries of polonium per kilogram of body weight show almost complete atrophy.

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