

THE EFFECTS OF SMALL AMOUNTS OF POLONIUM
ON RATS

MASTER

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and D. S. Anthony

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M o u n d L a b o r a t o r y

Miamisburg, Ohio

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L A B S T R A C T

Large groups of Sprague-Dawley rats were given either small single intravenous injections of 0.75, 0.5, or 0.25 microcurie of Po^{210} per kilogram body weight; or repeated doses designed to maintain a relatively constant polonium burden of 0.01 microcurie per kilogram body weight. Since the most sensitive criteria of polonium damage had been shown to be shortening of life span, or possibly increased tumor incidence, these two possible effects were given the most attention in observations continuing throughout the adult life of the animals.

Definite shortening of life span occurred in male rats that had received the single doses of 0.75 or 0.5 microcurie per kilogram body weight. Life span shortening at the 0.25 microcurie level was not as definite, though probable. None of the groups of females receiving single doses showed significant life span shortening, possibly in part because of the greater variance of the female populations.

A maintained polonium burden of 0.01 microcurie per kilogram body weight was completely without measurable effect.

No effect on tumor incidence was observed in any of the single or multiple dose groups. However, the tumor incidence in all groups of old injected and control rats was very high.



II. I N T R O D U C T I O N

Proposed reactors employing circulating molten bismuth create a potentially serious polonium toxicity hazard. Bi^{209} is converted to Bi^{210} by neutron irradiation and the Bi^{210} decays by β^- emission to Po^{210} . The polonium is a virtually pure alpha emitter of 138 day half life; hence, there is an acute toxicity hazard in the event of an accident releasing large amounts of this relatively short half life alpha particle emitter. The degree of hazard from single or repeated exposures such as might occur in routine operation of such reactors or related processing plants has been the subject of a series of "educated guesses". These guesses (1, 2, 3) have differed by more than an order of magnitude.

The first major attempt at providing experimental data useful in calculating the magnitude of hazard from polonium was the work at the University of Rochester as reported by Fink and co-workers in 1950 (4). This was a very good preliminary survey of the acute toxic effects of large amounts of polonium. In the past five years scattered reports in the open literature (5, 6) and in documents (7, 8) have begun to fill in the void of information on the sub-acute or chronic toxic effects of smaller amounts of polonium. The present report describes extensive experiments concerning the effects of very small doses of polonium in Sprague-Dawley rats.

The minimal effective single dose of polonium in the rat was established by the experiments reported here.



III. MATERIALS AND METHODS

SINGLE DOSE STUDIES

It was realized that an experiment dealing with minimal or sub-minimal effective doses of polonium in rats would require several dosage levels and a large number of animals; consequently, three dosage levels were chosen below the 0.9 and 1.0 microcurie of polonium per kilogram body weight level which had been established as definitely pathologic (7). The three doses chosen were 0.25, 0.5, and 0.75 microcurie per kilogram body weight. A group of 320 male and female Sprague-Dawley rats were divided into four groups (3 experimentals and one control), providing 40 rats of each sex per group. They were injected via the caudal vein with the activity solution or the carrier vehicle in the case of the control rats. They were then housed 10 rats to a cage, fed and watered ad libitum, and observed for the rest of their lives. The laboratory chow was obtained from Vitality Mills, Chicago, Illinois.

In order to accumulate 300 rats for this study at one time, it was necessary to utilize animals received in two separate shipments. The rats in these shipments had an age difference of 78 days for the males and 73 days for the females. Lacking critical information regarding the importance of this age difference to the experiment, the respective lots of rats, designated "young" and "old", were kept separate until this factor could be determined. They were randomly assigned to the four injection levels. Thus, each injection level contained four sub-groups of young and old, male and female animals.

Earlier studies indicated that shortening of life-span would be the only criterion of polonium effect sensitive enough to detect the toxicity of such low doses. Later, as this low dose study progressed, a frequent occurrence of tumors was observed. A study was then made to determine whether a correlation existed between tumor incidence and polonium dosage.

MULTIPLE DOSE STUDIES Since most of the maximum permissible exposure values given for humans assume continuous exposure to the toxic radioactive material, it was deemed advisable to study the effects of a maintained polonium burden in experimental animals. Accordingly, a low level multiple dose experiment was organized wherein 207 adult five and one-half month old female Sprague-Dawley rats (107 experimental and 100 control animals) were injected with small amounts of a polonium solution at 28 day intervals throughout their life span so that they maintained an average body burden of 0.01 microcurie per kilogram body weight. This dosage was selected for two reasons. Firstly, it was a dose which would serve as a test of the frequently used, though not officially accepted, human tolerance level of 0.2 microcurie of polonium per average 70 kilogram human, or approximately 0.003 microcurie per kilogram of body weight. By using approximately three times this dose as the maintained body burden in the rats it could be determined whether or not there was a modest safety factor in the human tolerance level.

Secondly, this dose, if maintained throughout the rats lifetime, would deliver a rep (roentgen equivalent physical) dose of less than any of the above single doses.

The average monthly replacement dose was 0.006 microcurie polonium per kilogram body weight. This dosage was calculated from the radiological decay of the body burden plus the excretion of polonium from the rats. Thus, all excreta was collected and analysed for polonium (described below), and this amount plus what activity that had decayed away within the animals body during the preceding 28-day period was considered the replacement dose.

The rats were housed five animals per metabolism cage and fed and watered ad libitum. The ration fed these rats was special synthetic diet (Table 1) in a wet mash form. Clean, fresh, food and water were offered daily. The routine experimental procedures and observations followed in this experiment consisted of daily measurements of food and water consumption and of urine and feces excretion by all of the rats in one cage. After measuring the volume of urine and mass of feces, the excreta from a given cage were pooled. The pooled samples for three days were combined to provide a sufficient number of alpha disintegrations for satisfactory polonium analysis. The physiological observations made and recorded in addition to food and water consumption and volume and weight of urine and feces excreted were body weight changes, tumor appearance and development, and general physical appearance.

Routinely, when an animal died, the carcass was digested and an analysis of the total body polonium content was made. If tumors were present, these were digested separately. Histopathological examination was made of all tumors present at the time of death, providing they were not necrosed or in a condition unsuitable for tissue sectioning.

In the multiple dose series, as in the single dose experiments, the criteria which were expected to give the most sensitive measure of polonium effect were the shortening of life-span and the correlation of tumor incidence with dose.

Table 1

SPECIAL RAT DIET FORMULA

Cellu-flour	120.0 g	Mazola Oil	200.0 g
Osborn-Mendel salts	40.0 "	Casein	160.0 "
Dried Brewers yeast	100.0 "	Corn starch	200.0 "
Wheat germ oil	10.0 "	Dextrin	190.0 "
Cod liver oil	10.0 "	Sucrose	200.0 "
Vitamin K	0.01 "	H ₂ O (Q-S)	2000.0 ml

IV. RESULTS

SINGLE DOSE The males receiving 0.75 or 0.5 microcurie per kilogram of body weight had significantly shorter life spans than their carrier controls (Table 2). The life spans of these groups were shorter by 108 and 85 days, respectively, than the 780-day life span of their controls. These differences in life span are statistically significant at the one per cent probability level. The males receiving 0.25 microcurie per kilogram lived an average of 62 days less than the controls. This difference was significant at the five per cent probability level and the presence of an effect must be termed still questionable, though probable.

The mean life span of female rats receiving 0.75, 0.5, or 0.25 microcurie of polonium per kilogram of body weight did not differ significantly from that of female carrier control rats. All of the polonium-injected female rats lived somewhat less, on the average, than did their controls, but the 44, 87, and 44 day shortening of life span at the 0.75, 0.5, and 0.25 microcurie levels, respectively, did not approach significance. The lack of significance was in part due to the large variation in life spans between individuals in a group.

Males definitely died sooner than females when injected with 0.75 microcurie of polonium per kilogram body weight. However, a sex difference could not be directly demonstrated for the 0.5 microcurie level because of the greater variability of the female population. Neither the animals at the 0.25 microcurie level nor the controls showed a sex difference in average life span.

The statistical analysis of life span data presented in Table 2 was complicated considerably by two extraneous factors. One was a pneumonia infection contracted by the rats at about 450 days post-injection. The rats were given a very large penicillin injection and the infection disappeared soon after. The other complicating factor was the existence of two age groups at the time of injection. These two groups, designated "young" and "old" have been described earlier in this report. An outline of the manner in which the effect of these two extraneous factors was determined or minimized is given below. Histograms (bar graphs) of life-spans were skewed to the left and were bimodal for all 16 groups of animals ("young" and "old" males and females for each of three injection levels and controls). The first mode was the result of the pneumonia infection. However, these histograms were successfully fitted by normal distributions by first transforming life-spans logarithmically and then smoothing off the first mode by omitting pneumonia deaths from the sample. The pneumonia apparently affected all groups alike, independent of sex or activity level. There were 58 rats discarded as pneumonia deaths. The total sample size remaining for testing the effects of the activity numbered 247 rats, with individual group numbers ranging from 13 to 19 rats per group. Since no tendency for the "young" or "old" (at time of injection) groups of a given sex and activity level to differ in mean life span was evident, except in the females at the 0.5 microcurie level, the age factor was disregarded in all other cases. The two groups were pooled in the interest of larger sample sizes, which then ranged from 27 to 33 rats.

Tumors occurred for the most part late in the rats life, and since the radioactivity had some effect on life-span in the higher injection levels (for the males, at least) some relationship was suspected between tumor incidence and radioactivity injection level. Such was not the case, either for the isolated sexes or for the total population (sexes combined): differences in tumor percentages were easily accountable to sampling error. An average of 30 per cent of the total population had tumors at death. However, the percentage of females with tumors at death (52%) was significantly greater than that of males (20%). Random samples of all tumors observed were sectioned and classified histologically. The ratio of benign to malignant tumors (with the sexes combined to provide adequate sample sizes of malignant tumors for a valid test) was independent of injection level. In fact, this ratio was almost identical in the three injection levels and controls. There were twice as many benign tumors as malignant tumors.

The most interesting feature of the tumor data was the high incidence of tumors in the control rats. This phenomenon is the subject of another report (9).

Table 2

THE EFFECT OF POLONIUM ON THE MEAN AGES OF RATS AT DEATH

Injection Level $\mu\text{c}/\text{Kg b. w.}$	Separate Groups				Sex Averages (Ages Pooled)		
	Group ^a	n	- x days	95% Fiducial Interval days	n	- x days	95% Fiducial Interval days
Carrier	Y male	18	786	730-847	33	780	742-820
	O male	15	772	717-833			
	Y female	17	800	719-892	31	786	736-839
	O female	14	769	710-833			
0.25	Y male	19	716	664-773	32	723 ^c	688-760
	O male	13	732	687-780			
	Y female	18	757	711-807	32	765 ^d	729-803
	O female	14	774	713-842			
0.5	Y male	15	683	646-722	30	684 ^b	658-712
	O male	15	686	645-730			
	Y female	16	718	657-787	33	723 ^{d, e}	
	O female						
0.75	Y male	13	686	644-731	27	709 ^b	684-735
	O male	14	731	703-759			
	Y female	15	777	715-845	29	763 ^d	725-804
	O female	14	749	699-804			

- a. Y animals were younger at time of injection and O animals were older. See description in text.
- b. Significantly lower than the mean of the corresponding carrier controls at the 1% probability level.
- c. Significantly lower than the mean of the corresponding carrier controls at the 5% probability level.
- d. Not significantly lower than the mean of the corresponding carrier controls.
- e. Y and O groups cannot be pooled because variances differ.

MULTIPLE DOSE A body burden of 0.01 microcurie of polonium per kilogram body weight maintained throughout adult life was apparently utterly without effect on a group of more than 100 female rats. The life span of the polonium-injected group was not significantly shorter than that of the controls. Tumor incidence also was not affected by the polonium injection. The statistical tests in the multiple dose series were straightforward and uncomplicated by the extraneous factors existing in the single dose series.



V. DISCUSSION

The minimal effective single dose of polonium in the male rat is approximately 0.25 microcurie per kilogram of body weight. The suggestive and apparently significant effect (shortening of life span) at 0.25 microcurie per kilogram body weight and the clearly significant effects at 0.5, 0.75, and at all higher doses (7) lead to this conclusion.

The rep dose to the kidney, which is the critical organ at slightly higher dose levels (if there really is a critical organ (10)), at 0.25 microcurie per kilogram body weight totaled 73 rep (11). The kidneys received an average of about 0.65 rep per day over the principal exposure period which was a little over 100 days. It is probable that the kidneys of the multiple dose animals received approximately 36 rep over a 600 day period. Two different studies (12, 13) have indicated that the polonium concentration in kidneys of rats averages about 20 times the concentration of that of the rest of the body. If this is true of the rats with a maintained body burden of 0.01 microcurie per kilogram body weight, then the above calculated rep dose value is correct.

Polonium was more toxic to males than females at the 0.75 microcurie level single dose. At lower doses, the variability of the female population was such that the difference between the females and males was not significant. One factor possibly contributing to the slightly greater toxicity in males was the slightly higher rep dose delivered to the vital organs of the males. Polonium doses were given on the basis of total weight, but the

polonium was soon concentrated almost entirely in the vital organs. The fraction of the males' total weight that is vital organs is lower than the similar fraction for females. Thus, the polonium was concentrated in a relatively smaller mass and the rep dose was proportionately higher.

The observed total lack of toxic effect from a body burden of 0.01 microcurie per kilogram body weight is in agreement with the findings of Stannard, Blair, and Baxter (14). They found, "life span is shortened only minimally at ---0.05 μ c/kg, slightly at ---0.23 μ c/kg, and definitely at two higher levels (0.59 and 2.43 μ c/kg)".

The results of the polonium studies raise serious questions about the wisdom of indiscriminate use of radioactive isotopes in humans without more rigorous experimental testing of each isotope than has been the case heretofore. The testing of radioactive isotopes, or other potentially toxic drugs, often is carried out for a time period that is only long enough to insure nearly complete elimination of the toxic substance from the body. It is assumed that if no manifestations of toxicity have become apparent in this time period none are likely to develop later when the toxic agent is no longer present. The polonium work suggests that this is a dangerous assumption. Although more than 93 per cent of a single dose of polonium is eliminated from a rat's body within 120 days through excretion and radioactive decay, a group of rats receiving this dose may show no effects of the treatment for several hundred days - until they die significantly earlier than their untreated controls. The treated animals that died early did not

have any characteristic or dramatic signs and symptoms. They died in exactly the same manner as their controls, but they died earlier.

If the results of polonium studies are used as a basis for calculation, extrapolation, etc., it should be borne in mind that there are some similarities between the radiation exposure from polonium and that from X or gamma radiation to the viscera. The polonium is rather evenly distributed throughout the viscera (12, 13) and is almost absent in the bone and muscle. The alpha radiation from polonium is virtually monoenergetic and is uncomplicated by any appreciable beta or gamma component.



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