Subacute Toxicity of Radioactive Phosphorus as Related to the Composition of the Diet.* (17723)

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The study of the toxicity of radioactive isotopes becomes more and more important with the increasing use of these substances both as a research tool and as a therapeutic agent. While the mechanism of the injurious action of radiation on living cells is probably the same for external and internal radiation sources, one may expect that, from the practical viewpoint, the effects of the introduction of isotopic compounds within the body will vary in relation to the physical characteristics of the isotope (such as the nature of the radiation, its energy and rate of decay) and to the behavior in the organism of the compound containing the radioactive atom (such as its absorption, distribution, intermediary metabolism and rate of excretion). Accordingly, conditions which enhance or attenuate the injurious effects of one isotope or isotopic compound, may be ineffective if a different radioactive material is used. It is thus apparent that the toxicity, as well as the factors which may modify it, should be investigated separately for each isotope and for each isotopic compound.

In the present investigation the subacute toxicity of P^{32}, given parenterally to mice (in one dose and in the form of Na_2HPO_4) has been studied in relation to the possibility that changes in the composition of diet may modify the action of the radioactive isotope. To our knowledge, the only data on the toxicity of P^{32} are those of Anthony and Snyder (1). On the basis of a few experiments on mice (presumably maintained on a stock diet), these authors suggest that the LD_{50} after one injection of P^{32} is around 4 microcuries per g of body weight. With this dose 4 out of 6 mice died between 16 and 21 days.

TABLE I.
Composition of Experimental Diets (% of the dry weight).

Diet No. *	34	29	31	35
Casein	10	25	10	25
Cod Liver Oil	1	1	2	2
Crisco	4	4	30	30
Dextrin	40	32	26	19
Sucrose	39	32	26	18

* In addition to the above components, the diets contained 2% Ruffex and a Salt Mixture (usually 4% of U.S.P. XII vitamin test mixture). A mixture of several B vitamins (Thiamine HCl 0.5 mg per 100 g of diet, Riboflavin 0.5, Pyridoxine HCl 0.5, Nicotinic acid 1.5, Ca pantothenate 2, Inositol 2, Paraaminobenzoic acid 2) was incorporated daily in the diets.

Experimental. Male mice (Rockland "all purpose" strain of approximately 20 g body weight) were transferred from a stock diet (Rockland Farms) to one of the experimental diets, the composition of which is recorded in Table I. The animals were divided in groups of 6, and each group kept in a metallic cage with raised screen floor. Water was given *ad libitum*. After 5 days on the experimental diet, the mice were injected intraperitoneally with a single dose (0.2-0.3 cc) of Na_2HPO_4 containing P^{32} (usually in the concentration of about 1 millicurie for 0.02 mg of P). The mice were then maintained on the same diet for 21 more days, during which the food consumption was recorded daily and the animals weighed at 4-5 day intervals. In each experiment, 6 to 12 groups of mice on

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1. Anthony, D. S., and Snyder, R. H., U.S. Atomic Energy Commission, Documents, 1947, MDDC-881, P. 1-14.

TABLE II.
Effects of Various Doses of P^{32} in Mice on a Low Protein, High Fat Diet (Diet 31).

P^{32} injected, $\mu c/g$	No. of mice	Avg change in body wt, g	Time of 50% deaths, days	% of survivors at the 21st day	Avg time of survival,* days
0	7	—1.8	>21	100	>21
2	6	+1.0	>21	100	>21
4	15	+2.5	>21	67	19 ± 0.8
6	39	—2.5	14	20	15 ± 0.6
8	23	—3.6	11	7	13 ± 0.8

* The values preceded by ± are the standard errors of the means.

3 to 6 different diets were used. As far as possible, the effects of changes in the composition of the diet were studied comparatively on groups run at the same time and injected with the same batch of isotopic phosphate.† As an indication of the effects of the isotope, the following criteria were used: (a) Time in days at which 50% deaths occurred; (b) Percentage of survivors at the 21st day; and (c) Average time of survival in days. Since in computing the averages a survival time of 21 days was used for the mice still alive at the end of the experiment, the figures are mostly lower than the true average survival times. In evaluating the results statistically, the probability P for a chance occurrence of the observed differences has been calculated, using the method of the χ^2 for the percentage of survivors and the t test of significance for the differences in the average survival times (3).

Results. Determination of the LD_{50} dose. The effect of increasing amounts of P^{32} introduced in mice maintained on a low protein, high fat diet (Diet 31) are shown in Table II. The differences between the groups injected with 4 and 6 microcuries of P^{32} per g are statistically significant ($P < 0.01$). It is apparent that the LD_{50} dose for mice on Diet 31 and for experiments of 21 days duration lies between 4 and 6 microcuries per g of body weight.

Fatty infiltration of the liver and lipotropic factors in the diet. It seemed possible that anatomic or functional alterations of the liver, such as those accompanying the fatty infiltration of this organ, might lower the resistance of the animals to the radiation injury. Accordingly, various doses of P^{32} (2-8 microcuries per g) were given to 35 mice on Diet 31 (which was high in fat and low in protein, *e.g.* deficient in lipotropic factors). The results were compared with those observed in 43 mice maintained on the same diet, supplemented with choline chloride (0.5%).‡ In other experiments, prior to the introduction of P^{32} 20 mice on Diet 31 received 2 or 3 injections of 0.10-0.15 CCl_4, as indicated by Barrett *et al.*(5), 10 other mice were treated identically except that choline chloride was

† This method of comparison should minimize the errors caused by variations in the conditions of the experiments (such as the season, room temperature, etc.) and in the concentration and chemical form of the P^{32} injected. The data on the radioactivity of the samples, supplied by the Oak Ridge National Laboratories with each shipment of P^{32}, were usually checked by us against a calibrated uranium source. However, only recently have we been aware of the possibility that some samples might have contained P^{32} in the form of acid-labile esters or pyrophosphate(2). This possibility could perhaps explain certain differences between the results of experiments, made under apparently identical conditions, but in which different batches of P^{32} had been used.

‡ On the basis of the data for the body weight and food consumption, the amounts of choline chloride ingested by our mice on the choline supplemented diets were 3 or 4 times greater than those which have been found adequate for complete protection against both fatty liver and renal damage of young rats on low protein, high fat diets(4).

2. Friedkin, M., and Lehinger, A. L., *J. Biol. Chem.*, 1949, v177, 775.

3. Fisher, R. A., *Statistical Methods for Research Workers*, London, 1936, 6th Edition.

4. Griffith, W. H., and Mulford, D. J., *J. Am. Chem. Soc.*, 1941, v63, 929.

5. Barrett, H. M., Best, C. H., MacLean, D. L., and Ridout, Jessie H., *J. Physiol.*, 1939, v97, 103.

TABLE III.
Effect of Varying the Protein and Fat Content n the Diet of Mice Injected with P^{32} (6 μc per g).

Diet No.	No. of mice	Food consumption, g/day	Change in body wt, g	Time of 50% deaths, days	% of survivors at 21st day	Avg time of survival,* days
34 (low fat, low protein)	80	3.6	—0.9	16	40	16 ± 0.4
31 (high fat, low protein)	39	1.7	—2.5	14	20	15 ± 0.6
29 (low fat, high protein)	37	3.6	+3.9	13	24	14 ± 0.8
35 (high fat, high protein)	70	2.4	+3.3	12	3	12 ± 0.2
Controls (Diet 35, not inj. with P^{32})	11	27	+4.2	>21	100	>21

* The values preceded by ± are the standard errors of the means.

TABLE IV.
Effect of Increases in the Phosphate Content of the Diet of Mice Injected with P^{32} (6 μc/g)

Series of exp. and diet No.*	No. of mice	Phosphate in diet (as H_3PO_4) %	Food consumption, g/day	Change in body wt, g	Time of 50% deaths, days	% of survivors at 21st day	Avg time of survival,† days
I (35)‡	24	1.3	2.5	+3.5	12	8	12 ± 0.6
(35)§	25	2.9	2.4	+2.1	13	28	14 ± 1.2
II (34)‡	53	1.3	3.7	—0.1	16	40	16 ± 0.5
(34)¶	29	3.2	4.1	+0.6	>21	64	18 ± 0.7
III (34)**	18	0.3	2.9	—4.0	14	11	15 ± 0.7
(34)††	18	2.9	3.2	—2.5	>21	61	19 ± 0.6

* No. of diet in parentheses.
† The values preceded by ± are the standard errors of the means.
‡ Salt mixture U.S.P. XI, 4 g % of diet.
§ Salt mixture U.S.P. XI, 3 g, K_2HPO_4 1.95 g, NaH_2PO_4 0.7 g, NaCl 0.35 g % of diet.
¶ Salt mixture U.S.P. XI 2 g, K_2HPO_4 2.6, NaH_2PO_4 0.9, NaCl 0.5 g % of diet.
** Salt mixture U.S.P. XI 1 g, KCl 1 g, NaCl 1 g, Ca lactate 0.8 g, $MgSO_4$ 0.2 g % of diet.
†† Salt mixture U.S.P. XI 1 g, K_2HPO_4 1.95 g, NaH_2PO_4 0.7 g, NaCl 1.35, Ca lactate 0.8 g, $MgSO_4$ 0.2 g % of diet.

added to the diet. From the data (which are omitted for brevity) no difference was detectable between the groups on either the choline deficient or the choline supplemented diet, whether or not poisoned with CCl_4.

Variations in the level of dietary fat and protein. Table III shows the results obtained in several experiments, in which the mice were maintained on 4 different diets, respectively low in protein and fat (Diet 34), high in protein and low in fat (Diet 29), low in protein and high in fat (Diet 31), high in both protein and fat (Diet 35). It is apparent that the susceptibility to the injurious action of P^{32} increased distinctly with the increase in the level of protein, or fat, or both. All differences in the values were highly significant ($P<0.01$), except for the difference between Diets 34 and 31 (which were not significant) and between Diets 34 and 29 (which were moderately significant for the average survival times only). During the 21 days after the injection of P^{32}, the average body weight of the mice on the low protein diets decreased, while it increased in the animals on the high protein diets. However, both decreases and increases in weight were not marked (less than 20% of the initial weight), and no correlation is apparent between these changes and the data on the toxicity of P^{32}.

Variations in the phosphate content of the diet. It was thought that by increasing the amount of phosphate ingested, the exchange between the P^{32} initially deposited in the bone and the non-isotopic phosphate circulating in the plasma might be enhanced, and that the increased rate of mobilization of P^{32} would shorten the effective time of exposure of the tissues, especially the bone marrow. Table IV shows that both the percentages and the times of survival were greater in the mice on phosphate enriched diets. The differences were quite significant in the II

and III series of experiments ($P<0.01$, except for the average survival times of series II in which $P<0.05$), but not quite significant in series I, in which the mice were on the high fat, high protein diet and the difference in the dietary level of phosphate between the test and control animals was less marked than in the other series.

Discussion. It should be pointed out that the present study concerns the acute or subacute toxicity of P^{32}. Any factor modifying the survival time figures in these experiments would act primarily by causing an increase or a decrease in the actual damage of the tissues, especially the hematopoietic tissues. This is probably the mechanism through which the enrichment of the diets with phosphate might cause a certain degree of protection. On the other hand, factors (such as the fatty infiltration of the liver or its partial damage) may probably impair or favor, directly or indirectly, the recovery of the damaged tissues. The action of such factors may not be detectable in experiments of acute toxicity, while it may become apparent in a study of the chronic effects of radiation, in which smaller doses of radioactivity would be used and the animals kept under observation for longer periods.

Our finding of an increased toxicity of P^{32} in mice maintained on high protein diets is in apparent discrepancy with the very recent results of Jennings(6), who described a greater susceptibility to external radiation in protein depleted rats. These animals had been on a diet with a protein content (1.9%) much lower than that of our "low protein" diets (10%), and were irradiated after more than 2 months on this diet, when they had lost 25% of their initial weight. On the other hand, our mice were fed the diets for a much shorter period, and even after the injection of P^{32}, little or no loss of body weight occurred. It seems therefore that an increased radiosensitivity becomes apparent only in a condition of severe protein depletion, whereas, a moderate decrease in the dietary protein to a low maintenance level would cause a decrease rather than an increase in the susceptibility toward radiation injury. In this respect, our results may also be compared with others in the literature, showing that mice fed thyroid(7), or kept at low temperatures (a condition accompanied by an increased oxygen consumption)(8), become more susceptible to external radiation. One might postulate that the acute or subacute effects of radiation parallel the intensity of the metabolism and that the greater toxicity of P^{32} in our mice on the high fat, high protein diet is, at least in part, related to the specific dynamic action of the dietary components (especially proteins).

Summary. In mice on a 10% casein, 32% fat diet, the LD_{50} dose at the 21st day lies between 4 and 6 microcuries per g of body weight. Neither the fatty infiltration of the liver (induced by a deficiency of lipotropic factors, with or without partial poisoning with CCl_4), or its prevention (by supplementing the diet with choline) changes the survival figures. The time of 50% deaths, the percentage of survivors at the 21st day and the average survival time of mice injected with P^{32} are highest with a diet low in fat and in protein, and are significantly decreased when the level of the fat, or of the protein, or both, are increased in the diet. Enrichment of the diets with inorganic phosphate seems to afford a moderate, but significant degree of protection against the injurious action of P^{32}.

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