

Progress Report of the work done on

TOXICITY OF P^{32} AS RELATED TO THE DIET AND TO THE FUNCTIONAL CAPACITY OF THE LIVER

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The present report covers the work done from March 16, 1950 to March 15, 1951. This work is a direct continuation of that summarized in a previous report of March 15, 1950.

1. General plan of the experiments.

During the past years, the study of dietary factors which may protect the tissues against the injurious effects of the isotope has been continued actively. Since radioactive phosphate exchanges rapidly with the inorganic components of the bones, it seemed likely that the damage to the bone marrow and the consequent impairment of its blood cell forming capacity represents the chief factor for the toxicity of P^{32} (as well as of other radioisotopes which are also deposited preferentially in the skeleton). Accordingly, it was thought that a more or less marked protection against the toxic action of inorganic P^{32} could be obtained by either of the following mechanisms: a) an increase in the rate of mobilization of the isotopic phosphate from the bones; this mobilization should shorten the time of the exposure of the bone marrow to radiation and consequently decrease the severity of tissue damage; b) an increase in the recovery and regeneration of the tissue, initially damaged. In this respect, it seemed likely that the maintenance of an adequate supply of the factors involved in normal hemopoiesis may be of great importance.

2. Enrichment of the diets with phosphate or citrate.

In experiments which we have reported previously, the beneficial effects of an increase in the phosphate content of the diet have been noted. These results have been now extended and the interpretation that phosphate is mobilized and excreted further confirmed by the actual demonstration of an increased elimination of the isotope in the urine. Several other series of experiments have been made with the addition of considerable amounts of sodium citrate to the diets. It was hoped that by increasing the concentration of citrate in blood and tissue, (thus reducing Ca^{++}), the mobilization of both calcium and phosphorus from the bones would be enhanced. However, the data on both the mortality of the animals and the rate of excretion of the isotope have been quite inconsistent, and no statistically significant difference was observed between the experimental groups and the controls.

3. Effects of parathyroid hormone.

Since the administration of parathyroid hormone is said to mobilize calcium from the bone and increase the excretion of calcium and phosphorus in the urine, several groups of mice have been injected with parathyroid hormone shortly before

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and immediately after the introduction of P^{32} . The experiments are still in progress, and therefore no definite statement can be made on this point. It is our impression, however, that little or no protection is apparent against the effects of doses of P^{32} in the higher range of the LD_{50} dose. We are planning to continue this study on animals injected with smaller amounts of the isotope.

4. Effects of folic acid and vitamin B_{12} on the survival of mice injected with P^{32} .

Among the hemopoietic factors which might affect the recovery from radiation injury, folic acid and vitamin B_{12} seemed to be worth investigating. Some beneficial effects of folic acid on the survival of animals irradiated by x-rays have been described. However, neither folic acid, nor vitamin B_{12} appeared to modify the hematological changes following total body irradiation. In preliminary experiments (mentioned in our last year's report), the addition of large amounts of folic acid or vitamin B_{12} to the diets did not give any significant protection against the toxic effects of lethal doses of P^{32} . During the past months, the investigation was continued with the following changes in the disposition of the experiments: a) the dose of P^{32} injected has been decreased to the lower range of the LD_{50} dose, and the length of the experiments increased from 3 to 8 weeks, so that the effects of the vitamins on the recovery of the animals (rather than on the actual damage to the tissue) could be studied; b) since large amounts of vitamins are probably synthesized by the intestinal flora, sulfasuxidine was added to the diets of the animals in an attempt to reduce this source of vitamins. Thus, the effects of the isotope on vitamin-deficient animals could be directly compared with those observed in animals receiving adequate amounts of the preformed vitamins in the diet; c) since several observations point to a synergistic action of mixtures of various vitamins, especially of vitamin B_{12} and folic acid, the isolated and combined administration of these and other vitamins has been tested. A total of 525 mice has been used in these experiments. In the following table we are summarizing the results of experiments in which the action of folic (FA), vitamin B_{12} , biotin (Bio), and vitamin K have been tested in mice on sulfasuxidine-containing diets.

Table I
Effects of Certain Vitamins on the Survival of Mice Injected with P^{32}
(5 μ g/g) and Maintained on a Sulfasuxidine-Containing Diet*

Supplements	No. of Mice	Avg food Consumption g/day	Avg change in body wt. g	Time of 50% deaths days	% of survivors		Avg time of survival † days
					21st day	56th day	
None	73	3.5	- 1.6	27	58	16	29 ± 2.0
FA	39	4.1	+ 5.0	20	49	31	30 ± 3.3
B_{12}	24	3.7	- 3.8	34	58	46	36 ± 4.0
FA + B_{12}	34	4.1	- 0.1	56	76	56	42 ± 3.5
FA, B_{12} , K, Bio	67	4.0	+ 1.1	54	72	49	41 ± 2.5
None †† (controls)	18	4.1	- 6.7	56	100	83	55 ± 0.8

Table I (continued)

- * Diet No. 34 (1) containing 5% sulfasuxidine. A basal B vitamin mixture was incorporated daily in the diet.
- + In the calculation of the avg. time of survival, a survival time of 56 days was ascribed to the mice still alive at the end of the experiments. Values preceded by + are the standard errors of the means.
- †† Not injected with P^{32} .

It should be pointed out that when the results of the control groups are compared with our previous data on the toxicity of P^{32} in mice on diets without sulfasuxidine, it is apparent that the inclusion of sulfasuxidine in the diet does not increase the susceptibility of the animals to the injurious action of the isotope. From the previous and present experiments, the following conclusions can be drawn: a) The addition of generous amounts of folic acid, or folic acid and vitamin B_{12} to diets without sulfasuxidine had no significant effect on the survival of mice injected with a dose of P^{32} in the higher range of the LD_{50} (21 days); b) Supplementation of the sulfasuxidine-containing diet with folic acid improved slightly the survival figures. Vitamin B_{12} alone was more effective. When, however, both vitamin B_{12} and folic acid were added to the diet, there was a significant improvement in the survival of the animals; c) The further addition of biotin and of a synthetic vitamin K-like product did not enhance the protection exerted by the mixture of folic acid and vitamin B_{12} .

The results of these experiments will be reported at the meeting of the Southeastern Section of the Society for Experimental Biology and Medicine to be held this coming April at Oak Ridge, Tenn. A complete manuscript has been submitted for publication in the Proceedings of the Society for Experimental Biology and Medicine.

5. Effects of folic acid and vitamin B_{12} on the hematological changes following the introduction of P^{32} .

These experiments were carried out with the purpose of studying if and to what extent the beneficial effects of vitamin B_{12} and folic acid on the survival of mice injected with P^{32} could be ascribed to a more rapid or complete recovery from the hematological changes caused by the isotope. In a number of experiments, several groups of animals were maintained on the same diets employed in the experiments of the preceding section. One animal in each group was sacrificed at regular intervals after the introduction of P^{32} , so that sufficient blood could be obtained for determinations of hemoglobin, red blood cells, white blood cells and hematocrit. After an initial fall, the hemoglobin, red cell, and white cell values rose progressively in all groups of animals. However, between the 12th and the 36th day after the administration of P^{32} , all these values in the blood of mice receiving the mixture of folic acid and vitamin B_{12} were consistently higher than in the blood of the other groups.

While the differences are perhaps not quite statistically significant, the data may be taken as at least suggestive of a relationship between the improved hematological picture and the prolonged survival of mice receiving the mixture of folic acid and vitamin B₁₂.

6. Other experiments.

Preliminary series of experiments have also been carried out in order to explore the possible protection exerted by other factors. Some of these factors, such as the administration of cysteine, have been claimed to be effective in the protection against total body irradiation. To date, our results on mice injected with P³² and cysteine are inconclusive. The affects of adding to the diets sodium bicarbonate (to make the diet alkalotic), or ammonium chloride (to make the diet acidotic) are being tested, but much more work is required before definite statements on this point can be made.

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Summary of the work from March 16, 1950 through March 15, 1951 on the project described as

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The influence of a number of dietary factors on the toxicity of P^{32} injected into mice as sodium phosphate has been studied. In an investigation of the importance of an adequate supply of B vitamins, sulfasuxidine has been included in the diet in order to reduce the synthesis of the vitamins by the microorganisms in the intestine. From a comparison between the survival of mice thus made vitamin-deficient and the survival of mice on the same diet supplemented with the vitamins, it appears that the combined administration of vitamin B_{12} and folic acid affords a quite significant protection against the injurious action of the radioisotope. It is suggested that such a protection is possibly due to the enhanced recovery and regeneration of the blood forming elements in the bone marrow.

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