Progress Report of the work done from July 1, 1949 to March 15, 1950 on the research project described as

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

sponsored by the Atomic Energy Commission under direct contract AT-(40-1)-288, Title III, with the Bowman Gray School of Medicine.

Principal Investigators: Dr. Camillo Artom, Dr. G.T. Harrell, Jr., and Dr. David Cayer.

According to the original plan of the investigation, the acute toxicity of P^{32} has been studied in relation to the possibility that changes in the functional capacity of the liver may modify the susceptibility of the animals toward the radiation effects. In the first series of experiments the role of a dietary deficiency of lipotropic factors and of a partial damage of the liver by poisons such as CCl_4 has been studied. While the results seemed to miminize such a role in the acute toxic effects of the isotope, they indicated definite differences in relation to the composition of the diets. Accordingly, the effects of quantitative variations in several dietary components have been investigated with results which seem of a certain interest.

The general disposition of the experiments, the points investigated, the results obtained and the plans for future experiments are summarized below:

1. General plan of the experiments. Mice of approximately 20 grams were transferred from a stock diet to experimental diets containing various amounts of protein (casein), fat, minerals, and vitamins. After 5 days on the experimental diets, the mice were injected intraperitoneally with a single dose of Na_2HPO_4 containing P^{32} (mostly 6 microcuries per g. of body weight). Food consumption and changes in weight were recorded and the mice maintained on the diets for three weeks or more after the injection of P^{32}. As an indication of the effects of the isotopes, the following criteria were used: a) Time in days at which 50% deaths occurred; b) Percent of survivors at the 21st day; and c) Average time of survival. The results have been evaluated statistically using the χ^2 and the t tests of significance. A total of more than 650 mice have been used in the experiments summarized below.

2. Determination of the LD_{50} dose. The LD_{50} dose was determined in experiments of 21 days duration, using 90 mice maintained on a 10% protein, 32% fat diet. In these conditions the LD_{50} dose lies between 4 and 6 microcuries per gram of body weight.

3. Fatty infiltration of the liver, CCl_4 poisoning and lipotropic factors in the diet. The results obtained in 35 mice on a low protein, high fat diet (which is deficient in lipotropic factors and causes an extensive fatty infiltration of the liver) were compared with those obtained in 43 mice, maintained on the same diet supplemented with generous amounts of choline. In another series of 30 mice, prior to introduction of P^{32} the liver was partially damaged by 2 or 3 subcutaneous injections of CCl_4. Part of these animals were then maintained on the deficient diet and others on the same diet supplemented with choline. However, no significant difference was detected between the groups on either the choline deficient or the choline supplemented diet, whether or not the liver had been previously damaged by CCl_4.

4. Variations in the level of dietary fat and protein. 280 mice were maintained on 4 different diets, respectively low in protein and fat, high in protein and low in fat, low in protein and high in fat, high in both protein and fat. The results clearly show that the susceptibility of the animals to the injurious action of P^{32} (6 microcuries per gram) increased distinctly with the increase in the level of protein, or fat, or both. Thus, with a dose of 6 microcuries of P^{32} per gram of body weight, the mortality of the mice on the high fat, high protein diet was 97% with 50% deaths at the 12th day, whereas in the mice on the low fat, low protein diet, the mortality at the 21st day was 60% with 50% deaths at the 16th day. It is tentatively suggested that the susceptibility to radiation injury parallels the rate of metabolism, and that the specific dynamic action of the dietary components (especially protein) may partly explain the results obtained.

5. Variations in the phosphate content of the diets. It was thought possible that by increasing the amount of phosphate in the diets, the mobilization of the P^{32}, initially deposited in the bone, would be enhanced and, consequently, the effective time of exposure to the isotope of the tissues (especially the bone marrow) would be shortened. Three series of experiments were made on a total of 167 mice, injected with 6 microcuries per gram of body weight. The results show a significantly decreased susceptibility in the mice on the phosphate enriched diets. Thus, in a group of mice on a diet high in phosphate (2.9% as H_3PO_4) a mortality of 39% at the 21st day was observed as compared with a mortality of 89% in the mice on a low phosphate diet (0.3% as H_3PO_4): the times of 50% deaths in these two groups were 23 and 14 days, respectively.

We have now in progress some additional experiments in which we are studying the rate of elimination of P^{32} in rats on diets with a low, medium and high phosphate content. The results obtained thus far seem to be in line with the data on the toxicity and therefore corroborate the interpretation we have suggested. Some of this data are reported here.

% Phosphate in Diet as H_3PO_4)	% of P^{32} eliminated in the urine		
	1st day	2 - 3 days	4 - 5 days
0.3	8.3	4.6	3.2
1.3	20.5	9.7	6.4
2.9	27.1	10.3	6.8

Additional experiments will soon be started, in which the action of other factors which might enhance the excretion of phosphate (such as parathyroid hormone, citrates, etc.) will be investigated.

6. Variations in the supply of certain vitamins. A protective action against external radiation by enriching the diet with folic acid has been described. We have reinvestigated this point on 160 mice injected with P^{32} and kept on various diets with or without additions of folic acid. The results thus far have been of doubtful significance. We are however, continuing our experiment with diets containing sulfasuxidine in an attempt to inhibit the bacterial flora in the intestine, which is an important source of several vitamins, including folic acid.

7. Future experiments. It is planned to use somewhat smaller doses of radioactivity and to keep the animals under experiment for longer periods. Indeed, it seems likely that in experiments of acute or subacute toxicity, the percentage and time of survival

are primarily influenced by factors which might enhance or attenuate the actual damage to the tissues (as for instance, an increased phosphate content of the diet). Factors (such as the partial damage of the liver, or an increased supply of certain vitamins) may act, more probably, by impairing or favoring, directly or indirectly, the recovery of the damaged tissues. The action of such factors may not be detectable in experiments of short duration and with high doses of the isotope, while their role may become apparent in studies on the chronic effects of radiations.

The results summarized above have been reported or are on the programs of the following scientific meetings:

Southeastern Section of the Society of Experimental Biology and Medicine, Durham, N. C., January 1950.

Southern Society for Clinical Research, New Orleans, La., March 1950.

Federation of the American Biological Societies, Atlantic City, N. J., April 1950.

A more extended paper, "Subacute Toxicity of Radioactive Phosphorus as Related to the Composition of the Diet", by W. E. Cornatzer, G. T. Harrell, Jr., David Cayer, and Camillo Artom has been accepted for publication in the Proceedings of the Society for Experimental Biology and Medicine.

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In mice maintained on a 10% casein, 32% fat diet and injected intraperitoneally with a single dose of P^{32} as Na_2HPO_4, the LD_{50} dose at the 21st day lies between 4 and 6 microcuries per gram of body weight.

Neither the fatty infiltration of the liver (induced by a deficiency of lipotropic factors, with or without partial damage of the organ by 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 survival 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 significant degree of protection against the injurious action of P^{32}.

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