Effects of Pantothenic Acid and Its Analogs in Radiation Injury by P^{32}.* (21039)

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In the first paper of this series it has been shown that the severity of the damage caused by the administration of P^{32} can be modified by changes in the levels of dietary protein, fat, or phosphate(1). Subsequently, we investigated the role of certain B vitamins, a deficiency of which is less likely to occur under ordinary nutritional conditions. Beneficial effects were observed with the administration of vit. B_{12} and folic acid(2), or of pyridoxin(3), to mice deficient in these vitamins. The results of the present investigation, which concludes this phase of our study, indicate that pantothenic acid (PA) also exerts a significant protection against the injurious effects of the radioisotope. The sulfonic acid analog of PA, pantoyltaurine (PT), was found to be almost as active in this respect as the natural vitamin, whereas no protection was apparent with ω-methyl pantothenate (MP), an analog in which the pantoyl moiety of PA has been modified.

Experimental. The basal diet (Diet 45) contained: Casein 12 p.p. 100, dextrin 26, sucrose 25, Crisco 25, cod liver oil 5, Ruffex 2, Salt Mixture (U.S.P. No. 2) 4, sulfasuxidine 1, or 1.5. To each 100 g portion of the diet, B vitamins were added in the following amounts: Thiamine HCl 0.4 mg, riboflavin 0.8 mg, pyridoxine HCl 0.4, niacin 5.0, folic acid 0.1, menadione 0.5, biotin 0.05, B_{12} 0.02, inositol 10. Other supplements which were added to the diet of some of the animals only, were: Ca pantothenate (PA) 5 mg p. 100 g, p-aminobenzoic acid (PABA) 2.5 mg, pantoyltaurine (PT) 100 mg, taurine (T) 30-100 mg, ω-methyl-pantothenate (MP) 25 mg. Male albino mice, with an initial average weight of 20 g, were distributed in groups of 8 or 9, and each group housed in a metallic cage with raised screen floor. The diet, to which vitamins and supplements were freshly added, was given 3 times a week, and food consumption was determined. The number of mice alive in each day of experiment and the weekly changes in body weight were also recorded. Seventeen series of experiments were carried out on a total of about 2000 animals, each series including several groups under different nutritional conditions. After a period varying between 5 and 10 days on the experimental diet, some groups were injected intraperitoneally with a solution of phosphate (pH 7.4), containing 0.5 millicuries/ml. The doses injected in different series varied between 4.5 and 5.5 μc/g of animal. Control groups were injected with an identical volume of Ringer solution. Since, in preliminary experiments, the control animals on the PA-deficient diet for more than 4 weeks exhibited a high mortality, in the subsequent series PA was added to the diet of all groups, beginning with the 21st day after the injection of P^{32}. Administration of supplements other than PA was also discontinued at the same date. Under these conditions, very few deaths, or no deaths at all, occurred among the controls, not injected with P^{32}. The mice were kept on the diet and observed for 6 weeks after the injections.

Results. As in previous papers(1-3), the time of 50% deaths, the % of survivors at the 21st day, and the average length of survival were taken as indications of the damage caused by the isotope, a 42-day survival being ascribed to the mice which were still alive at the end of the experiment. All data have been corrected for the mortality of the control groups, not injected with P^{32}.

In Tables I-IV, the average values obtained in several series of experiments from the

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TABLE I. Effects of Pantothenic Acid (PA) on Survival of Mice Injected with P^{32}.

Series	Dietary supplement PA	No. of mice	Time of 50% deaths, days	Survivors 21st day, %	Avg length of survival, days*
1- 7†	—‖	172	16	32	23 ± .8
	+	167	>42	69	32 ± 1.0
8-13‡	—‖	163	20	49	26 ± 1.0
	+	153	>42	62	30 ± 1.1
14-15§	—‖	39	17	41	22 ± 1.9
	+	40	29	65	30 ± 2.0

* Values preceded by ± are stand. errors of the means.
† Diet 45 + sulfasuxidine 1.5%. PABA included in vit. sol.
‡ *Idem.* PABA excluded from vit. sol.
§ Diet 45, without sulfasuxidine. PABA included in vit. sol.
‖ PA was added beginning 21st day after inj. of P^{32}.

groups receiving, or not receiving, a given supplement, have been compared. For such a comparison only the data obtained from groups, run simultaneously and injected with the same batch of P^{32}, were used. This should minimize the effects of slight changes in the conditions of the various experimental series, and also of possible variations in the actual potency and chemical purity of the isotopic solutions injected. From Table I it is apparent that administration of PA increased the survival of mice on the basal diet, supplemented with sulfasuxidine. The differences between the groups receiving, or not receiving, PA were somewhat smaller in series 14-15 in which sulfasuxidine had been omitted from the diet, and even more so in series 8-13, in which PABA had not been included in the vitamin mixture. The latter finding was further investigated in experiments, in which groups of mice receiving, or not receiving, either or both vitamins were run simultaneously. However, from the results of these directly comparable experiments (which are omitted for brevity sake), it does not appear that the protective action of PA is modified appreciably by the presence or absence of PABA in the vitamin mixture. Table II shows that PT, added to the PA-deficient diet, exerts a protection which is approximately of the same degree as that exerted by the natural vitamin. The values for the survival of mice receiving both PA and PT were about the same as, or higher than, the corresponding figures for the groups receiving PA only. The possibility that the protective action exhibited by PT might be due, at least partly, to the S-containing moiety of PT was tested in experiments in which free T was added to the diet. The data of Table III suggest that the compound might be effective, although at a slight degree only. On the other hand, no protection was apparent with the administration of MP, another analog of PA in which the pantoyl moiety has been modified (Table IV). In the amounts we used, the compound did not seem to be toxic, since no deaths occurred in the control groups, receiving MP, with or without PA. The extremely low acute toxicity of MP has been noted by others(12).

Many of our data have been appraised statistically, using the χ^2 method for the number of survivors at the 21st day, and the t test of significance for the average survival time. The most pertinent results of this appraisal are recorded in Table V.

Discussion. The results of the present

TABLE II. Effect of Pantoyltaurine (PT) on Survival of Mice Injected with P^{32}.

Series*	Dietary supplements PA	PT	No. of mice	Time of 50% deaths, days	Survivors 21st day, %	Avg length of survival, days†
1, 3, 5, 8, 11, 12	—‡	—	136	17	34	22 ±10
	—‡	+§	123	41	61	31 ± 1.1
	+	—	131	>42	57	30 ± 1.1
1, 3, 8, 12	+	—	96	19	47	28 ± 1.4
	+	+§	83	27	55	27 ± 1.8

* Diet 45 with sulfasuxidine 1.5%.
† Values preceded by ± are stand. errors of means.
‡ PA added beginning 21st day after inj. of P^{32}.
§ PT discontinued after 21st day from inj. of P^{32}.

TABLE III. Effects of Taurine (T) on Survival of Mice Injected with P^{32}.

Series*	Dietary supplements PA	T	No. of mice	Time of 50% deaths, days	Survivors 21st day, %	Avg length of survival, days†
4, 5, 11, 13	—‡	—	106	16	39	22 ± 1.2
	—‡	+§	91	20	47	26 ± 1.3
	+	—	101	>42	55	29 ± 1.5

* Diet 45 with sulfasuxidine 1.5%.
† Values preceded by ± are stand. errors of means.
‡ PA added beginning 21st day after inj. of P^{32}.
§ Admin. of T discontinued after 21st day from inj. of P^{32}.

study indicate that the injurious effects of internal radiation by P^{32} are more severe in mice on a PA deficient diet and that administration of this vitamin protects the animals to a significant degree. The finding of a protection by PT was unexpected, since the latter compound is an efficient PA displacer for several microorganisms(4-6). A deficiency syndrome has been described in mice receiving PT(7), but this finding was not duplicated by other workers in mice and hamsters(8), or in rats(9). As far as protection against internal radiation is concerned, PT, in the doses used by us, not only did not behave as an antagonist of PA, but seemed to be able to substitute for the latter quite efficiently. It is possible, however, that the effect of PT is partly due to the taurine moiety, since free T seemed to give some protection. The other analog of PA which we have tested, MP, is quite active in preventing growth of microorganisms which require preformed PA(10) and can also induce a PA deficiency in mice(11). Unlike PT, in our experiments MP did not give any protection against, or perhaps made more severe, the effects of P^{32} in PA deficient mice.

Since PA is a component of Coenzyme A (CoA), it may be postulated that its protective action is due chiefly to the maintenance of an adequate level of CoA in tissues. In this respect, our results can be compared with the finding that, on a molar basis, CoA exerts a greater protection against X irradiation than β-mercaptoethylamine(13).

The results of our present and previous papers indicate that PA, pyridoxin, or the association of folic acid and B_{12} increase significantly the survival of vitamin-deficient mice, injected with P^{32}. A similar effect could not be demonstrated with the administration of other dietary factors, such as choline(1), vit. K and biotin(2), or PABA (this paper). Likewise, in unpublished experiments from this laboratory, α-tocopherol did not alleviate, and, at higher doses, even increased the severity of the damage by the radioisotope. These results closely resemble those obtained by others(14) after whole body X irradiation of mice. It seems, therefore, that certain vitamins play a greater, or a more specific, role in protecting against, or in favoring the recovery from, the injurious effects of radiation.

Our findings may also have some practical significance for the treatment of radiation

TABLE IV. Effect of ω-Methylpantothenate (MP) on Survival of Mice Injected with P^{32}.

Series*	Dietary supplements PA	MP	No. of mice	Time of 50% deaths, days	Survivors 21st day, %	Avg length of survival, days†
15-17	—‡	—	35	30	60	26 ± 1.3
	—‡	+‖	54	24	52	23 ± 1.6
	+	—	26	39	78	33 ± 2.0
	+§	+‖	31	33	62	28 ± 2.5

* Diet 45 with sulfasuxidine 1%.
† Values preceded by ± are stand. errors of means.
‡ PA added beginning 21st day after inj. of P^{32}.
§ These groups received 10 mg/100 g diet, reduced to 5 mg after 21st day from inj. of P^{32}.
‖ MP discontinued after 21st day from inj. of P^{32}.

TABLE V. Statistical Appraisal of Protection by Pantothenic Acid (PA), Pantoyltaurine (PT) or Taurine (T) against Toxic Effects of P^{32}.

Dietary supplement	No. of mice	Survivors at 21st day Chi²	P*	Avg length of survival R†	P*
PA‡	339	61.4	<.001	7.1	<.001
PA§	316	5.5	<.02	2.9	<.01
PA‖	79	4.6	<.05	3.0	<.01
PT	259	19.1	<.001	5.9	<.001
T	197	1.4	>.05	2.4	<.02

* Probability for a chance occurrence.
† Ratio of difference between adjusted means to its stand. error.
‡ PABA included in vit. mixture.
§ " excluded from vit. " .
‖ " included in vit. " . Sulfasuxidine not added to diet.

injury. It seems unlikely that a deficiency of PA, pyridoxine, B_{12}, or folic acid will develop in humans under ordinary conditions, in view of the wide occurrence of these vitamins in natural foods, as well as of their extensive synthesis by the bacterial flora, perhaps by the tissues also. It is possible, however, that, in the radiation syndrome, diminished food intake, impaired absorption from the intestine, severe damage to other tissues, and, in addition, administration of large amounts of antibiotics might lead to an actual deficiency. In such a condition there would be a definite indication for the administration of generous amounts of those vitamins which, in our experiments, appeared to exert a beneficial action.

Summary. A significant protection against the injurious effects of P^{32} was observed by the administration of pantothenic acid to mice on a deficient diet. Pantoyltaurine was almost as effective in this respect as the natural vitamin. ω-methyl pantothenate was totally ineffective.

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Performance of Acclimatized Mice at Altitude. (21040)

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One of the most impressive experiences that one has who journeys to high altitudes and remains there for awhile is the increasing subjective sensation of well being from that of initial feeling of distress. Each day physical work becomes a little easier and mental attitudes become a little less confused. It is well established even in the transient sojourner at natural high altitudes that acclimatization is an important factor in his ability to perform well(1). There are, however, some questions which are not completely answered. How much does acclimatization at a lower altitude increase performance at a