Beneficial Effects of Vitamin B_{12} and Folic Acid on Recovery from Internal Radiation by P^{32}.* (18553)

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In a previous paper the toxicity of P^{32} has been investigated in relation to the content of lipotropic factors, protein, fat and phosphate in the diets(1). In a continuation of such a study, the effect of varying the supply of certain vitamins has now been tested. Damage to the bone marrow is a constant occurrence in radiation injury and should be of even greater importance after administration of radioactive compounds, such as isotopic phosphate which is deposited preferentially in the skeleton. Under this condition, one might expect that the recovery of the animals will be affected markedly by a deficiency of hematopoietic factors such as folic acid and vit B_{12}. Some beneficial effects of folic acid on the anemia and leukopenia following radiation therapy in human beings have been reported(2,3). An increase in the % of survivors and in the survival time of x-ray irradiated mice receiving generous amounts of either pyridoxine or folic acid has also been described(4). However, no significant effect of these vitamins on the hematological changes of the animals was detected. Folic acid also failed to prevent such changes after total body irradiation with x-rays of rats(5), cats(6), and swine(7), and after introduction of P^{32} in human beings, or Sr^{89} in rats(8). Likewise, vit B_{12} failed to modify the anemia and leukopenia of rats exposed to x-rays(9).

To our knowledge, no study has yet been reported concerning the effectiveness of the combined administration of folic acid and vit B_{12} on the recovery from radiation injury: a point which is directly suggested by the close relationships in the biological actions of these two compounds. Moreover, since large amounts of these and other vitamins are synthesized by the intestinal flora, an attempt to reduce such a synthesis by the ingestion of poorly absorbed antibacterial agents, would tend to give a more accurate estimation of the value of the vitamins.

Experimental. Four series of experiments were carried out on a total of 525 mice under conditions identical to those of our previous experiments(1), except as indicated below. In series I and II, 172 mice were maintained on experimental diets without added sulfasuxidine and injected with 6 μc/g of P^{32}. Large doses of folic acid (20 mg/100 g of diet) or of a mixture of folic acid (20 mg/100 g) and vit B_{12} (0.75 mg) were added to the diet of part of these animals. In Series III and IV the low fat, low protein diet (Diet No. 34) (1) contained 5% sulfasuxidine and a basal mixture of B vitamins (Thiamine HCl 0.5 mg, riboflavin 0.5 mg, pyridoxine HCl 0.5 mg, Ca

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TABLE I. Effects of Certain Vitamins on Survival of Mice Injected with P^{32} (5 μc/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	
0	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
0‡ (controls)	18	4.1	—6.7	>56	100	83	55 ± 0.8

* Diet No. 34(1) containing 5% sulfasuxidine. A basal B vit. mixture (see text) 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}.

TABLE II. Statistical Comparison of the Survival of Mice Injected with P^{32} (5 μc/g) and Maintained on a Sulfasuxidine-containing Diet.

Supplements	Avg time of survival			No. of survivors at 56th day	
	n	t	P	χ^2	P
FA	110	0.23	>.05	2.32	>.05
B_{12}	95	1.67	>.05	7.56	<.01
FA + B_{12}	105	3.19	<.01	15.70	<.01
FA, B_{12}, K, Bio.	138	3.85	<.01	15.92	<.01

* n = Degrees of freedom; t = According to Fisher(10); P = Probability for a chance occurrence.

pantothenate 2 mg, inositol 2 mg). In addition, some groups received also, as "supplements" to each 100 g of the diet: folic acid alone (FA = 1.5 mg), or vit B_{12} alone (B_{12} = 0.45 μg), or both folic acid and B_{12} (FA + B_{12}; 1.5 mg and 0.45 μg, respectively), or a mixture (FA,B_{12},K,Bio) of folic acid (1.5 mg), vit B_{12} (0.45 μg), 2,methyl-1,4-naphthoquinone (Menadione, 15 μg), and biotin (1.2 μg). Three groups of 6 mice each were maintained for the whole period of the experiments on the sulfasuxidine-containing diet without supplements and were not injected with the isotope. All other animals were maintained on the diets, unsupplemented or supplemented, for 10 days before the introduction of P^{32}, and for 56 more days thereafter. Each of these mice received a single injection of P^{32} (5 μc/g): from our previous data this dose was estimated to be slightly below the LD_{50} (at the 21st day and for a low-protein, low-fat diet)(1). In the experiments of Series IV, one animal in each group was sacrificed by decapitation immediately before and at regular intervals after the introduction of P^{32}, so that blood could be obtained for determinations of the hemoglobin content, red blood cell and white blood cell counts, and hematocrit.

Results. In the experiments of Series I and II the diets did not contain sulfasuxidine, and therefore notable amounts of vitamins were probably still available to the animals through synthesis in the intestine. Supplementation of such diets with folic acid, or folic acid and vit B_{12}, did not appear to cause any significant effect on the survival of mice injected with a dose of P^{32} in the higher range of the LD_{50} (at the 21st day)(1). Data of the experiments of these series (and of Series IV also) are omitted for brevity. Only the data on the survival of mice on the diets containing sulfasuxidine and the results of a statistical treatment of these data are recorded in Tables I and II, respectively. A few of

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the mice not injected with P^{32} died after several weeks on the low protein diet with added sulfasuxidine. On the other hand, in the groups of mice injected with 5 μc/g of P^{32}, the % of survivors at the 21st day and the time of 50% deaths were the same as, or greater than the values previously observed in mice injected with a smaller dose of P^{32} (4 μc/g) and maintained on a diet without added sulfasuxidine (Diet 31, which differed from Diet 34 only in a higher fat content) (1). It seems therefore that the inclusion of sulfasuxidine in the diet did not enhance the susceptibility of the animals to the injurious action of the isotope.† Supplementation of the sulfasuxidine-containing diet with folic acid alone increased the % of survivors at the 56th day, but the increase was not statistically significant ($P>0.05$). Vit B_{12} seemed more effective, however only the increase in the % of survivors was significant. On the other hand, the combined administration of folic acid and vit B_{12} offered a marked protection with a high degree of significance for both % of survivors and survival time. The further addition of menadione and biotin did not enhance the beneficial action of the mixture of folic acid and vit B_{12}. Altogether our results suggest that none of the vitamins tested modify appreciably the damage caused by the introduction of P^{32}, but that an adequate supply of both vit B_{12} and folic acid may be an important factor for the recovery of the damaged tissues.

The hematological changes following the introduction of P^{32} in mice on the sulfasuxidine-containing diet showed approximately the same general behavior in all groups of Series IV. After an initial fall, the values rose progressively and often declined again at the longest intervals. However, between the 12th and the 36th day, that is, during the recovery period after the initial fall, the % hemoglobin and the red and white cell counts in the blood of mice on the diet supplemented with both folic acid and vit B_{12} were all higher than in the other groups. Because of the limited number of determinations, the differences observed can only be taken as a suggestive indication for a relationship between the improved hematological picture and the prolonged survival of mice receiving the mixture of the two vitamins.

Summary. The addition of generous amounts of folic acid, or folic acid and vit B_{12}, to experimental diets had no significant effect on the survival of mice injected with a dose of P^{32} in the higher range of the LD_{50} (21st day). On the other hand, when sulfasuxidine was added to the diet and the mice were injected with a dose of P^{32} slightly below the LD_{50} (21st day), the administration of vit B_{12} and folic acid increased significantly the time of 50% deaths, the average time of survival and the % of survivors (at both the 21st and the 56th day).

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† Several recent reports suggest that antibiotics may even be beneficial in the treatment of radiation injury by preventing general or localized infections. For example, streptomycin and penicillin effectively reduced the mortality or prolonged the survival of rats injected with P^{32}(11).

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Development and Secretion of the Blood Group Factor O in the Newborn. (18554)

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As part of a series of investigations on the development and secretion of blood group properties, we have investigated the development and secretion of the O property in the newborn, child, and adult. The development of the O property has been studied by determining the agglutinability and group-specific absorbing power of red blood cells, using