B₁₂ aids in the maintenance of mitochondrial integrity.

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Effect of Orotic Acid on Vitamin B₁₂-deficient Rats

STUDIES in this laboratory^{1,2} have recently shown that orotic acid is a factor for growth and survival in rats depleted of the animal protein factor of casein. Also, vitamin B₁₂ has a protective effect on the deficiency in animal protein factor³. This indicates a relationship, from a nutritional point of view, between vitamin B₁₂ and orotic acid. In the present communication the direct influence of orotic acid upon the deficiency of vitamin B₁₂ is reported.

To this end, thirty-six weanling rats, male and female in equal number, of the Sprague-Dawley strain weighing 40-45 gm. were employed. The animals were divided into three groups, all of which were fed the basal vitamin B₁₂-deficient corn-soybean meal ration proposed by Register *et al.*⁴, namely, whole ground yellow corn 46.35 gm., commercial soybean meal 46.35 gm., corn oil 5 gm., cystine 0.3 gm., calcium hydrogen phosphate 0.92 gm., calcium carbonate 0.6 gm., sodium chloride 0.44 gm., crystalline manganese sulphate 0.04 gm., thiamine 0.3 mgm., riboflavin 0.3 mgm., niacin 2 mgm., pyridoxine 0.2 mgm., pantothenic acid 2 mgm., folic acid 0.025 mgm., biotin 0.01 mgm., inositol 10 mgm., choline 100 mgm., *p*-aminobenzoic acid 25 mgm.; vitamins A and D were administered weekly. Iodinated casein or desiccated thyroid were omitted.

Group 1 served as control and received only the basal ration. Group 2 were fed the basal ration supplemented with vitamin B₁₂ (2γ/100 gm. of diet). Group 3 received the basal ration supplemented with orotic acid (1,000γ/100 gm. of diet).

The animals were maintained on their respective ration for 4 weeks; at the end of this period weights were recorded and the liver betaine-homocysteine transmethylese activity was determined, on 6 animals

Table 1

Group No.	Supplement to 100 gm. basal ration	Body-weight after 4 weeks (gm.)	Transmethylese activity, γ methionine formed per hr. per gm. liver
1	None	95 (77-113)*	300 (288-322)*
2	2γ vitamin B ₁₂	120 (104-155)	510 (425-680)
3	1,000γ orotic acid†	117 (107-133)	440 (383-517)

* Range of individual values.

† Orotic acid manufactured by Biochemical Inc., Co., Cleveland, Ohio.

for each group, by the method of Williams *et al.*⁵. It is known, in fact, that in vitamin B₁₂-deficiency growth is retarded, and the liver betaine-homocysteine transmethylese activity is decreased⁵.

The results are shown in Table 1.

From these results it appears that orotic acid has a growth-promoting action on the B₁₂-deficient rats; moreover, orotic acid is a factor stimulating liver betaine-homocysteine transmethylese activity.

These data suggest a metabolic relationship between orotic acid and vitamin B₁₂.

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Glass Surface and Blood Coagulation

PLATELET-FREE, citrated plasma, collected and handled in silicone-treated apparatus, has a very long clotting time on recalcification. The exposure to glass surface greatly shortens the clotting time, and various hypotheses have been advanced to explain this phenomenon¹⁻⁴. The present study is an attempt to examine certain aspects of this reaction by the exposure of various normal and pathological plasma specimens and plasma fractions to a standard glass surface in the form of glass 'ballotini' spheres of 0.12 mm. average diameter. The activity may be assessed either by recalcifying the activated specimen directly or by adding a sample to a standard volume of silicone-collected plasma and recording the recalcified clotting time of the mixture. The use of this silicone plasma as a diluent is essential for testing samples which are devoid of one or more clotting factors (cf. Table 1). (For reasons to be discussed elsewhere, in some experiments 'silicone plasma' containing 2,000-10,000 platelets/c.mm. was used as the diluent for the activated, platelet-free samples.)

The results of such experiments are shown in Table 1, and it will be seen that the ability to respond to glass contact is not lost after heating at 56°C. or after absorption with aluminium hydroxide and is retained in the 30-50 per cent saturated ammonium sulphate fraction. These properties suggest that the substance concerned is not identical with any known plasma-clotting factor. The activation by glass contact could also be demonstrated in hæmophilic and christmas disease plasma and in the plasma of patients treated with dindivan, which indicates that the factors deficient in these samples cannot be directly concerned with contact activation. By adding small samples of glass-contacted plasma or fractions to platelet-rich plasma in siliconed containers, the prothrombin consumption of the latter was found to be markedly increased and thrombin generation greatly accelerated. These findings are consistent with past observations, based on different methods of approach⁴⁻⁶. Control samples of the