Effect of Vitamin B_{12} on Nucleic Acid Metabolism of the Rat.* (19439)

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The relation between vit. B_{12} and nucleic acids has attracted considerable attention. The desoxyribosides of a number of purines and pyrimidines have been found to be active in replacing the vit. B_{12} requirement for *Lactobacillus leichmannii*(1-3), whereas the ribosides and free bases are inactive(4,5). Other studies indicated that desoxyribose-1-phosphate was not active in replacing vit. B_{12} for *L. leichmannii* when tested in the presence or absence of orotic acid, ureidosuccinate or subminimal levels of vit. B_{12}(5). The ribonucleic acid content of livers from rats fed a vit. B_{12} deficient diet was shown to be lower (decrease in basophilia) than that for rats fed a supplemented diet(6).

These experiments suggested that vit. B_{12} may function in the synthesis of nucleic acids, possibly in the synthesis of the nucleosides or their precursors. In the present report, studies on the effect of a vit. B_{12} deficiency on the amounts of ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) in rat liver, and other organs, and on the rate of incorporation of N^{15} labelled glycine into the purines of RNA are presented. A preliminary report on certain phases of this study has been published(7).

Methods. Rats that had been fed a corn-soybean oil meal-iodinated casein ration with or without a crystalline vit. B_{12} supplement (24 μg/kg ration) were used in this study. The detailed composition of the rations, and feeding regimen have been described previously(8,9).‡ In the initial experiment, the animals were sacrificed by decapitation after 2 weeks on experiment and the amounts of RNA and DNA in the livers were determined. The method of Schneider(10) was used in Exp. 1 and 2 for the RNA and DNA determinations with the orcinol and diphenylamine methods of analysis.§ In all cases the amounts of RNA and DNA were determined for each liver. In Exp. 2 and 3 the nucleic acid content of the spleens and kidneys was determined. In Exp. 3 a modification of the method of Ogur and Rosen(11) was used and the nucleic acids determined by their absorption at 260 mμ in the Beckman spectrophotometer. The tissue to be analyzed was homogenized in 10 volumes of cold 0.2 N $HClO_4$ and washed 2 times with this acid solution. After 2 washes with 95% ethanol the residue was suspended in 10 volumes of 1.5 N $HClO_4$ and kept at 4-6°C for 24 hours with occasional stirring. Extraction of RNA was complete and essentially no diphenylamine reacting material (DNA) was extracted in kidney or liver. Spleen could not be handled in this way since the DNA was also extracted by this treatment. The DNA was then extracted from the washed liver or kidney residue with 5 volumes of 1 N $HClO_4$ at 80° for 30 minutes. This procedure was repeated once. For the colorimetric analysis, ribose and DNA were used as the reference standards for RNA and DNA, respectively, while for the spectral analyses the extinction coefficients cited by Ogur and Rosen(11) were used as the basis for calculating the amounts of RNA and DNA in the tissues.

Results. The results (Table I) showed that the DNA content per g of liver was reduced by approximately 20% in the livers from the vit. B_{12} deficient animals as compared to the results for the supplemented group while the RNA was reduced by approximately 10% in Exp. 1. No differences were observed in the percentage of dry weight

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TABLE I. Effect of Vit. B_{12} Deficiency on RNA and DNA Content of Rat Liver.

Duration*	DNA (mg/g)			RNA (mg/g)		
	$-B_{12}$	$+B_{12}$	F value†	$-B_{12}$	$+B_{12}$	F value†
2 wk	2.56	3.08	15.1	6.6	7.1	
4 wk	2.37	2.99	20.3	5.1	6.9	25.6
4 wk	2.04	2.58‡	60.8	7.9	9.7‡	12.4

* 8 rats per group in Exp. 1; 7 rats in Exp. 2; and 5 rats in Exp. 3.

† F values required for significance at the 1% level were 8.68, 9.07, and 10.56 in Exp. 1, 2, and 3, respectively.

‡ Livers from rats fed supplemented diet, but with food intake restricted to that of the deficient diet were found to contain an average of 2.67 mg DNA and 9.9 mg RNA/g.

TABLE II. Effect of Dietary Treatment on Amount of RNA and DNA Per Nucleus in Rat Liver.

Dietary regimen	Body wt, g	Liver wt, g	No. nuclei per g, $\times 10^6$	mg DNA per cell, $\times 10^{-8}$	mg RNA per cell, $\times 10^{-8}$
$-B_{12}$	170	11.2	138	1.49	5.78
$+B_{12}$	240	12.4	170	1.54	5.82
$+B_{12}$ (food restricted)	172	8.5	176	1.52	5.51

or total nitrogen of the livers for the 2 groups (*i.e.*, an average of 3.70% N and 3.61% N for the deficient and supplemented group, respectively).

The results for liver in Exp. 2 and 3 substantiated those obtained in the first study (Table I). The reduction in the RNA content of the liver was greater, however, for the tests conducted for 4 weeks. These data were analyzed statistically by the analysis of variance method. The F values observed and those required for significance at the 1% level ($P = .01$) are also shown in Table I. The highly significant differences observed clearly demonstrate the constancy of the data for the animals within each group. Further, the percentage difference as well as the level of statistical significance were similar for the data obtained by the 2 methods of RNA and DNA analysis (Table I).

As in the first studies, no differences in the percentage of dry weight or nitrogen were observed in the livers for the deficient and supplemented groups. The liver weight was greater per unit of body weight, however, for the deficient animals as compared to the controls(9). The rate of gain per week for the deficient animals averaged 21 g as compared to 39 g for the supplemented rats.

In view of other observations that the amount of DNA per nucleus of the liver is constant when the test animal has been subjected to a variety of dietary stresses(12-15), it was important to determine the number of cells per g of liver for the different groups. In these tests, samples of liver from each animal in Exp. 3 (5 in each group) were homogenized (Potter-Elvehjem) and suitable aliquots stained with methyl green and the number of nuclei counted with a hemocytometer(15,16). These results (Table II) show that the number of nuclei per g was lower for the deficient group, while the food restricted group showed no reduction in the number of nuclei per g of liver. These results show that the reduction in the amounts of DNA and RNA and the number of nuclei per g of liver could be attributed to feeding the vit. B_{12} deficient diet and not to the associated reduction in the food intake. The amounts of DNA and RNA per cell were shown to be the same for the 3 groups. Therefore, the basic unit for comparison of the nucleic acid values would appear to be the cell, rather than the amounts of RNA and DNA per unit of fresh weight or dry weight of liver or liver nitrogen. It is recognized that the liver cells of the deficient animals may have been higher in fat which would dilute the other constituents of the cells. Although fat estimations were not made, gross observations and the dry weight and nitrogen determinations did not indicate that the fat content of the livers was different for the deficient and supplemented animals. One may conclude, therefore, that in a vit. B_{12} deficiency the reduction in nucleic acids per g of liver is a reflection of a decrease in the number of cells, although the amount of protein per cell was increased. These findings could be explained by the hypothesis that limiting the rate of nucleic acid synthesis by a vit. B_{12} deficiency might in turn have limited the rate of cell divisions. That vit. B_{12} de-

TABLE III. RNA and DNA Content of Spleen and Kidney from Vit. B_{12} Deficient and Supplemented Rats.

Organ*	DNA (mg/g)		RNA (mg/g)	
	$-B_{12}$	$+B_{12}$	$-B_{12}$	$+B_{12}$
Spleen	16.3	15.9	4.23	4.53
Kidney	3.14	3.24	2.76	2.90

* Six animals in each group. Spleens and kidneys from 2 animals in each group were pooled prior to analysis.

TABLE IV. Incorporation of N^{15} Glycine Into RNA and Protein of Rat Liver.*

Dietary treatment	Rat No.	Atom % excess N^{15} in RNA		Atom % excess N^{15} in liver protein
		Adenine	Guanine	
Basal	50	.042	.041	.073
	51	.049	.060	.083
Basal + vit. B_{12}	55	.095	.081	.100
	56	.088	.064	.099

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ficiency may have reduced the rate of mitosis, thus limiting nucleic acid synthesis is also a possibility. The results for the cell counts in interpreting these experiments show the usefulness of this procedure in other studies on the influence of different nutritional regimens on the nutrient content of the liver from the test animals. These measurements would not only avoid being misled in interpreting the results, but would aid in clarifying the significance of the biochemical changes observed.

Studies on the nucleic acid content of the kidney and spleen showed no differences attributable to dietary treatment. The data for the amount of DNA and RNA in these organs are shown in Table III for Exp. 2.

In an attempt to elucidate further the effect of a vit. B_{12} deficiency on the apparent rate of synthesis of nucleic acids by the rat, N^{15} labeled glycine (31.5 atom % excess) was fed to 2 vit. B_{12} deficient and 2 supplemented rats at a level of 40 mg per 100 g body weight. The glycine was thoroughly mixed with the basal ration and fed in 2 feedings; the second feeding was provided 6 hours after the first. The animals were sacrificed by decapitation 18 hours after the last feeding, the livers removed, and the adenine and guanine from the isolated RNA and DNA analyzed for N^{15}. The adenine and guanine were isolated by ion exchange chromatography(17), and the absorption maxima and minima were in good agreement with those reported by Hotchkiss (18). The residual protein remaining after removal of RNA and DNA(10) was also retained for isotopic analysis. The pertinent data are presented in Table IV. No significant incorporation of N^{15} occurred in the DNA in these studies.

Although the results show that the rate of synthesis of the purines of RNA from N^{15} labeled glycine for the supplemented animals was approximately twice that for deficient animals, an increase in incorporation of glycine into liver protein also occurred for the supplemented animals. With the protein data in Table IV as a base line of comparison, the resultant small differences in the rate of incorporation of glycine into the RNA purines are of doubtful significance with respect to the effect of vit. B_{12}. This conclusion seems warranted also in view of the difficulties that are associated with the use of animals of different body weights and of different liver weight to body weight ratios. A subsequent experiment was conducted and the difference in the rate of incorporation of N^{15} glycine in the RNA purines of the livers from vit. B_{12} deficient rats as compared to supplemented rats was not any greater than in the first experiment.

It may be concluded, therefore, that although differences in the RNA and DNA content of the livers, and in the rates of synthesis of RNA purines could be shown that were attributable to the vit. B_{12} deficiency, these differences could be largely explained by taking into consideration the number of nuclei per g of liver of the rats in the 2 groups.

Summary. 1. The amounts of desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in the livers of rats fed a vit. B_{12} deficient diet were significantly lower per g of fresh or of dry liver or of liver nitrogen than those for the supplemented rats. No difference was observed, however, in the amounts of RNA and DNA per cell for the deficient or supplemented rats. The amount of nucleic acids per g of spleen or kidney was the same

for the deficient and supplemented group. 2. Other studies indicated that the rates of incorporation of N^{15} glycine into the purines of RNA were lower for the deficient animals. 3. These studies suggest that in a vit. B_{12} deficiency the reduced rate of nucleic acid synthesis in the liver reduced the rate of cell divisions.

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Coccidioides immitis Spherule Antigen in a Complement Fixation Test For Experimental Coccidioidomycosis. (19440)

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Prior to 1937 few investigators were successful in demonstrating an immune response to *Coccidioides immitis* in patients with coccidioidal granuloma. Cooke(1) obtained a positive precipitin test with serum from a patient using antigens prepared from dried powdered cultures, and extracts of pus-containing spherules. Davis(2) obtained a positive complement fixation test with serum from a patient using an antigen prepared from a macerated mycelial culture. Chipman and Templeton(3), however, were the first to use successfully a broth filtrate of *Coccidioides immitis* as an antigen in a complement fixation test. This type of antigen has since proven effective and is now widely used. C. E. Smith and associates(4) have used the broth filtrate, coccidioidin, as antigen in a precipitin test and complement fixation test for diagnosis and prognosis in coccidioidomycosis.

In a preliminary report(5) it was shown that the spherules or tissue phase of *Coccidioides immitis* could be obtained in abundance by inoculation of the yolk sac of chick embryos with arthrospores of *Coccidioides immitis*. The use of the spherules as antigen in the complement fixation test is presented in this paper.

Methods. The yolk sacs of 10-day-old embryonated hens' eggs were inoculated with the arthrospores of *Coccidioides immitis* as described in a previous report(5). Up to 0.5 ml of egg yolk from the initially inoculated embryos was passed to the yolk sac of a second series of 10-day-old embryos. After 9 days incubation the yolk of the second transfer eggs was harvested and treated with 4 parts of 10% NaCl, and allowed to stand overnight in the refrigerator. The suspension of yolk and spherules was centrifuged at 3000