Incorporation of Radiophosphorus into Nucleic Acids of Vitamin B₁₂-Deficient Rat Tissues

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In an earlier study (Venkataraman & Friedell, 1954) it was observed that rats which received iodinated casein in the diet to accentuate the effect of dietary vitamin B₁₂ deficiency incorporated more ³²P into the ribonucleic acid (RNA) of liver than did normal controls. Administration of vitamin B₁₂ to such rats resulted in a fall in the ³²P concentration of the RNA to values comparable with those of normal controls. These findings could be due to the combined effects of the altered thyroxine and vitamin B₁₂ levels in the tissues. Various thyrotoxic substances have been shown to produce a marked decrease and alteration in the structure of the cytoplasmic basophilia of the liver tissue (Stenram, 1953; Pirozynski & Bertalanffy, 1953). They also influence the total content of RNA of rat liver and its regenerative capacity on partial removal (Canzanelli, Rapport & Guild, 1949; Drabkin, 1950). Therefore it became important to produce an uncomplicated vitamin B₁₂ deficiency in order to be able to assess the influence of the vitamin B₁₂ on the incorporation of ³²P into tissue nucleic acids. This was accomplished by using animals obtained from mothers who had been kept on a soya-bean oil-corn meal diet deficient in vitamin B₁₂ during the last 10 days of pregnancy and during the lactation period. The main objective of the present study has been to examine, with the aid of appropriate dietary controls, whether vitamin B₁₂ will exert any influence *per se* on the incorporation of ³²P into the nucleic acids of rat liver, intestine and spleen. The body of evidence presented here indicates that vitamin B₁₂ appears to have a direct role in the incorporation of ³²P into deoxyribonucleic acid (DNA) of the rat spleen.

EXPERIMENTAL

Animals and diets

Weanling rats (black-back strain, bred in the laboratory of Professor M. O. Schultze, College of Agriculture, University of Minnesota) 25–30 days' old, born to mothers maintained on vitamin B₁₂-deficient diet (A) during the last 11–12 days of pregnancy and during lactation, were used in these experiments. Diet A consisted of ground yellow-corn

meal 45.5 g., soya-bean-oil meal (solvent-extracted, 44% of protein) 45.5 g., corn oil 3 g., wheat-germ oil 2 g., salts (U.S.P. XIII) 2 g. and a sucrose-vitamin mixture (Register, 1954) 2 g. The mothers during pregnancy and lactation received 2 drops of oil percomorphum weekly. The young had access to diet A until they were weaned, after which they were placed on the deficient diet B. This diet consisted of soya-bean-oil meal 72 g., gelatin 5 g., pure lard 4 g., sucrose 13 g., salts (U.S.P. XIII) 4 g. and a sucrose-vitamin mixture 2 g. The sucrose-vitamin mixture used in diet B contained choline chloride 100 mg., thiamin hydrochloride 0.6 mg., pyridoxine hydrochloride 0.6 mg., riboflavin 1.0 mg., calcium pantothenate 5 mg., niacin 4 mg., folic acid 0.02 mg., biotin 0.02 mg., *p*-aminobenzoic acid 30 mg., inositol 40 mg., tocopherol 0.5 mg. and menadione 0.6 mg. In addition, the animals received 2 drops of oil percomorphum weekly, diluted 1:4 with corn oil. The diets were prepared at frequent intervals and stored in the cold room. Attempts to feed diet B to pregnant mothers resulted in 70% mortality of the young within 48 hr. after birth, evidently due to severe vitamin B₁₂ deficiency (Halverson & Schultze, 1950; Dryden, Hartman & Cary, 1952), whereas with diet A the infant mortality was only about 30–40%.

Treatment of animals

Weanling rats, of both sexes, born to mothers on diet A were distributed into three groups, care being taken to ensure that each group was composed predominantly of males. All three groups received the deficient diet B. Group II was pair-fed against group I, but in addition received vitamin B₁₂ intraperitoneally three times weekly in amounts to provide 0.6 µg. of the vitamin/rat/day. In the place of the vitamin, group I received similar injections of normal saline. Group III received similar amounts of vitamin B₁₂ in the same way as group II but was fed *ad lib*. All animals were housed in individual cages with raised-screen bottoms in an air-conditioned room. At the end of 8 weeks and on the last day of the experiment, after a previous fasting for 12–14 hr., the animals were injected intraperitoneally with approximately 0.5 µCi of radioactive inorganic phosphate (supplied as H₃³²PO₄ by the Oak Ridge National Laboratory, on allocation from the U.S. Atomic Energy Commission) in normal saline/g. of body wt. At specified intervals of time, after injecting ³²P, the animals were anaesthetized with ether and exsanguinated. The tissues were rapidly removed and dropped into beakers chilled in ice. In experiments involving the isolation of DNA from liver (48 hr. period), the animals were injected with ³²P and had access to food and water, except for 14 hr. before killing.

Preparation of tissues and methods of analysis

Liver. The livers were blotted to remove adhering blood, weighed on a torsion balance and passed through a tissue press. The pressed tissue was homogenized and fractionated according to the procedure of Barnum and co-workers (Huseby & Barnum, 1950; Barnum, Nash, Jennings, Nygaard & Vermund, 1950; Barnum & Huseby, 1950; Vermund, Barnum, Huseby & Stenstrom, 1953). The samples obtained were the nuclear fraction (1400 g for 4 min.), the microsomal fraction (23 000 g for 85 min.), the ultracentrifugable particles (95 000 g for 60 min.) and the non-sedimentable supernatant of this fraction.

From each of these fractions RNA was isolated and purified. In the 48 hr. experiment for the isolation of DNA, the homogenate was spun at 1400 g for 4 min. and the cytoplasmic supernatant was discarded. The sediment, without any further treatment, was used directly for the isolation of DNA (Vermund *et al.* 1953). Portions of the homogenate were used for the determination of total RNA and DNA by the procedure of Schneider (1945), and for isolation of the acid-soluble phosphorus.

Intestine. The small intestine, from the duodenum to the end of the ileum, was removed from each animal and any extraneous tissue was peeled off. The intestine was cut into four pieces and washed gently with 0.9% NaCl (pH 7.2) from a syringe. The pieces were pressed between the folds of a sterile gauze, cut with scissors into very small pieces, and weighed rapidly on a torsion balance and homogenized with 4 vol. of ice-cold 10% trichloroacetic acid (TCA). Portions (1 ml.) were removed for estimation of RNA and DNA (Schneider, 1945). Duplicate portions of the homogenate (6–10 ml.) were pipetted into 15 ml. centrifuge tubes for isolation of acid-soluble phosphorus, RNA and DNA. These samples were allowed to stand in the ice-box for 1 hr. and then spun. The supernatant was saved for the determination of the specific activity of acid-soluble phosphorus. The sediments were washed three times with cold 5% TCA and the washings were pooled with the supernatant of the first extract. The residue was rapidly stirred with 5–10 ml. of cold 0.9% NaCl (pH 7.2) and centrifuged in the cold. The supernatant was discarded and the residue was freed from the phospholipids by extracting three times with boiling ethanol-ether (3:1, v/v). The phospholipid-free residue was washed once with ether and the nucleoprotein residue was dried in a desiccator. RNA and DNA were isolated from the dry material by the procedure of Vermund *et al.* (1953).

Spleen. After being weighed on a torsion balance, the tissue was extracted with 4 vol. of 10% TCA and treated in the same manner as the intestine for isolation of acid-soluble phosphorus, RNA and DNA without duplicates. No portions were drawn for the determination of total RNA and DNA.

Measurements of ³¹P and ³²P. The phosphorus content and radioactivity were measured as described previously (Barnum & Huseby, 1950).

Calculations. The specific activity of each sample was calculated (Schulman & Falkenheim, 1950) as the biological concentration coefficient as follows:

$$\frac{(\text{counts/min./m.-mole of P}) \times 100}{\text{Administered counts/min./g. of body wt.}}$$

Since the biological concentration coefficient of the acid-soluble phosphorus of the various tissues at the specified time intervals showed no statistically significant difference

between the three groups of rats, the values reported in this paper refer to the relative specific activity, which is defined as follows:

$$\frac{\text{Biological concn. coefficient of organic compound} \times 100}{\text{Biological concn. coefficient of acid-soluble phosphorus}}$$

This relative specific activity served as the basis of comparison between the different samples to evaluate the effect of vitamin B₁₂ on the incorporation of ³²P into organic compounds. Since the possible variations of different parameters under different dietary and time conditions are being examined, the analysis of variance has been used in evaluating the effects of vitamin B₁₂ statistically.

RESULTS AND DISCUSSION

Body weights, tissue weights and the concentration of deoxyribonucleic and ribonucleic acids in tissues

In Table 1 data concerning the gains in body weights, tissue weights and the concentration of DNA and RNA in tissues are summarized. The significant increase ($P < 0.01$) in the growth rate of vitamin B₁₂-supplemented rats fed *ad lib.* over those of deficient and pair-fed supplemented groups has to be ascribed largely to the increased food intake (Black & Bratzler, 1952) and a rise mainly in the body fat content (Knoebel & Black, 1952). From our observations, it appears that vitamin B₁₂ has no growth-promoting activity in the pair-fed rats. Since the differences in the growth rates of the deficient and supplemented groups fed *ad lib.* are not as large as those observed by Ling & Chow (1952), we feel that our observations are pertinent to a relatively mild state of vitamin B₁₂ deficiency. The weights of liver, intestine and spleen expressed/100 g. of body wt. showed no statistically significant difference between the groups, although in the deficient animals (group I) the liver weight tended to be heavier. Vitamin B₁₂ deficiency appears to have no effect on the concentration of DNA and RNA of the tissues examined, whether the results are expressed per unit wt. of tissue or per organ adjusted to 100 g. of body wt. The values for spleen DNA were calculated from the phosphorus content of DNA samples isolated for evaluation of specific activity, as sufficient material was not available for determination of total DNA. It has been observed by Rose & Schweigert (1952) that vitamin B₁₂ has no effect on the concentration of DNA and RNA per unit weight of the spleen. Our observations that vitamin B₁₂ deficiency has no effect on the concentration of liver RNA and DNA is supported by the experiments of Bruemmer, O'Dell & Hogan (1955) on vitamin B₁₂-deficient young rats. Recently Wong & Schweigert (1956) pointed out that, whether the vitamin deficiency was uncomplicated or produced by the aid of iodinated casein, there was a significant reduction in both liver DNA and RNA per unit weight of the tissue. In the absence of

Table 1. *Growth, tissue weights and nucleic acid content of vitamin B₁₂-deficient and -supplemented rats*

Values are the averages for the numbers of animals shown in parentheses.

	Deficient (group I)	Pair-fed (group II)	Fed <i>ad lib.</i> (group III)	Values of <i>F</i> from analysis of variance*	
				Observed	Required for significance at 0.01 level
Vitamin B ₁₂ supplement ...	0	+	+		
Gain in body wt. (g.)	114 (14)	101 (11)	154 (15)	16.03†	5.21
Liver (g./100 g. of body wt.)	3.25 (14)	2.95 (11)	3.08 (14)	1.81	5.25
Intestine (g./100 g. of body wt.)	2.00 (10)	2.31 (7)	1.79 (12)	3.86	5.45
Spleen (mg./100 g. of body wt.)	245 (10)	265 (7)	244 (12)	1.07	5.45
<i>RNA content</i>					
Liver (mg./g. fresh wt.)	8.12 (13)	8.23 (11)	8.67 (15)	2.77	5.25
Liver (mg./100 g. of body wt.‡)	26.4	24.3	26.7	—	—
Intestine (mg./g. fresh wt.)	6.88 (10)	6.28 (8)	6.26 (12)	<1	5.49
Intestine (mg./100 g. of body wt.‡)	13.8	14.5	11.2	—	—
<i>DNA content</i>					
Liver (mg./g. fresh wt.)	2.53 (13)	2.68 (11)	2.75 (15)	3.19	5.25
Liver (mg./100 g. of body wt.‡)	8.22	7.91	8.47	—	—
Intestine (mg./g. fresh wt.)	12.39 (10)	11.79 (8)	11.76 (12)	<1	5.49
Intestine (mg./100 g. of body wt.‡)	24.8	27.2	21.1	—	—
Spleen (mg./g. fresh wt.§)	6.15 (10)	7.20 (7)	7.19 (9)	1.99	5.66

* The means, under each heading, for the three different groups were tested for equality with the analysis of variance.

† This observed *F* value is sufficiently significant to warrant comparison of the differences between pairs of means. Group I against II, *P* > 0.05; group I against III, *P* < 0.01; group II against III, *P* < 0.01.

‡ These values were obtained by multiplying the mean nucleic acid content/unit wt. of tissue by the mean tissue wt./100 g. of body wt.

§ These values were calculated from the phosphorus content of DNA isolated for evaluation of specific activity. Only about 40% of the DNA of spleen was isolated.

cytochemical data on various tissues in the present work, it appears futile to attempt to examine the disparity between our results and those of Wong & Schweigert (1956).

Incorporation of ³²P into ribonucleic acid

It may be seen in Table 2 that liver RNA, derived from other fractions than the microsomes, did not show significant differences between the three experimental groups when compared by an analysis of variance. With microsomal RNA the analysis indicated border-line significance and suggested a possible difference between groups I and III, but not between groups I and II. These equivocal conclusions with regard to the effect of vitamin B₁₂ on liver RNA metabolism may be contrasted with the marked inhibition of ³²P incorporation into liver nuclear RNA in folic acid deficiency in the monkey (Lowe & Barnum, 1952). If vitamin B₁₂ deficiency is affecting the metabolism of liver RNA at all it certainly does not appear to be causing an increased incorporation of ³²P. Thus it would appear that the previous observations (Venkataraman & Friedell, 1954) on the increased incorporation of ³²P into liver RNA of vitamin B₁₂-deficient animals fed on iodinated casein was probably due to induced hyperthyroidism (Fraenkel-Conrat & Li, 1949).

In sharp contrast with liver, the incorporation of ³²P into the RNA of intestine and spleen (Tables 3 and 4) in vitamin B₁₂ deficiency (group I) is inhibited significantly, by 30–50% during the time intervals studied, in comparison with the animals fed *ad lib.* (group III). Likewise the rate of incorporation of ³²P into the RNA of the intestine and spleen of pair-fed animals (group II) was significantly reduced in comparison with group III but did not, however, differ from the deficient rats (group I). It may be pointed out that the animals of group III consumed considerably more food daily and also exhibited a greater gain in body weight than groups I and II during the experimental period. These results therefore indicate that vitamin B₁₂ probably acts indirectly in maintaining the normal turnover of RNA in intestine and spleen rather than being directly involved in some manner with RNA turnover. If the latter were the case, a more rapid rise in incorporation of ³²P into the RNA of the tissues would have been expected in the pair-fed rats (group II).

Incorporation of ³²P into deoxyribonucleic acid

The relative specific activity of liver DNA, 48 hr. after the administration of ³²P, reveals no difference among the three groups of rats (Table 2). The failure to observe any differences in the radio-

Table 2. *Effect of vitamin B₁₂ deficiency and supplementation on the relative specific activity of deoxyribonucleic acid phosphorus and ribonucleic acid phosphorus derived from various cell fractions of the rat*

For calculation of relative specific activity see text. Group I was vitamin-deficient, group II was pair-fed and group III was fed *ad lib*.

					Values of <i>F</i> from analysis of variance*	
Group no.	Vitamin B ₁₂ supplement	Time (hr.)			Observed	Required for significance at 0.01 level
		1	2	48		
<i>RNA from nuclear fraction</i>						
I	0	11.4 (3)	30.7 (3)	—	1.40	6.93
II	+	19.5 (2)	33.2 (3)	—		
III	+	14.2 (4)	36.4 (3)	—		
<i>RNA from microsomal fraction</i>						
I	0	0.09 (3)	0.57 (3)	—	6.84†	6.93
II	+	0.13 (2)	0.70 (3)	—		
III	+	0.13 (3)	1.15 (3)	—		
<i>RNA from ultrasedimentable particles</i>						
I	0	0.10 (3)	0.75 (3)	—	2.24	6.93
II	+	0.31 (2)	1.05 (3)	—		
III	+	0.18 (4)	1.47 (3)	—		
<i>RNA from non-sedimentable supernatant</i>						
I	0	1.38 (3)	3.60 (3)	—	4.37	6.93
II	+	1.32 (2)	4.25 (3)	—		
III	+	1.48 (4)	6.15 (3)	—		
<i>DNA</i>						
I	0	—	—	4.10 (3)	<1	10.92
II	+	—	—	3.99 (3)		
III	+	—	—	3.96 (3)		

* Differences among the means of the relative specific activities of the RNA of various liver-cell fractions were tested for significance by the analysis of variance with unweighted means for a two-way classification (diet × time). Variations caused by time differential were removed from the analysis. Since no time variation is involved in DNA figures, the differences among the means of the three groups were evaluated as indicated in Table 1.

† This *F* value is near enough to being significant to warrant comparison by groups: group I against II, $P > 0.01$; group II against III, $P > 0.01$; group I against III, $P < 0.01$.

activity of liver DNA, in contrast with the observations on intestine and spleen, might be explained on the basis that under conditions of relatively mild deficiency, as was obtained in the present work, more vitamin B₁₂ was retained in the liver than in the spleen. The fact that liver binds vitamin B₁₂ in a tenacious manner is well known (Schilling, 1955). Moreover, the mitotic activity of liver is very low compared with that of intestine or spleen and the effect of vitamin B₁₂ deficiency on DNA synthesis might well be more pronounced in tissues with a high mitotic rate.

As seen in Table 3 there is a significant reduction, about 40% at 4 hr., in the relative specific activity of intestinal DNA in the deficient group in comparison with the group fed *ad lib*. However, in the pair-fed rats, the relative specific activity did not differ significantly from the deficient group. This implies an indirect effect of vitamin B₁₂ associated with consumption and utilization of food rather than a direct effect on DNA synthesis. The interpretation of the relative specific activity of the

intestinal DNA is complicated by the fact that this tissue is composed of cells with highly different rates of division and the cells are destroyed on a large scale. It has been shown that essentially all the DNA synthesis occurs in the crypts, and that the cells of the epithelium are continuously replaced by cells that have been newly formed in the crypts (Leblond, Stevens & Bogoroch, 1948).

The effect of vitamin B₁₂ deficiency and supplementation on the relative specific activity of the spleen DNA is presented in Table 4. In the deficient animals the relative specific activity, at all the time intervals studied, is 5–8 times ($P < 0.01$) lower than the pair-fed group and the group fed *ad lib*. (II and III), which showed no difference in their relative specific activities. The very high specific activity of the DNA in the pair-fed group and group fed *ad lib*. is evidently associated with rapid cell division in the lymphopoietic cells (Jolly & Rosello, 1909; Andreasen, 1943; Andreasen & Ottsen, 1945).

A direct correlation between the renewal of DNA phosphorus and cell division has been implicated

(Stevens, Daoust & Leblond, 1953; Daoust, Bertalanffy & Leblond, 1954; Barnum, Huseby & Vermund, 1953). The decreased relative specific activity of spleen DNA in the deficient group in comparison with the pair-fed controls, and in intestinal DNA in the deficient group in comparison with the controls fed *ad lib.*, with no apparent change in DNA content or in the specific activity of acid-soluble phosphorus suggests that vitamin B₁₂ deficiency has in some way (directly or indirectly) interfered with DNA synthesis to a considerable degree in these tissues. This view is consistent with the observations of Schweigert, Scheid & Downing (1954) on the impaired cell replacement in vitamin B₁₂-deficient rats subjected to partial hepatectomy. It is also in line with the suggestions of Rasch, Swift & Schweigert (1955) on mitotic blockstemming

from the cytochemical studies on the livers of vitamin B₁₂-deficient and -supplemented rats. Furthermore, O'Dell, Bruemmer & Hogan (1956) observed that in new-born rats from vitamin B₁₂-deficient dams the rate of phosphorus 'turnover' in DNA of the liver was lowered in comparison with that in rats from the vitamin B₁₂-supplemented dams, although the concentration of DNA per nucleus in either group was unchanged. However, the biochemical observations presented here on the renewal of DNA and RNA in spleen and intestine in vitamin B₁₂ deficiency will have to be correlated by histological and cytochemical studies.

The present evidence on the renewal of spleen DNA suggests that vitamin B₁₂ exerts a direct effect on the incorporation of ³²P into the DNA of rat spleen. As previously mentioned, such ³²P

Table 3. *Effect of vitamin B₁₂ deficiency and supplementation on the relative specific activity of ribonucleic acid phosphorus and deoxyribonucleic acid phosphorus isolated from the rat intestine*

For calculation of relative specific activity see text. Group I was vitamin-deficient, group II was pair-fed, and group III was fed *ad lib.*

was fed *ad lib.*

Group no.	Vitamin B ₁₂ supplement	Time (hr.)			Values of <i>F</i> from analysis of variance*		Significance of difference between pairs of means
		1	2	4	Observed	Required for significance at 0.01 level	
<i>RNA phosphorus</i>							
I	0	2.89 (3)	5.59 (3)	24.8 (4)	13.76†	5.93	{ I against II, <i>P</i> > 0.05 I against III, <i>P</i> < 0.01 II against III, <i>P</i> < 0.01
II	+	3.26 (2)	7.82 (3)	25.6 (3)			
III	+	6.12 (4)	11.00 (2)	34.0 (4)			
<i>DNA phosphorus</i>							
I	0	0.93 (3)	2.21 (3)	6.92 (4)	6.33†	5.93	{ I against II, <i>P</i> > 0.05 I against III, <i>P</i> < 0.01 II against III, <i>P</i> < 0.05 - > 0.01
II	+	0.70 (2)	3.25 (3)	7.73 (3)			
III	+	1.00 (4)	3.33 (3)	11.2 (4)			

* See first footnote to Table 2.

† Differences among the means of the three groups were significant and they were compared in pairs.

Table 4. *Effect of vitamin B₁₂ deficiency and supplementation on the relative specific activity of ribonucleic acid phosphorus and deoxyribonucleic acid phosphorus isolated from rat spleen*

For calculation of relative specific activity see text. Group I was vitamin-deficient, group II was pair-fed and group III was fed *ad lib.*

Group no.	Vitamin B ₁₂ supplement	Time (hr.)			Values of <i>F</i> from analysis of variance*		Significance of difference between pairs of means
		1	2	4	Observed	Required for significance at 0.01 level	
<i>RNA phosphorus</i>							
I	0	13.2 (3)	19.4 (3)	30.7 (4)	16.94†	6.01	{ I against II, <i>P</i> > 0.05 I against III, <i>P</i> < 0.01 II against III, <i>P</i> < 0.01
II	+	10.1 (2)	21.1 (3)	30.5 (3)			
III	+	14.2 (4)	42.7 (2)	46.8 (3)			
<i>DNA phosphorus</i>							
I	0	0.86 (3)	3.42 (3)	8.76 (3)	30.96†	6.01	{ I against II, <i>P</i> < 0.01 I against III, <i>P</i> < 0.01 II against III, <i>P</i> > 0.05
II	+	5.78 (2)	17.8 (3)	42.0 (3)			
III	+	6.88 (4)	16.6 (2)	32.2 (3)			

* See first footnote to Table 2.

† Differences among the means of the three groups were significant and they were compared in pairs.

incorporation is best correlated at present with DNA synthesis, and thus vitamin B₁₂ deficiency would appear drastically to inhibit DNA synthesis in the spleen. The finding of Downing & Schweigert (1956) that vitamin B₁₂ was involved in the biosynthesis of deoxyribose, or a precursor, in *Lactobacillus leichmannii* appears pertinent to our observations on the effects of vitamin B₁₂ on DNA synthesis, at least in the spleen.

SUMMARY

1. The role of vitamin B₁₂ in nucleic acid synthesis was investigated by measuring the incorporation of radioactive phosphate, into rat liver, intestine and spleen. The nucleic acid content and the relative specific activities of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) at various time intervals after the administration of [³²P]-phosphate were determined in vitamin B₁₂-deficient rats and in rats injected with vitamin B₁₂ which were either pair-fed against the deficient group or fed *ad lib*.

2. The DNA and RNA content of the tissues were unaffected by the vitamin deficiency.

3. The metabolic pattern and the relative specific activities of the RNA of liver-cell fractions were not significantly altered by lack of administered vitamin B₁₂.

4. The incorporation of ³²P into the RNA of intestine and spleen was inhibited both in the deficient and the pair-fed groups when compared with animals which ate *ad lib*. This observation was interpreted to indicate an indirect action of the vitamin associated with increased food consumption and efficient utilization for purposes of growth.

5. The incorporation of ³²P into liver DNA appears to be unaffected by the absence of administered vitamin B₁₂.

6. The results on the relative specific activity of intestinal DNA suggest an indirect action of vitamin B₁₂, as with intestinal and spleen RNA, since there was no significant difference between the deficient and pair-fed groups. The relative specific activity of spleen DNA, however, was increased by vitamin B₁₂ in both pair-fed rats and rats fed *ad lib*., suggesting a direct role of vitamin B₁₂ in the synthesis of spleen DNA.

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