Alterations in Rat Serum Proteins in Folic Acid and Vit. B_{12} Deficiency.* (22851)

ANAND G. MULGAONKAR AND A. SREENIVASAN (Introduced by Sam C. Smith)
Department of Chemical Technology, University of Bombay, Bombay.

The known participation of folic acid (PGA) and vit. B_{12} in various single carbon addition reactions implicates these vitamins in the metabolism of glycine, serine, threonine, tryptophane, histidine, and possibly other amino acids and, therefore, in general protein metabolism. This is also inferable from the demonstration that, in a vit. B_{12} deficiency, there is impairment in carbohydrate and lipid metabolism(1) and from the fact that optimal utilization of amino acids depends upon caloric adequacy. It was of interest therefore to study the influence of a deficiency of PGA and vit. B_{12} on the electrophoretic pattern of serum proteins.

Materials and methods. Young male rats (Wistar strain), 50 g in weight were reared on a purified ration consisting of (in g per 100 g of diet): vitamin-free casein, 10; iodinated casein ('Protomone': Cerophyl Laboratories, Kansas City, Mo.), 0.15; sesame oil, 6; shark liver oil, 2; salt mixture (U.S.P. No. 2), 4; sucrose, 9.85; and maize starch, 68; with the following vitamin additions (in mg kg of diet): thiamine hydrochloride, 6; pyridoxine hydrochloride, 6; calcium pantothenate, 20; riboflavin, 10; biotin, 1; α-tocopherol, 50; nicotinic acid, 30; choline chloride, 500; inositol, 500; and vit. K (Menadione, Merck), 10. The vitamin additions provided in this basal diet were considered adequate for the hyperthyroid condition. At the end of 4 weeks, the animals were divided into 2 groups, one of which continued to receive the deficient diet modified by the withdrawal of iodinated casein and substitution by 2% succinyl sulphathiazole. The second group was fed on the modified diet with additions of PGA and vit. B_{12} at 5 mg and 50 μg respectively per kg of diet. The rats were sacrificed after a further period of 5 weeks and blood was collected from the inferior vena cava. Livers were quickly excised and chilled in cracked ice.

Serum vit. B_{12} was determined by the method as described by Ross(2) using *Euglena gracilis*. Liver vit. B_{12} was determined after liberation from the tissue by overnight incubation under toluene with papain (B.D. H., 25 mg/g of liver) in 0.1 M acetate buffer of pH 4.6; the samples were then autoclaved, homogenized, neutralized and made to volume. Assays were made with *Lactobacillus leichmannii* (ATCC 7830) by a turbidimetric adaptation of the U.S.P. method(3). Liver folic acid was determined after autolysis in 0.2 M phosphate buffer of pH 7.6 using *Streptococcus faecalis R* (ATCC 8043) and the assay medium of Mitbander and Sreenivasan (4).

Electrophoresis of the separated serum was carried out on Whatman No. 3 paper strips 5 x 34 cm in a horizontal, open, strip-type cell (5) with barbital buffer of pH 8.6 and ionic

TABLE I. Blood and Liver Picture in Folic Acid and Vit. B_{12} Deficiency (Averages of 4 Determinations).

Group	Leucocytes/mm³ (× 10,000)	Hemoglobin, %	Erythrocytes/mm³ (× million)	Liver PGA, μg/g	Liver B_{12}, mμg/g	Serum B_{12}, $\mu\mu$g/ml
Casein (10%) diet without PGA and vit. B_{12}	.47	11.6	3.05	1.8	35.4	170
Casein (10%) diet with PGA and vit. B_{12}	.76	14.6	6.25	6.3	100.8	850

* This work was supported by grant from Williams-Waterman Fund of Research Corporation, N. Y. City.

TABLE II. Fractionation of Rat Serum Proteins in Folic Acid and Vit. B_{12} Deficiency. 7 rats per series.

Group	Total serum proteins	Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin	Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin
	g/100 ml serum						% of total proteins				
Stock diet (20% proteins)	6.93 ± .11	2.79 ± .09	1.34 ± .07	.38 ± .10	1.14 ± .04	1.27 ± .11	40.3 ± 1.4	19.4 ± 1.1	5.5 ± 1.5	16.5 ± .6	18.3 ± 1.7
Casein (10%) diet with PGA and vit. B_{12}	5.35 ± .31	2.13 ± .05	.91 ± .07	.36 ± .07	.97 ± .08	.97 ± .10	39.8 ± .9	17.1 ± 1.4	6.8 ± 1.3	18.1 ± 1.5	18.2 ± 2.0
Casein (10%) diet without PGA and vit. B_{12}	4.16 ± .40	1.38 ± .15	.53 ± .10	.37 ± .05	1.12 ± .14	.76 ± .09	33.1 ± 3.7	12.8 ± 2.5	8.8 ± 1.4	26.9 ± 3.3	18.4 ± 2.2

strength 0.075. Separation was effected at a constant current of 2.5 mA/strip for 16 hours at room temperature (28°C). The subsequent procedure employed was essentially that described by Jencks *et al.*(6). The strips were scanned after immersion in hot liquid paraffin, draining for two hours and blotting off excess paraffin. An 'EEL' Scanner (Evans Electroselenium Ltd., Harlow, Essex) based on the device by Latner *et al.*(7) was used. Total protein was determined by the biuret method(8). Patterns were also obtained for sera from normal adult rats maintained on the laboratory stock diet (20% protein) consisting of (in g/100 g of diet): whole wheat flour, 60; whole milk powder, 10; defatted peanut meal, 11; brewer's yeast, 4; wheat bran, 4; dried fish meal, 3; sesame oil, 4; sodium chloride, 2; and calcium carbonate 2.

Results. In Table I are given the results of blood and liver analyses for the normal and deficient groups of animals at the time when they were sacrificed for fractionation of serum proteins. The latter results are summarized in Table II. Typical profiles for each group are also reproduced in Fig. 1.

It is seen (Table II, Groups 1 and 2) that protein level in the diet alters chiefly the serum concentrations of albumin and α_1-globulin, and, to a less extent, of γ-globulin; their proportions to the total serum protein remain unchanged. In a deficiency of PGA and vit. B_{12} (Group 3), there is a significant reduction in total serum proteins and in serum albumin, α_1-globulin and γ-globulin, although the percentage of total protein as γ-globulin is the same.

Discussion. It has been reported that vit. B_{12} in liver tissue is loosely bound to a protein fraction resembling serum β-globulin in electrophoretic mobility, and that normally, serum vit. B_{12} bound to β-globulin is assayable as free vitamin while that bound to α-globulin is unavailable to assay organisms except after protein denaturation by heating (9). The observed high proportion of β-globulin in group 3 (Table II and Pattern 3) may point to an increased concentration of free vit. B_{12} in the deficient condition. It

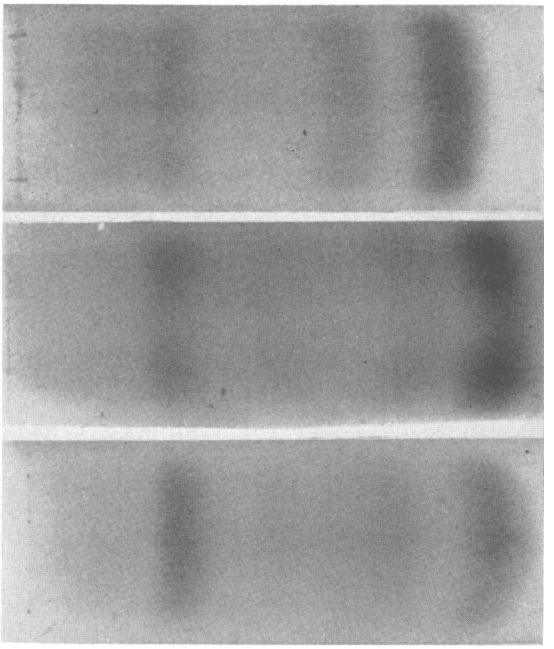

FIG. 1. Alterations in electrophoretic pattern in PGA and vit. B_{12} deficiency. The bands reading from the starting line are, in order γ-globulin, β-globulin, α_2-globulin, α_1-globulin and albumin. There is a decrease in serum levels of albumin, α_1-globulin and γ-globulin with rise in β-globulin level in the deficient animals (Pattern 3). Vit. B_{12} and folic acid supplementation brings these levels to normal (Pattern 2). The serum picture of rats maintained on the stock diet (Pattern 1) has been taken as standard for comparison.

would seem that in a vit. B_{12} deficiency there is increased mobilization of this fraction from the liver to the serum. The increased serum β-globulin observed in the choline-deficient rat(10) may perhaps be similarly explained as due to a greater channelling of vit. B_{12} in this condition. In the present work, the effects of deficiencies of both folic acid and vit. B_{12} on serum proteins were studied in view of their well known metabolic inter-relationships. Experiments are in progress to ascertain their effects singly.

Summary. 1. In a deficiency of vit. B_{12} and folic acid in rats, there is a significant reduction in total serum proteins and in serum albumin, α_1-globulin and γ-globulin. An increase in the proportion of β-globulin is probably indicative of higher serum level of free vit. B_{12} in the deficient state. 2. Dietary protein level influences serum concentrations of albumin, α_1-globulin and, to a lesser extent, γ-globulin.

1. Ling, C. T., and Chow, B. F., *J. Biol. Chem.*, 1954, v206, 797.

2. Ross, G. I. M., *J. Clin. Path.*, 1952, v5, 250.

3. Rege, D. V., Ph.D. Thesis, Univ. of Bombay, 1953.

4. Mitbander, V. B., and Sreenivasan, A., *Arch. fur Miokrobiolo.*, 1955, v21, 60.

5. Grassmann, W., and Hannig, K., *Z. Physiol. Chem.*, 1952, v290, 1.

6. Jencks, W. P., Jetton, M. R., and Durrum, E. L., *Biochem. J.*, 1955, v60, 205.

7. Latner, A. L., Braithwaite, F., and Nunn, R. A., *ibid.*, 1952, v51, x.

8. Gornall, A. G., Bardawill, C. J., and David, M. M., *J. Biol. Chem.*, 1949, v177, 751.

9. Pitney, W. R., Beard, M. F., and VanLoon, E. J., *ibid.*, 1954, v207, 143; 1955, v212, 117.

10. Fisher, M. A., and Garrity, G. C., *ibid.*, 1953, v204, 759.

Received September 12, 1956. P.S.E.B.M., 1957, v94.