Protective Action of Vitamin B_{12} in the Hyperthyroid Rat

SINCE thyroxine is known to decrease the efficiency with which phosphorylation is coupled to respiration[1], the possibility suggests itself that vitamin B_{12} may function in hyperthyroidism by restoration, in part at least, of the energy-requiring functions of impaired cells. This has now been examined by a study of the following conjugation systems: acetylation of *p*-aminobenzoic acid, benzoylation of glycine, and synthesis of glutathione.

Groups of six rats (Wistar strain), 120–140 gm. in weight, were maintained on the following laboratory stock diet:

Whole wheat flour	69 parts	Shark liver oil	4 parts
Casein	10 ,,	Sesame oil	4 ,,
Whole milk powder	2 ,,	Calcium carbonate	2 ,,
Dried fish meal	3 ,,	Sodium chloride	2 ,,
Wheat bran	4 ,,		

with modifications as shown (Table 1). Iodinated casein ('Protomone': Cerophyl Laboratories, Kansas City, Mo.) was at 3 per cent level and replaced an equal amount of casein in the diet. Vitamin B_{12} ('Cobionne': Merck) was administered intraperitoneally at 10 γ per rat per day. The animals receiving iodinated casein (group 3) in the diet lost 7–10 per cent body-weight during two to three weeks when they were considered hyperthyroid[1]. Acetylating ability was determined during this period by the method of Bratton and Marshall[2] after intraperitoneal administration of *p*-aminobenzoic acid (1 mgm./300 gm. body-weight) followed by urine collection for the next 24 hr. A small amount of the *p*-aminobenzoic acid was apparently excreted as *p*-aminobenzoyl glycine, which was determined separately in aliquots of the urine[3]. Corrections were made in either case for endogenous excretions.

Blood samples were collected from the tail veins for determinations of erythrocyte glutathione after hæmolysis, plasma being nearly devoid of this metabolite.

At the end of three weeks, the animals were killed; livers and adrenals were quickly excised and chilled for immediate determinations of glutathione[4] and for the *in vitro* synthesis of benzoyl glycine by the liver enzymes. For the latter purpose, the procedure employed was as follows: 0·5 c.c. of 1 : 5 liver homogenate in 0·1 *M* phosphate buffer, *p*H 7·5, was added to a mixture of glycine (0·01 *M*), benzoic acid (0·0025 *M*), potassium dihydrogen phosphate (0·037 *M*), magnesium sulphate (0·0008 *M*), sodium citrate (0·01 *M*) and sodium fumarate (0·0015 *M*) in the concentrations indicated in a final volume of 5 c.c., *p*H being adjusted to 7·5 with dilute potassium hydroxide. All flasks were gassed with oxygen for 10 min. prior to incubation for 3 hr. at 37° with frequent shaking. Reaction was stopped by addition of 0·2 c.c. of *N* sulphuric acid and the benzoyl glycine formed estimated[3]. Values reported in Table 1 represent increments after the incubation period over corresponding zero-time digests prepared similarly.

The results, which are averages for the six animals in each group, show that in the hyperthyroid animals there is a marked reduction in acetylating ability as well as in tissue glutathione as compared to the control group. Vitamin B_{12} supplementation counteracts this condition appreciably.

An observation of interest emerging from this study is the increase in blood glutathione during thyrotoxicosis and its reversal by vitamin B_{12}. This inverse relationship between blood- and liver-levels of glutathione has since been confirmed in other studies now under progress, and is also suggested from the report[5] that there is an increase in erythrocyte glutathione in methionine deficiency.

Iodinated casein has been reported to interfere with vitamin B_{12} absorption[6]. Since vitamin B_{12} is involved in the activation of soluble and protein sulphydryl, its role in carbohydrate and lipid metabolism has been demonstrated[7]; it is also involved in the synthesis of porphyrins and respiratory pigments[8]. It seems, therefore, that the primary manifestation of thyrotoxicosis is a deficiency of vitamin B_{12}, and consequent interference in the generation and utilization of energy-rich bonds coupled with electron transport.

Table 1

Group	Per cent gain in weight (during 3 weeks)	Glutathione in: Liver (mgm./gm., fresh basis)	Glutathione in: Adrenals (mgm./gm., fresh basis)	Glutathione in: Blood (mgm./100 c.c.)	Synthesis *in vitro* of benzoyl glycine (mgm./gm. dry liver)	Per cent urinary conversion of *p*-aminobenzoic acid to: *p*-aminobenzoyl glycine	Per cent urinary conversion of *p*-aminobenzoic acid to: N-acetyl *p*-aminobenzoic acid
1. Basal (stock diet)	30·5±7·4*	1·30±0·15*	1·82±0·12*	11·6±1·8*	0·87±0·13*	9·6±0·5*	64±13*
2. Basal+vitamin B_{12}	28·2±7·6	1·40±0·10	1·87±0·18	13·4±3·0	1·10±0·11	12·6±1·2	86± 8
3. Basal with iodinated casein	−8·5±2·3	0·82±0·02	0·97±0·06	33·1±1·4	0·43±0·10	4·8±0·3	32± 5
4. Basal with iodinated casein+vitamin B_{12}	−1·8±3·7	1·11±0·04	1·44±0·03	16·9±2·8	0·63±0·07	7·9±1·5	48± 5

* Standard error.

This work was supported by a grant from the Indian Council of Medical Research.

PREMA FATTERPAKER
URMILA MARFATIA
A. SREENIVASAN

Foods Section,
Department of Chemical Technology,
University of Bombay.
Feb. 24.

[1] DuToit, C. H., "Phosphorus Metabolism", 2, 597 (Johns Hopkins Press, 1952).
[2] Riggs, T. R., and Hegsted, D. M., *J. Biol. Chem.*, **172**, 539 (1948).
[3] Chantrenne, H., *J. Biol. Chem.*, **189**, 227 (1951).
[4] Grunert, R. R., and Phillips, P. H., *Arch. Biochem. and Biophys.*, **30**, 217 (1951).
[5] Mortensen, R. A., *J. Biol. Chem.*, **204**, 240 (1953).
[6] Kano, A. K., Anderson, J. A., Hougham, D. F., and Charkey, L. W., *Proc. Soc. Exp. Biol. and Med.*, **86**, 8 (1954).
[7] Ling, C. T., and Chow, B. F., *J. Biol. Chem.*, **206**, 797 (1954).
[8] Oginsky, E. L., Smith, P. H., Tonhazy, N. E., Umbreit, W. M., Lichstein, H. C., and Carson, S. F., *J. Bact.*, **61**, 581 (1951).