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THE VITAMIN B₁₂ GROUP OF FACTORS

BY

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Progress in the vitamin B₁₂ field has been so rapid that it seems already appropriate to supplement the review published in the *Journal* a year ago (Lester Smith, 1949). In particular, it has been found possible to obtain in crystalline form two further members of the vitamin B₁₂ group of factors—namely, vitamins B_{12c} and B_{12d}—as recently announced by workers in these laboratories (Anslow *et al.*, 1950). These factors have been submitted to extensive clinical trials, which form the subject of other papers in this issue.

Characteristics of the New Factors

These new factors were isolated from *Streptomyces griseus* fermentation liquors and were separated by partition chromatography. Vitamin B_{12c} can be distinguished from the other factors by its characteristic absorption spectrum and partition behaviour. The absorption spectrum of vitamin B_{12d} is identical with that of vitamin B_{12b}, but they can be separated chromatographically. In addition, vitamins B_{12c} and B_{12d} behave differently from the other factors in the microbiological plate assay technique; they tend to give fuzzy rings of bacterial growth, whereas vitamins B₁₂ and B_{12b} give sharp clear-cut rings.

An American team has shown that vitamin B_{12b} is probably identical with vitamin B_{12a}, the reduction product of vitamin B₁₂ (Brockman *et al.*, 1950). We have confirmed this observation (Anslow *et al.*, 1950). Thus it seems that the new factors are not conjugates of vitamin B₁₂ with peptides (like the folic-acid conjugates), but represent slight chemical modifications of the vitamin B₁₂ molecule.

Recent simultaneous publications from the Organon and Merck Laboratories describe a surprising new feature of the vitamin B₁₂ molecule. It contains a cyanide group co-ordinated with the cobalt atom. Vitamin B_{12b} differs only in the absence of this cyanide group, and is readily converted into vitamin B₁₂ by treatment with potassium cyanide (Veer *et al.*, 1950; Brink *et al.*, 1950b; and Kaczka *et al.*, 1950).

Further progress has been made in elucidating the chemical structures of vitamins B₁₂ and B_{12c} (see Buchanan *et al.*, 1950a, 1950b; Brink *et al.*, 1950a; Wolf *et al.*, 1950; Chargaff *et al.*, 1950). It is now possible to write a partial formula for vitamin B₁₂.

The latest advance was made by Todd and his colleagues at Cambridge; they isolated intact a large portion of the molecule comprising 5:6-dimethylbenziminazole in glycoside linkage with a molecule of ribose

phosphorylated at C₂ or C₃. The cobalt-containing moiety is attached to this phosphorus atom; it represents about two-thirds of the molecule, and its structure has not yet been elucidated. In addition, ammonia and two molecules of D_L-1-amino-2-propanol are attached somewhere.

Further light has been thrown on the problem of Castle's intrinsic and extrinsic factors. There is now little doubt that the extrinsic factor is simply vitamin B₁₂ itself. When administered orally to cases of pernicious anaemia the pure vitamin is ineffective, except in enormous doses, but it is strongly potentiated by normal

gastric juice (Berk *et al.*, 1948; Bethell *et al.*, 1949; Hall *et al.*, 1949; Campbell *et al.*, 1949). Ungley (1950) and Ternberg and Eakin (1949) have shown that vitamin B₁₂ combines with a factor in normal gastric juice—presumably the intrinsic factor—to yield a complex which is microbiologically inactive and non-dialysable. On autoclaving the complex is split, to yield a dialysable active substance, presumably regenerated vitamin B₁₂. These findings have been confirmed by Shaw (1950).

Activities of Vitamin B₁₂

There are indications that vitamin B₁₂ has a number of functions in living organisms. A growth-promoting action has been clearly demonstrated in pigs, chicks, and turkeys. There is also a suggestion that vitamin B₁₂ may in some instances augment the growth of backward children (Wetzel *et al.*, 1949). It has been shown that vitamin B₁₂ is concerned in transmethylation reactions. Thus chicks on a deficient diet can utilize

homocystine as an alternative to methionine only when vitamin B₁₂ is given (Jukes, Stokstad, and Broquist, 1950; see also Schaefer *et al.*, 1950). Dubnoff (1950) has shown that vitamin B₁₂ augments the reduction of S-S compounds in liver slices.

Jukes, Broquist, and Stokstad (1950) have put forward an ingenious scheme relating the activities in bacterial metabolism of vitamin B₁₂, certain deoxyribosides, folic acid, and "citrovorum factor" ("folinic acid"; see Bardos *et al.*, 1949).

Traina (1950a, 1950b) found that vitamin B₁₂ has an antihistamine and anti-anaphylactic effect, but we have failed to confirm the former (Sharpe, Woollett, and Cuthbertson, 1950).

Swendseid *et al.* (1947) showed that liver extract diminishes the excessive urinary excretion of phenols which occurs in pernicious anaemia. Abbott and James (1950) have confirmed this phenomenon with crystalline vitamin B₁₂. This may be connected with the suggested toxic factor of pernicious anaemia, production of which ceases on administration of vitamin B₁₂. In due time these observations will doubtless be welded together into a coherent theory of the mode of action of vitamin B₁₂, but as yet they provide little clue to the mechanism of its haemopoietic activity, beyond its suggested function in the building of nucleic acids.

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EFFECT OF VITAMIN B₁₂^c IN PERNICIOUS ANAEMIA AND SUBACUTE COMBINED DEGENERATION OF THE CORD

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The isolation in crystalline form of several factors related to vitamin B₁₂ is discussed by Lester Smith in the preceding paper, and differences between vitamin B₁₂, B₁₂^b, B₁₂^c, and B₁₂^d are defined. The work has a significant bearing upon the treatment of pernicious anaemia, if only because the widely used vitamin B₁₂ concentrates derived from bacterial cultures may contain more than one member of this group of red pigments. In the case of vitamin B₁₂ itself, quantitative methods have already been applied to dose-response relationships and to its effects in subacute combined degeneration of the cord. Here similar methods are applied to one of the new compounds with the object of determining its activity relative to vitamin B₁₂.

Methods

The methods adopted were those used for the study of vitamin B₁₂ (Ungley, 1949a, 1949b). Vitamin B₁₂^c was given intramuscularly as a single dose. Doses were graded logarithmically—10, 20, 40, 80, and 160 µg. Doses smaller than 10 µg. were not used, because in tests with vitamin B₁₂ they had given less predictable responses than larger doses. Four of the patients had a second test, making 28 tests in all. Conditions governing the inclusion of responses in patients who had received a small transfusion or who had relapsed after

TABLE I.—Initial Responses to Vitamin B₁₂^c

Case No.	Dosage in µg.	Retic. Response %	Red Blood Cell Counts (Millions/c.mm.) at Days Indicated						
			0	5	10	15	20	25	30
1	10	24.6	1.28	1.67	2.88	2.61			
2	10	31.2	1.37	1.47	3.09	3.81	2.58		
3	10	P.I.	1.64	1.79	2.85	2.38			
4	10	9.6	2.46	2.72	3.53	3.71	3.63		
2a	10	P.I.	2.58	2.97	3.30	4.11	3.89		
1a	10	"	2.61	2.57	3.74	3.71			
5	20	24.8	1.38	1.58	2.74	2.58	3.39	3.48	3.48
6	20	20.6	1.49	1.95	2.56	2.62	2.60	3.23	2.96
7	20	24.6	1.54	1.81	3.11	3.66	3.34		
8	20	23.2	1.75	1.96	2.54	2.96	3.61		
9	20	7.0	2.48	2.64	3.97	4.54	4.06		
10	40	19.6	1.29	1.63	2.43	3.08	2.89		
11	40	29.2	1.58	1.71	3.02	3.25	3.44	3.29	
12	40	24.8	2.13	2.26	3.11	3.75	3.80	3.64	
13	40	12.0	2.13	2.23	2.81	3.66	3.84	3.61	
10a	40	P.I.	2.89	4.09	3.99	4.11	4.08		
14	80	P.T.	0.94	1.09	3.07	2.97	3.19	2.87	
15	80	{ P.T. P.I. }	1.07	1.15	2.75	3.20	3.17		
16	80	24.8	1.24	1.58	2.90	3.27	3.47	4.24	3.99
17	80	26.0	1.27	1.46	2.84	2.68			
18	80	28.6	1.29	1.52	3.10	3.75	4.46	4.25	
19	80	16.6	1.79	1.96	3.01	3.90	3.76		
20	80	12.4	2.75	2.99	3.62	3.63			
21	160	P.T.	1.38	1.52	2.43	2.85	2.87		
22	160	31.2	1.42	2.19	3.02	4.08	4.45	4.82	5.10
23	160	27.4	1.70	1.54	3.10	3.53	3.99	3.84	
24	160	20.0	1.84	2.42	3.52	3.63	3.68	3.46	
21a	160	P.I.	2.87	—	—	4.87	5.05		

The highest count is in bold figures. P.I. = previous injection followed by relapse after period without treatment. P.T. = small transfusion before treatment.

Three counts were made a day before or after the day indicated; none of these was a 15-day count.