carbon dioxide plus soya-bean oil (ratio 2:1) grew poorly, and all died during the first half of the experimental period.

4. The animals fed on a mixture of PT 006 or PT 006 plus distillate–soya-bean oil (ratio 2:1) grew throughout the whole experimental period (36 weeks), although significantly more slowly than the controls. No neoplastic tissue was found at autopsy. Histological examination revealed pathological changes in the kidneys (abundance of calculi, hyaline casts, uncalcified degenerations of the tubules and necrosis of the papilla), liver (degeneration of the central zone of lobules) and the small intestine (hyperplastic chronic enteritis).

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Vitamin B_{12} and protein metabolism

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In the last few years numerous papers have linked vitamin B_{12} in a general way with the metabolism of proteins in different animals.

We reported some years ago (Henry & Kon, 1951) that addition of vitamin B_{12} increased the biological value of casein for the vitamin B_{12}-deficient rat. Indian authors (Marfatia & Sreenivasan, 1951; Baliga & Rajagopalan, 1954; Baliga, Balakrishnan & Rajagopalan, 1954) obtained similar increases for vegetable proteins with normal rats. There was, however, a growing indication of a more specific connexion between vitamin B_{12} and biosynthesis of the essential amino-acid methionine (see, for example, Schaeffer, Salmon & Strength, 1949; Oginsky, 1950) through its effect on the formation of methyl groups (see review by Arnstein, 1955). We have since investigated the bearing of these findings on our original observation, and the results are set out below.

EXPERIMENTAL

Plan of the experiments

Three groups of experiments were done and their general plan is summarized in Table 1.

Table 1. *Protein source and amount of protein in, and nitrogen content of, the experimental diets*

Exp. no.	Protein source: Description	Amount in diet (%)	N in diet (%)
	Experiments with vitamin B_{12}-deficient rats		
1	Casein*	9·0	1·212
2	Casein*	8·5	1·234
	Casein + 0·5 % DL-methionine*	8·5	1·235
3	Casein*	8·5	1·181
	Casein + 0·5 % DL-methionine*	8·5	1·181
5	Casein	8·6	1·175
	Casein + 1 % DL-methionine	8·6	1·146
	Casein + 1 % homocysteine + 0·5 % choline†	8·6	1·178
6	Casein	8·6	1·172
	Casein + 1 % DL-methionine	8·6	1·173
	Casein + 1 % homocysteine + 0·5 % choline	8·6	1·181
8	Casein	8·6	1·177
	Casein + 1 % DL-methionine	8·6	1·159
	Casein + 1 % homocysteine*	8·6	1·170
10	100 % extraction wheat flour*	63·7	1·398
	100 % extraction wheat flour + 0·5 % DL-methionine	63·7	1·395
11	100 % extraction wheat flour*	63·7	1·398
	100 % extraction wheat flour + 0·5 % DL-methionine	63·7	1·395
	100 % extraction wheat flour + 1 % L-lysine*	60·0	1·404
	100 % extraction wheat flour + 1 % L-lysine + 0·5 % DL-methionine*	60·0	1·393
	Experiments with normal rats		
4	Casein*	8·5	1·201
	Casein + 0·5 % DL-methionine*	8·5	1·202
7	Casein	8·6	1·211
	Casein + 1 % DL-methionine	8·6	1·121
	Casein + 1 % homocysteine + 0·5 % choline*	8·6	1·190
9	Casein	8·6	1·201
	Casein + 1 % DL-methionine	8·6	1·189
	Casein + 1 % homocysteine*	8·6	1·192
12	100 % extraction wheat flour*	61·4	1·370
	100 % extraction wheat flour + 0·5 % DL-methionine*	61·4	1·287
	100 % extraction wheat flour + 1 % L-lysine*	58·2	1·364
	100 % extraction wheat flour + 1 % L-lysine + 0·5 % DL-methionine*	58·2	1·274
13	Soya-bean meal*	14·8	1·211
	Soya-bean meal + 1·6 % DL-methionine*	14·8	1·213
14	Casein (8 % protein)*	8·6	1·343
	Casein (16 % protein)*	17·5	2·408

* One group of rats without vitamin B_{12}, another with 0·2 μg vitamin B_{12} daily.
† Rats received 0·2 μg vitamin B_{12} daily.

Effect of vitamin B_{12} on the biological value of casein

The effect of additions of vitamin B_{12} or methionine or both on the biological value of casein was studied with vitamin B_{12}-deficient (Exps. 1–3) and normal (Exp. 4) rats. Both kinds of rat were used also to test the possibility that vitamin B_{12} is involved in transmethylation processes, and for this purpose the effect of additions of choline and homocysteine to casein with or without vitamin B_{12} was studied (Exps. 5–7). Finally, to test the now accepted view that vitamin B_{12} is involved in the synthesis of methyl groups, the effect of the addition of vitamin B_{12} to casein supplemented with homocysteine was determined with deficient (Exp. 8) and with normal (Exp. 9) rats. In all these experiments protein constituted 8% of the diet.

Effect of vitamin B_{12} on the utilization of vegetable proteins

With deficient rats two experiments were done. In one (Exp. 10) the effect of vitamin B_{12} on the biological value of the proteins of 100% extraction wheat flour was compared with that of added methionine. In the other (Exp. 11) the protein-efficiency ratio of the same flour with the above supplements was determined, and, in addition, the effect of the vitamin on the protein-efficiency ratio of the flour with supplements of lysine or of lysine and methionine. Exp. 11 was repeated on normal rats (Exp. 12); an extra group of rats was included, for which the flour was supplemented simultaneously with methionine and vitamin B_{12}. A second experiment with normal rats (Exp. 13) dealt with the effect of vitamin B_{12} on the biological value of soya protein alone or supplemented with methionine.

In Exps. 10–13 the 8% protein level was again used.

Effect of vitamin B_{12} on the biological value of casein at 8% and at 16% levels of protein intake

Vitamin B_{12} is said to stimulate the growth of rats and mice on high-protein diets (Hartman, Dryden & Cary, 1949*a*, *b*; Bosshardt, Paul & Barnes, 1950). It has long been established that the biological value of proteins decreases as the concentration of protein in the diet increases (Mitchell, 1923–4*a*). The purpose of this experiment (Exp. 14) was to determine with normal rats whether vitamin B_{12} would counteract the depression in biological value associated with higher levels of protein.

Diets

Diets used for depleting rats of vitamin B_{12}

Two vitamin B_{12}-deficient diets were used: diet A was based on that described by Emerson (1949), diet B was diet RB 12S/4 of Cuthbertson & Thornton (1952). Table 2 gives their composition.

Experimental diets

The basal diet used in all experiments had the following percentage composition: rice starch 64, sugar 12, potato starch 10, margarine fat 10, salts (de Loureiro, 1931) 4. The protein source to be tested was added in place of an equal weight of rice starch;

sugar was omitted from the diets containing flour (Exps. 11 and 12). Table 1 gives the nitrogen content of the diets and the amount of the protein source replacing starch. In the experiments with vitamin B_{12}-deficient rats each animal was given daily, on a separate dish, 0·5 ml. of the mixture of water-soluble vitamins described by Campbell & Kosterlitz (1948). The normal rats were given 0·3 ml. of an aqueous extract of brewer's yeast (Osborne & Wakeman, 1919) equivalent to 0·3 g dried yeast. Vitamins A and D were supplied as two drops daily of cod-liver oil per rat.

Table 2. *Percentage composition of the vitamin B_{12}-deficient diets*

Component	Diet A*	Diet B†
Soya-bean meal	60	—
Soya flour (full fat)	—	72
Glucose	25·5	—
Lactose	—	22
Salts (de Loureiro, 1931)	4	4
Groundnut oil (semi-hardened)	10	—
Vitaminized arachis oil‡	—	2
Dried thyroid	0·5	—
Vitamin additions (mg/kg diet):		
Thiamine	10	30
Riboflavin	20	30
Pyridoxin	10	8
Calcium pantothenate	100	100
Nicotinic acid	100	100
Inositol	50	220
p-Aminobenzoic acid	300	75
Choline hydrochloride	1000	—
Biotin	0·5	0·2
Folic acid	2	1
Menaphthone	142	—

* Based on the diet of Emerson (1949).
† Diet RB 12S/4 of Cuthbertson & Thornton (1952).
‡ Vitamin A 2×10^5 i.u., vitamin D_2 10^5 i.u., α-tocopheryl acetate 14·2 g, menaphthone 100 mg and arachis oil to 1000 g.

In all metabolic experiments with depleted rats the ether-extracted whole egg used in the diet in the standardizing periods (see Mitchell & Carman, 1926) was from eggs of hens deficient in the animal protein factor (Black, Getty, Coates, Harrison & Kon, 1949).

Rats

Hooded Norwegian litter-mate rats of our own breeding were used. In accordance with our usual procedure, females were used in the balance experiments with normal animals. The experiments with vitamin B_{12}-deficient rats and the measurements of the protein-efficiency ratio were done with males.

Experimental procedure

Experiments with normal rats

Exps. 4, 7, 9, 13 *and* 14. The biological value and true digestibility of the test proteins were determined by the method of Mitchell (Mitchell, 1923–4*b*; Mitchell & Carman, 1926). Three litters of four young growing female rats were used in each experiment;

each rat received each diet in turn according to a Latin-square design. Full experimental details will be found in our earlier publications (Henry, Kon & Watson, 1937; Henry, Kon, Lea & White, 1948). In Exp. 9 the periods for the collection of excreta were reduced from 6 to 4 days on the advice of Drs A. P. de Groot and C. Engel of Utrecht (private communication), who found the procedure satisfactory. It had the advantage of appreciably reducing the length of the experiment.

Vitamin-free casein (Glaxo Laboratories Ltd) was used in Exp. 4 and vitamin-low casein (Genatosan Ltd) in Exps. 7, 9 and 14. Heated soya grits were used in Exp. 13.

Exp. 12. The protein-efficiency ratio of 100% extraction wheat flour with and without supplements (see Table 1) was determined by the growth method of Osborne, Mendel & Ferry (1919). Six litters of eight newly weaned male rats were used; further details were as given by Henry *et al.* (1948).

Experiments with vitamin B_{12}-deficient rats

Litters of male rats were weaned on to one of the vitamin B_{12}-deficient diets (Table 2) and kept on it for a minimum depletion period of 4 weeks. Diet A was used in Exps. 1 and 2 only. It contained dried thyroid, which caused some deaths among the animals. In all other experiments diet B was used. We have found in this laboratory (unpublished results) that rats vary markedly in their growth rate on vitamin B_{12}-deficient diets. When available, therefore, more litter-mates than finally required were weaned on to diets A or B, to make possible selection of the more uniform animals for the metabolic tests. Animals given vitamin B_{12} received 0·2 μg/day, as we found (unpublished results) that with this dose deficient rats grow satisfactorily. Our normal Latin-square design, in which all rats receive each diet in turn, could not be used in these experiments with diets with and without vitamin B_{12}. The arrangement of rats in each experiment and the necessary slight variations in experimental details between experiments are given below.

Exp. 1. Six pairs of male rats from four litters were used. The casein ('grain curd') was prepared in the laboratory by the method of Clark, Zoller, Dahlberg & Weimar (1920) and was shown by chick tests to be free from the animal protein factor.

Exp. 2. Six litters of four male rats were used, one rat from each litter being given one of the four diets (Table 1). DL-Methionine (0·5%) was added to the casein, as previous work on milk (Henry & Kon, 1953) and the data of Block & Mitchell (1946–7) led us to consider that this addition should cover the sulphur amino-acid deficiency of casein. Glaxo vitamin-free casein was used.

Exp. 3. Three litters of four male rats were used, and there were two experimental periods. One animal from each litter received one of the diets (Table 1) during the first experimental period. During the second the animal that had been given the casein diet now received the casein diet supplemented with methionine, and vice versa; similarly, the animals given the vitamin B_{12} supplement were changed over. Glaxo vitamin-free casein was again used, but was first extracted three times with 70% ethanol to remove any traces of vitamin B_{12}. As in Exp. 2, the casein was supplemented with 0·5% DL-methionine.

Exps. 5 *and* 6. Six litters of three male rats were used in each experiment. In both,

one rat from each litter was given the diet containing casein, casein + methionine or casein + homocysteine and choline. In Exp. 5 the last group received a further supplement of vitamin B_{12}. In these and subsequent experiments the methionine supplement to casein was increased to 1% as, contrary to expectation, the lower supplement did not significantly increase in Exps. 2 and 3 the biological value of the casein for the deficient rats (see Table 3). Genatosan low-vitamin casein was used.

Exp. 8. Six litters of four male rats and Genatosan low-vitamin casein were used. The experimental period was reduced to 4 days as in Exp. 9 (see p. 43).

Exp. 10. Six litters of three male rats were used for the tests with 100% extraction wheat flour.

Exp. 11. Five litters of seven male rats were used and the protein-efficiency ratio of 100% extraction wheat flour with or without various supplements (Table 2) was determined by the growth method of Osborne *et al.* (1919).

RESULTS

Effect of vitamin B_{12} on the biological value of casein

The results of these experiments (nos. 1–9) are given in Table 3. They show that with vitamin B_{12}-deficient rats the addition of the vitamin had no consistent effect on the biological value of casein. Thus, in Exp. 1 there was a significant increase, in Exp. 2 there was no effect and in Exp. 3 the increase was not statistically significant. Somewhat surprisingly the addition of 0·5% methionine to casein did not significantly increase its biological value for the deficient rats (Exps. 2 and 3), although the increase was significant for the normal rats (Exp. 4). In Exp. 3, but not in Exp. 2, the addition of the vitamin to casein supplemented with methionine significantly increased the biological value. For the normal rats (Exp. 4) the biological value of casein alone or with methionine was not affected by the addition of vitamin B_{12}. As already mentioned, in Exps. 5–7 the methionine addition to casein was increased to 1% and the biological value of casein was significantly increased, by some 12%, both with the normal and the deficient animals; a similar increase was observed for both groups when 1% homocysteine and 0·5% choline were added to casein with or without a supplement of vitamin B_{12}. In Exp. 8 (deficient rats) homocysteine had no effect on the biological value of casein unless vitamin B_{12} was given, when the increase did not differ significantly from that observed with the methionine supplement; for the normal rats a similar increase was obtained with homocysteine, whether alone or with vitamin B_{12} (Exp. 9); in this experiment the effect of the addition of 1% methionine was greater than in Exps. 5–8.

It will be noticed that the biological values obtained for the unsupplemented casein differed markedly from experiment to experiment. We periodically observe such differences between experiments, and consider that they are due to differences in response between different batches of rats. Relative values are, however, reproducible, and, for this reason we normally include a reference protein in all experiments. To facilitate comparisons between experiments, percentage differences from the biological value of casein caused by the various supplements are given in parentheses in Table 3.

Table 3. *Effect on the biological value and true digestibility of casein of added methionine, or homocysteine and choline or homocysteine alone, each with or without a supplement of 0·2 μg vitamin B_{12} daily. Experiments with vitamin B_{12}-deficient and normal rats*

		Vitamin B_{12}-deficient rats				Normal rats			
Addition to casein	Vitamin B_{12} supplement	Exp. no.	No. of rats	Biological value	True digestibility	Exp. no.	No. of rats	Biological value	True digestibility
None	−	1	6	83·0 (100)	99·9				
None	+		6	90·0 (108)	99·3				
			S.E. (3 d.f.)	± 1·51	± 0·50				
None	−	2 and 3	6	84·3 (100)*	100·0*	4	12	79·4 (100)	97·8
				77·7 (100)†	98·8†				
None	+		6	83·6 (99)*	100·0*		12	78·4 (99)	97·3
				82·4 (106)†	97·3†				
0·5 % DL-methionine	−		6	89·1 (106)*	99·9*		12	85·5 (108)	98·3
				81·5 (105)†	98·0†				
0·5 % DL-methionine	+		6	89·0 (106)*	100·0*		12	86·8 (109)	98·2
				89·5 (115)†	97·3†				
			S.E. (15 d.f.)	± 1·75*	± 0·04*		S.E. (18 d.f.)	± 1·17	± 0·43
				± 1·81†	± 0·48†				
None	−	5 and 6	6	84·9 (100)‡	98·7‡	7	12	76·7 (100)	98·8
				85·4 (100)§	99·1§				
1 % DL-methionine	−		6	95·1 (112)‡	99·7‡		12	85·5 (112)	99·6
				95·5 (112)§	99·5§				
1 % homocysteine, 0·5 % choline	−		6	—	—		12	85·5 (112)	99·3
				94·0 (110)§	99·7§				
1 % homocysteine, 0·5 % choline	+		6	94·3 (111)‡	99·9‡		12	84·3 (110)	99·3
			S.E. (10 d.f.)	± 1·10‡	± 0·39‡		S.E. (18 d.f.)	± 1·22	± 0·29
				± 1·79§	± 0·27§				
None	−	8	6	68·5 (100)	98·7	9	12	74·0 (100)	99·3
1 % DL-methionine	−		6	78·0 (114)	99·6		12	86·6 (117)	99·7
1 % homocysteine	−		6	70·2 (102)	99·9		12	81·8 (111)	99·7
1 % homocysteine	+		6	76·3 (111)	98·8		12	81·4 (110)	99·2
			S.E. (15 d.f.)	± 1·88	± 0·49		S.E. (18 d.f.)	± 1·09	± 0·34

Figures in parentheses are values referred to casein = 100.
* Exp. 2.
† Exp. 3.
‡ Exp. 5.
§ Exp. 6.

Table 4. *Effect on the biological value, true digestibility and protein-efficiency ratio of* 100% *extraction wheat flour or of soya-bean meal of added amino-acids, with and without a supplement of* 0·2 *μg vitamin* B_{12} *daily. Experiments with vitamin* B_{12}*-deficient and normal rats*

		Vitamin B_{12}-deficient rats					Normal rats				
Addition to protein source	Vitamin B_{12} supplement	Exp. no.	No. of rats		Biological value	True digestibility	Protein-efficiency ratio	Exp. no.	No. of rats	Biological value	True digestibility	Protein-efficiency ratio
					100% extraction wheat flour							
None	−	10 and 11	6*	5†	63·9	91·5	1·64	12	6	—	—	1·43
None	+		6*	5†	62·7	91·1	1·90		6	—	—	1·51
0·5% DL-methionine	−		6*	5†	64·4	91·0	1·72		6	—	—	1·56
0·5% DL-methionine	+		S.E. (10 d.f.) ± 1·54			± 1·03	—		6	—	—	1·54
1% L-lysine	−			5†	—	—	2·07		6	—	—	2·01
1% L-lysine	+			5†	—	—	2·44		6	—	—	2·12
1% L-lysine, 0·5% DL-methionine	−			5†	—	—	2·13		6	—	—	2·20
1% L-lysine, 0·5% DL-methionine	+			5†			2·22		6	—	—	2·33
						S.E. (22 d.f.) ± 0·07					S.E. (35 d.f.) ± 0·06	
					Soya-bean meal							
None	−		—	—	—	—	—	13	12	72·3	85·0	—
None	+		—	—	—	—	—		12	72·2	84·6	—
1·6% DL-methionine	−		—	—	—	—	—		12	81·7	86·3	—
1·6% DL-methionine	+		—	—	—	—	—		12	81·7	85·2	—
									S.E. (18 d.f.) ± 1·92		± 1·20	

* Exp. 10. † Exp. 11.

Effect of vitamin B_{12} on the utilization of vegetable proteins

The results of these experiments are given in Table 4. They show that for the vitamin B_{12}-deficient rats (Exps. 10 and 11) the biological value of 100% extraction wheat flour was not affected by supplements of methionine or of vitamin B_{12}. The protein-efficiency ratio of the flour, alone or supplemented with lysine, was, however, significantly increased when vitamin B_{12} was given. With normal rats (Exp. 12) vitamin B_{12} had no effect on the protein-efficiency ratio of the flour, either alone or supplemented with methionine or lysine or with both, but the addition of methionine caused a significant increase with the flour supplemented with lysine.

With normal rats (Exp. 13), the addition of vitamin B_{12} had no effect on the biological value of soya alone or supplemented with methionine. Methionine, as would be expected, caused a significant increase.

Effect of vitamin B_{12} on the biological value of casein at 8% and at 16% levels of protein intake

The results of this experiment (Exp. 14) are shown in Table 5. Vitamin B_{12} had no effect on the biological value of casein at either level of intake.

Table 5. *Biological value and true digestibility of casein at 8% and 16% levels of protein intake with or without a supplement of 0·2 μg vitamin B_{12} daily. Experiment with normal rats*

Exp. no.	No. of rats	Level of casein	Vitamin B_{12} supplement	Biological value	True digestibility
14	12	8% protein	−	79·8	99·1
	12	8% protein	+	82·2	99·2
	12	16% protein	−	65·6	98·8
	12	16% protein	+	64·4	98·3
			S.E. (18 d.f.)	±1·22	±0·33

Vitamin B_{12} and true digestibility of protein

In none of the metabolic experiments (1–10, 13 and 14) was the true digestibility altered by the addition of vitamin B_{12}.

DISCUSSION

Our most significant finding was that homocysteine increased the biological value of casein in the presence of vitamin B_{12}, but not in its absence (Exps. 8 and 9). With the vitamin 1% homocysteine was as effective as 1% methionine, showing that, in the presence of a suitable acceptor, vitamin B_{12} is involved in the synthesis of methyl groups. Our quantitative study of protein metabolism in rats thus provides further support to the earlier evidence of this specific function of vitamin B_{12}, which has been demonstrated in growth experiments (Stekol & Weiss, 1950; Jukes, Stokstad & Broquist, 1950; Young, Norris & Heuser, 1954; Chang & Johnson, 1955; Johnson, Firth & Mistry, 1955), with radioactive tracers (Stekol, Weiss & Weiss, 1952;

Arnstein & Neuberger, 1953, Arnstein, 1955) and microbiologically (Johnson, Holdsworth, Ford, Porter & Kon, 1955).

It is now generally agreed (Arnstein & Neuberger, 1953; Firth, Mistry, James & Johnson, 1954; Mistry, Vadopalaite, Chang, Firth & Johnson, 1955; Stekol, Weiss, Smith & Weiss, 1953; Young *et al.* 1954) that vitamin B_{12} plays no part in transmethylation. The results of Exps. 5–7 support this view. They show that addition of 1% homocysteine and 0·5% choline increased the biological value of casein for the deficient rat to the same extent as 1% methionine. Hence vitamin B_{12} is not necessary for the transfer of methyl groups from choline to homocysteine.

In Exp. 1, already briefly reported (Henry & Kon, 1951), the biological value of casein was significantly improved by a supplement of vitamin B_{12}, but we could not confirm this finding in two experiments with deficient rats (Exps. 2 and 3). We must conclude, therefore, in agreement with our other findings and with present views, that vitamin B_{12} is not involved in protein utilization as such.

It has been mentioned (p. 44) that in Exps. 2 and 3 the increase in the biological value of casein caused by the addition of 0·5% methionine was significant for normal rats (Exp. 4), but not for the deficient rats. It is not possible to say whether this finding indicates a slightly higher methionine requirement by the vitamin B_{12}-deficient rat or whether it is due to differences in response between batches of rats (cf. p. 44). The latter is perhaps the more likely explanation, as with normal rats a greater increase in the biological value of casein resulted from addition of 1% methionine in Exp. 9 than in Exp. 7.

The remaining experiments described in this paper further demonstrate that vitamin B_{12} does not specifically improve the utilization of proteins (cf. Henry & Kon, 1951). Exps. 10–12 (Table 4) were done to test its effect on the utilization of a protein deficient in lysine, namely that of 100% extraction wheat flour. In these experiments the protein-efficiency ratio of flour, alone or supplemented with lysine, was significantly improved by vitamin B_{12} (Exp. 11), but this apparent increase was not confirmed in a balance experiment done at the same time (Exp. 10). As the protein-efficiency ratio expresses the gain in weight per unit protein ingested, its significance hinges on the composition of the weight gain. Several authors (Black & Bratzler, 1952; Knoebel & Black, 1952; Ling & Chow, 1952) have shown that deficient rats given vitamin B_{12} gain weight largely by deposition of fat, probably because of increased synthesis of choline (cf. Arnstein, 1955). It is therefore probable that the effect of vitamin B_{12} on the protein-efficiency ratio in Exp. 11 was spurious and should be discounted. In the experiment with normal rats (Exp. 12), when vitamin B_{12} would not be expected to stimulate growth, the protein-efficiency ratio of flour, whether alone or supplemented with amino-acids, was not improved by its further addition. In this experiment methionine significantly increased the protein-efficiency ratio of the flour supplemented with lysine, but not that of the unsupplemented flour. This finding indicates that, as one would expect, methionine, which with valine is the secondary limiting amino-acid in flour, only becomes an effective supplement when the primary lysine deficiency has been at least partly overcome.

We can sum up the results of these fourteen experiments as providing further

evidence that vitamin B_{12} is involved in the synthesis of methyl groups, but that it does not play a part in transmethylation. There is no proof that it is in any other way involved in protein metabolism.

SUMMARY

1. The effect of vitamin B_{12} on protein metabolism has been studied in a series of experiments with vitamin B_{12}-deficient and normal rats.

2. For deficient rats the biological value of casein was increased to the same extent by the addition of 1% methionine as by the addition of 1% homocysteine and a vitamin B_{12} supplement. Homocysteine was ineffective in the absence of the vitamin. It is concluded that vitamin B_{12} is involved in the synthesis of methyl groups.

3. With deficient rats the addition of 1% homocysteine, together with 0·5% choline, in the presence or absence of vitamin B_{12}, increased the biological value of casein to the same extent as 1% methionine. This finding demonstrates that the vitamin plays no part in transmethylation.

4. In two out of three experiments the addition of vitamin B_{12} had no effect on the biological value of casein determined on deficient rats.

5. For deficient rats vitamin B_{12} did not increase the biological value of the protein in wheat flour of 100% extraction. The protein-efficiency ratio of the flour alone, or supplemented with lysine or with both lysine and methionine, was increased by the addition of vitamin B_{12}; it is considered that this finding may be spurious, as the method hinges on the composition of the weight gain in relation to protein intake.

6. With normal rats the addition of vitamin B_{12} had no effect on the protein-efficiency ratio of 100% extraction flour alone or supplemented with lysine or methionine or both.

7. With normal rats vitamin B_{12} did not increase the biological value of soya or of casein at a high (16%) level of protein intake.

8. It is concluded that vitamin B_{12} plays a part in the synthesis of methyl groups, but that it neither functions in transmethylation nor is involved in protein utilization as such.

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