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# The experimental production of vitamin $B_{12}$ deficiency in the baboon (*Papio cynocephalus*). A 2-year study

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1. The development of vitamin $B_{12}$ deficiency, as indicated by the serum and liver vitamin $B_{12}$ levels and the excretion of methylmalonic acid, was studied over a 2-year period in baboons (*Papio cynocephalus*) given a diet deficient in vitamin $B_{12}$. The effects of partial hepatectomy and the inclusion of either ampicillin or sodium propionate in the diet on the rate of development of the deficiency were also studied.

2. The baboons had previously been fed on a mainly vegetarian diet. Their serum vitamin $B_{12}$ levels were less than 100 ng/l and the mean liver vitamin $B_{12}$ concentration was 0·56 $\mu$g/g. Similar serum and liver vitamin $B_{12}$ levels were found in baboons given a purified diet supplemented with 1 $\mu$g vitamin $B_{12}$/d, and marked increases in the serum and liver vitamin $B_{12}$ levels occurred when the daily intake was increased to 2 $\mu$g.

3. The serum vitamin $B_{12}$ levels decreased to less than 20 ng/l in all baboons given a vitamin $B_{12}$-deficient diet.

4. The liver vitamin $B_{12}$ concentration also decreased in all baboons given a deficient diet. At 9 months the lowest levels (0·20 $\mu$g/g) were found in partially hepatectomized baboons but subsequently baboons given the diet containing ampicillin had the lowest levels (0·11 $\mu$g/g).

5. The excretion of methylmalonic acid after a valine load was found to be inversely related to the liver vitamin $B_{12}$ concentration. In the early part of the study, partially hepatectomized baboons excreted the highest amount but subsequently baboons given a diet containing ampicillin excreted the highest amount.

6. Increased formiminoglutamic acid excretion after a histidine load was observed in two baboons given a vitamin $B_{12}$-deficient diet and in both baboons the liver folic acid concentration was low.

7. No haematological or neurological symptoms of the vitamin $B_{12}$ deficiencywere observed.

In man, a deficiency of vitamin $B_{12}$ most commonly occurs as a result of impaired absorption, e.g. in pernicious anaemia, gastrectomy, or ileal resection. The deficiency is characterized by either megaloblastic anaemia or subacute combined degeneration of the spinal cord, or both. The development of these symptoms as a result of inadequate dietary intake of the vitamin is much rarer, although cases have been reported (Smith, 1962; Stewart, Roberts & Hoffbrand, 1970).

Biochemically vitamin $B_{12}$ is known to function as a coenzyme in only two reactions in mammals. As 5′-deoxyadenosyl-cyanocobalamin it is required for the isomerization of methylmalonyl-CoA to succinyl-CoA (Gurnani, Mistry & Johnson, 1960) and as methyl-cyanocobalamin it is required for the methylation of homocysteine to form methionine (Sakami & Ukstins, 1961; Buchanan, Elford, Loughlin, McDougall & Rosenthal, 1964). Theories have been proposed as to how a disturbance of these reactions might result in the haematological (Herbert & Zalusky, 1962) and neurological changes (Cardinale, Carty & Abeles, 1970) seen in man, but conclusive proof is still awaited. Perhaps the most likely way of obtaining such evidence is by a comparative

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Table 1. *Composition of vitamin $B_{12}$-deficient diets given to baboons (g/baboon per d)*

| | Basal diet | Sodium propionate-containing diet |
|---|---|---|
| Starch | 110 | 110 |
| Sucrose | 50 | 50 |
| Vitamin-free casein | 25 | 50 |
| Salt mixture* | 10 | 10 |
| Cellulose | 8 | 8 |
| Maize oil | 25 ml | 3 ml |
| Vitamin supplement | † | † |
| Sodium propionate | — | 10 |
| α-Tocopheryl acetate | — | 60 mg |

* Composed of (g): 335 NaCl; 150 $CaHPO_4$; 1033 $KH_2PO_4$; 600 $CaCO_3$; 20 $MgSO_4.7H_2O$; 18·25 MgO; 22·8 $FeSO_4.7H_2O$; 1·6 KI; 0·7 $MnSO_4.4H_2O$; 0·5 $ZnCl_2$; 0·6 $CuSO_4.5H_2O$.

† Each baboon received (mg/d): thiamin 1; calcium pantothenate 3; nicotinic acid 5; ascorbic acid 25; choline chloride 25; *myo*-inositol 100; *p*-aminobenzoic acid 100; pyridoxine 2; riboflavin 2; menaphthone 0·2; biotin 0·1; pteroylmonoglutamic acid 1; retinol 1; ergocalciferol 0·008.

biochemical study of a vitamin $B_{12}$-deficient experimental animal and its normal counterpart. Unfortunately it has been found that the production of the deficiency is difficult and, in animals in which a deficiency has been produced, the haematological changes seen in man have not been observed (reviewed by Stokstad, 1968).

In the present study it was found that the feeding of a vitamin $B_{12}$-deficient diet to the baboon resulted in the development of a subclinical deficiency of the vitamin without producing any clinical signs. Possible reasons for these findings are discussed.

## EXPERIMENTAL

### *Animals and treatments*

Fifteen sub-adult male baboons (*Papio cynocephalus*), which had been captured in Kenya and subsequently given a diet of fresh fruit and vegetables supplemented with high-protein biscuits, were used. They were housed individually in cages with wire floors that allowed almost all excreta to fall out of reach, and were given free access to tap-water. The baboons were divided at random into five groups each of three animals. Three control baboons were given the basal diet (Table 1) supplemented with vitamin $B_{12}$ at the rate of 1 μg/baboon per d for the first 9 months and thereafter 2 μg/d. The remaining twelve baboons were given vitamin $B_{12}$-deficient diets and divided into four groups as follows: (1) three baboons were given the basal diet, (2) three baboons were given the basal diet, but at the beginning of the study approximately 70% of the total liver mass was removed, (3) three baboons were given the basal diet with the addition of 50 mg ampicillin (Penbritin, Beecham Research Laboratories, Brentford, England) per kg diet, (4) three baboons were given a diet low in fat and containing sodium propionate (Table 1). The protein content of this diet was increased both to partly compensate for the lower energy content and also to raise the requirement for vitamin $B_{12}$. Liver biopsy specimens were taken at intervals from animals anaesthetized with diethyl ether.

### *Analytical methods*

*Vitamin assays.* Vitamin $B_{12}$ was measured microbiologically with *Lactobacillus leichmannii* (Skeggs, Nepple, Valentik, Huff & Wright, 1950; Coates, Ford, Harrison, Kon & Porter, 1953; Spray, 1955) and folic acid with *Lactobacillus casei* (Waters & Mollin, 1961; Chanarin, Hutchinson, McLean & Moule, 1966).

*Methylmalonic acid estimation.* The concentration of methylmalonic acid in 24 h urine samples collected into 10 ml concentrated HCl was measured by the semi-quantitative method of Gutteridge & Wright (1970). By extracting the urine with diethyl ether for 7 h using a downward-displacement liquid–liquid extractor, it was found that saturation of the urine with ammonium sulphate was unnecessary. The method of Williams, Spray, Newman & O'Brien (1969) was tried but proved unsatisfactory due to the presence of interfering compounds in the urine.

*Formiminoglutamic acid estimation.* The concentration of formiminoglutamic acid in urine samples collected into 15 ml 1 M-HCl was determined by the method of Chanarin & Bennett (1962). Increased levels were verified electrophoretically (Kohn, Mollin & Rosenbach, 1961).

*Haematology.* Blood was obtained from the femoral vein and all counts were done on a Coulter Counter model ZBI except for that of the platelets, which were counted manually. Bone-marrow aspirates were obtained from the sternum and stained with either May–Grunwald–Giemsa or Leishman–Giemsa stain.

### *Statistical analysis*

All the values for the baboons given the vitamin $B_{12}$-supplemented diet and the results for liver vitamin $B_{12}$ concentrations in the four vitamin $B_{12}$-deficient groups are presented as group means. Analysis of variance of the values for weight gain, serum vitamin $B_{12}$ and methylmalonic acid excretion showed that the differences between the four vitamin $B_{12}$-deficient groups were either not significant or only achieved significance at a single time. Values for these groups were averaged, and the *mean* for all vitamin $B_{12}$-deficient baboons is given.

From about 9 months the variation between animals in the vitamin $B_{12}$-supplemented group was not homogeneous with the variation between animals in the vitamin $B_{12}$-deficient groups for the serum and liver vitamin $B_{12}$ and methylmalonic acid excretion. In these instances, means for the vitamin $B_{12}$-supplemented group are given with individual standard errors with two degrees of freedom. At times there was also evidence of heterogeneity of the variations between animals within the vitamin $B_{12}$-deficient groups for serum and liver vitamin $B_{12}$ and methylmalonic acid excretion, but there was no consistent trend in its occurrence. Except for the standard errors for the vitamin $B_{12}$-supplemented group referred to above, the other standard errors were derived from the pooled variation between animals within groups with ten degrees of freedom if the vitamin $B_{12}$-supplemented group was included and eight degrees of freedom if the vitamin $B_{12}$-supplemented group was not included.

Table 2. *Increase in body-weight (kg) of baboons given either a vitamin $B_{12}$-deficient or a vitamin $B_{12}$-supplemented diet*

(Mean values for one group of three baboons given a vitamin $B_{12}$-supplemented diet and for four groups of three baboons given a vitamin $B_{12}$-deficient diet (see p. 220). The initial body-weights, mean values with their standard errors, were (kg): vitamin $B_{12}$-supplemented, 7·50±0·75; vitamin $B_{12}$-deficient group 1, 7·50±0·90; group 2, 7·83±1·30; group 3 8·08±0·58; group 4, 7·75±1·38)

| | Period (months) | | | |
|---|---|---|---|---|
| | 0–6 | 0–12 | 0–18 | 0–24 |
| Vitamin $B_{12}$-supplemented | 2·38 | 4·82 | 7·87 | 9·28 |
| Vitamin $B_{12}$-deficient* | 2·09 | 4·51 | 6·25 | 7·14† |
| SE of difference (10 df) | 0·527 | 0·776 | 1·034 | 0·852 |

* Differences between the four vitamin $B_{12}$-deficient groups were not significant.
† Significance of difference of mean value from that of vitamin $B_{12}$-supplemented group, $P < 0·05$.

Table 3. *Haematological results for twelve baboons given a vitamin $B_{12}$-deficient and three baboons given a vitamin $B_{12}$-supplemented diet for 2 years*

(Mean values with their standard errors)

| | Vitamin $B_{12}$-deficient | | Vitamin $B_{12}$-supplemented | |
|---|---|---|---|---|
| | Mean | SE | Mean | SE |
| Red cell count ($10^{12}$/l) | 5·43 | 0·14 | 5·13 | 0·24 |
| Haemoglobin (g/l) | 147 | 4·6 | 144 | 4·6 |
| Packed cell volume | 0·44 | 0·01 | 0·42 | 0·02 |
| Mean corpuscular volume (fl) | 81 | 1·4 | 82 | 0·6 |
| Leucocytes ($10^9$/l) | 8·61 | 1·22 | 7·33 | 0·35 |
| Platelets ($10^9$/l) | 323 | 78·5 | 314 | 2·9 |

## RESULTS

*Clinical signs.* The baboons given the vitamin $B_{12}$-deficient diets remained healthy and active throughout the 2-year study. During the 1st year the mean weight gain of the baboons given the vitamin $B_{12}$-deficient diets was similar to that of the vitamin $B_{12}$-supplemented baboons, but decreased significantly during the 2nd year (Table 2). No significant differences in rate of weight gain were found between the four groups of baboons given the vitamin $B_{12}$-deficient diets, although the mean weight gain of the partially hepatectomized baboons and the baboons given the diet containing sodium propionate tended to be lower than that of the baboons given the basal diet and the baboons given the diet containing ampicillin. Haematological investigations were carried out at regular intervals and throughout the study the values in the baboons given the vitamin $B_{12}$-deficient diets were found to be very similar to those in the baboons given the supplemented diet. Table 3 shows values obtained at the end of the study. Also there were no apparent differences in the bone marrow morphology of the deficient and supplemented animals. Detailed neurological studies were not done, but there were no physical signs of impaired neurological function in any of the baboons.

*Serum vitamin $B_{12}$ levels.* Before the start of the study the baboons had been given

Table 4. *Serum vitamin $B_{12}$ levels (ng/l) in baboons given either a vitamin $B_{12}$-deficient or a vitamin $B_{12}$-supplemented diet*

(Mean values with their standard errors for one group of three baboons given a vitamin $B_{12}$-supplemented diet and for four groups of three baboons given vitamin $B_{12}$-deficient diets, see p. 220)

| | Time (months) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 6 | 9 | 12 | 18 | 24 |
| Vitamin $B_{12}$-supplemented | | 61 | 73 | 162 ± 27 (2 df) | 219 ± 40 (2 df) | 264 ± 67 (2 df) |
| Vitamin $B_{12}$-deficient† | 67 ± 5‡ (10 df) | 41* | 25*** | 22 ± 1*§ (8 df) | 17 ± 2* (8 df) | 15 ± 2 (8 df) |
| SE of difference (10 df) | — | 7·4 | 6·2 | — | — | — |

Significance of difference of mean value from that of vitamin $B_{12}$-supplemented group: * $P < 0.05$; *** $P < 0.001$.

† Differences between the four vitamin $B_{12}$-deficient groups were not significant except at 12 months.

‡ Differences between the groups were not significant, see p. 221.

§ The mean level in the vitamin $B_{12}$-deficient group 1 (14 ng/l) was less than the mean of the other three vitamin $B_{12}$-deficient groups (25 ng/l) ($P < 0.01$).

Table 5. *Liver vitamin $B_{12}$ concentrations ($\mu$g/g) in baboons given either a vitamin $B_{12}$-deficient or a vitamin $B_{12}$-supplemented diet*

(Mean values with their standard errors for one group of three baboons given a vitamin $B_{12}$-supplemented diet and four groups of three baboons given vitamin $B_{12}$-deficient diets, see p. 220)

| | Time (months) | | |
|---|---|---|---|
| | 9 | 18 | 24 |
| Vitamin $B_{12}$-supplemented | 0·51 | 1·07 ± 0·20 (2 df) | 0·96 ± 0·12 (2 df) |
| Vitamin $B_{12}$-deficient | | | |
| 1 | 0·29*** | 0·18** | 0·19** |
| 2 | 0·20*** | 0·18** | 0·20** |
| 3 | 0·26*** | 0·13** | 0·11** |
| 4 | 0·29*** | 0·21** | 0·20** |
| SE of group mean (10 df) | 0·022 | — | — |
| SE of a group mean for the vitamin $B_{12}$-deficient groups (8 df) | — | 0·022 | 0·017 |

Significance of difference of mean value from that of vitamin $B_{12}$-supplemented group: ** $P < 0.01$, *** $P < 0.001$.

a mainly vegetarian diet and as a result the initial serum vitamin $B_{12}$ levels were low (< 100 ng/l). The levels decreased in all baboons given a vitamin $B_{12}$-deficient diet and in general the different treatments had no effect on the rate of decrease (Table 4). In the baboons given the vitamin $B_{12}$-supplemented diet the levels remained fairly constant for the 1st 9 months during which time each baboon was receiving 1 $\mu$g vitamin $B_{12}$/d. Subsequently the daily intake per baboon was increased to 2 $\mu$g and this resulted in a marked rise in the serum levels.

*Liver vitamin $B_{12}$ levels.* The initial liver vitamin $B_{12}$ concentration was not measured. However, in other baboons given the mainly vegetarian diet the liver vitamin $B_{12}$

Table 6. *Methylmalonic acid excretion (mg/24 h) after a valine load in baboons given either a vitamin $B_{12}$-deficient or a vitamin $B_{12}$-supplemented diet*

(Mean values for one group of three baboons given a vitamin $B_{12}$-supplemented diet and for four groups of three baboons given vitamin $B_{12}$-deficient diets, see p. 220)

| | | Time (months) | | | | |
|---|---|---|---|---|---|---|
| | 4 | 9 | 12 | 18 | 24 | 24† |
| Vitamin $B_{12}$-supplemented | 2 | 1 ± 0·3 (2 df) | 1 ± 0 (2 df) | 1 ± 0·3 (2 df) | 0 ± 0 (2 df) | 0·3 ± 0·3 (2 df) |
| Vitamin $B_{12}$-deficient‡ | 4 | 16 ± 1·7*** (8 df) | 36 ± 4·1*** (8 df) | 58 ± 8·5*** (8 df) | 70 ± 9·1*** (8 df) | 11 ± 2·0*** (8 df) |
| SE of difference (10 df) | 2·0 | — | — | — | — | — |

Significance of difference of mean value from that of vitamin $B_{12}$-supplemented group: ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

† No valine load.

‡ Differences between the means for four vitamin $B_{12}$-deficient groups were not significant except at 24 months when group 3 (127 mg/24 h, SE 26·7) was significantly ($P < 0{\cdot}05$) higher than group 1 (45 mg/24h, SE 13·2), group 2 (57 mg/24 h, SE 8·8) and group 4 (52 mg/24 h, SE 19·2).

concentration was found to range from 0·39 to 0·79 μg/g with a mean of 0·56 μg/g. If this figure is assumed to be representative of the initial levels in the baboons used in this study, then, as the results in Table 5 show, giving a vitamin $B_{12}$-deficient diet for 9 months caused a decrease in the liver vitamin $B_{12}$ concentration of approximately 50%. At this time the lowest levels (0·20 μg/g) were found in the partially hepatectomized baboons. However, there was no further decrease in the levels in these baboons during the remainder of the study. Similarly, the levels in the baboons given the basal diet and the diet containing sodium propionate decreased to approximately 0·20 μg/g and thereafter remained constant. Only in the baboons given the diet containing ampicillin did the levels show a continual decrease and at the end of the study the levels in these baboons were significantly lower than in the other vitamin $B_{12}$-deficient groups. The liver vitamin $B_{12}$ concentration of the three vitamin $B_{12}$-supplemented baboons took a similar course to that of the serum levels. Thus, after 9 months, during which time each animal received 1 μg vitamin $B_{12}$/d, the levels were similar to those found in baboons given a mainly vegetarian diet, but they increased twofold when the daily vitamin $B_{12}$ intake was increased to 2 μg.

*Methylmalonic acid excretion.* The results in Table 6 show the amount of methylmalonic acid excreted after a loading dose of valine. At 4, 9 and 12 months, 10 g DL-valine was given orally but, owing to difficulties encountered in ensuring that the dose was taken, the results at 18 and 24 months were obtained after giving 5 g L-valine intraperitoneally. In general, the excretion of methylmalonic acid tended to reflect the liver vitamin $B_{12}$ concentration. The amount excreted by the vitamin $B_{12}$-supplemented baboons remained very low throughout, whereas in the vitamin $B_{12}$-deficient baboons, it increased with time. At 9 months the partially hepatectomized baboons excreted the highest amount (23 mg/24 h, SE 1·5) but at 24 months the baboons given the diet containing ampicillin excreted significantly more than those in the other groups. Under normal dietary conditions, i.e. without a valine load, the amount

excreted was much lower and the baboons receiving the diet containing sodium propionate excreted the highest amount (mean value, 18 mg/24 h, SE 6·5).

*Liver folic acid levels and formiminoglutamic acid excretion.* At the end of the study the folic acid concentration in the liver and the excretion of formiminoglutamic acid in 24 h urine samples collected after giving 5 g L-histidine intraperitoneally were measured. In the three baboons given the vitamin $B_{12}$-supplemented diet the mean liver folate concentration was 7·5 $\mu$g/g, SE 1·40 and the mean formiminoglutamic acid excretion was 24 mg/24 h, SE 5·2. Similar liver folate levels and formiminoglutamic acid excretion were found in ten of the twelve baboons given the vitamin $B_{12}$-deficient diets. However, one of the baboons in group 1 had a liver folate concentration of 2·5 $\mu$g/g and excreted 587 mg formiminoglutamic acid and one of the baboons in group 3 had a liver folate concentration of 3·5 $\mu$g/g and excreted 550 mg formiminoglutamic acid.

## DISCUSSION

A decreased growth rate is probably the only feature common to all experimental animals fed on a vitamin $B_{12}$-deficient diet (Coates, 1968). A decreased growth rate was also found in the present study although it only occurred during the 2nd year. Since the lowest liver vitamin $B_{12}$ levels were found in baboons given a diet containing ampicillin, it might be expected that the growth rate of these animals would be the lowest. Many workers have shown, however, that the feeding of antibiotics increases growth rate and this probably offsets to some extent the effect of the deficiency. Also the lower weight gain of the baboons given the diet containing sodium propionate was probably due in part to the lower energy content of this diet and also to the growth-retarding effect of sodium propionate (Hogue & Elliot, 1964). No other clinical signs of the deficiency were observed. None of the baboons became anaemic and, although detailed neurological studies were not undertaken and the possibility of histopathological changes in the spinal cord or peripheral nerves cannot be ruled out, hind leg paralysis of the kind described by Oxnard & Smith (1966) in monkeys and attributed to a deficiency of vitamin $B_{12}$ was not observed. The feeding of a vitamin $B_{12}$-deficient diet did, however, result in the development of a subclinical deficiency as shown by the low serum and liver vitamin $B_{12}$ levels and the increased excretion of methylmalonic acid.

Measurement of the serum concentration of vitamin $B_{12}$ provides a useful index of vitamin $B_{12}$ status. In normal healthy people the concentrations range from 110 to more than 1000 ng/l with a mean of about 450 ng/l (Halstead, Carroll & Rubert, 1959; Mathews, 1962). In untreated pernicious anaemia the levels are low and, in general, values below 160 ng/l are considered suggestive of vitamin $B_{12}$ deficiency. On this basis, baboons given a vitamin $B_{12}$-deficient diet, a vegetarian diet, or a diet supplemented with 1 $\mu$g vitamin $B_{12}$/d have serum levels indicative of vitamin $B_{12}$ deficiency. It would appear, though, that on a comparable dietary intake the levels in the baboon tend to be lower than those in man. Thus the levels in the baboons given a vitamin $B_{12}$-deficient diet were low even when compared with those in untreated pernicious anaemia, and the levels in baboons given a mainly vegetarian diet tended to be lower than the reported values for humans taking a similar diet (Banerjee

& Chatterjea, 1960; Mehta, Rege & Satoskar, 1964; Ellis & Montegriffo, 1970). Stewart *et al.* (1970) found that in a patient suffering from a dietary deficiency of vitamin $B_{12}$ the feeding of 1 $\mu$g vitamin $B_{12}$/d increased the serum levels from 40 to 164 ng/l, whereas in baboons given 1 $\mu$g/d the serum levels were below 100 ng/l and even on 2 $\mu$g/d the levels were below normal by human standards. The low levels found in the baboons are unlikely to be due to experimental technique since the values we obtained with human serums were similar to those reported by other workers.

As the liver is the main storage organ for vitamin $B_{12}$, assay of the liver vitamin $B_{12}$ concentration provides a direct measure of vitamin $B_{12}$ status. In man the normal range is from 0·6 to 1·5 $\mu$g/g wet tissue (Joske, 1963). Levels within this range were found in baboons receiving 2 $\mu$g vitamin $B_{12}$/d but the levels were slightly lower in baboons given a vegetarian diet or a diet supplemented with 1 $\mu$g vitamin $B_{12}$/d. In baboons given a vitamin $B_{12}$-deficient diet the levels decreased and initially the decrease was most rapid in partially hepatectomized baboons. In most of the deficient baboons the levels decreased to approximately 0·20 $\mu$g/g and thereafter remained constant. This was most apparent in the partially hepatectomized baboons in which there was no further decrease after 9 months although in these animals this might in part be due to a redistribution of vitamin $B_{12}$ among the tissues. The lowest levels were found in baboons given the diet containing ampicillin, their liver concentration of vitamin $B_{12}$ was similar to that found in patients with untreated pernicious anaemia. The possibility that these low levels were due to the presence of ampicillin, an inhibitor of the growth of *L. leichmannii* (Powell, Thomas, Mandal & Dignam, 1969), in the liver homogenates was discounted by finding that the recovery of vitamin $B_{12}$ added to the homogenates was between 90 and 109% and that assays at different dilutions gave similar results.

An increased excretion of methylmalonic acid occurs in vitamin $B_{12}$ deficiency due to a reduction in the rate of the vitamin $B_{12}$-dependent conversion of methylmalonyl-CoA to succinyl-CoA. Gompertz, Jones & Knowles (1967) showed that a loading dose of valine increased the amount excreted by deficient patients but had no effect in controls. This test is relatively specific for vitamin $B_{12}$ deficiency, for the only other known condition in which increased quantities of methylmalonic acid are excreted is a rare inborn error of metabolism in which the ability to synthesize either the coenzyme form of vitamin $B_{12}$ or the isomerase enzyme is lacking (Scriver, 1970). In the present study, the amount of methylmalonic acid excreted was inversely related to the liver vitamin $B_{12}$ concentration. In baboons given a vitamin $B_{12}$-supplemented diet the amount excreted remained low, whereas in baboons given a deficient diet there was an increase with time. At the end of 2 years the highest amount was excreted by the ampicillin-fed baboons, thus confirming the finding of lower liver vitamin $B_{12}$ levels in these baboons.

The observation that the vitamin $B_{12}$ deficiency was more severe in baboons given a diet containing ampicillin suggests that the intestinal flora may play a part in the vitamin $B_{12}$ nutrition of the baboon. Many of the intestinal micro-organisms are known to produce vitamin $B_{12}$ and the amount they produce is in excess of the host's requirements (Mickelsen, 1956). However, in non-ruminants most if not all of the

bacterial synthesis occurs in the large intestine and the products are not available to the non-coprophagous animal. The mean concentrations of vitamin $B_{12}$ in the faeces of the three baboons given the vitamin $B_{12}$-deficient diet, the three baboons given the vitamin $B_{12}$-deficient diet containing ampicillin and the three baboons given the vitamin $B_{12}$-supplemented diet were 7·8, 6·4 and 6·6 $\mu$g/g dry weight respectively, showing that the ampicillin was not affecting the production of vitamin $B_{12}$ in the large intestine. In the small intestine the ampicillin might exert an effect either by sterilizing the gut or by altering the composition of the flora so that vitamin $B_{12}$-utilizing micro-organisms become predominant. Vitamin $B_{12}$ deficiency due to abnormal intestinal flora has been reported, and it generally occurs as a result of bacterial over-growth in intestinal diverticula (Mollin, Booth & Baker, 1957). Studies with germ-free animals have neither confirmed nor disproved the possible role of the intestinal flora in the vitamin $B_{12}$ nutrition of the host. For example, Oace & Abbott (1972) found that, although the feeding of a vitamin $B_{12}$-deficient diet depressed weight gain more in germ-free than in conventional rats, the deficient conventional animals excreted more methylmalonic acid. They suggested that the latter finding may be due to the production of propionic acid, a precursor of methylmalonic acid, by the intestinal flora.

Assays performed on the various components of the diets used in this study showed that both the drinking-water and the vitamin-free casein contained *L. leichmannii* growth-promoting activity. The amount in the drinking-water was very low, being less than 1 ng/l (calculated as vitamin $B_{12}$) and often zero whereas the casein contained approximately 0·004 $\mu$g/g. If this activity was due to vitamin $B_{12}$ then each baboon was receiving 0·1 $\mu$g vitamin $B_{12}$/d which could in part explain the apparent ability of the baboons to maintain minimal tissue levels of vitamin $B_{12}$. The enterohepatic circulation of vitamin $B_{12}$ could also play a part in the maintenance of these levels in animals with normal vitamin $B_{12}$ absorption. Considerable quantities of vitamin $B_{12}$ are excreted in the bile but most is reabsorbed (Gräsbeck, Nyberg & Reizenstein, 1958). When absorption is impaired, as in pernicious anaemia, the vitamin $B_{12}$ is not reabsorbed but is excreted in the faeces.

In man, the anaemia of vitamin $B_{12}$ deficiency is morphologically indistinguishable from that of folic acid deficiency and it also responds to large doses of folic acid. Herbert & Zalusky (1962) proposed that a deficiency of vitamin $B_{12}$ results in a secondary deficiency of the folic acid coenzymes due to a reduction in the rate of the vitamin $B_{12}$-dependent conversion of *N*-5-methyltetrahydrofolate to tetrahydrofolate. The absence of anaemia in the baboons in the present study may have been due to the high dietary intake of folic acid, 1 mg/baboon per d. Even so there was evidence of disordered folic acid metabolism in two of the deficient baboons as shown by the increased excretion of formiminoglutamic acid. The liver folate concentration was also subnormal in these two baboons. Low liver folate levels have been found previously in vitamin $B_{12}$-deficient experimental animals (Dawbarn, Hine & Smith, 1958), and more recently Thenen & Stokstad (1973) have found that there was a marked reduction of the folate di-, tri- and pentaglutamates in the livers of vitamin $B_{12}$- and methionine-deficient rats.

Two other factors which cannot be discounted as possible reasons for the absence of anaemia in the baboons are a species difference in the response to the deficiency and the duration of the deficiency. The present study extended over a period of only 2 years, whereas in patients with total gastrectomies, who therefore cannot absorb vitamin $B_{12}$, a megaloblastic anaemia due to vitamin $B_{12}$ deficiency very rarely occurs within 2 years and can take as long as 10 years to develop (Adams, 1968).

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