Vitamin B_{12} nutrition and metabolism in the baboon (*Papio cynocephalus*)

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1. Measurement of the vitamin B_{12} content of baboon tissues showed that the liver contained the highest concentration, followed by the pituitary, kidney, heart, spleen and pancreas.

2. The dietary vitamin B_{12} requirement of the baboon for the maintenance of satisfactory body stores was between 1 and 2 μg/d.

3. Satisfactory liver vitamin B_{12} stores were invariably associated with serum levels above 125 pg/ml, whereas liver levels were usually low when the serum level was below 50 pg/ml.

4. Increased methylmalonic acid (MMA) excretion after a valine load occurred when the liver vitamin B_{12} level was less than 0·40 μg/g. L- and DL-valine were approximately equally effective as precursors of MMA, whereas sodium propionate, whether given orally or intraperitoneally, was less effective.

5. The distribution of radioactivity along the wall of the intestinal tract after an oral dose of [^{57}Co]cyanocobalamin suggested that the distal half of the small intestine was the main site of vitamin B_{12} absorption. However, the utilization of vitamin B_{12} put direct into the middle part of the small intestine was much lower than that of an oral dose.

6. The unsaturated vitamin B_{12}-binding capacity of baboon serum was not related to the serum vitamin B_{12} level. There was a significant difference between the unsaturated vitamin B_{12}-binding capacities of the two subspecies of baboon (*Papio cynocephalus cynocephalus* and *P. cynocephalus anubis*) studied.

Few studies have been carried out on the nutrition and metabolism of vitamin B_{12} in non-human primates. Oxnard (1964) found that, by comparison with man, the serum vitamin B_{12} levels were low in primates fed on vegetarian diets, whereas Huser & Beard (1969) found that in primates receiving 3·5–15·7 μg vitamin B_{12}/d, the serum levels were within the normal human range or higher. Wilson & Pitney (1955) found that the serum levels were low in rhesus monkeys (*Macaca mulatta*) receiving 1 μg vitamin B_{12}/d, but increased when the daily intake was increased to 10 μg. Boass & Wilson (1963) studied the intestinal absorption of vitamin B_{12} in the rhesus monkey and found that the ileum was the main site of absorption.

Vitamin B_{12}-deficiency studies in baboons (Siddons, 1974) showed that, although the feeding of a vitamin B_{12}-deficient diet resulted in the development of low serum and liver vitamin B_{12} levels, no haematological changes occurred. This is in contrast to man where vitamin B_{12} deficiency commonly causes megaloblastic anaemia, and it was suggested that it may be due to a species difference in the metabolism of the vitamin. The results in the present paper, however, show that in many ways the nutrition and metabolism of vitamin B_{12} in the baboon is similar to that in man.

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EXPERIMENTAL

Animals and diets

Two subspecies of male baboons, *Papio cynocephalus cynocephalus* and *P. cynocephalus anubis*, weighing between 10 and 20 kg, were studied. They were given either a mainly vegetarian diet consisting of fresh fruit and vegetables supplemented with high-protein biscuits, or a purified diet consisting of starch, sucrose, vitamin-free casein and maize oil, plus salt and vitamin supplements (Siddons, 1974). Baboons given the purified diet were housed individually in cages with wire-mesh floors which allowed excreta to fall out of reach, whereas baboons given the mainly vegetarian diet were housed communally in cages with concrete floors, in which it was possible for the food to become contaminated with faeces.

Analytical methods

Vitamin B_{12} was measured microbiologically with *Lactobacillus leichmanii* (Spray, 1955). Tissue vitamin B_{12} concentration was measured after digestion of the sample with papain (Chanarin, 1969*a*).

Methylmalonic acid (MMA) was measured by thin-layer chromatography as described by Gutteridge & Wright (1970) except that the urine was not saturated with ammonium sulphate and diethyl ether extraction was done for 7 h using a liquid–liquid extractor.

The unsaturated vitamin B_{12}-binding capacity (UBBC) of the serum was determined by incubating 1 ml serum with 1 ml 0·1 M-sodium phosphate buffer, pH 7·4, containing 5 ng [^{57}Co]cyanocobalamin, for 1 h at 37°. The unbound cyanocobalamin was removed by dialysis against distilled water for 60 h at 4° and the amount of radioactivity remaining in the bag was measured using a well-type scintillation counter (Ekco Electronics Ltd, Southend-on-Sea, Essex).

The site of vitamin B_{12} absorption was determined by studying the distribution of radioactivity along the intestinal tract after an oral dose of [^{57}Co]cyanocobalamin. The baboon was killed with an intravenous injection of sodium pentobarbitone, and the intestinal tract was removed and divided into sections 90–120 mm in length. Each section was slit open, washed three times with 0·15 M-sodium chloride, and gently blotted dry with a piece of gauze. The mucosa was scraped off, weighed, and the amount of radioactivity measured. Whenever contents were present in the intestinal lumen, the radioactivity content of a weighed quantity was determined.

RESULTS

Tissue vitamin B_{12} levels

The concentration of vitamin B_{12} in various tissues obtained from two baboons immediately after death is shown in Table 1. Both baboons were of the subspecies *P. cynocephalus anubis*, and baboon 64 had been given the mainly vegetarian diet and baboon 129 the purified diet. Although in the majority of tissues from baboon 64, the concentration was lower than that in the corresponding tissue from baboon 129, the

Table 1. *Vitamin B_{12} concentrations ($\mu g/g$) in tissues from two baboons* (Papio cynocephalus anubis)

(Total vitamin B_{12} (μg/organ) in parentheses)

	Baboon no. 129		Baboon no. 64	
Liver	0·720	(153·4)	0·392	(125·8)
Pituitary	0·192		0·084	
Kidney	0·145	(5·2)	0·069	(3·1)
Heart	0·125	(8·5)	0·062	(6·6)
Spleen	0·090	(1·3)	0·110	(2·2)
Pancreas	0·075	(1·0)	0·081	(1·5)
Lymph nodes	0·070		0·045	
Stomach	0·057		0·036	
Duodenum	0·055		0·023	
Ileum	0·047		0·049	
Brain	0·034	(5·0)	0·016	(2·6)
Colon	0·029		0·031	
Caecum	0·026		0·034	
Thyroid	0·025		0·014	
Tongue	0·025		0·006	
Spinal cord	0·023		—	
Testis	—		0·013	(1·0)
Bladder	0·014		0·011	
Lung	0·011		0·019	
Muscle	0·008		0·002	
Skin	0·001		0·001	

Table 2. *Serum and liver vitamin B_{12} levels in baboons given diets containing different amounts of vitamin B_{12}*

(Mean values with their standard errors: no. of baboons in parentheses)

	Vitamin B_{12}					
	Serum (pg/ml)			Liver ($\mu g/g$)		
Diet	Mean	SE		Mean	SE	
Purified diet						
+2 μg vitamin B_{12}/d	217	20	(8)	0·87	0·07	(8)
+1 μg vitamin B_{12}/d	66	6	(8)	0·63	0·09	(8)
No supplement	17	2	(15)	0·20	0·02	(15)
Vegetarian diet	59	3	(49)	0·56	0·04	(12)

distribution between the tissues was similar in both baboons. The liver contained the highest concentration with the pituitary, kidney, heart, spleen and pancreas containing the next highest amount, although the concentration in these organs was considerably lower than that of the liver. Skin and muscle contained very small amounts of vitamin B_{12}.

Serum and liver vitamin B_{12} levels and MMA excretion

The mean serum and liver vitamin B_{12} levels in groups of baboons given diets containing different amounts of vitamin B_{12} for at least 6 months, and in the instance of the vitamin B_{12}-deficient, purified diet, for at least 2 years, are shown in Table 2. The lowest levels were found in baboons given the vitamin B_{12}-deficient, purified diet and

Table 3. *Relationship between serum and liver vitamin B_{12} concentrations in baboons given diets containing different amounts of vitamin B_{12}*

(Measurements for twelve baboons given a mainly vegetarian diet and twenty-three baboons given a vitamin B_{12}-deficient or a vitamin B_{12}-supplemented (1 or 2 μg/d) purified diet)

	Serum vitamin B_{12} (pg/ml)		
Liver vitamin B_{12} (μg/g)	0–50	51–125	> 125
0–0·20	19	0	0
0·21–0·29	13	0	0
0·30–0·40	8	4	0
0·41–0·60	3	3	1
> 0·60	0	5	24
Total no. of measurements	43	12	25

Table 4. *The excretion of methylmalonic acid (mg/24 h) after giving valine or sodium propionate orally or intraperitoneally to vitamin B_{12}-deficient baboons*

(Mean values with their standard errors for three baboons)

	Oral		Intraperitoneal	
	Mean	SE	Mean	SE
No load	12	4	—	—
5 g L-valine	133	18	137	20
5 g DL-valine	77	3	102	21
5 g sodium propionate	52	14	62	10
10 g L-valine	220	12	—	—
10 g DL-valine	247	7	—	—
10 g sodium propionate	75	18	—	—

the highest in baboons receiving 2 μg vitamin B_{12}/d. The levels in baboons given 1 μg vitamin B_{12}/d were intermediate and were similar to the levels in baboons given the mainly vegetarian diet. The relationship between the serum and liver vitamin B_{12} levels is shown in Table 3. In general, serum levels greater than 125 pg/ml were associated with a liver concentration greater than 0·6 μg/g, whereas those less than 50 pg/ml were associated with a liver concentration less than 0·3 μg/g.

Baboons with low liver vitamin B_{12} levels excreted increased amounts of MMA in the urine. Under normal dietary conditions the amount excreted was low, but increased when a loading dose of a metabolic precursor of MMA was given. To assess the effectiveness of L-valine, DL-valine and sodium propionate as metabolic precursors of MMA, loading doses of these compounds were given either orally or intraperitoneally to three vitamin B_{12}-deficient baboons and the excretion of MMA measured. The results are shown in Table 4. All three compounds caused an increase in MMA excretion, and the amount excreted increased as the loading dose was increased. L- and DL-valine were approximately equally effective as precursors of MMA, whereas the response to sodium propionate was lower.

The amount of MMA excreted after a valine load was found to be inversely related

Table 5. *The relationship between liver vitamin B_{12} levels and the amount of methylmalonic acid (MMA) excreted in the baboon after a loading dose of 5 g* L- *or* DL-*valine administered either orally or intraperitoneally*

(Measurements for twenty-three baboons given a vitamin B_{12}-deficient or a vitamin B_{12}-supplemented (1 or 2 μg/d) purified diet)

	MMA excretion (mg/24 h)			
Liver vitamin B_{12} (μg/g)	0–5	6–20	21–50	> 50
0–0·20	0	1	12	17
0·21–0·30	0	8	8	3
0·31–0·40	1	4	1	0
> 0·40	37	2	0	0
Total no. of measurements	38	15	21	20

to the liver vitamin B_{12} concentration, i.e. the lower the liver vitamin B_{12} concentration the higher the MMA excretion (Table 5). When the liver vitamin B_{12} concentration was greater than 0·4 μg/g, the amount of MMA excreted was nearly always less than 5 mg/24 h.

Vitamin B_{12} absorption

The intestinal absorption of vitamin B_{12} in the baboon was studied using [^{57}Co]-cyanocobalamin. After an oral dose of 0·5 μg [^{57}Co]cyanocobalamin, radioactivity was found in the plasma within 1·5 h and reached a maximum between 4 and 8 h (Fig. 1 *a*). The increase in the amount of plasma radioactivity was much lower when [^{57}Co]-cyanocobalamin was given direct into the middle part of the small intestine, by laparotomy under diethyl ether anaesthesia, than when the same dose was given orally (Fig. 1 *b*). This suggested a lower utilization of the cyanocobalamin when given direct into the intestine, and was confirmed by measuring the amount of radioactivity excreted in the faeces. After the oral dose, 30% of the total radioactivity given was excreted in the faeces, as compared with 72% when the [^{57}Co]cyanocobalamin was given direct into the middle part of the small intestine.

The site of vitamin B_{12} absorption was determined by studying the distribution of radioactivity along the wall of the intestinal tract after an oral dose of [^{57}Co]cyanocobalamin. The results obtained for three baboons are shown in Fig. 2. Baboons 164 and 85 were given 0·6 μg [^{57}Co]cyanocobalamin and killed 3 h later. In both baboons very little radioactivity was found in the wall of the stomach, duodenum and proximal jejunum. However, in baboon 164 the radioactivity was located mainly in the wall of the ileum with a small amount in the distal jejunum, whereas in baboon 85 the greatest amount of radioactivity was found in the wall of the distal jejunum and proximal ileum with none in the distal ileum. This apparent discrepancy between the two baboons was explained by the fact that radioactivity could not be detected in the contents from the distal ileum of baboon 85, showing that in the 3 h period between giving the [^{57}Co]cyanocobalamin and killing the baboon, the radioactive vitamin B_{12}

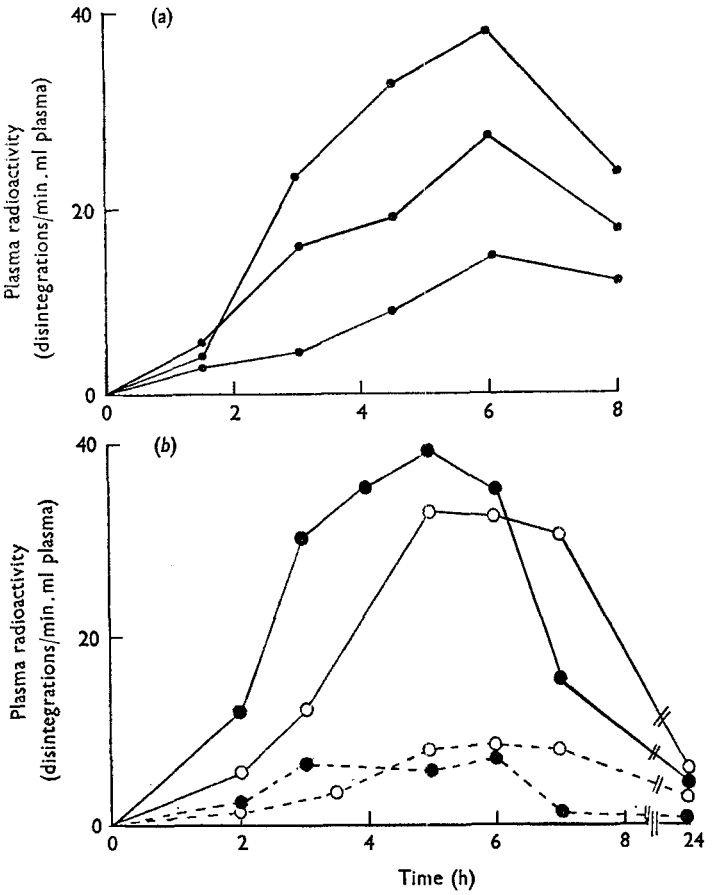

Fig. 1. (*a*) The increase in the amount of plasma radioactivity (disintegrations/min per ml plasma) for three baboons after an oral dose of 0·5 μg [^{57}Co]cyanocobalamin; (*b*) the comparative increase in the amount of plasma radioactivity (disintegrations/min per ml plasma) of two baboons after either an oral dose of 0·25 μg [^{57}Co]cyanocobalamin (——) or after administration of 0·25 μg [^{57}Co]cyanocobalamin direct into the middle part of the small intestine (– –).

had not traversed the length of the small intestine. In order to overcome this problem, baboon 133 was given a total of 1 μg [^{57}Co]cyanocobalamin in four equal doses at hourly intervals and killed 1 h after the final dose. Under these conditions the amount of radioactivity in the wall of the ileum was again higher than in the stomach, duodenum and jejunum, and was higher in the proximal ileum than in the distal ileum.

Serum vitamin B_{12}-binding capacity

Under the experimental conditions employed the UBBC of serums obtained from twenty-two baboons ranged from 0·800 to 2·431 ng/ml with a mean of 1·474 ng/ml. Two subspecies of baboon were studied and the UBBC of *P. cynocephalus anubis* was significantly ($P < 0{\cdot}001$) higher than that of *P. cynocephalus cynocephalus* (Table 6). In both subspecies, the UBBC for baboons given a vitamin B_{12}-deficient diet was

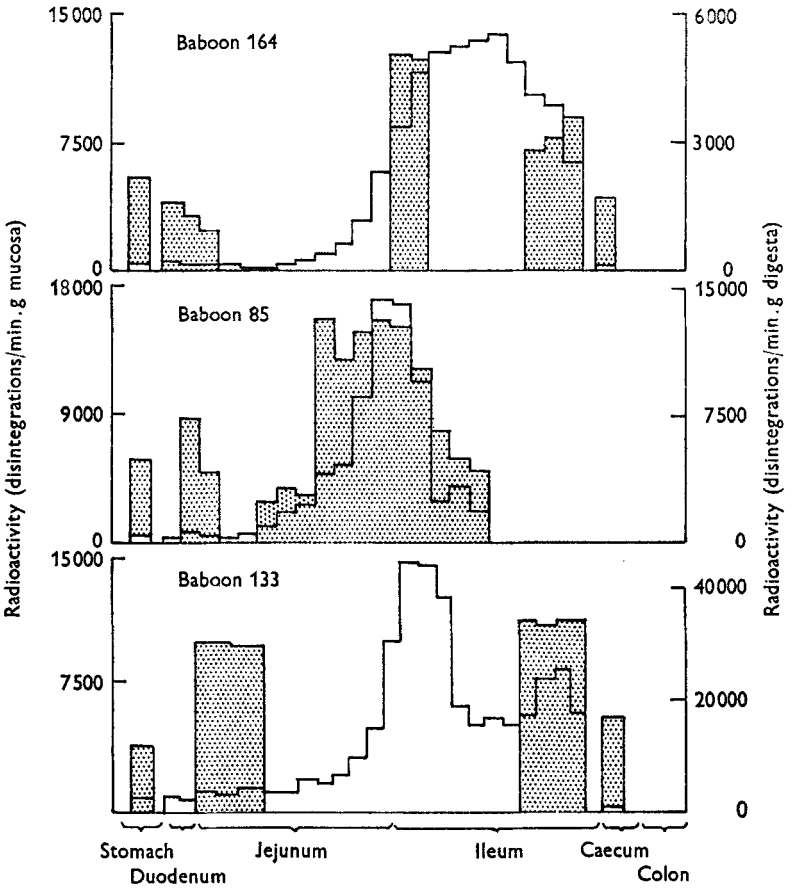

Fig. 2. The distribution of radioactivity along the wall of the intestinal tract (□) and in the digesta (▦) of three baboons after an oral dose of 0·6 μg [^{57}Co]cyanocobalamin.

Table 6. *The unsaturated vitamin B_{12}-binding capacity (UBBC) and total vitamin B_{12}-binding capacity (TBBC)(ng vitamin B_{12} bound/ml serum) of serum from two subspecies of baboon,* Papio cynocephalus cynocephalus *and* Papio cynocephalus anubis, *which had been given either a vitamin B_{12}-deficient or a vitamin B_{12}-supplemented diet*

(Mean values with their standard errors calculated from the variation between baboons within groups: no. of animals in each group in parentheses)

	P. cynocephalus cynocephalus		*P. cynocephalus anubis*	
	UBBC	TBBC	UBBC	TBBC
Vitamin B_{12}-deficient	1·201 ± 0·100	1·213 ± 0·100 (11)	1·881 ± 0·166	1·913 ± 0·166 (4)
Vitamin B_{12}-supplemented (2 μg vitamin B_{12}/d)	1·240 ± 0·192	1·592 ± 0·191 (3)	1·992 ± 0·166	2·308 ± 0·166 (4)

similar to that for baboons given a vitamin B_{12}-supplemented diet. Since the serum vitamin B_{12} levels were higher in baboons given a vitamin B_{12}-supplemented diet, the total vitamin B_{12}-binding capacity of the serum (UBBC + serum vitamin B_{12}) was also higher, but the difference between the vitamin B_{12}-deficient and the supplemented groups was not significant.

DISCUSSION

The distribution of vitamin B_{12} in the tissues of the baboon was similar to that found in man (Hsu, Kawin, Minor & Mitchell, 1966; Rapazzo, Salmi & Hall, 1970). In fact, the concentration in the various tissues of baboon 129, with the exception of the spleen, in which the levels tended to be higher in the baboon than man, was very similar to the mean human values quoted by Rapazzo *et al.* (1970). In both man and baboon the liver contained by far the highest concentration of vitamin B_{12}.

As would be expected, the vitamin B_{12} status of the baboon was related to the dietary intake. Serum and liver vitamin B_{12} levels were higher in baboons receiving 2 μg vitamin B_{12}/d than in baboons receiving 1 μg, with the lowest levels in baboons given a vitamin B_{12}-deficient diet. It is difficult to state with certainty the vitamin B_{12} content of the mainly vegetarian diet because there was the possibility of bacterial and faecal contamination of the food. The high-protein biscuits supplied approximately 0·25 μg vitamin B_{12}/baboon per d, but the serum and liver vitamin B_{12} levels would suggest a daily intake in the region of 1 μg.

MMA excretion studies showed that when the liver vitamin B_{12} level for the baboon was below 0·4 μg/g, there was an increase in the amount of MMA excreted after a valine load. This suggests that a liver vitamin B_{12} level greater than 0·4 μg/g is required for the maintenance of normal metabolic function. In general, baboons receiving 1 μg vitamin B_{12}/d had liver levels in excess of 0·4 μg/g but occasionally lower levels were found. On 2 μg vitamin B_{12}/d the liver levels were well in excess of 0·4 μg/g, which would indicate that the daily dietary vitamin B_{12} requirement of the baboon for the maintenance of satisfactory body stores is between 1 and 2 μg. In man, it has been suggested that the minimum daily requirement is 0·1 μg since this amount, when given parenterally to deficient, anaemic subjects, will produce a gradual return to haematological normality. Whole-body turnover studies, on the other hand, suggest that the daily requirement is 0·5–2·5 μg (Herbert, 1968). Chanarin (1969*b*) estimates that, to maintain the physiological status quo, the dietary vitamin B_{12} intake of man should be between 3 and 7 μg/d. Thus, on a body-weight basis, the vitamin B_{12} requirement of the baboon appears to be similar to that of man.

The concentration of vitamin B_{12} in the livers of baboons given 2 μg vitamin B_{12}/d was similar to the normal human values, whereas the serum levels were lower. In the baboon, serum levels greater than 125 pg/ml were associated with liver levels greater than 0·6 μg/g and low liver levels were found when the serum level was less than 50 pg/ml. In man a liver level greater than 0·6 μg/g is invariably associated with a serum level greater than 180 pg/ml, and if the serum level is less than 120 pg/ml it is indicative of low liver levels (Joske, 1963; Stahlberg, Radner & Norden, 1967).

In vitamin B_{12} deficiency there is an increased excretion of MMA (Cox & White, 1962). Valine, a metabolic precursor of MMA, caused an increase in the amount of MMA excreted by baboons which had liver vitamin B_{12} levels below 0·4 μg/g, but had no effect when the liver vitamin B_{12} levels were greater than 0·4 μg/g. Sodium propionate also caused an increase in MMA excretion in vitamin B_{12}-deficient baboons, but was less effective than valine even when given intraperitoneally. This would suggest that the limiting factor in the response to propionate is its metabolic conversion to methylmalonyl-CoA.

The intestinal absorption of physiological amounts of vitamin B_{12} is a complex process involving its combination with intrinsic factor, the uptake of the intrinsic factor–vitamin B_{12} complex by the intestinal cell, the intracellular dissociation of the complex and, finally, the release of the vitamin B_{12} into the blood. Thus, some time elapses between the ingestion of vitamin B_{12} and its appearance in the bloodstream. In the baboon, maximum level of radioactivity in the plasma was found 4–8 h after an oral dose of radioactive vitamin B_{12} as compared with 8–12 h in man (Booth & Mollin, 1956). The shorter time taken for the radioactivity to appear in the blood in the baboon may simply reflect the time taken for the vitamin B_{12} to reach the site of absorption. Evidence suggesting that the distal half of the baboon small intestine is the area of maximal vitamin B_{12} absorption was obtained by measuring the intramural distribution of radioactivity along the intestinal tract after an oral dose of [^{57}Co]cyanocobalamin. The distal part of the small intestine has also been shown to be the site of vitamin B_{12} absorption in man (Booth & Mollin, 1959). The finding that vitamin B_{12} put into the middle part of the small intestine of the baboon was less well utilized than when given orally was probably due to a deficiency of intrinsic factor in this part of the intestine. However, the fact that some of the dose was absorbed is of interest, since it suggests that if vitamin B_{12} is produced by micro-organisms in the lower part of the small intestine, it might be available to the host.

Although vitamin B_{12} in the serum is bound to protein, the ability of serum to bind vitamin B_{12} in vitro is much greater than the normal serum vitamin B_{12} level. The extra binding capacity is termed the UBBC and has been found to vary considerably between different animal species (Rosenthal & Austin, 1962). In the baboon the UBBC ranged from 0·800 to 2·431 with a mean of 1·474 ng/ml, which is similar to the values obtained with human serums under similar experimental conditions (Miller, 1958). However, in the present study two subspecies of baboon were used and the UBBC of *P. cynocephalus anubis* was significantly higher than that of *P. cynocephalus cynocephalus*. The serum vitamin B_{12} levels in the two subspecies were not significantly different, which is in contrast to the findings in man, in which the higher UBBC of West Indians and Nigerians as compared to Europeans is associated with higher serum vitamin B_{12} levels (Fleming, 1968; Low-Beer, McCarthy, Austad, Brzechwa-Ajdukiewicz & Read, 1968). The UBBC of baboons given a vitamin B_{12}-deficient diet was similar to that of baboons given a vitamin B_{12}-supplemented diet, but the total vitamin B_{12}-binding capacity (UBBC + serum vitamin B_{12}) was lower in the deficient animals. Similar results were obtained by Heller, Epstein, Cunningham, Henderson & Yakulis (1964) in their studies on vitamin B_{12}-deficient human subjects, whereas

Soraya & Chopra (1973) found that as the serum vitamin B_{12} level decreased, the UBBC increased.

It is possible that a species difference in the nutrition and metabolism of vitamin B_{12} may explain the variation in the deficiency signs between species and why it has not so far proved possible to produce in lower animals the megaloblastic anaemia seen in vitamin B_{12} deficiency in man. The results of this study show that in many ways, the nutrition and metabolism of vitamin B_{12} in the baboon is similar to that in man. So far, however, no haematological changes have been found in baboons maintained on a vitamin B_{12}-deficient diet.

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