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# Vitamin $B_{12}$ metabolism in the fruit bat (*Rousettus aegyptiacus*). The induction of vitamin $B_{12}$ deficiency and its effect on folate levels

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1. Vitamin $B_{12}$ metabolism was studied in bats of the species *Rousettus aegyptiacus*, which live on an all-fruit diet in the wild.

2. There was a wide range in values for the serum vitamin $B_{12}$ concentration of newly captured bats, but bats captured in the early spring had significantly higher mean serum vitamin $B_{12}$ levels than bats captured in the early autumn.

3. There was an exponential decrease in serum vitamin $B_{12}$ concentration with time in captivity for bats fed on a vitamin $B_{12}$-deficient, all-fruit diet; the biological half-life was 80 d in serum, 109 d in liver and 164 d in kidney.

4. The main storage organ for vitamin $B_{12}$ in the bat was the liver, mean content 1067 ng vitamin $B_{12}$. After 50 d, injected [$^{57}$Co]cyanocobalamin had equilibrated with body vitamin $B_{12}$ stores, and 17% of the retained radioactivity was present in the liver. From these results it was calculated that the total body vitamin $B_{12}$ content of the bat was *c.* 6500 ng.

5. The biological half-life of injected [$^{57}$Co]cyanocobalamin was 70–88 d and the calculated daily requirement was 50–60 ng (0·2 $\mu$g/kg body-weight per d).

6. As serum vitamin $B_{12}$ levels decreased, serum folate levels increased. The erythrocyte folate concentration increased significantly after 130 d on the all-fruit diet and then decreased to the initial values after 190 d.

7. Vitamin $B_{12}$ metabolism in the fruit bat is similar in many respects to that of man, but on a 'weight-for-weight' basis the bat has a 5- to 15-fold greater requirement for this vitamin.

8. Vitamin $B_{12}$ deficiency may be induced fairly rapidly in fruit bats fed on an all-fruit diet.

There is a continual quest for suitable animal models for the study of human diseases. Amongst these we need a model to study the effects of vitamin $B_{12}$ deficiency. Although there have been numerous attempts, no animal showing the complete picture of vitamin $B_{12}$ deficiency as seen in man has been identified (Prichard, 1968). In most animal species, vitamin $B_{12}$ balance is maintained by the mutually favourable factors of dietary sufficiency and a highly efficient absorptive mechanism. Omnivorous habits, coprophagy and vitamin synthesis by gut flora in ruminants, either singly or severally, provide the dietary source of vitamin $B_{12}$ in different species.

Because of their eating habits, none of these possible sources of vitamin $B_{12}$ is available to the fruit bat, the Egyptian or Nile bat (*Rousettus aegyptiacus*), which subsists on an all-fruit diet (Novick, 1969). Fruit is rich in folate, but is devoid of vitamin $B_{12}$ (McCance & Widdowson, 1960). The natural dietary source of vitamin $B_{12}$ for these animals is unknown, but they probably obtain their vitamin $B_{12}$ either by the inadvertent ingestion of insects present in or on the fruit they eat, or by drinking stagnant water contaminated with vitamin $B_{12}$-producing micro-organisms (Robbins,

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Hervey & Stebbins, 1950). These potential sources of vitamin $B_{12}$ are easy to exclude in captivity and we have reported the fairly rapid induction of vitamin $B_{12}$ deficiency in fruit bats, without significant alteration in their natural diet. Vitamin $B_{12}$-deficient bats develop neurological changes that are similar to those found in the vitamin $B_{12}$-deficient man, with subacute combined degeneration of the spinal cord. However, haematological changes, apart from leucopaenia, are not present (Green, van Tonder, Cole, Oettlé & Metz, 1975).

Because of the fruit bat's unusual predisposition towards the development of vitamin $B_{12}$ deficiency in captivity, and its potential use as an animal experimental model to study the effects of such deficiency, we undertook the present studies of vitamin $B_{12}$ metabolism in normal bats, and the changes which occur in serum and tissue levels of vitamin $B_{12}$ and folate when bats are kept on a diet deficient in vitamin $B_{12}$ but rich in folate.

## EXPERIMENTAL

### *Animals and diets*

The bats studied (the Egyptian or Nile bat) were of the suborder Megachiroptera, which feed on fruit and flowers (Novick, 1969) and are not known to prey on animals. Bats were caught in the Matlapitsi Cave in the Northern Transvaal, and transported to the laboratory for study. In the wild, these bats show a preference for litchis (*Nephelium litchi* Camb.), bananas, papayas (*Carica papaya*) and other cultivated tropical fruits (N. H. G. Jacobsen, personal communication), but the wild fig (*Ficus capensis*) provides the bulk of the food that is consumed throughout the year. Other wild fruits eaten include water pear (*Syzygium gerrardii*), water berry (*Syzygium cordatum*) and bitter amandel (*Pygeum africanum*). Ants and other pests have been found in these fruits (N. H. G. Jacobsen, personal communication), and these could provide a dietary source of vitamin $B_{12}$ in the wild.

In captivity, bats were fed on an all-fruit diet consisting only of first-grade bananas, papayas, pears and oranges fed *ad lib.* All fruit was washed in tap-water, peeled and cut to ensure that it was insect- and pest-free. Fresh tap-water was provided daily, and the cement floor of the housing cage was cleaned daily to decrease faecal contamination of the diet. No vitamin $B_{12}$ supplement was given and all experiments excluding observations on the induction of vitamin $B_{12}$ deficiency were completed within 100 d of capture.

### *Experimental procedures*

*Reagents.* All chemical reagents were analar grade and solutions were made up with glass-distilled, de-ionized water.

*Blood sampling.* Bats were bled by cardiac puncture, and the volume of blood taken was approximately 15 $\mu$l/g body-weight. Previous studies with $^{51}$Cr-labelled erythrocytes have shown that the total blood volume of this species averages 72 $\mu$l/g body-weight (J. Keegan & D. Hart, unpublished results).

*Serum vitamin $B_{12}$.* Serum vitamin $B_{12}$ concentrations were measured by radioisotope dilution (RID) assay using chicken serum as the vitamin $B_{12}$-binding protein (Green, Newmark, Musso & Mollin, 1974). Assays were done using 0·1 ml serum;

volumes of standard cyanocobalamin, [$^{57}$Co]cyanocobalamin buffers and chicken serum used in the conventional serum assay were scaled down proportionately. For this and other procedures involving the use of radioactive cyanocobalamin, high specific activity (100–300 $\mu$Ci/$\mu$g) [$^{57}$Co]cyanocobalamin (Radiochemical Centre, Amersham, Bucks., UK) was used. The serum vitamin $B_{12}$ concentration was measured in two groups of male bats within 1 week of capture, one group caught during the month of October (early spring) and the other group caught during April (early autumn). Serum vitamin $B_{12}$ levels were also measured in surviving bats at various times after being kept on the experimental all-fruit diet.

*Liver and kidney vitamin $B_{12}$ concentration.* The vitamin $B_{12}$ concentration was measured in samples of liver and kidney removed from bats which had been killed at the time of capture and at various intervals after they were placed on the experimental diet. An RID assay was used (S. V. van Tonder, J. Metz & R. Green, unpublished results) which was a modification of the serum method of Green *et al.* (1974).

*Biological half-life ($T_{0.5}$) of vitamin $B_{12}$.* To determine $T_{0.5}$ for vitamin $B_{12}$, bats were given an intramuscular injection of 2 ng high specific activity [$^{57}$Co]cyanocobalamin, and total body radioactivity was measured using a human whole-body gamma spectrometer which consisted of an 8 m$^3$ room made of 110 mm thick laminated steel plates and lined with 3 mm monitored lead. The detector was a single cylindrical thallium-activated sodium iodide crystal measuring 228 × 102 mm. Total whole-body radioactivity was measured for bats placed individually in a 400 ml cardboard carton at various intervals up to 48 d after injection of the [$^{57}$Co]cyanocobalamin. The first measurement, recorded immediately after the dose had been given, was taken as the 100% reference and the reduction in the number of counts with time was expressed as a percentage of this initial value.

*Equilibration of injected [$^{57}$Co]cyanocobalamin with body stores.* In order to establish whether injected radioactive cyanocobalamin equilibrates with the body vitamin $B_{12}$ store, the specific activities of two organs (liver and kidney) were compared at different times after the injection of [$^{57}$Co]cyanocobalamin.

Two bats were each given 2 ng high specific activity [$^{57}$Co]cyanocobalamin by intramuscular injection. The bats were killed after 21 and 50 d. The liver and kidneys were removed, weighed and the amount of radioactivity in these organs was measured using a well-type gamma spectrometer (Autogamma Model 3003; Packard Instrument Co., Downer's Grove, Illinois, USA). Appropriate corrections were made for differences in sample volumes. The vitamin $B_{12}$ content of each organ was measured by the RID assay and expressed as ng vitamin $B_{12}$/g wet weight. Blank tubes, to which no [$^{57}$Co]cyanocobalamin was added, were included in the assay to correct the value obtained for the amount of radioactivity in each sample for endogenous [$^{57}$Co]-cyanocobalamin present in the tissue as a result of the injected radioactive material.

From the recorded radioactivity (counts/min per g organ) and the total amount of measured vitamin $B_{12}$ (ng/g organ) the specific activity of [$^{57}$Co]cyanocobalamin in each organ was calculated and expressed as counts/min per ng vitamin $B_{12}$.

*Organ distribution of injected [$^{57}$Co]cyanocobalamin.* To determine organ distribution, five bats were given an intramuscular injection of 2–5 ng [$^{57}$Co]cyanocobalamin

and were killed at intervals up to 47 d after the injection. Various organs were removed, homogenized in saline (9 g NaCl/l) and the radioactivity measured and expressed as a percentage of the radioactivity present in the whole animal. The homogenate was placed in a test-tube and the amount of organ radioactivity was determined using the well-counter. Since carcass radioactivity was determined using a whole-body counter, a correction factor was applied to compensate for differences in instrumentation and geometry. The correction factor was derived by determining the ratio between the measured radioactivity using the whole-body counter (400 ml) and that using the well-counter (1·0 ml) for the same amount of [$^{57}$Co]cyanocobalamin.

*Total body vitamin $B_{12}$.* The total body vitamin $B_{12}$ content was estimated in newly captured bats by the method of Adams & Boddy (1971). This was done by applying the value for the mean percentage of retained [$^{57}$Co]cyanocobalamin present in the liver 47 d after its injection to the value for mean total liver vitamin $B_{12}$ content in normal bats. Equilibration of the tracer with body vitamin $B_{12}$ was assumed to have taken place after 47 d. Total body vitamin $B_{12}$ was therefore given by the expression: total body vitamin $B_{12}$ (ng) = (liver vitamin $B_{12}$ (ng) × 100) ÷ percentage of retained [$^{57}$Co]cyanocobalamin present in liver.

*Folate measurement.* Serum folate estimation was done using 0·1 ml serum by the method described by Herbert (1961) and by Waters & Mollin (1961). Erythrocyte folate estimation was done using haemolysates prepared from 0·2 ml whole blood by the method of Hoffbrand, Newcombe & Mollin (1966).

### *Statistical analysis*

Student's *t* test was used to compare the significance of differences between mean values as well as correlation coefficients. Regression lines were calculated by the least-squares method, with logarithmic transformation of ordinate values in the instance of exponential factors.

## RESULTS

*Serum vitamin $B_{12}$.* The mean serum vitamin $B_{12}$ concentration with its standard error for sixty-one bats captured during October was 1900 ± 123 pg/ml. This was significantly greater than the corresponding value of 1129 ± 201 pg/ml for seventeen bats captured during April ($t_{76}$ 3·5, $P < 0{\cdot}001$). Changes in serum vitamin $B_{12}$ concentrations of the bats captured during October which were kept on the vitamin $B_{12}$-deficient, all-fruit diet are shown in Fig. 1. There was an exponential decrease in the serum vitamin $B_{12}$ concentration with the period of time in captivity.

The equation for the regression line representing the decrease in serum vitamin $B_{12}$ concentration (pg/ml) ($y$) with time in captivity (d) ($x$) was: $\log y = -0{\cdot}0038x + 3{\cdot}24$. The value for $T_{0{\cdot}5}$, calculated from the regression line, was 80 d. The over-all decrease in serum vitamin $B_{12}$ concentrations was highly significant ($r_{114}$ −0·79, $P < 0{\cdot}001$).

*Liver vitamin $B_{12}$.* The liver vitamin $B_{12}$ concentrations for three newly captured bats were 358, 320 and 215 ng/g tissue. Corresponding values for total liver vitamin $B_{12}$ were 1248, 1065 and 889 ng (mean 1067 ng). Liver vitamin $B_{12}$ concentration was

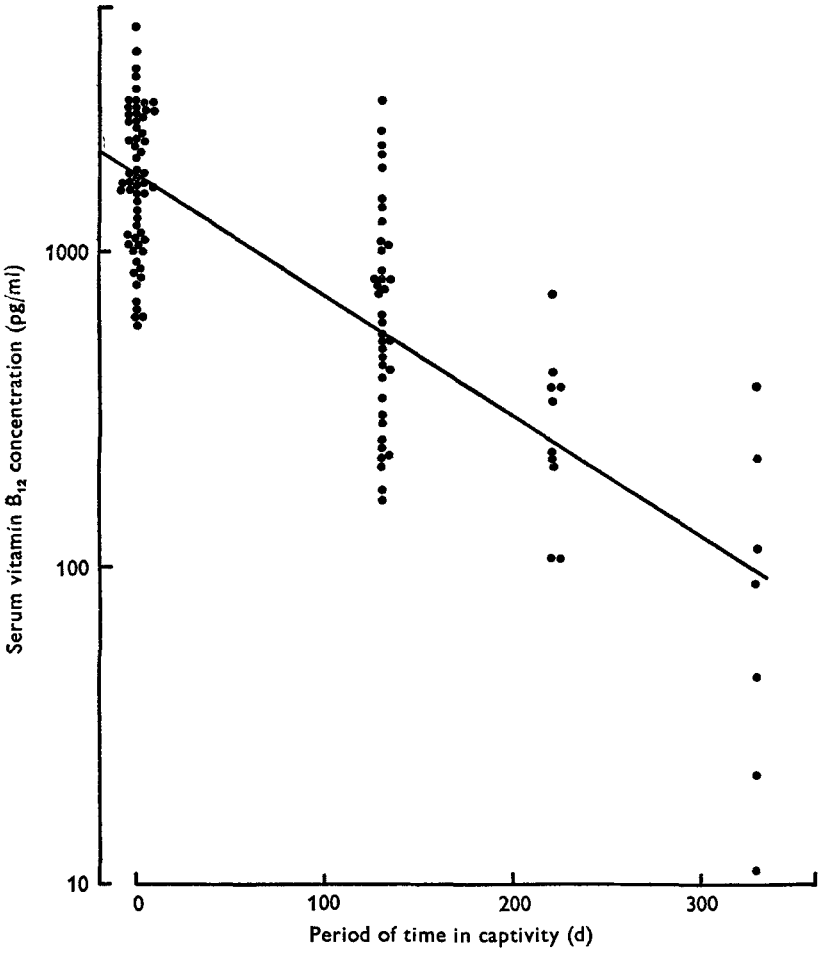


Fig. 1. Decrease in serum vitamin $B_{12}$ concentration (log scale) with time in captivity for fruit bats (*Rousettus aegyptiacus*) fed on a vitamin $B_{12}$-deficient, all-fruit diet (for details, see p. 398). The equation for the regression line is $\log y = 0{\cdot}0038x + 3{\cdot}24$, where $y$ is the vitamin $B_{12}$ concentration (pg/ml) and $x$ is the period of time in captivity (d). The biological half-life, calculated from the regression line, was 80 d. The decrease was statistically significant ($r_{114}$ −0·79, $P < 0{\cdot}001$).

measured also in twenty-eight bats killed at different intervals after they were first given the vitamin $B_{12}$-deficient, all-fruit diet. The log liver vitamin $B_{12}$ concentrations varied inversely with the period of time in captivity (Fig. 2), indicating an exponential decrease in liver vitamin $B_{12}$ concentration with time on the experimental all-fruit diet. The equation for the regression line representing the decrease in liver vitamin $B_{12}$ concentration (ng/g) ($y$) with time in captivity (d) ($x$) was: $\log y = -0{\cdot}0028x + 2{\cdot}49$. The value for $T_{0{\cdot}5}$ calculated from this regression line, was 109 d. The over-all decrease in liver vitamin $B_{12}$ concentration was highly significant ($r_{26}$ −0·90, $P < 0{\cdot}001$).

An estimate of the mean liver vitamin $B_{12}$ concentration at the time of capture was

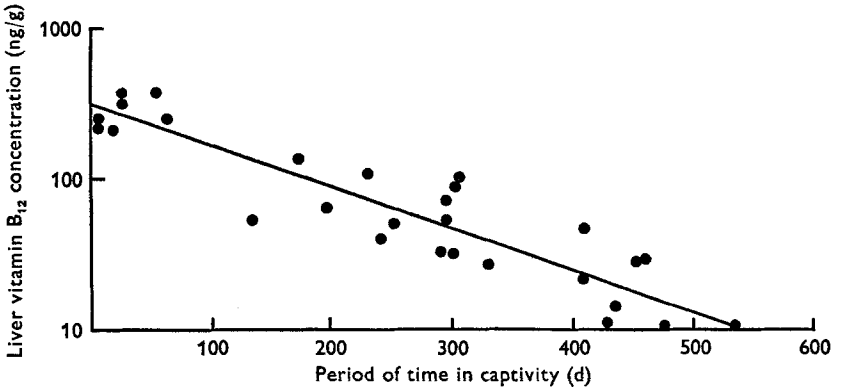


Fig. 2. Liver vitamin $B_{12}$ concentrations (log scale) for twenty-eight fruit bats (*Rousettus aegyptiacus*) killed at different intervals after they were first given a vitamin $B_{12}$-deficient, all-fruit diet (for details, see p. 398). The equation for the regression line is $\log y = -0{\cdot}0028x + 2{\cdot}49$, where $y$ is the vitamin $B_{12}$ concentration (ng/g) and $x$ is the period of time in captivity (d). The biological half-life, calculated from the regression line, was 109 d. The decrease was statistically significant ($r_{26}$ −0·90, $P < 0{\cdot}001$). The $y$-axis intercept for the regression line extrapolated to zero time gave a value of 309 ng/g, which was taken to represent the average liver vitamin $B_{12}$ concentration at the time of capture.

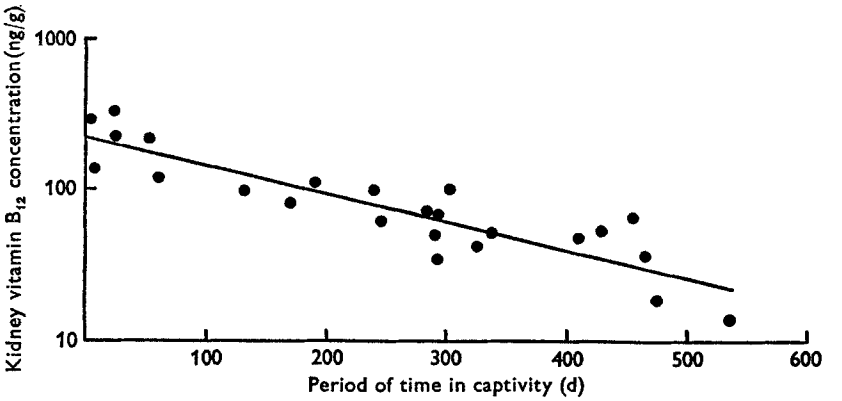


Fig. 3. Kidney vitamin $B_{12}$ concentrations (log scale) for twenty-four fruit bats (*Rousettus aegyptiacus*) killed at different intervals after they were first given a vitamin $B_{12}$-deficient, all-fruit diet (for details, see p. 398). The equation for the regression line is $\log y = -0{\cdot}0018x + 2{\cdot}34$, where $y$ is the vitamin $B_{12}$ concentration (ng/g) and $x$ is the period of time in captivity (d). The biological half-life, calculated from the regression line, was 164 d. The decrease was statistically significant ($r_{22}$ −0·89, $P < 0{\cdot}001$). The $y$-axis intercept of the regression line extrapolated to zero time gave a value of 219 ng/g, which was taken to represent the average kidney vitamin $B_{12}$ concentration at the time of capture.

obtained by extrapolating the regression line to zero time; the value of its intercept on the $y$ axis was 309 ng/g.

There was a good correlation between the concentration of vitamin $B_{12}$ in the serum of eighteen bats taken less than 10 d before they were killed, and the liver vitamin $B_{12}$ concentration ($r_{34}$ 0·85, $P < 0{\cdot}001$).

*Kidney vitamin $B_{12}$.* Kidney vitamin $B_{12}$ concentrations for twenty-four bats at different intervals after capture also decreased exponentially with time, but the rate of decrease was slower than that for liver vitamin $B_{12}$ (Fig. 3). The equation for the

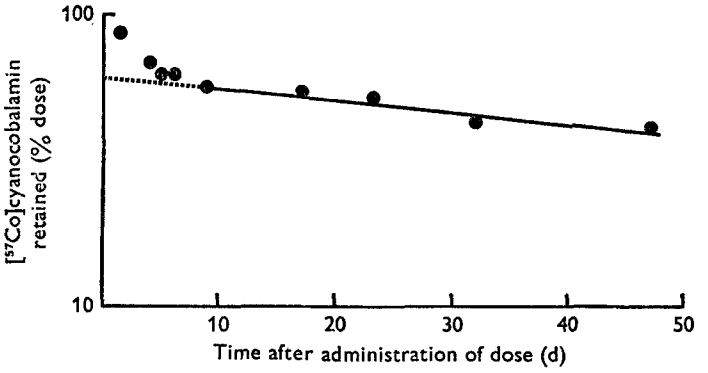


Fig. 4. Representative pattern of retained radioactivity in a fruit bat (*Rousettus aegyptiacus*) given 2 ng [$^{57}$Co]cyanocobalamin by intramuscular injection. After 10 d, the decrease in the amount of radioactivity fitted an exponential model. (——), The slower exponential component (for explanation, see below) (half-life, 71 d), which is the regression line through values obtained from the 10th day after injection. In two other bats the patterns were similar, and the half-lives were 72 and 88 d respectively.

regression line representing the decrease in kidney vitamin $B_{12}$ concentration (ng/g) ($y$) with time in captivity (d) ($x$) was $\log y = -0{\cdot}0018x + 2{\cdot}34$. This decrease, also, was significant ($r_{22}$ $-0{\cdot}89$, $P < 0{\cdot}001$). The value for $T_{0{\cdot}5}$, calculated from the regression line, was 164 d. By extrapolation of the line, the calculated mean kidney vitamin $B_{12}$ concentration at the time of capture was found to be 219 ng/g.

*$T_{0{\cdot}5}$ for [$^{57}$Co]cyanocobalamin.* In three bats which received an injection of [$^{57}$Co]-cyanocobalamin, all were found to have a similar pattern of decrease in total whole-body radioactivity with time after the injection. The semi-log plot of radioactivity retained *v.* time indicated two components, an initial rapid decrease taken to represent mixing of the tracer with vitamin $B_{12}$ stores, and a slower exponential decrease (Fig. 4). From the regression line of best fit through the values obtained from the 10th day after injection, $T_{0{\cdot}5}$ for [$^{57}$Co]cyanocobalamin was found to be 71, 72 and 88 d in the three bats. The turnover rates for [$^{57}$Co]cyanocobalamin, calculated from the $T_{0{\cdot}5}$ for the slower component (turnover rate (%) $= 100 \times \ln 2 \div T_{0{\cdot}5}$), were 0·98, 0·96 and 0·79 %/d respectively. Assuming equilibration of the tracer with body vitamin $B_{12}$ stores, this value for turnover would reflect the rate of loss of vitamin $B_{12}$ from the body.

*Equilibration of injected [$^{57}$Co]cyanocobalamin with body stores.* Values for specific activities of the liver and kidney are given in Table 1. The specific activity of the liver vitamin $B_{12}$ was lower than that of the kidney for bat no. 1 after 21 d (191 counts/min per ng vitamin $B_{12}$ compared with 247 counts/min per ng vitamin $B_{12}$). In bat no. 2 after 50 d, the specific activities of these organs were more similar; the value for the liver was slightly greater than that for the kidney (156 and 137 counts/min per ng vitamin $B_{12}$ respectively).

*Organ distribution of injected [$^{57}$Co]cyanocobalamin.* The amount of radioactivity in the organs from five bats at the time of killing is given in Table 2. The percentage amount of radioactivity in the liver and the kidney increased until 13 d after injection and then remained constant. At 47 d the kidney contained 13 and 17 % of the total

Table 1. *Specific activities for liver and kidneys from two fruit bats* (Rousettus aegyptiacus) *at days* 21 *and* 50 *respectively after an intramuscular injection of* 2 *ng* [$^{57}$*Co*]*cyanocobalamin*

| Bat no. | Time after injection (d) | Organ | Organ radioactivity (counts/min per g) | Tissue vitamin $B_{12}$ concentration (ng/g) | Organ specific activity (counts/min per ng vitamin $B_{12}$) |
|---|---|---|---|---|---|
| 1 | 21 | Liver | 12328 | 64·7 | 191 |
| | | Kidney | 29807 | 120·7 | 247 |
| 2 | 50 | Liver | 10597 | 68·0 | 156 |
| | | Kidney | 13682 | 101·0 | 137 |

Table 2. *Mean organ distribution* (% *administered dose*) *of radioactivity for five fruit bats* (Rousettus aegyptiacus) *killed at various intervals after an intramuscular injection of* 2–5 *ng* [$^{57}$*Co*]*cyanocobalamin*

| | Time after injection (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 4 | 13 | 47 |
| Liver | 9 | 8 | 8 | 16 | 17 |
| Kidneys | 7 | 7 | 11 | 14 | 13 |
| Gut | 12 | 8 | 9 | 4 | 7 |
| Brain and spinal cord | 1 | 1 | 3 | 3 | 4 |
| Heart | 1 | 1 | 1 | 2 | 2 |
| Lungs | 1 | 2 | 2 | 1 | 1 |
| Spleen | 2 | 2 | 1 | 1 | 2 |
| Remainder of carcass | 65 | 68 | 64 | 58 | 50 |

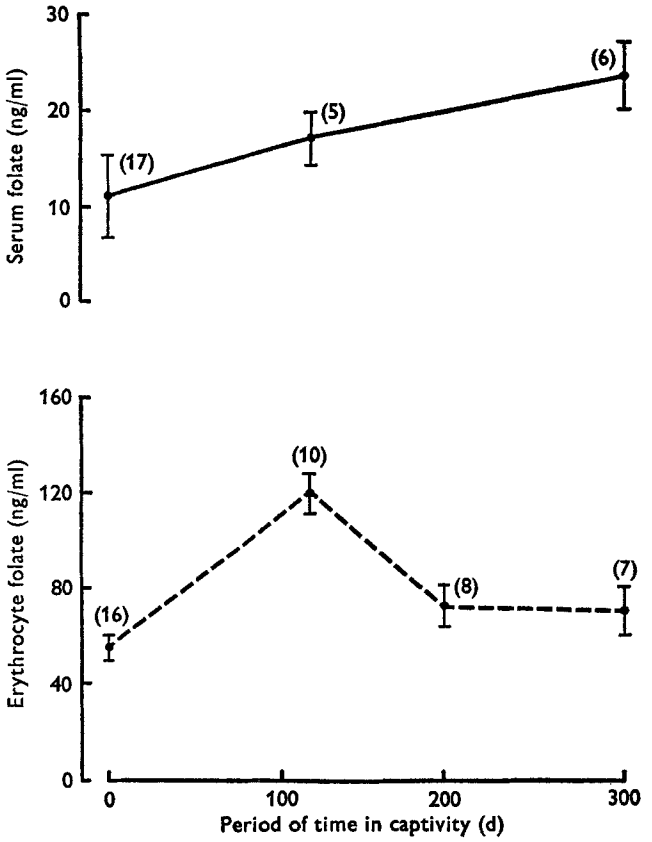


Fig. 5. Changes in serum (—) and erythrocyte (– – –) folate concentration in fruit bats (*Rousettus aegyptiacus*) fed on a vitamin $B_{12}$-deficient, all-fruit diet (for details, see p. 398). Each point represents the mean value for the number of determinations given in parentheses; the vertical bars represent the standard errors. There was a continuous increase in serum folate concentration, which was statistically significant ($r_{26}$ 0·67, $P < 0·001$). The mean erythrocyte folate concentration increased significantly after 130 d ($t_{24}$ 7·2, $P < 0·001$) and decreased after 190 d to a level which was not significantly different from the initial mean value.

body radioactivity respectively. Assuming that the injected [$^{57}$Co]cyanocobalamin had equilibrated with the total body vitamin $B_{12}$, 17% of the total vitamin $B_{12}$ store was contained in the liver after 47 d.

*Total body vitamin $B_{12}$.* The estimated total body vitamin $B_{12}$ for the bat, assuming equilibration of injected [$^{57}$Co]cyanocobalamin with body stores, was 6500 ng. This estimate was based on the mean liver vitamin $B_{12}$ content for three bats (1067 ng) and the percentage of retained [$^{57}$Co]cyanocobalamin present in the liver 47 d after injection (17%).

*Serum folate.* At the time of capture the mean serum folate with its standard error for seventeen bats was 11·0 ± 1·0 ng/ml (range 5·0–23·0 ng/ml). This level increased progressively during maintenance on the vitamin $B_{12}$-deficient, all-fruit diet (Fig. 5). The over-all increase was significant ($r_{26}$ 0·67, $P < 0·001$).

Although the serum folate concentration increased as the serum vitamin $B_{12}$

concentration decreased, there was no significant inverse correlation between these measurements ($r_{54}$ −0·346, 0·3 > $P$ > 0·2).

*Erythrocyte folate.* The initial erythrocyte folate concentration for sixteen bats measured at the time of capture ranged from 22 to 95 ng/ml (mean and SE 55 ± 4·9). After 130 d on the vitamin $B_{12}$-deficient, all-fruit diet the mean value for ten bats had increased to 119 ± 8·0 ng/ml (range 81–160 ng/ml) (Fig. 5). This value was significantly different from the initial value ($t_{24}$ 7·2, $P$ < 0·001). After 190 d the mean erythrocyte folate level for eight bats had decreased to 72 ± 9·4 ng/ml (range 50–123 ng/ml), and remained unchanged at 300 d when it was 70 ± 10·3 ng/ml (range 37–116 ng/ml). The values at 190 and 300 d were both significantly lower than that at 130 d ($P$ < 0·005), but neither value differed significantly from the initial value ($P$ > 0·1).

## DISCUSSION

Considerable variation in the serum vitamin $B_{12}$ concentration has been found between different animal species under normal conditions, but the factors responsible for these variations have not been established (Couch, Olcese, Witten & Colby, 1950; Doctor & Couch, 1952; Rosenthal & Brown, 1954; Kato, 1960; Miller & Sullivan, 1961; Huser & Beard, 1970; Hippe, 1971). Serum vitamin $B_{12}$ levels may not reflect body stores, because although there is a wide variation in serum levels (Hippe, 1971), values for liver vitamin $B_{12}$ concentration are similar for most animal species. The relationship between serum levels and tissue stores of vitamin $B_{12}$ therefore varies between species. These variations could be related to either genetic or dietary factors. In addition to the marked inter-species variation in serum vitamin $B_{12}$ concentration there is also a considerable variation within any particular species (Simnet & Spray, 1961). Such variation is likely to be due to nutritional, rather than to genetic factors.

The range of serum vitamin $B_{12}$ concentrations in fruit bats at the time of capture is consistent with the considerable intra-species variation, and may be explained in part by seasonal variability in the dietary supply of the vitamin in the wild. Bats captured in the late spring had higher serum vitamin $B_{12}$ levels than those captured in early autumn. During the summer months the bats obtain a plentiful diet of cultivated fruit from orchards and, because of the widespread practice of pest control by the use of insecticide-spraying, these fruits contain few insects. The intake of vitamin $B_{12}$ during this period might therefore be expected to be low, and since the normal level of vitamin $B_{12}$ in plasma is in part maintained by an inflow of vitamin $B_{12}$ from the gut, this could result in a progressive decrease in the serum vitamin $B_{12}$ concentration during the summer months. During the winter and early spring, uncultivated wild fruit is the only available food, and the bats have been observed to feed on fruits such as wild figs. These figs contain large numbers of insects such as fruit flies and ants which would contain vitamin $B_{12}$ and presumably are ingested by the bats. During the winter, vitamin $B_{12}$ intake would therefore tend to be higher and serum levels would increase.

The exponential decrease in serum, liver and kidney vitamin $B_{12}$ concentrations in bats kept on the experimental diet reflected depletion of the body vitamin $B_{12}$ stores.

The rate of decrease in serum vitamin $B_{12}$ levels ($T_{0.5}$ 80 d) was more rapid than in the liver ($T_{0.5}$ 109 d), which was more rapid than in the kidney ($T_{0.5}$ 164 d). This implies that either there are differences in the rates of mobilization of vitamin $B_{12}$ from various storage compartments, or there is a redistribution of vitamin $B_{12}$ between these compartments when stores become depleted.

Grossowicz, Jablonska, Izak & Rachmilewitz (1970) found that the vitamin $B_{12}$ concentration of liver, kidney and serum of rats given a vitamin $B_{12}$-deficient diet decreased very rapidly within the first 5–10 d on the diet. This suggests that $T_{0.5}$ for vitamin $B_{12}$ in the rat is very short and a value of 46 d has been calculated by Gräsbeck, Ignatius, Järnefelt, Linden & Mali (1961). In man $T_{0.5}$ for vitamin $B_{12}$ in liver is approximately 1 year (Glass, 1954; Meyer, Berlin, Jiminez-Casado & Arkun, 1956; Schloesser, Deshpande & Schilling, 1958; Heinrich & Pfau, 1962). Grossowicz *et al.* (1970) suggested that the higher turnover rate for vitamin $B_{12}$ in the rat may be associated with the increased metabolic rate of small animals or may be specific for the rat. The findings reported in this study, indicate that $T_{0.5}$ for vitamin $B_{12}$ in the bat, while it is slower than in the rat, is of a similar order of magnitude.

After the initial sharp decrease in liver and kidney vitamin $B_{12}$ concentration in the rat, Grossowicz *et al.* (1970) found no further decrease in these organs although the serum vitamin $B_{12}$ concentration continued to decrease. They suggested that because rats lose only a fraction of their liver vitamin $B_{12}$, while appreciable stores are maintained, there may be two forms of vitamin $B_{12}$ in the liver, one readily dissociable and the other firmly bound, and they suggested that the dissociable form is lost more rapidly with the vitamin $B_{12}$-deficient diet.

Our finding that in bats the serum vitamin $B_{12}$ concentration decreases more rapidly than liver vitamin $B_{12}$ is in agreement with those of Grossowicz *et al.* (1970) for the rat. However, Nygaard, Killander, Myhre & Helsingen (1966) found a more rapid decrease in vitamin $B_{12}$ levels in the liver than in the serum of gastrectomized rats and suggested that the level of vitamin $B_{12}$ in the serum is maintained by mobilization from stores. The fact that Nygaard *et al.* (1966) used gastrectomized rats could account for the faster decrease which they found in liver vitamin $B_{12}$ concentrations, because after gastrectomy biliary vitamin $B_{12}$ would not be absorbed through the normal enterohepatic mechanism for vitamin $B_{12}$ reabsorption (Gräsbeck, Nyberg & Reizenstein, 1958; Green, van Tonder, Kew & Metz, 1975). In the normal rat and bat subjected to dietary deprivation of vitamin $B_{12}$, reabsorption of biliary vitamin $B_{12}$ would occur, and this might prevent the hepatic vitamin $B_{12}$ from decreasing as rapidly as in a gastrectomized animal.

It has been suggested that the time interval between the administration of radioactive cyanocobalamin and its equilibration with body stores is dependent on the mass of the dose, the route of administration and the species studied (Gräsbeck *et al.* 1961). Adams & Boddy (1968) have found in man that equilibration occurs by 5–10 d after the intravenous administration of 0·1 $\mu$g radioactive cyanocobalamin. However, 70–84 d elapse after administration of a 5000 $\mu$g dose before equilibration takes place. In relation either to body-weight or to the calculated total body vitamin $B_{12}$ store of a bat (*c.* 6500 ng) an injected dose of 2 ng vitamin $B_{12}$ is comparable to a dose of 0·3 $\mu$g

in man. It might therefore be anticipated that equilibration with body stores should occur after an interval of 10 d after the injection of [$^{57}$Co]cyanocobalamin.

There are several pools of body vitamin $B_{12}$ in man (Reizenstein, Ek & Matthews, 1966), but because the rate of decrease of a tracer dose of injected radioactive cyanocobalamin may be described by a single exponential equation, it has been assumed that these pools behave as a single pool (Heysell, Bozian, Darby & Bell, 1966; Adams & Boddy, 1968).

To test this assumption in the bat, the specific activities of cyanocobalamin were compared in the liver and kidney of bats killed 21 and 50 d after the injection of 2 ng [$^{57}$Co]cyanocobalamin. There was an appreciable difference in the specific activities of these organs after 21 d, but only a small difference after 50 d. This continuing small difference could indicate that the injected [$^{57}$Co]cyanocobalamin had not fully equilibrated with body stores of vitamin $B_{12}$, but could also represent the summation of small inaccuracies in the techniques used to determine the specific activity. It seemed reasonable to assume that equilibration of [$^{57}$Co]cyanocobalamin with body stores occurs within 50 d of the injection of 2 ng labelled cyanocobalamin.

The turnover rate of vitamin $B_{12}$ in the bat (0·79–0·98 %/d) calculated from $T_{0.5}$ for injected [$^{57}$Co]cyanocobalamin is four to ten times greater than the range reported in man (Boddy & Adams, 1968). Based on the calculated total body vitamin $B_{12}$ content of a vitamin $B_{12}$-replete bat, this turnover rate implies a daily requirement of approximately 50–60 ng vitamin $B_{12}$ for the bat to remain in vitamin $B_{12}$ balance. The average weight of an adult bat is approximately 150 g, so that on a 'weight-for-weight' basis, the daily requirement for vitamin $B_{12}$ in the bat is between five and fifteen times greater than that for man (2–6 $\mu$g for a 70 kg man) (Adams & Boddy, 1971). The bat's calculated daily requirement for vitamin $B_{12}$ (0·2 $\mu$g/kg body-weight per d) is, however, similar to that reported for other animals such as the rat (Jaffé, Indacochea & Embden, 1957) chick (Stokstad, Page, Pierce, Franklin, Jukes, Heinle, Epstein & Welch, 1948) pig (Nesheim, Krider & Johnson, 1950) and sheep (Smith, Koch & Turk, 1951). In these species, the requirement varies from 0·2 to 0·8 $\mu$g/kg body-weight per d.

In most animal species studied the liver is the main site of vitamin $B_{12}$ storage. In the rat, the kidney is the main storage organ (Rosenblum, Chow, Condon & Yamamoto, 1952). Based both on tissue vitamin $B_{12}$ concentration and on the organ distribution of injected [$^{57}$Co]cyanocobalamin, the liver appears to be the main storage site in the bat.

In man vitamin $B_{12}$-deficiency is associated with a variable serum folate level which is usually increased above the normal range (Waters & Mollin, 1961, 1963; Herbert & Zalusky, 1962). A progressive increase in the serum folate concentration in the bat occurred as the bats became vitamin $B_{12}$-deficient. These findings in the bat agree with the finding that vitamin $B_{12}$-deficient rats had higher serum folate levels than control animals (Vitale & Hegsted, 1967). Increased serum folate concentrations in vitamin $B_{12}$ deficiency may be explained on the basis of either the 'methyl-folate trap' hypothesis (Herbert & Zalusky, 1962; Noronha & Silverman, 1962) or the hypothesis that methyl folate transport into cells is impaired in vitamin $B_{12}$ deficiency (Das & Hoffbrand, 1970; Tisman & Herbert, 1973). It should be pointed out that a further

factor which might have contributed to the increased serum folate concentration in the bats kept on the vitamin $B_{12}$-deficient diet was their continuously high dietary folate intake. This could also be the explanation for the initial increase in erythrocyte folate concentration. After the initial increase, erythrocyte folate concentrations decreased in vitamin $B_{12}$-deficient bats although there was a continued increase in serum folate levels. This is in accord with the finding that folate levels in erythrocytes tend to be low in vitamin $B_{12}$ deficiency, and low liver folate levels have been described in vitamin $B_{12}$-deficient sheep and rats (Dawbarn, Hine & Smith, 1958; Kutzbach, Galloway & Stokstad, 1967).

These findings are compatible with the hypothesis proposed by Das & Hoffbrand (1970) and Tisman & Herbert (1973) that transport of reduced folate across cell membranes is impaired in vitamin $B_{12}$ deficiency.

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