VITAMIN $B_{12}$ DEFICIENCY IN CAPTIVE MONKEYS
AND ITS EFFECT ON THE NERVOUS SYSTEM
AND THE BLOOD

by

C. E. OXNARD*

W. THOMAS SMITH

and

I. TORRES†

Departments of Anatomy and Pathology,
The Medical School, University of Birmingham,
Birmingham, 15

SUMMARY

Monkeys fed vegetarian diets develop neurological and haematological abnormalities; paralysis can occur though the neural lesions (cerebral degeneration, posterior and lateral column degeneration of the spinal cord, segmental and wallerian degeneration of the peripheral nerves) are usually not evident during life. It is clearly important that the diet given to captive animals should contain an adequate amount of vitamin $B_{12}$, and that newly captive monkeys should not be used for research purposes unless shown to have normal serum levels of the vitamin.

The use of animals in experimental situations, particularly those involving prolonged captivity, necessitates maintenance of normal biological parameters. When animals used for any experiment are abnormal, conclusions drawn from the data obtained may be invalid. Recent work has shown that in 40 specimens of 8 genera of Old World primates fed adequate diets, the serum vitamin $B_{12}$ levels were of the same order as is found in man (Huser & Beard, 1969); also that various biological changes, not manifest in obvious illness, may be due to a deficiency of this same vitamin in some primates maintained on vegetarian diets (reviewed by Oxnard, 1967).

Rhesus monkeys when recently captive showed amounts of vitamin $B_{12}$ in the serum that resembled those found in man (150-600 pg/ml—Krohn, Oxnard

*Present address: Department of Anatomy, The University of Chicago, Chicago, Illinois 60637, U.S.A.
†Present address: Departamento de Morfologia, Universidad de Oriente, Ciudad Bolivar, Venezuela.
When kept in captivity under the conditions previously existing in the primate colony at the University of Birmingham, the amount of the vitamin in the serum gradually fell to levels as low as 20 pg/ml after 2 years (Oxnard, 1964). The evidence of Wilson & Pitney (1955) in Northwestern University and of Das Gupta, Chatterjea, Ghosh & Banerjee (1955) in India, when viewed in retrospect, suggests that this phenomenon was not confined to the Birmingham colony. It seems to be associated with the vegetarian diet (supplemented with iron and with vitamins other than B<sub>12</sub>) that was, until recently, commonly given to monkeys in captivity. Hypovitaminosis B<sub>12</sub> probably does not occur when monkeys are given animal-food products, vitamin B<sub>12</sub> supplements, or commercial pelleted diets which contain food of animal origin or vitamin B<sub>12</sub> additives.

When allowed to become deficient, rhesus monkeys do not often show obvious changes; for instance, the macrocytic anaemias that occur in man do not appear, individual animals sometimes have greater amounts of haemoglobin, higher red cell counts and higher serum iron levels than are found in recently captive animals, and obvious macrocytosis has not been noted (Oxnard & Smith, 1966). Anaemias that have been found from time to time in isolated animals are microcytic and hypochromic. Macrocytic anaemias reported in rhesus monkeys have been shown to respond to folic acid (and sometimes ascorbic acid) rather than to treatment with vitamin B<sub>12</sub> (reviewed by Smith, 1965).

Neuropathies are also well known in man with vitamin B<sub>12</sub> deficiency associated with pernicious anaemia, in malabsorption syndromes (Cooke & Smith, 1966), and in true vegetarians; subacute combined degeneration of the cord may be the sole manifestation of the deficiency. Similarly, in a few monkeys that have been maintained on vegetarian diets and have been found to have low levels of the vitamin in the serum, paralysis of the hindlimbs and tail can occur. A continued finding over many years has been the association of some cases of ‘cage paralysis’ with demyelination of the posterior (mainly) and lateral columns of the cord (reviewed by Innes & Saunders, 1962). Recent evidence indicates that hypovitaminosis B<sub>12</sub> is a causative factor in certain cases of cage paralysis, and that widespread occult histological lesions also occur in a high percentage of non-paralysed animals (Oxnard & Smith, 1966). These occult lesions include: spongiform degeneration of the posterior and lateral columns of the spinal cord; patchy demyelination of peripheral nerves of the hindlimb; and, occasionally, spongiform degeneration of the cerebral white matter and demyelination of the optic nerves. In some monkeys, lesions were found after less than 8 months in captivity on a vegetarian diet; the earliest onset of paralysis was after 14 months.

Lesions of the spinal cord and brain accompanied by loss of axons are
presumably irreversible. Recent evidence suggests, however, that when the lesions involve the peripheral nerves treatment can result in functional improvement, paralyses in affected monkeys becoming less severe (Oxnard & Smith, 1966). In non-paralysed monkeys segmental remyelination of peripheral nerves has been observed following administration of vitamin $B_{12}$ (Oxnard, Smith & Torres, 1967), though in the present paper we shall show that such remyelination is also found in animals with untreated vitamin $B_{12}$ deficiency.

The purpose of this present paper is to assess in some further detail the extent and nature of the neurological lesions in a large number of animals with levels of vitamin $B_{12}$ that are low compared with those of treated and recently captive animals, and also to assess whether or not clinical neurological and haematological examination can reveal prior signs of the condition.

**MATERIALS AND METHODS**

*Neuropathological investigation*

Some 43 monkeys in the Birmingham colony were studied: there were 40 rhesus monkeys (*Macaca mulatta*), 2 patas monkeys (*Erythrocebus patas*) and 1 baboon (*Papio anubis*). Of these, 12 (including the baboon and 1 patas monkey) had been maintained on wholly vegetarian diets for periods varying from 11 months to 10 years, (Group I); 14 monkeys (including the other patas monkey) kept in captivity from 11 months to more than 18 years, and originally given vegetarian diets, were later given a series of injections of vitamin $B_{12}$ followed by a normal diet (Group II); and 17 recently captive monkeys had been given a normal diet since arriving in the colony (Group III). Of the Group III monkeys, 14 had been in captivity for less than 21 days, and the other 3 for 6, 7 and 14 months. In Group I, 10 animals had low serum vitamin $B_{12}$ levels ($<100$ pg/ml); in Group II, 13 animals had high serum levels of the vitamin (500 pg/ml); and in Group III the serum levels were normal except in 2 estimations made in pregnancy, which is frequently associated with reduced levels (Oxnard, 1964). 5 animals in Groups I and II were paralysed; all of the others were clinically normal.

The brains, spinal cords and peripheral nerves were examined in all cases by routine neuropathological techniques. In 29 animals (10 from Group I, 10 from Group II and 9 from Group III) single nerve fibres from the proximal sciatic, proximal tibial and common peroneal, and distal tibial nerves were studied after fixing individual nerves in 4 per cent formaldehyde in saline and staining with 1 per cent osmium tetroxide; the fibres were then teased apart in glycerine under a dissecting microscope.
Clinical examination of the nervous system

Neurological examination presented considerable difficulties and could not be carried out on the full range of animals. Full clinical status was assessed in 9 animals of Group I, 7 animals of Group II, and 5 animals of Group III, and this included observations of the behaviour of the animals while in their cages, and examinations out of the cage. In 22 animals it was only possible to observe the animals from outside their cages. During observation of the monkeys, special attention was paid to the coordination, precision and strength of their spontaneous movements and alterations in their normal behaviour. The examination included a search for trophic changes and the testing of passive movements, muscle power and tendon reflexes. Tests for more subtle reflexes, such as the placing reaction, together with electrophysiological examination could not be carried out. The recording of objective phenomena was somewhat problematical and it is likely that only the most gross signs were recognised.

Haematological investigation

Detailed haematological examinations were carried out on 2 further groups of monkeys. The first consisted of 25 recently captive animals that had normal levels of vitamin B$_{12}$ in the serum (Group IV); the second consisted of 15 rhesus monkeys that had been maintained in the colony on a vegetarian diet for longer than 2 years, and of which all save 1 had levels of the vitamin below 60 pg/ml (Group V). The specimen of serum in the exceptional case was heavily haemolysed so that the level of the vitamin was not known.

The 15 deficient rhesus monkeys (Group V) were given, at intervals of about 10 days, intramuscular injections of 1000 μg of vitamin B$_{12}$. Serum determinations after the injections showed that the animals did indeed receive the vitamin. Repetition of treatment was carried out because previous experience showed that in severely deficient animals serum levels fall rapidly after single injections of the vitamin. Repeated assays confirmed that such a regimen was sufficient to maintain high serum levels in all animals.

Blood was drawn from the short saphenous vein with minimal stasis and, for haematological examination, anticoagulated with EDTA.

The following haematological values were estimated using the methods given by Dacie & Lewis (1963).

Haemoglobin (Hb): the oxy-haemoglobin method was used with a colorimeter (Evans Electroselenium Ltd, Harlow, Essex) which was standardised daily with a neutral gray screen of 0.475 density (Ilford Ltd, 4 New Oxford Street, London, W.C.1.).

Haematocrit (PCV): the Hawksley micro-haematocrit was used with the usual precautions. The capillary tubes were centrifuged for 5 minutes and no allowance was made for 'trapped plasma'.
VITAMIN B₁₂ DEFICIENCY IN MONKEYS

Red cell count (RBC): erythrocytes were counted on an electronic cell counter (Model A, Coulter Electronics Ltd, High Street South, Dunstable, Bedfordshire) using the ‘threshold value’ for the machine estimated from rhesus monkey red cells suspended in an albumin-saline solution. As many thousands of cells are counted in a few minutes by electronic counters, the accuracy achieved is greater than with manual methods. Thus, the derived values (e.g. MCH) are of greater significance.

Derived values: mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) were calculated from the usual formulae.

All the above values were estimated at least twice as a check on the accuracy of the techniques.

Details of diets

Vegetarian diet

This consisted of steamed potatoes, carrots and other raw vegetables, fresh fruit, plus a slice or two of white bread covered with ‘Bemax’ (Bemax Ltd, 23 Upper Mall, London, W.6)—which contains vitamins B and E but not B₁₂—as well as supplements of vitamins A, C and D. The diet for adults is, therefore, exclusively vegetable; small infants received additional milk. The total daily consumption was about 500-600 g (700-800 cal, 2 900-3 300 J) and the various items per adult animal per day on this regime were estimated to be as follows (Eckstein & Kelly, 1966):

- carbohydrate—30 per cent of diet—170 g (680 cal, 2 850 J)
- protein: total—3.5 per cent of diet—20 g (80 cal, 330 J)
  - animal—0 per cent of diet
  - vegetable—100 per cent of total protein
- fat—trace
- vitamins: B complex (added as ‘Bemax’)
  - B₁₂—trace
  - E—2 mg (128 mg/lb ‘Bemax’)
- iron—occasional supplement
- ash—2 per cent

The actual diet consumed probably varied somewhat. On days when root vegetables were not adequately cleaned, vitamin B₁₂ may have been imbibed from that in adherent soil or root hairs.

Normal diet

The adult animals received pelleted diet (‘Oxoid’ 41B, ‘Oxoid’ Ltd, Southwark Bridge Road, London, S.E.1), together with fresh occasional fruit.
and vegetables, about 200 g per day (500 cal, 2100 J); the various items per adult animal per day were estimated to be as follows:

- carbohydrate—49 per cent of diet—90 g (350 cal, 1470 J)
- protein: total—13.5 per cent of diet—25 g (100 cal, 420 J)
  - animal—40 per cent of total protein
  - vegetable—60 per cent of total protein
- fat—3.5 per cent of diet—6.5 g (55 cal, 230 J)
- vitamins: B complex present
  - B<sub>12</sub> about 0.5 μg
  - E about 0.15 mg
- iron—occasional supplements
- ash—3 per cent

This diet thus contains less vitamin B complex and less vitamin E than vitamin B<sub>12</sub>-deficient diet. Again it must be emphasised that the quantity consumed was variable, so that the estimations can only be regarded as approximate.

RESULTS

Neuropathological investigation

Histological sections

Groups I and II (26 animals): 23 animals (including all 5 paralysed animals) showed degeneration of the posterior and lateral columns of the spinal cord similar to that previously described by Oxnard & Smith (1966), that closely resembled human subacute combined degeneration resulting from vitamin B<sub>12</sub> deficiency. Such degeneration was exceptionally severe in 3 of the 5 paralysed animals, in contrast to the mild to moderate severity found in all 18 of the 21 non-paralysed animals showing cord changes. The posterior nerve roots showed demyelination, or the posterior root ganglia showed secondary neuronal degeneration (chromatolysis, neuronophagia), or both, in 9 of 24 animals in which they were examined. The peripheral nerves showed demyelination in frozen sections in 21 of the 26 animals, being most marked in 3 paralysed and 3 non-paralysed animals. The cerebral white matter showed spongiform degeneration in 3 of the 5 paralysed animals and in 2 of the 21 non-paralysed animals (Table 1). In Group II, 3 non-paralysed animals did not show spinal cord degeneration; the posterior nerve roots showed degeneration in 1 of these, the peripheral nerves showed degeneration in another, and the posterior roots and peripheral nerves were normal in the 3rd.
Group III (17 animals): 1 animal showed slight degeneration of the cord, 2 showed slight degeneration of the peripheral nerves and 1 showed slight degeneration of the cord and posterior roots and ganglia, and moderate peripheral nerve degeneration (Table 1).

Table 1. Incidence of neurohistological lesions in the monkeys studied.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of para-</th>
<th>Number paralysed</th>
<th>Normal peripheral nerve lesions</th>
<th>Abnormal spinal cord lesions</th>
<th>Abnormal posterior root &amp;/or ganglion lesions</th>
<th>Cerebral lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I deficient</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>II deficient, then treated</td>
<td>14</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>III recently captive controls</td>
<td>17</td>
<td>0</td>
<td>13</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NP* non-paralysed; P paralysed; the posterior roots and/or ganglia were not examined in 2 cases in Group I, neither was the brain-stem in 1 case in Group II.

Teased preparations of peripheral nerves

These results were mentioned briefly and illustrated by Torres, Smith & Oxnard (1969).

Groups I and II—teased preparations were examined from 20 of the 26 animals: 15 animals showed lesions. Segmental demyelination was seen in every affected case, in 13 cases it was the most pronounced abnormality and was always associated with clear evidence of segmental remyelination. Wallerian (axonal) degeneration was present in only 4 affected animals, in 3 of which there was evidence of axonal regeneration and remyelination. In 2 animals the wallerian degeneration was more pronounced than the segmental demyelination.

Group III—nerve fibres from 9 of the 17 animals were teased: 3 animals showed segmental demyelination and remyelination, and in 1 of these there was limited evidence of wallerian degeneration. The 3 animals with segmental demyelination were the same 3 animals that showed degeneration in routine histological sections of peripheral nerves.

There was no evidence in any of the 3 groups that the lesions were more pronounced in the distal parts of the nerves; the distribution pattern suggested random involvement.
Examination in life

The animals of Group III appeared healthy, with brisk, easily elicited reflexes. Those in Groups I and II, when not paralysed, did not show trophic lesions and also appeared healthy. But in these groups, though one must interpret such a finding with caution, it did seem that reflexes in the hindlimbs were reduced, difficult to elicit, or even absent. It was also an impression that these captive rhesus monkeys were more aggressive and morose; however this remains to be tested by some objective method.

The 5 animals with chronic paralysis showed, in general, considerable flexion deformity, trophic changes on the heels and toes, and loss of hair and skin on the tail. These lesions appeared to be painless; however, hyperesthesia seemed to be caused in some animals by stroking or rubbing the digits or sole, and by pressure on the muscles and tendons of the leg. The worst of these cases presented complete paralysis; the less affected showed impaired mobility so that they could not climb, or climbed with difficulty. A typical posture for these animals was to sit with the toes curled and with only the lateral aspect of the foot resting lightly on the bars or wires of the cages. There seemed to be considerable resistance to bearing the body weight on the soles of the feet. The reflexes were reduced or absent.

The 2 patas monkeys both developed their first attack of paralysis within a few months of captivity, and were consequently examined frequently during the course of the disease. As there were 4 other patas monkeys also available in the colony, there was good material for comparative observations.

First the affected patas monkeys merely appeared unwell and with reduced mobility. Gradually over subsequent days they showed a marked disinclination or inability to jump up to their perches, and sat for considerable periods of time with the limbs held inverted so the plantar surfaces did not touch the substrate. Touching or stroking the feet elicited withdrawal and signs of discomfort from the animals—this was worse on one side. In 1 animal the hand showed a tremor when holding a nut or when held in a sustained posture against gravity. Clinical examination of the 2 animals showed extreme hyperreflexia in the hindlimbs, greater on the more affected side. The forelimbs appeared to show normally brisk reflexes. 1 of the animals became very much worse and was killed. The other was treated and showed definite improvement in motor activities. Over the succeeding days the reflexes gradually became normally brisk. Treatment with parenteral cyanocobalamin was not maintained, though the animal was put on a pellet diet which included animal products and presumably, therefore, vitamin B₁₂. Certainly the serum level of the vitamin was maintained. 7 months later the animal again became unsteady; at this time it showed a picture more like that of the other paralysed animals.
Haematological investigation

The comparison of 25 recently captive monkeys (Group IV) with 15 that had been maintained on deficient diets for more than 2 years (Group V), confirmed that for haemoglobin and for haematocrit there were no significant differences at the probability level of 1 in 20 (Table 2). But the RBC was significantly lower in the deficient animals as compared with the recently captive animals (P=0.05-0.02). Though the MCH and MCHC did not differ significantly between the 2 groups, the MCV was significantly lower in the recently captive (P=0.05-0.02; the means differed by 5.4 \( \mu m^3 \)).

Table 2. Haematological findings on recently captive monkeys (Group IV), compared with those maintained for more than 2 years on a vegetarian diet both before (Group V) and after (Group V+) treatment with vitamin B\( _{12} \).

<table>
<thead>
<tr>
<th></th>
<th>Group IV</th>
<th>Group V</th>
<th>Group V+</th>
<th>Significant differences* between:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (per cent)</td>
<td>85.1±7.9</td>
<td>82.8±5.8</td>
<td>89.2±7.6</td>
<td>V and V+</td>
</tr>
<tr>
<td>PCV (per cent)</td>
<td>42.7±3.4</td>
<td>41.0±2.4</td>
<td>43.0±3.1</td>
<td>V and V+</td>
</tr>
<tr>
<td>RBC (millions/mm(^3))</td>
<td>4.93±0.16</td>
<td>4.65±0.5</td>
<td>5.1±0.5</td>
<td>IV and V; V and V+</td>
</tr>
<tr>
<td>MCHC (per cent)</td>
<td>29.0±3.2</td>
<td>29.1±2.1</td>
<td>30.1±1.6</td>
<td>...</td>
</tr>
<tr>
<td>MCV (pm)</td>
<td>86.7±9.4</td>
<td>92.1±10.4</td>
<td>86.1±5.9</td>
<td>IV and V; V and V+</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>25.3±2.9</td>
<td>26.3±2.9</td>
<td>26.1±2.8</td>
<td>...</td>
</tr>
</tbody>
</table>

*Differences significant at the 5 per cent level or less. Differences between IV and V+ not significant.

Table 2 also shows measurements made on the 15 Group V monkeys approximately 6 weeks after treatment with B\( _{12} \). Here all parameters save the MCH and MCHC were significantly different. The haemoglobin was raised by 6.4 per cent (P=0.01) and the PCV by 2 per cent (P=0.05-0.02). The RBC was raised by nearly half a million cells per mm\(^3\) (P=0.01-0.001) and the MCV was correspondingly reduced by 6 \( \mu m^3 \) from 92 to 86 (P=0.05-0.02).

DISCUSSION

Groups I and II were considered together because both consisted of long-stay animals that had been fed vegetarian diets for long enough to have induced neurological lesions; Oxnard & Smith (1966) detected early histological changes after 8 months and clinical paralysis after 14 months. Group II
was originally distinguished from Group I because all animals in Group II had been given vitamin \( \text{B}_{12} \) injections followed by a normal diet for periods varying from 6 months to 4 years before death, in an attempt to assess the effect of replacement therapy. We have not yet found histological evidence of reparative changes attributable to such therapy in either central or peripheral nervous systems. The evidence of functional improvement previously noted (Oxnard & Smith, 1966) probably resulted from recovery of temporarily suppressed nerve function, after therapy had arrested the degeneration due to deficiency. In the present series, active sudanophilic degeneration of myelin was still seen in the cord of an animal that had received a normal diet continuously for 35 months after 43 months on a deficient diet; and in another animal paralysis recurred after 6 months of treatment.

The minor changes found in 4 of the animals in Group III can be explained in several ways. 2 of these animals were pregnant and had very low levels, and 1 non-pregnant animal had a low-normal serum-vitamin \( \text{B}_{12} \) level, after only 10, 13 and 19 days respectively on a non-vegetarian diet (the vitamin \( \text{B}_{12} \) level was not estimated in the 4th animal). This suggests that low levels due to pregnancy or a previously inadequate diet may have already affected the nervous system before the animals arrived in the Birmingham colony. Advancing age and peripheral nerve trauma can also result in minor peripheral nerve lesions similar to those shown in 3 of 4 of these animals, but unfortunately both the age and the previous history of the animals prior to their arrival in the colony was unknown. These findings should be borne in mind when recently captive monkeys are used for neurological research, particularly when the peripheral nerves are under investigation.

Unless the animals developed overt paralysis, there was no evidence during life of the lesions found on histological examination. The hyporeflexia that was found in most of the animals in Groups I and II was only evident when recently captive animals were available for comparison, and even then the finding must be treated with caution. It seems likely that tests for more subtle reflexes such as the placing reaction, or investigation of motor nerve conduction velocities, might provide prior evidence of the disease process.

Routine examination of blood films, Hb, PCV, MCHC and MCV is probably of little value in the diagnosis of simian vitamin \( \text{B}_{12} \) deficiency. Oxnard & Smith (1966) found no significant differences between deficient animals and recently captive animals. The results reported here seem to be a little at variance in that the RBC and MCV now differed significantly. However, this can perhaps be attributed in part to somewhat larger samples and perhaps also to the increased sensitivity of the RBC as determined with the Coulter counter (previous determinations were done by hand). In fact, in the previous work the RBC and MCV were just not significantly different at the \( 1 \) in 20 level, and in the present study they just attained that level of significance.
The results obtained from the longitudinal haematological investigation of the same animals before and after treatment show that although in most parameters a statistically significant improvement did occur, the changes were small enough to pass undetected in the routine examination of the animals. Certainly the somewhat larger size of the red blood cells in the animals before treatment (i.e. when deficient) could not be labelled ‘macrocytosis’. As in the previous studies qualitative examination of blood films did not reveal a single case that was consistent with a macrocytic anaemia. Nevertheless the improvement on treatment was sufficient to produce mean values resembling those of the more normal recently captive animals.

Recently Huser & Beard (1969) in a small series of 5 rhesus monkeys, which had received diets adequate in vitamin B$_{12}$ for at least 2 years, found haematological parameters which showed even better figures than were found here after treatment. Thus, for instance, their mean RBC was 5.5 millions (range 5.2-5.9) and their mean MCV was as low as 77 µm$^3$. This suggests the possibility that even more marked improvements might have been found in the animals in this study had the experiments been carried further. It also suggests that the animals accepted as normal in this study (either because they had been recently imported and therefore had not had time to develop the vitamin B$_{12}$ deficiency on the vegetarian diet, or because they were receiving supplements) were still not in an optimal nutritional state.

It is clear, then, that unless captive monkeys are fed diets containing natural vitamin B$_{12}$ or are given supplements of the vitamin, many of them will develop neural lesions. The progression of such lesions may be arrested by treatment, though the restoration of disordered function that ensues is likely to be due to cessation of degeneration and release of inhibition, rather than to remyelination and regeneration. Evidence of remyelination after segmental demyelination, and regeneration after wallerian (axonal) degeneration, was found in the peripheral nerves in animals with untreated vitamin B$_{12}$ deficiency, a state of affairs not unlike that in experimental lead poisoning (Fullerton, 1966); treatment did not appear to influence the degree of segmental remyelination and axonal regeneration.

Little is known about the long-term effects of vitamin B$_{12}$ deficiency on the growth and weight of monkeys. Flinn & Oxnard (1966) found there were significant increases in the growth of infant monkeys studied for about 2 years after post-weaning treatment with vitamin B$_{12}$, as compared with the growth patterns in untreated monkeys. But this was a retrospective study based on body weights previously recorded, and could not be correlated with other desirable data such as radiological studies.

It is possible that other nutritional factors were also deficient in the monkeys studied, though the similarity of the lesions to those of human 'subacute combined degeneration', and the functional improvement previously reported after
treatment with vitamin B₁₂ (Oxnard & Smith, 1966) suggest that deficiency of this vitamin was the most important factor. The practical point that emerges is that vegetarian-fed monkeys should not be used for neurological or psychological research, in view of the high incidence of neural lesions found in this investigation. In addition, recently captive animals may be suspect unless serum assays confirm their normal nutritional status for the vitamin.

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