Phosphatase activity in the limb bones of monkeys (*Lagothrix humboldti*) with hyperparathyroidism

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SYNOPSIS The paper reports a study of the distribution of phosphatases in the femora of three specimens of Humboldt's woolly monkey (*Lagothrix humboldti*) suffering from chronic hyperparathyroidism. Bone structure ranged from the apparently normal to extreme osteitis fibrosa. Most marked changes were found in the distribution of alkaline phosphatase, which reached at least 10 times the normal levels in the bone of the second monkey in the series, dropping to levels still well above normal in that of the most severely affected animal. Very high concentrations were found in the deeper layers of hypertrophied growth cartilage and in the osteoblasts lining poorly calcified trabeculae, and high concentrations in the fibre bone of the third animal. Lack of mineralization and the development of osteitis fibrosa are thus associated with a marked increase in alkaline phosphatase activity. Osteoclasts reacted strongly for acid phosphatase but were negative for alkaline phosphatase. Acid phosphatase levels were comparatively high in fibre bone, but overall levels ranged from 1/20 to less than 1/100 those of alkaline phosphatase. Some slow staining for acid phosphatase probably represents residual activity at acid pH of the markedly increased alkaline phosphatase. There may be some association between a failure of mineralization and the presence of acid phosphatase in osteoclasts and osteoid.

The aetiology of the monkeys' condition is discussed. It seems likely that the parathyroid hypertrophy and rachitic changes were caused by low blood calcium dependent on a low calcium diet and lack of vitamin D, in which the requirements of New World monkeys are reputedly high.

In 1959-60, the Bristol Bone Tumour Register received, through Mr. J. A. Dall, the bodies of five monkeys from Paignton Zoo. Four animals had a severe bone disorder, and the parathyroid glands appeared markedly enlarged in all five. The animals are listed in Table I. All but the green monkey were South American species. The three specimens of Humboldt's woolly monkey formed a series in which the condition of the bones ranged from apparently normal to extreme deformity. A study of the distribution of phosphatases in their femora forms the subject of this paper.

A brief résumé of the history of simian bone disease given by Ruch (1959) is relevant to the condition of these animals. Workers early in this century reported a condition in captive monkeys characterized by thickened skull bones, enlarged jaw bones, and tumour-like swellings of the nasal bones, identifying it with the human tropical disease goundou, a rare tertiary lesion of yaws. It was later

realized that this facial deformation was only one manifestation of a generalized bone disorder of captivity, which was identified by Corson White (1922b) with Paget's osteitis deformans of bone, though, even while making this identification, he drew attention to the similarity to osteitis fibrosa cystica and also to the calcium-deficient diet of the affected animals. Later workers suggested that the condition resembled von Recklinghausen's disease.

Ruch suggests that the disease is one of dietary insufficiency: 'A factor probably more powerful than words was that the pseudo-goundou-Paget's disease entity disappeared from collections of captive monkeys and apes as a result of improved diets introduced widely at this time.' Again, 'Throughout the period of 1895-1935 almost no one gave serious consideration to the possibility that some of the processes which result in rickets and osteomalacia in man manifest themselves in monkeys in an unusual fashion. This oversight was repeated even though many of the animals simultaneously showed obvious signs of rickets or osteomalacia.'

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TABLE I
PARATHYROID AND BONE CONDITION IN MONKEYS FROM PAIGNTON

Animal	Sex	Weight (kg.)	Parathyroids		Bone Condition
			(mg.)	(mg./kg.)	
A BTR/1277 Humboldt's woolly monkey, adolescent	M	2.698	29	10.7	Bones well calcified and apparently normal
B. BTR/1254 Humboldt's woolly monkey, adolescent	M	4.536	44	9.7	Bones soft and flexible, several fractures, hypertrophic growth cartilages, mandible could be cut with knife
C BTR/1215 Humboldt's woolly monkey, adolescent	F	1.927	91	47.2	Bones very soft; advanced and extensive osteitis fibrosa cystica, with large and microfractures. Hypertrophic growth cartilages, mandible easily cut with knife
BTR/1278 Capuchin, adult	F	1.474	38	25.1	Bones generally softened, callus formation from past incomplete fractures, and healing fracture of femur mandible readily cut with knife
BTR/1280 Green monkey, adult	M	6.397	30	4.7	Bones slender and somewhat softened, fractures of humerus and radius, mandible soft, but too hard to cut

Yet the pathologic picture in some animals was sufficiently different from rickets and osteomalacia of man to prompt the thought that the monkey differs from man in some important way in osseous function, mineral metabolism, dietary requirements, or endocrine make-up. There is always the possibility that, in the decay of interest in simian bone pathology, an interesting and important aspect of physiology and pathology has been lost sight of.

Although osseous lesions resembling Paget's osteitis deformans of mammals other than man have been described, closer investigation has in our hands shown characteristic differences, and there are good reasons to suggest that osteitis deformans is a skeletal disease entirely restricted to the human race. Osteitis fibrosa in many mammals affects first the jaw and skull bones. 'Rubber-jaw' of dogs is a late manifestation of hyperparathyroidism secondary to leptospiral nephritis (Platt, 1951); 'big-head' of equines is an extra-European disease associated with comparative excess of phosphorus in the diet, and manifesting acid urine with hyperphosphaturia, slightly high serum P, and slightly low serum Ca (Hutyra, Marek, and Manninger, 1946). The probability that in the monkeys from Paignton Zoo the hyperparathyroid state was secondary to rickets will be considered in the discussion.

MATERIALS AND METHODS

The three specimens of Humboldt's woolly monkey were all adolescent, with epiphyses still open. They have been designated A, B, and C in order of increasing severity of bone changes. Brief notes of necropsies performed by Dr. C. H. G. Price are given below.

A (BTR/1277) A male, weighing 2.698 kg., which died suddenly on 15 January 1960. The body was received on 17 January and necropsy performed on 20 January. The animal appeared relatively healthy, though thin, and the cause of death was not found. The lymph nodes were

slightly enlarged; the kidneys were gritty to the knife suggesting some metastatic calcification; otherwise the viscera appeared normal. The bones were firm and well calcified, without deformities or fractures. Two parathyroid glands were found, weighing 14 and 15 mg. making a total of 29 mg., or 10.7 mg. per kg. body weight. This was the mate of the animal C (BTR/1215), which died on 15 February 1959 when it also was thought likely to die from the same condition. By July 1959, however, it had fully recovered. A sample of blood serum was obtained on 15 October 1959 which was analysed as follows:—Calcium, 12.1 mg./100 ml.; phosphorus, 9.9 mg./100 ml.; alkaline phosphatase, 296 King-Armstrong units/100 ml.; acid phosphatase, 23.6 K-A. units/100 ml. The serum was markedly haemolysed, which might explain the high value obtained for acid phosphatase since there is a high concentration of this enzyme in erythrocytes. It would not, however, explain the very high level of alkaline phosphatase.

B (BTR/1254) A male, weighing 4.536 kg., was killed on 16 July 1959 and necropsied on 17 July. This animal showed generalized lymphadenopathy. The liver and kidneys were congested, but otherwise normal. Bones tended to be soft and flexible, particularly the distal bones; there were fractures in the right femur and tibia, the left tibia and fibula. The mandibles were very soft and easily cut with a knife through the horizontal ramus. Four parathyroid glands were found, weighing 7, 13, 13, and 16 mg., making a total of 44 mg. or 9.7 mg. per kg. body weight. The glands were of very loose texture, slightly acinar, and composed of transitional, chief, and clear cells. No adenomata were found.

C (BTR/1215) A female, weighing 1.927 kg., which died on 15 February 1959 and was necropsied on 16 February. The body was generally in good condition, though rather poorly nourished. Lymph nodes were enlarged; the kidneys showed marked cloudy swelling and widespread mild tubular nephritis, with cellular proliferation in the glomeruli. There were extensive areas of necrosis with abscess formation in the liver. The bones showed well-advanced and very extensive osteitis fibrosa cystica, with both large and microfractures and much soft-tissue swelling (e.g., of periosteum), with

wispy new bone laid down in large amounts but poorly mineralized. Several growth cartilages were distorted and deformed, with poorly developed primary spongiosa. The marrow was generally hyperplastic, rather congested and with areas of thrombosis; fatty marrow was generally replaced by swollen myxoid fibrous tissue. The mandible was very soft, and easily cut transversely with a knife. Two parathyroids were found, with a total weight of 91 mg., giving a ratio of 47 mg. per kg. body weight. The glands were nodular, acinar, with chief and clear cells and transitional cells. No adenomata were found.

QUANTITATIVE PHOSPHATASE ESTIMATIONS The bones of the right limbs were dissected out and stored in the freezing chamber of a domestic refrigerator before examination. Quantitative estimations were carried out as in a previous study of normal rabbit bones (Jeffree, 1959). Bones were cleaned, weighed, and drawn to scale, then cut longitudinally, and half or a quarter of each bone divided into portions on an anatomical basis; each portion was finely chopped and weighed. Separate portions were well ground in a glass or porcelain mortar with a little acid-washed silver sand and a measured quantity of distilled water, to give known dilutions of bone (the sand, being insoluble, can be neglected in the calculation). Initial dilutions varied from 1 in 10 to 1 in 50, according to the amount of bone available. The suspensions were left in the refrigerator overnight, then shaken and centrifuged; the supernatant fluid was used for phosphatase estimations, higher dilutions of the original extract being made where necessary for estimations of alkaline phosphatase. Para-nitrophenyl phosphate was used as substrate, according to the modification of the method of Bessey, Lowry, and Brock (1946) described by Jeffree (1959). Distribution maps are comparable with those in the latter paper.

HISTOLOGICAL DEMONSTRATION OF PHOSPHATASES Portions of bone removed from the freezing chamber were fixed overnight in the body of the refrigerator in absolute ethanol, 10% formol saline (approximately 3.5% formaldehyde), or 5% formol saline buffered at pH 7.2. These were then dehydrated and embedded in paraffin wax, or double-embedded in low-viscosity nitrocellulose and paraffin according to the method of Ball (1957), whose technique for cutting undecalcified sections was used for most of the slides illustrated, though with the soft bones of monkeys B and C it was possible to cut sections of single-embedded tissue on a Cambridge rocking microtome. The results of the two methods may be compared in Fig. 3. Fig. 3B shows material which was double-embedded and cut on a base sledge microtome, with Sellotape support for the section, by the method of Ball, whereas the softer bone of Fig. 3C was single-embedded and cut on a Cambridge rocking microtome without support; even in this very soft tissue the cartilage cells have been displaced in the process of sectioning.

A few sections were stained for phosphatase according to the methods of Gomori (1946, 1950) as quoted by Pearse (1960) but it was found preferable to use the method of Burstone (1958a and b), which avoids false positive reactions in nuclei, cartilage matrix, and undecal-

cified bone, and shows the relationship of phosphatase staining to mineralized structures which take the haemalum counterstain.

RESULTS

QUANTITATIVE ESTIMATIONS OF ALKALINE PHOSPHATASE Fig. 1 shows the distribution of alkaline phosphatase in the right femora of the three monkeys and in that of a 5-month-old rabbit. The femur of A is roughly comparable to this rabbit bone, but shows somewhat higher overall values and not such a marked concentration of the enzyme below the epiphyseal plates. This appears to be a normal or nearly normal bone, with alkaline phosphatase levels perhaps somewhat raised (I have no means of assessing the species differences). The bones of the other two animals show marked divergence from the normal. Levels of alkaline phosphatase in B are enormously high, being about 10 times those in A, as will be seen by comparison with the inset figure, in which phosphatase values have been shown at one tenth their actual level, giving a picture very similar to A; the femur of B, in fact, shows a roughly normal distribution of alkaline phosphatase at 10 times the normal level. The distribution in C is markedly different. There are raised values (though not as high as in B) throughout the bone, except in the periosteum and the fractured head, where very low values are found. The most marked deviation from normal is in the shaft, where the thickened, fibrous bone shows a concentration of the enzyme very much greater than in a normal compact shaft wall.

QUANTITATIVE ESTIMATIONS OF ACID PHOSPHATASE Fig. 2 shows the distribution of formaldehyde-stable acid phosphatase. Estimations were made in the presence of 0.5% formaldehyde in order to minimize the contribution of erythrocyte acid phosphatase and to give values comparable with those of the previous study of rabbit bones (Jeffree, 1959). It should be noted that, in view of the smaller concentrations of this enzyme, shadings in this diagram represent only one tenth the activity shown by similar shadings for alkaline phosphatase. The values of acid phosphatase in the femur of A, like those of alkaline phosphatase, are somewhat higher than in that of the 5-month-old rabbit, except below the growth cartilages, where they are lowered rather than raised. The femur of B shows similar values but a slightly different distribution, with raised concentrations below epiphyseal and articular cartilages and in the endosteum. Acid phosphatase in the femur of C is generally lower than in the other two animals with a more even distribution throughout the bone, except for the fractured head which shows extremely low levels of the enzyme.

FIG. 1 Alkaline phosphatase of monkey femora.

FIG. 2. Formaldehyde-stable acid phosphatase of monkey femora.

FIG. 3A

FIG. 3. *Alkaline phosphatase in epiphyseal plates of femora.*

Incubations at room temperature and pH 8.3.

Substrate Naphthol AS-TR phosphate.

Diazotate Fast Red TR salt.

FIG. 3A. *Four and a half hours' incubation. $\times 120$.*

FIG. 3B

FIG. 3A and 3B. *Fixed cold formol saline buffered at pH 7.2; double-embedded and cut on base sledge microtome with Sellotape support for sections.*

FIG. 3B. *One and a half hours' incubation. $\times 60$.*

FIG. 3C

FIG. 3C. *Fixed cold formol saline; paraffin embedded and cut on Cambridge rocking microtome.*

FIG. 3C. *Three hours' incubation. $\times 60$.*

HISTOCHEMICAL STAINING OF ALKALINE PHOSPHATASE
This enzyme is demonstrated in four main sites:— 1 In the deeper layers of cartilage cells of both articular and epiphyseal cartilage; 2 in the osteoblasts on trabecular surfaces; 3 in the inner layer of the periosteum; and 4 in the fibrous tissue between trabeculae in the affected bones of B and especially C.

Table II gives comparisons between the three femora. The growth cartilage in both B and C is markedly hypertrophied, and irregular in depth, with a tendency to separate into tongues as in typical rickets (Fig. 3). In A there is also some irregularity in depth, but the cartilage is much narrower and shows a well-mineralized zone of provisional calcification. In the metaphysis, wide osteoid zones are apparent in B and C (Fig. 4). Osteoclasts are in evidence only in B, and here only in moderate numbers. Fibre bone is a noteworthy feature of C and a few fibres are found between the trabeculae of B but A shows well-formed cortical bone which appears to be fully mineralized.

The very high levels of alkaline phosphatase found quantitatively in B are reflected to some extent in the shorter periods of staining required for the bones of this animal.

HISTOCHEMICAL STAINING FOR ACID PHOSPHATASE
With some exceptions, staining these bones for phosphatase at pH 5 to 5.4 gives a picture very similar to that obtained at pH 8.3, suggesting that this staining may be largely due to residual activity of alkaline phosphatase at the acid pH. Allen and Hunter (1960) found, on zymograms of epididymal

enzymes, two bands reactive for phosphatase at pH 6, of which one represented acid phosphatase *per se* and the other the residual activity of alkaline phosphatase. Bone sections stained for alkaline phosphatase were incubated at room temperature, but for acid phosphatase at 37°C. and generally for considerably longer periods, so that like intensity of staining by no means represents comparable enzyme activity. Under these conditions the residual activity of alkaline phosphatase, which quantitatively may be 100 times as active as the acid phosphatase, is a factor to be considered. However, there are some differences in the distribution of staining at acid pH which are likely to represent the activity of acid phosphatase proper.

1 *Articular and epiphyseal cartilage* In sections incubated for a moderate length of time, acid phosphatase is demonstrated in and around the cells in the deeper layers of the columns, with a distribution similar to that of alkaline phosphatase (Fig. 5B, 1) but in some sections incubated over longer periods, staining is seen throughout the depth of the epiphyseal cartilage in both cells and matrix (Fig. 5B, 2). It is possible that this weak activity represents diffusion of the enzyme during the long period of storage in the refrigerator before fixation.

2 *Trabeculae* The presence of acid phosphatase in osteoblasts on the trabecular surfaces (Fig. 6B) is in contradistinction to the findings of Burstone (1958c) but may well be explained by the very high values of alkaline phosphatase in these bones and the long periods of staining at pH 5.4, permitting a residual reaction from this enzyme. Staining in the

TABLE II

DISTRIBUTION OF ALKALINE PHOSPHATASE IN MONKEY FEMORA

Region	Animal A	Animal B	Animal C
Epiphyseal cartilage	Short columns of cells staining weakly for alkaline phosphatase in lower levels. Stronger staining round degenerate cells of well-mineralized zone of provisional calcification Fig. 3A	Cartilage markedly hypertrophied and irregular in depth, tending to separate into tongues. Strong staining for alkaline phosphatase to a depth of 20 to 30 of lower cells, very little mineralization Fig. 3B	Cartilage markedly hypertrophied with tendency to split into tongues. Alkaline phosphatase staining through a deep zone of cells, moderate degree of mineralization Fig. 3C
Trabeculae of metaphysis	Wide well-calcified trabeculae with narrow osteoid seams bordered by narrow layer of alkaline phosphatase-positive osteoblasts Osteoclasts not in evidence Fig. 4A	Narrow trabeculae with wide osteoid seams covered by a layer of strongly alkaline phosphatase-positive osteoblasts (Fig. 4B) A fair number of osteoclasts, which are negative for alkaline phosphatase (Fig. 4BO)	Trabeculae poorly formed, partly mineralized, with wide seams of osteoid sometimes on one side only Some surfaces lined by cells which are positive for alkaline phosphatase Osteoclasts not seen Fig. 4C
Subperiosteal region	In all three animals the inner layer of the periosteum shows a zone of variable width of alkaline phosphatase-positive cells. The cells are elongated and appear similar to those of fibre bone Figs. 4A, 4B, and 4C		
Fibre bone	May be represented by an increased width of subperiosteal tissue in some places Fig. 4A	A few alkaline phosphatase-positive fibres are found between the trabeculae Fig. 4B	An extensive network of fibres which are positive for alkaline phosphatase connects osteoid seams of trabeculae and islets of osteoid Fig. 4C

FIG. 4A. *One and a half hours' incubation. $\times 60$.*

FIG. 4B. *Three quarters of an hour's incubation. $\times 60$.*

FIGS. 4A, 4BO, and 4C. *Fixed cold formol saline buffered at pH 7.2; double-embedded and cut on base sledge microtome, with Sellotape support for sections.*

FIG. 4B. *Fixed cold formol saline; paraffin embedded and cut on Cambridge rocking microtome.*

FIG. 4C. *Twenty-four hours' incubation. $\times 60$.*

FIG. 4BO. *One and a half hours' incubation. $\times 227$.*

FIG. 4. *Alkaline phosphatase in metaphyses. Incubations at room temperature and pH 8.3.*
 Substrate *Naphthol AS-TR phosphate.* Diazotate *Fast red TR salt.*

FIG. 5B.1. Four hours' incubation. $\times 60$.

FIG. 5B.2. Eighteen hours' incubation. $\times 60$.

FIG. 5. Acid phosphatase in epiphyseal plate of femur. Bone fixed in cold formol saline, buffered at pH 7.2: double-embedded and cut on base sledge microtome, with Sellotape support for sections. Incubations at 37°C. and pH 5.4. Substrate Naphthol AS-TR phosphate. Diazotate Fast Bordeaux OL salt.

FIG. 6B. Two hours' incubation at pH 5.4. $\times 60$.

FIG. 6C. Twenty-four hours' incubation at pH 5.0. $\times 60$.

FIG. 6B. Fixed in cold formol saline, buffered at pH 7.2: double-embedded and cut on base sledge microtome, with Sellotape support for sections. Incubation at 37°C. and pH 5.4.

FIG. 6C. Fixed in cold ethanol; paraffin embedded and cut on Cambridge rocking microtome. Incubation at 37°C. and pH 5.4.

Substrate Naphthol AS-TR phosphate. Diazotate Fast Bordeaux OL salt.

FIG. 6. Acid phosphatase in metaphyses.

osteoclasts, however, indicates the presence of acid phosphatase proper, since these cells show no reaction for alkaline phosphatase.

3 *Subperiosteal region* Staining here may be entirely due to a residual reaction of the alkaline phosphatase (Fig. 6).

4 *Fibre bone* The fibre bone (Fig. 6c) of animal C stains strongly for acid phosphatase on long incubation and the staining extends into the osteoid seams of the skimpy trabeculae and the islets of osteoid. Since this distribution is somewhat different from that of alkaline phosphatase, it may represent a real association between the presence of acid phosphatase and a failure of mineralization.

DISCUSSION

These three monkeys form a series with bone structure ranging from the apparently normal condition in A to extreme osteitis fibrosa in C. There are changes suggesting rickets in B and C and the parathyroids of all three animals are greatly enlarged by human standards and by comparison with the meagre data available for monkeys.

Gilmour and Martin (1937) give the mean weights of histologically normal parathyroid glands as 120.8 mg. for men and 139.7 mg. for women. Assuming, after Brody (1945), a mean body weight of 70 kg. for men and 60 kg. for women, the normal human ratios will be 1.72 mg. and 2.33 mg. per kg. body weight. Gilmour and Martin's highest value in a 'normal' human was 388 mg. of parathyroids in a stout man; if, on a conservative estimate, we assume his weight to have been 80 kg., the ratio in this extreme instance was 4.85 mg. per kg. body weight, or just half the lowest value in this series of monkeys.

The only figures available for monkeys are those given by Baker (1942) for parathyroid volumes in a macaque, *Macaca mulatta* (the rhesus monkey). His ratios for normal animals are 0.276 c.mm. per kg. body weight for males and 0.344 c.mm. for females, measuring the external parathyroids only. According to Arndt (1923), the external parathyroids in apes correspond to the inferior glands in man, which are slightly heavier than the superior pair, and internal glands may be present or absent in different individuals of the same species. It is thus a generous estimate to assume the total parathyroid tissue in these animals to be double that of the external glands. If, following Gilmour and Martin (1937), we take the specific gravity of the parathyroids as 1.1, we can convert Baker's figures for the macaque to approximate ratios of total parathyroid weight to body weight on the following basis:—

Males $0.276 \times 2 \times 1.1 = 0.608$ mg. per kg. body weight
Females $0.344 \times 2 \times 1.1 = 0.756$ mg. per kg. body weight

These are less than half the normal human ratios, making it improbable that, even in a different species of monkey, the normal ratios would be grossly above those for man.

Parathyroid hypertrophy may thus safely be assumed in all three animals, though it is most extreme in C, the animal with the most severely affected bones, with a ratio of 47.2 mg. per kg. body weight. A similar hypertrophy was found in the parathyroids of the other two monkeys from Paignton, whose bones are not included in this study. These were both adult animals. One was a female Capuchin (*Cebus*), also a South American monkey, which had softened bones with evidence of past incomplete fractures, and a healing pathological fracture in the left femur. The mandible was markedly softened and cut fairly readily with a knife through the horizontal ramus. There were four parathyroids with a total weight of 38 mg. in an animal weighing 1.474 kg., giving a ratio of 25.8 mg. per kg. body weight.

The other animal was an Old World monkey, a male green monkey (*Cercopithecus*), weighing 6.397 kg., with less bone disease than any except animal A of this series. The bones were somewhat softened and deformed and there was an incomplete fracture of the lower shaft of the left humerus, but it was not possible to cut the horizontal ramus of the mandible completely with a knife. Only two parathyroids were found, after an extensive search, with a combined weight of 30 mg., giving a ratio of 4.7 mg. per kg. body weight, the lowest in the series, and indeed within Gilmour and Martin's extreme limit for the normal human, though well above the human mean and still further above that for monkeys.

Parathyroid hypertrophy is thus present in at least four, and probably in all five animals, and the bone changes in the green monkey and the Capuchin with some softening of all bones, but most notably of the mandible, are suggestive of hyperparathyroidism. In the series of three woolly monkeys, the mandible was found to be very soft in both B and C, but normal in A, in which no bone lesions were found. The bones of animals B and C showed changes similar to rickets, with typically hypertrophied and irregular epiphyseal cartilages and wide osteoid seams in the metaphysis, and in there was extreme osteitis fibrosa.

The hyperparathyroidism may well be secondary to the rachitic condition, but the cause of this is still somewhat uncertain. The condition was at first thought to be a renal rickets consequent upon a kidney infection, like the disease of dogs known

as 'rubber jaw', in which hyperparathyroidism follows phosphate retention by kidneys damaged by leptospiral infection (Platt, 1951). However, in view of the mild kidney damage, even in the most severely affected animal C, this aetiology seems improbable for the monkey lesion. A more likely explanation is a low calcium rickets, dependent on an absolute or relative calcium deficiency in the diet, or on lack of vitamin D, or a combination of both causes. Parathyroid hypertrophy may have been initiated by the low blood calcium.

Corson White (1922a) described osteomalacia in New World monkeys on a diet deficient in both calcium and vitamins and stated that this disease was rare in Old World macaques. He also recorded three New World monkeys with so-called 'osteitis deformans', which from his description was almost certainly osteitis fibrosa (Corson White, 1922b). The food was similar in both instances—two apples and/or two bananas, six small sweet potatoes, and a lump of boiled rice the size of an egg—and Corson White himself had no doubt that calcium and vitamin deficiency was the underlying cause of both disorders. He stated that the diet could be rendered adequate for rats by the addition of leafy vegetables and fresh milk and that one at least of his animals with 'osteitis deformans' showed a marked avidity for lime, and seemed more comfortable when treated with a salt mixture containing calcium phosphate and calcium lactate, though no other change was noted in its condition. The choice of calcium phosphate was perhaps unfortunate, as this would do little to redress a calcium/phosphorus imbalance, but it is also possible that, as in man, once parathyroid hypertrophy was established, the condition would be autonomous, even after removal of the original cause. Fiennes (1961) has found that osteodystrophy in monkeys rarely responds to vitamin D therapy; but the probability is that any treatment, short of parathyroidectomy, would be ineffective once the condition was fully established.

Table III gives the calcium and phosphorus content and the Ca/P ratio of a number of foods. All those listed by Corson White have a low Ca/P ratio and lack vitamin D, which would have assisted in the utilization of the available calcium. Sherman (1941) states that 'observations upon several species have indicated that during rapid growth and calcification it is well that the food as a whole should have a Ca/P ratio somewhere between 1 and 2. When adult maintenance only is involved, the calcium requirement is lower, both absolutely and relatively to the phosphorus requirement'.

The food of the monkeys at Paignton Zoo during 1959-60 is stated to have been a mixture of root vegetables and fruit, mincemeat, egg, and milk.

TABLE III
CALCIUM AND PHOSPHORUS IN FOODS¹

Food	Ca (mg./100 g.)	P (mg./100 g.)	Ca/P Ratio
Apples	7	11	0.64
Bananas	8	28	0.29
Lean beef	13	204	0.06
Bread, white	(5)	(10)	(0.5)
Bread, whole wheat	(6)	(37)	(0.16)
Brussels sprouts	25	105	0.24
Cabbage (headed)	45	28	1.61
Cabbage (loose leaf or greens)	429	72	5.96
Carrots	42	40	1.05
Maize	29	281	0.10
Dates	72	60	1.20
Eggs (whole)	58	224	0.26
Grapefruit	17	18	0.95
Grapes	17	21	0.81
Kale	181	67	2.70
Lettuce	54	31	1.74
Cow's milk	118	93	1.27
Mutton	15	208	0.07
Oatmeal	81	365	0.22
Onions	32	44	0.73
Oranges (or orange juice)	25	19	1.32
Parsley	193	84	2.30
Parsnips	57	80	0.71
Pears	13	16	0.81
Peanuts	66	392	0.17
Peas, dry	73	397	0.18
Peas, fresh	22	122	0.18
Potatoes	13	53	0.25
Radishes	37	31	1.19
Raisins	55	110	0.5
Rice, entire	68	336	0.20
Rice, white	9	92	0.10
Sweet potato	33	52	0.64
Tomatoes (seeds included)	11	27	0.41
Turnip	51	32	1.59
Turnip tops	254	58	4.38
Watercress	168	41	4.10

¹Data from Sherman (1941)

This sounds a reasonably balanced diet, but may still have had a low Ca/P ratio if the proportions of meat and egg, which are both very high in phosphorus content, were large compared with that of milk, whereas a sufficiency of milk would supply both calcium and vitamin D. The amount of sunlight received by the animals while in the zoo is uncertain; although many of the monkey cages have outside runs, some get sunlight only through glass, and it is not certain which cages these animals occupied. However, it is probable that much of the damage antedated the animals' arrival at the zoo. In animal C at least, which was purchased from dealers only 10 weeks before its death in February 1959, the condition of the bones and the extreme hypertrophy of the parathyroids suggest a much longer history. Its mate, animal A, was at this time thought to be suffering from a similar condition, but had apparently made a full recovery by July 1959 and at death, in January 1960, its bones were well calcified and appeared normal, despite the parathyroid hypertrophy. Animal B was purchased from dealers in

TABLE IV
SERUM ANALYSIS AND SIZE OF PARATHYROIDS ESTIMATED IN OCTOBER 1959

Animal	Ca (mg./100 ml.)	P (mg./100 ml.)	Alkaline Phosphatase (K-A. units/100ml.)	Acid Phosphatase (K-A. units/100ml.)	Parathyroids (mg. per kg. body weight)
Woolly monkey A (adolescent)	12.1	9.4	296	23.6	10.7 (at death Jan. 1960)
Green monkey (adult)	11.1	6.6	328	6.6	4.7
Capuchin (adult)	17.0	—	310	31.4	25.1

June 1958 and survived at the zoo till July 1959 when it was killed on account of its poor condition.

No blood analyses were made for animals B and C, but blood was obtained from animal A in October 1959, and also from the green monkey and Capuchin which died at this time. Analyses are shown in Table IV. In all three animals the level of alkaline phosphatase is extremely high and this is no doubt associated with the bone condition; but little reliance can be placed on the raised figures for acid phosphatase, as the sera were markedly haemolysed and hence contaminated with erythrocyte acid phosphatase. Serum calcium in A is slightly but not markedly high; serum phosphorus is definitely raised. In the green monkey also serum phosphorus is raised, but serum calcium is normal. In the Capuchin, serum calcium is markedly raised, but unfortunately serum phosphorus was not estimated owing to insufficiency of serum. These figures are compatible with the hypothesis that the initial cause of the condition was a low calcium intake due to hypovitaminosis D or a Ca/P imbalance in the diet, leading to raised blood phosphorus and initially lowered blood calcium. Parathyroid hypertrophy, initiated by the lowered blood calcium, would restore this to normal, whilst the blood phosphorus remained high. As will be seen from Table IV, the level of serum calcium increases with increasing parathyroid hypertrophy.

It is probable that some at least of these monkeys were kept in cages lacking direct sunlight, and hence suffered from avitaminosis D. Ruch (1959) mentions the reputedly high requirements of New World monkeys in vitamin D. Cowdry and Scott (1936) found two specimens of a New World monkey (*Cebus fatuellus*) unaffected by large doses of vitamin D which produced kidney damage and calcification of the arteries in macaques. Woolly monkeys in nature are predominantly if not purely fruit-eaters, though the Capuchins also eat insects (Beddard, 1902). Vitamin D requirements must normally be met principally by synthesis with the aid of sunlight; it may be that these monkeys do not utilize vitamin D in food as readily as the Old World species. It was the opinion of a keeper at Bristol Zoo that natural sunlight is of far greater importance in the

prevention of bone disease in these animals than either vitamin supplements or artificial ultra violet. A lack of sunlight, either in the hands of dealers or in covered cages in the zoo, may possibly be the greatest single factor in the aetiology of this condition.

Harrison and Fraser (1960a) record experiments on calcium and vitamin D deficiency in rats which are pertinent to this discussion. A diet deficient in calcium, but with adequate vitamin D, led to osteoporosis, without formation of osteoid; serum calcium and phosphorus were low, but alkaline phosphatase levels were not affected. On diets deficient in both calcium and vitamin D wide osteoid seams were found in the bones though, probably because these rats failed to grow, rachitic changes at the epiphyses were not clearly shown. Serum calcium levels were still lower than in the rats lacking only calcium; serum phosphate and alkaline phosphatase were considerably raised. Osteoclastic activity in the bones was not prominent in either condition.

In a subsequent paper (Harrison and Fraser, 1960b) the effect of calcium deficiency on the parathyroids was studied. After three months the parathyroids of calcium-deficient rats were found to be grossly hypertrophied and between two and three times the size of those from control rats of the same age, with significantly fewer nuclei per unit area. Unfortunately, the effect on the parathyroids of the diet deficient in both calcium and vitamin D was not investigated.

Assuming a similar physiological response in the monkey, the condition of the bones in animals B and C suggests both calcium and vitamin D deficiency. Serum analysis, for the three monkeys in which it was made, agrees with Harrison and Fraser's findings for calcium- and vitamin D-deficient rats in respect of phosphorus and phosphatase levels. The normal or raised serum calcium in the monkeys may be explained by the parathyroid hypertrophy.

In animal A the structure of the bones appeared normal despite the enlarged parathyroids, and it seems probable that the dietary deficiencies which initiated the hypertrophy had been made good by the time of the animal's death. Phosphatase distribution in these bones was not very different from

that in the young rabbit, though the somewhat greater concentrations in the metaphysis, particularly of alkaline phosphatase, may be related to the parathyroid hypertrophy. In B the level of alkaline phosphatase was extremely high throughout the femur, which accords with the clinical experience of high serum alkaline phosphatase in osteitis fibrosa and rickets, and indeed, according to Albright and Reifenstein (1948), in any condition where matrix is being laid down in excess. It is noteworthy that, in an electrophoretic analysis of extracts from the bones of animals B and C performed by Miss S. F. Greene of the Royal Free Hospital, the alkaline phosphatase migrated as a band in the beta-globulin region corresponding with that shown by the alkaline phosphatase of normal human serum, supporting the assumption that the serum enzyme is in the main derived from that of bone. The extremely high levels of alkaline phosphatase in B were associated with thinning of the trabeculae and a marked insufficiency of mineralization. In C, where the whole bone had a largely fibrous structure, the values were still well above those of normal bone, but this may be partly due to the greater cellularity of this fibre bone.

The hypertrophied epiphyseal cartilage of B and C reacted strongly for alkaline phosphatase, suggesting that this is only one factor in mineralization, though there appears to have been more calcification in the subepiphyseal region than elsewhere in these bones, wide osteoid seams appearing lower down the metaphysis in both animals, along with much fibre bone in C. These fibres again, and the osteoblasts covering poorly mineralized trabeculae, reacted strongly for alkaline phosphatase; failure of mineralization is associated with excess rather than lack of this enzyme.

The presence of acid phosphatase in high concentration in osteoclasts has already been observed by a number of workers (Gomori, 1941; Changus, 1957; Pepler, 1958; Schajowicz and Cabrini, 1958; Burstone, 1958c). This, together with the local increase in acidity found around these cells by Cretin (1951), may be significant in relation to their role in bone resorption. It is open to speculation whether the acid phosphatase found in fibre bone and in osteoid seams and islets of osteoid may bear some relation to the failure of calcification, even in the presence of concentrations of alkaline phosphatase of the order of 100 times as great.

What local differences in pH, or in concentrations of activators or inhibitors, may affect the activities of the two enzymes, are at present unknown.

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