EARLY SKELETAL AND VASCULAR CHANGES IN RATS FED ON
SWEET PEA (LATHYRUS ODORATUS) SEEDS

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The disease caused by the copious ingestion of leguminous food was first recognised by Ramazzini, who described an outbreak of lathyrism in Modena in 1690, but it is probable that it had been known by Hippocrates (Stockman 1929). In 1868 Irving referred to the prevalence of the illness in India and described its main features, particularly muscle spasm; he found that 7 per cent of the population under review were affected. Acton (1922) studied the disease in the population of the town of Ravat (India) and found that about 6 per cent of the children showed signs of lathyrism. Vivanco and Jiménez Diaz (1951) described similar findings in Spain. Stockman (1929) reported his results of feeding guinea pigs on sweet pea meal; all died within one to five weeks. He believed that the cause was not so much poisoning as a deficiency state due partly to scurvy.

The deleterious effects of leguminous diet have been ascribed to an alkaloid (devicine) by Anderson, Howard and Simonsen (1925), isolated by them from Lathyrus odoratus, the sweet pea.

Lee (1950) found that the acid of the stomach destroyed this alkaloid, thus excluding the probability that it caused lathyrism due to an ingested leguminous diet. He also exonerated enzymes, as the toxic factor persisted after boiling. Dupuy and Lee (1954) reported the isolation of a crystalline substance, later identified as B (Y-L glutamyl) aminopropionitrile by Schilling and Strong (1954).

Ponseti and Baird (1952) and Ponseti and Sheppard (1954) reported the first experimental observations on the disease process with special reference to its effect on the skeleton and the rest of the mesodermal system. They described a widespread lesion of the epiphysial plates and a loosening and detachment of ligamentous and tendinous insertions causing many varied lesions and deformities, some of the latter probably being sequelae and not part of the disease entity. They believed that the active principle contained in the lathyrus pea, producing the skeletal changes, was B-aminopropionitrile.

The present work has been undertaken in order to study the earliest changes of the disease in the skeleton, and to detect the role played by blood vessels in the affected regions. It was also hoped to determine whether the changes were reversible or not.

METHOD

Twelve thirty-five-days-old albino rats taken from the same litter were used in the experiment and were divided into three groups of four, A, B and C. Group A was used as a control, group B was fed on a pea-meal diet‡; and the last group, C, was fed on the pea-meal diet for ten days and then returned to a normal diet (standard laboratory N.41 diet). Radiographs of the anaesthetised animals were taken at intervals. One rat from each group was killed by an injection of Nembutal at intervals of fourteen, eighteen, twenty-two and twenty-six days.

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† British Council Scholar.
‡ Diet No. 3 as used by Ponseti and Sheppard (1954):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
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<tbody>
<tr>
<td>Pea meal</td>
<td>50</td>
</tr>
<tr>
<td>Corn flour</td>
<td>28</td>
</tr>
<tr>
<td>Sugar</td>
<td>6</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>4</td>
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<tr>
<td>Wheat germ oil</td>
<td>2 ml. 100 gr.</td>
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<tr>
<td>Dried yeast</td>
<td>10%</td>
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<tr>
<td>Halibut liver</td>
<td>2 ml. 100 gr.</td>
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after the start of the experiment, and was injected immediately with a mixture of equal parts of 10 per cent barium sulphate and Berlin blue and prepared for examination by the routine method described in previous papers from this Centre (Trueta, Barclay, Daniel, Franklin and Pritchard 1947; Trueta and Harrison 1953). The Gomori (1946) azo dye method was used to estimate the alkaline phosphatase content of the bone.

RESULTS

The pea-meal diet retards growth and appears to be a systemic poison, as all animals on the experimental diet were much smaller and less active than the controls (Figs. 1 to 3).

After twenty-two and twenty-six days of diet the mean differences in weight were:

<table>
<thead>
<tr>
<th>Group A</th>
<th>130 grammes</th>
<th>180 grammues</th>
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<tbody>
<tr>
<td>Group B</td>
<td>100 grammues</td>
<td>75 grammues</td>
</tr>
<tr>
<td>Group C</td>
<td>110 grammues</td>
<td>130 grammues</td>
</tr>
</tbody>
</table>

Note that the rats in group B were actually losing weight.

Radiological appearances—The earliest and most obvious radiographic changes after ten days’ diet in groups B and C were the lack of skeletal growth and a narrowing of the growth plate. Early signs of osteoporosis were present. These changes became more evident after twenty-two and twenty-six days in group B; there was well marked patchy osteoporosis, most evident in the femora, vertebral bodies, sacrum and scapulae, and the texture of the skeleton as a whole became indefinite, especially in the region of the metaphyses (Fig. 2). The cortical bone was much thinner. The difference in size of the individual bones was still marked, and areas of
Epiphysial plate showing the split in the growth plate between the zone of expanding cells and the "calcified" layer. The germinal layer is much thinner than normal. (× 500.)

The widening of the epiphysial plate of the tibia, and part of that of the fibula, which is caused mainly by non-ossification at the metaphysial end. (× 40.)
Cortex and periosteum showing (A) cortex, (B) new bone formation three times the width of the original cortex, and (C) raised periosteum. (×100.)

Increased alkaline phosphatase activity in the region (B) shown in Figure 6. (×100.)
raised periosteum were present. At the insertion of the deltoïd, biceps and quadriceps muscles and the calcaneal tendon large exostoses were raised from bones.

In the later radiographs the growth plates had widened and were irregular.

In the rats of group C, to which a normal diet was given after ten days, growth was seen rapidly to resume a normal rate and the above radiological findings did not develop (Fig. 3). **Histology**—The earliest changes appeared after two weeks and consisted of a transverse split in the growth plate at the junction of the segment of hypertrophied chondrocytes and the segment of large, degenerating cartilage cells (Fig. 4). This area contained blood vessels and extravasated blood. There was a slight generalised osteoporosis.

After eighteen days bone rarefaction was evident in group B; the lamellae were thin and scanty both in the metaphysis and in the epiphysis. The periosteum was well attached to the cortex and there were no histological changes visible in the marrow cavity. The growth plate was slightly widened (Fig. 5) and showed metachromasia. The columns of chondrocytes were becoming disorganised near their metaphysial end, and the transverse splits noted above were now more evident. The increased width of the plate was due to hypertrophy of individual cells and lengthening of the cell columns by delayed ossification on the metaphysial side of the plate. It was not the result of hyperplasia in the germinal layers.

The changes were very pronounced after twenty-two days of pea-meal diet. and even more so after twenty-six days. Bone rarefaction, with sparse and attenuated lamellae, was present and the cortex was reduced to half the size of that of the control animals. In regions where the periosteum had become detached from the cortex new bone was laid down, occasionally to three times the normal width (Fig. 6). A marked increase in alkaline phosphatase activity was noted (Fig. 7).

In the metaphysis the trabeculae were so thin and far apart that there was often loss of continuity of the cortex. The periosteal detachment was advanced in some bones, and new bone was being laid down rapidly. The epiphysial cartilage became more than double its normal width, and the transverse defect previously mentioned was more in evidence, sometimes spreading right across the plate. These spaces were filled with new blood vessels and extravasated blood. Metachromasia was pronounced. The cells were large in all layers and the degenerate portion was much wider than normal. The normal columnar arrangement was absent, the cells having a haphazard arrangement with little or no intercellular matrix, or with a change in direction as if they were bent by a shearing force. In the lower end of the femur the growth plate widened to many times its normal size, and no ossification was taking place (Figs. 8 and 9). In other bones the upper end of the metaphysis was most irregular and there was complete absence of the orderly laying down of new bone on a calcified cartilaginous matrix (Figs. 10 and 11). In places the new bone marrow extended right up to the growth plate; islands of cartilage cells were scattered in the marrow. The mounting of these sections presented technical difficulties because of separation of the epiphysis from the shaft, and friability due to osteoporosis.

**The blood vessels**—The smaller blood vessels and capillaries were seriously affected.

The injection mass filled the vessels in the control rats without damage to their walls; in the rats on the pea-meal diet numerous "bursts" were present at the growing ends of the bones where the capillaries were most numerous (Figs. 12 to 14). In group C, "bursts" were also present but they were not so numerous.

Blood vessels were present in the growth plate at the site of the fissures—that is, at the junction of the zone of hypertrophic cells with the zone of calcification.

In the metaphyses of the rats in group B the capillary loops were thin and very attenuated when compared with those of the control group. They showed no orderly orientation in relation to the columns of cartilage cells and did not all reach the same level; thus the line formed by the vascular loops was always very irregular and sometimes was completely absent from whole sections. The altered line of vascular loops corresponded with the irregularity in the
FIG. 8
The lower end of the femur in a control rat, a litter mate of that shown in Figure 9. (×12.)

FIG. 9
The lower end of the femur of a rat after twenty-six days on pea-meal. The growth plate is wide and there is generalised decalcification. (×12.)
FIG. 10
The growth plate of a control rat showing the orderly columnar arrangement and bony trabeculae in the metaphysis. (× 300.)

FIG. 11
The growth plate showing the effect of pea-meal. Note the loss of the columnar arrangement and the complete disorientation near the metaphysis. (× 325.)
plate, which had become wider from an accumulation of the "degenerating" cells, sometimes appearing as broad finger-like processes directed towards the centre of the metaphysis, sometimes involving one-half of the metaphysis.

Bursts were numerous in the epiphyses also, and extravasated blood was present in the deeper layers of the articular cartilage. Similarly, bleeding was present under the periosteum. **Alkaline phosphatase**—Alkaline phosphatase was present in excessive amounts in the nuclei of the degenerating cells and in the metaphyseal region. It was abundant in the subperiosteal region when new bony trabeculae had been freshly laid down (Fig. 15).

**DISCUSSION**

In the course of this work it was found that the administration of pea-meal diet caused interference with growth, lesions of the smaller blood vessels, raising of the periosteum, formation of exostoses, and osteoporosis. Alkaline phosphatase activity was increased.

The early manifestations of the disease have been studied in order to avoid confusion with their sequelae. **Growth**—Interference with growth manifested itself in a failure of the rats to gain weight and increase in size when compared with the controls, and also in narrowing of the growth plates as seen in the radiographs taken at ten days. This may be due to a lack of stimulus of the growth hormone of the pituitary on the proliferative layer of the cartilage columns, or to a generalised metabolic disorder, or to both (Ray, Evans and Becks 1941). It may also have some connection with the early vascular damage we have detected.

Failure to gain weight persisted to the end; some animals were losing weight by the fourth week. The widening of the growth plate in the later stages was due to increase in size of the cells in the hypertrophic layer and to an accumulation of the cells of the degenerating zone, and not to hyperplasia in the proliferating zone, where the number of cells was diminished.

The plate becomes broken at a level that appears to be brittle in other conditions as well. Thus Harris (1950) found that it is at the zone between the hypertrophic and the degenerating layers of the columns that stress causes greatest damage. It may be that the split that appears...
in this zone is due to a tearing or distracting force exerted by the expanding cells at the level where their increase in size appears to be most rapid. The new blood vessels enter this zone through splits between the cells of the degenerating layer.

The disorganisation of the columnar arrangement in the plate seems to be caused by an absence or decrease of the intercellular substance, and by the absence of the regular honeycomb arrangement of calcified cartilage matrix laid down by the normal growth plate. Serious interference thus occurs in ossification.

The abnormal blood vessels alone could also be responsible for the widened growth plate, as it has been shown conclusively (Trueta 1957) that cutting off the metaphyseal blood supply causes ossification to stop. The trabeculae are few in number and they are thin and weak. Slipping of the epiphysis occurs easily and contributes to deformity.

![Fig. 15](image)

A section of the metaphysis and epiphysis of a rat in group B showing the alkaline phosphatase activity under the raised periosteum and in the metaphysis (see also Fig. 7). Note the generalised osteoporosis. (× 20.)

**The periosteum**—The raising of the periosteum may be caused by several factors acting singly or in combination.

**Bleeding**—There is extravasation of blood under the periosteum, but it is uncertain whether it is due to friability of the blood vessels or whether it is the effect of avulsion of the periosteum from the bone.

**Loose Sharpey's fibres**—The periosteum is bound down to bone by Sharpey's fibres. These are fixed to the cortex by a cement substance and, if this is deficient, they become loose, allowing the periosteum to be pulled away from the cortex by normal muscle pull.

**Abnormal muscle pull**—Muscle spasm and spastic paraplegia have been reported as a predominant clinical feature of the disease in man (Anderson, Howard and Simonsen 1925; Acton 1922; Stockman 1929), but no obvious signs of paraplegia were noted in our rats.
Exostosis—The large exostoses at the deltoid tuberosity and other areas may be due to the deficiency of cement substance binding down the tendinous fibres to the periosteum and to bone. Loosening of these attachments allows the periosteum to be avulsed from the cortex, with a laying down of new bone in the direction of the line of pull.

Osteoporosis—Bone rarefaction is both focal and general. Some bones—especially the femur, scapula, sacrum and vertebrae—show severe rarefaction.

The work of Geiser and Trueta (1958) shows that osteoporosis is closely linked with the lack of transmission of muscle stresses through the bone. Detachment of the calcaneal tendon caused local bone absorption with marked changes in the vascular pattern. In rats in group B the loose muscular connections and the detached periosteum must lead to a diminished muscle pull.

Furthermore, at the metaphysis, interference with the transmission of weight and stress from epiphysis to diaphysis leads to displacement. The forces normally acting in this region which influence bony structure and density are therefore disrupted. Finally, the blood vessels, being affected, would of themselves influence bone density.

The interference with the production of cement substance is not peculiar to lathyism. Stockman (1929) believed the changes to be scorbatic, and the resemblance of the syndrome to scurvy is striking. Alkaline phosphatase concentration in scurvy is, however, known to be low, and rats in group B showed a higher concentration than normal. In scurvy again the collagen fibres are affected, whereas in lathyism Ponseti and Shepard (1954) saw normal fibres with the electron microscope.

Although it is unlikely that the two conditions are one and the same, there is a common link—the interference with the synthesis of cement substance.

Most are agreed that the cement substance in cartilage is the mucopolysaccharide chondroitin sulphate, but not much is known about the cement substance elsewhere.

Cortisone and compound F are known to influence the synthesis of chondroitin sulphate (Bourne 1956), and this in turn is influenced by the other hormones, chiefly by the growth hormone of the pituitary. That the growth hormone of the pituitary, and possibly also that of the parathyroid, may be involved in the changes detected in lathyism has been suggested. The increased weight of the suprarenals of male rats (Dasler 1954) and the decreased spermatogenesis reported by Ponseti and Shepard (1954) point to an endocrine disturbance. It may be that the main effect of lathyism is either a direct one on the suprarenal cortex, or an indirect one through the growth hormone of the pituitary. However much the local effects on the skeleton are due to an endocrine disorder, our findings show that direct vascular damage plays an important part in the disorganisation of the epiphysial cartilage.

CONCLUSIONS

The main findings in this experimental work on rats fed on lathyrus odoratus (sweet-pea) meal are as follows:

1. Growth is retarded.
2. The growth plate is disorganised and normal ossification at the metaphysis is interfered with.
3. The small blood vessels are seriously affected and probably contribute quite largely to the disorganisation and lack of calcification.
4. Alkaline phosphatase activity is increased.
5. Raising of the periosteum and laying down of new bone result in exostoses.

The possible underlying etiology and the role of cement substance, endocrine factors and the blood vessels are discussed.

We wish to thank Professor J. Trueta for his unfailing advice and encouragement in our work, and for all the facilities he placed at our disposal.

This work was done with the co-operation of Mr D. W. Charles and of Mr A. Mann, to whom we express our thanks.
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