Rates of Growth and of Remodelling as Factors in the Genesis of Vascular and Osseous Lesions of Odoratism in Rats

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WITH ONE PLATE

INTRODUCTION

Since the original report by Geiger, Steenbock, & Parsons (1933), the syndrome of experimental odoratism, including aortic aneurysms and ruptures, skeletal deformities and herniae, has been reported as reproducible with considerable consistency in weanling rats fed either the seeds of the sweet pea (Lathyrus odoratus) or the extractable active nitriles (Strong, 1956). The vascular lesions have recently received special attention by a number of investigators (Bachuber & Lalich, 1955; Churchill et al., 1955; Walker & Wirtschafter, 1956; Menzies & Mills, 1957), the general consensus of present opinion being that the dramatic aortic aneurysms and ruptures are primarily attributable to 'elastinolysis'. While Walker & Wirtschafter (1956) considered the aortic lesions to be '...one sign of a generalised metabolic disorder which at the histological level is basically elastolytic', neither they nor other workers, to our knowledge, have attempted to interpret, within a single framework all the above-mentioned lesions of odoratism, nor have attempts yet been made to explain particularly the marked variations in susceptibility to the odoratus toxin of weanlings as compared with adult rats.

Yet several studies have already shown that the lesions in odoratism seem somehow to be bound up with age or the stage of development of affected animals (Ponseti & Shepard, 1954; Chang et al., 1955) and even that connective tissues may be diffusely involved throughout the organism (Dasler, 1954). However, the reasons underlying these influences of age and/or of developmental activity on the susceptibility of individuals to the toxic effects of odoratus seeds or to the extractable toxic nitriles have not been discussed at any length by others.

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Our own orientation to this problem was initially from the standpoint of collagen synthesis and related biochemical changes during wound healing, although our initial histological studies of healing wounds in odoratus-intoxicated rats have not yet revealed easily detectable differences from the normal. However, we anticipate that the quantitative chemical analyses of this problem, presently being undertaken, may perhaps be more revealing. We had, nevertheless, already disclosed indications that the synthesis of elastic tissue and of collagen were intimately related with ground substance metabolism in maintaining the integrity of healthy vascular elastic membranes (VEM's) (Gillman, Hathorn, & Penn, 1957). We have also found that, following injury, the regeneration and/or repair of VEM's is closely comparable in many respects with the serial histological and histochemical events observable in the healing of cutaneous wounds. The massive accumulations of metachromatic and ferrocyanide-positive polysaccharides in the aortic wall, detectable even within 14 days of initiating odoratus intoxication, led us to consider that it was the remodelling and associated lysis and reconstruction of VEM's and of long bones, during periods of active growth, which made young animals so particularly susceptible to the toxic effects of sweet-pea seeds. This encouraged us to re-examine the literature as well as to undertake further original studies both of odoratism and of the post-natal growth of normal rats. Our initial findings, relating only to some aspects of the aortic and osseous lesions, here reported in outline, are believed to lend some support to this basis for accounting for both the vascular and the osseous lesions in odoratus intoxication.

If these views can be substantiated by quantitative radio-active tracer studies, then a new orientation may be provided for further analyses of several types of vascular and osseous lesions and of some forms of vascular sclerosis and especially for the 'pseudo-elastic tissue' accumulations associated with ageing or following chronic injury in man (Gillman et al., 1955; Gillman, Hathorn, & Penn, 1957). Even more important, perhaps, is the possibility that lesions, hitherto regarded as peculiar to the arteries of ageing individuals, may yet be shown to be simply the late sequelae of disturbances in the growth and remodelling of the vascular tree, especially during that period of active growth which characterizes adolescence (Tanner, 1955).

Such possibilities may also underline the need previously stressed (Gillman, Hathorn, & Penn, 1957; Gillman & Hathorn, 1957), for regarding sclerosis and other alterations in many connective tissues, however specialized they may at first seem to be, in terms of modern knowledge of the changes in and the factors regulating connective tissue regeneration or repair which have recently been disclosed by studies of healing cutaneous injuries (Kodicek & Loewi, 1955; Abercrombie et al., 1954, 1956, 1957; Dunphy et al., 1955, 1956; Udupa et al., 1956; Gillman & Penn, 1957).
In our studies of odoratism we have fed both weanling and adult rats with sweet-pea seeds. As originally shown by Geiger et al. (1933), and later by Ponseti & Shepard (1954), adults are also susceptible to the toxic actions of the active principle, albeit less so than weanlings and in less dramatic ways.

In our first experiment, 10 male weanling Wistar-strain rats aged 23–25 days (mean initial weight 38 g.) and 10 male adult rats aged about 2½ months (mean initial weight 211 g.) were fed our stock diet with the addition of 50 per cent. ground sweet-pea seeds. Equal numbers of weanling and adult male control rats were fed the stock diet alone. Two experimental and 2 control rats, in both the weanling and adult groups, were sacrificed by ether anaesthesia at approximately fortnightly intervals, with the exception of 2 weanling rats on the sweet-pea seed diet (out of 4 remaining at the time) which died spontaneously of aortic ruptures at 52 and 53 days respectively.

In the second experiment, 10 male weanling rats aged 26–27 days (mean initial weight 48 g.) and 10 male adult rats aged about 3 months (mean initial weight 235 g.) were fed the above sweet-pea seed diet, an equal number of control rats being simultaneously fed the stock diet. In this experiment, however, the animals were sacrificed only when moribund or paralysed or at the end of the experiment lasting 216 days.

Immediately after sacrifice and macroscopic post-morten examination, the animals were fixed in toto in 10 per cent. neutral formalin and, 1 week later, specimens of heart, aortic arch, thoracic and abdominal aortae, humerus, elbow-, wrist-, and knee-joints, and the upper end of the femora were taken for decalcification (in the case of bones), dehydration, and subsequent paraffin embedding and sectioning. As described in other studies (e.g. Gillman, Hathorn, & Penn, 1957) serial sections of all specimens were stained with haematoxylin and eosin, a modified Masson method, the periodic-acid Schiff (PAS) routine, alcoholic toluidine blue, a ferrocyanide method for mucopolysaccharides (Rinehart & Abul-Haj, 1951), and the Wilder reticulin method counterstained with Van Gieson.

**OBSERVATIONS**

**General**

Text-fig. 1 illustrates the weight changes occurring in each group of animals. It will be seen that the weanling rats on the *L. odoratus* diet continued to grow, albeit at a slower rate than the weanling controls, until about 100 days, when their weight curve tended to plateau. The aortic ruptures that occurred at 52 and 53 days (in 2 of the 4 rats still surviving at this stage of the first experiment) nevertheless took place almost immediately after a period of rapid growth. The other interesting fact illustrated by Text-fig. 1 is that, over the period of the
experiment, the rats which were started on the odoratus diet when they were already adults grew hardly at all as compared with the adult controls.

Fatal aortic ruptures occurred only in those animals which were initially 23–25 days old when started on the odoratus diet. The fact that aortic ruptures did not occur in the second group of weanling rats may have been due to their greater initial ages (26–27 days) at the time they commenced consuming this diet. This is in conformity with the findings of Ponseti & Shepard (1954) who showed that the incidence of aortic ruptures dropped sharply in weanling rats which started eating toxic diets when more than 28 days old. However, large and fibrosed aortic aneurysms were detected in 2 of the weanlings in the second experiment, when they were killed 216 days after starting the diets. Early changes in the spines, humeri, and especially at the knee- and wrist-joints, were striking—particularly in initially young rats (Plate, figs. A–E). Several animals were sacrificed because they developed lower limb paralyses caused by gross spinal deformities.
Microscopic findings

We consistently found both histologically and histochemically detectable changes in the aortic walls of weanling rats even within the first 14 days of the experiment. The most striking of these initial changes was the accumulation of excessive amounts of metachromatic mucopolysaccharides (MMP's) initially located primarily around the inner 2 or 3 aortic VEM's—findings confirming those of Churchill et al. (1955) and later of Menzies & Mills (1957). Such accumulations of MMP's were found by us to be progressive during the first 50–60 days of the experiment, during which time they became more widespread within the aortic walls and also appeared in the smaller arteries. This held only up to but not beyond the 80th day of the experiments. This reaction is consistent, being found in all weanlings, whether or not aortic ruptures supervene. In the later stages of the experiments (after 40–60 days on the diet) almost the entire thickness of the aortic wall was heavily laden with MMP's which was deposited, for the most part, immediately around but to some extent also between the collagen-like 'cores' of the VEM's previously described (Gillman, Hathorn, & Penn, 1957). Associated histochemical changes, to be described in detail elsewhere, were alterations in the amounts and distribution of resorcin-fuchsin and/or orcein-positive 'elastin', PAS-positive and ferrocyanide-positive polysaccharides, and reticulin. Elastic membranes, once ruptured, did not regenerate easily and tended to be replaced by fibrous (scar) repair of the injured areas. This observation, regarding the apparent difficulty in elastic fibre regeneration, thus seems to hold not only for arteries (Crawford, 1956; Gillman, 1957b; Gillman & Hathorn, 1957) but, as we have previously shown, also for the skin (Gillman & Penn, 1956).

Fibroblastic proliferations, around sites of frank or incipient aortic ruptures, seemed to be characterized by initial delay in both fibrin deposition and in subsequent fibre formation. Our findings, in this regard, are in conformity with those of other workers, who, although they do not specifically remark on this absence of fibres, nevertheless all stress the 'cellularity' of the connective tissue reactions in injured areas, at least in vessels (Bachuber & Lalich, 1955) and in bones (Robinson & Bast, 1934; Ponseti & Shepard, 1954). Fibrin depositions were notably scanty at sites of frank aortic ruptures. This absence of fibrin may perhaps have been attributable to the binding of fibrinogen by circulating excess of mucopolysaccharides in a manner described by Smith & von Korff (1957). This seems all the more likely in view of the excessive amounts of histochemically demonstrable ferrocyanide-positive mucopolysaccharide seen by us in sections, lying within the lumen of blood-vessels, and particularly since we have also demonstrated a notable increase of marrow mast-cells in odoratus-fed rats (Gillman, 1957a). Moreover, Selye & Bois (1957) noted, in passing, that ‘... it was obvious at autopsy that clot formation was greatly delayed’ (in nitrile intoxicated rats).
GENESIS OF LESIONS OF ODORATISM

These disturbances in healing (viz. defects in fibrillogenesis) seem reminiscent of those found in wound healing in scorbutic guinea-pigs or in animals fed a protein-deficient diet (Udupa et al., 1956) or treated with cortisone. However, the lesions in odoratism seem to differ from those found in these other experimental situations in that, in the latter, sulphation of mucopolysaccharides is apparently suppressed, whereas in odoratism it seems to be highly active. The block in fibrillogenesis, in odoratism, would thus seem to occur after that phase which is characterized by sulphation of those mucopolysaccharides which accumulate in connective tissue ground substance at sites of repair.

We wish to suggest a pathogenesis for the aortic ruptures different from the 'elastolytic' view maintained by others. We have accumulated evidence indicating that, during its growth the aorta normally undergoes remodelling analogous in many respects to the remodelling of long bones during their growth. The latter process is known to involve carefully integrated endosteal bone resorption occurring simultaneously with the deposition of new bone perichondrially and endochondrially at the epiphyses. Thus, although elastolysis of vascular elastic membranes does occur in weanling odoratus-intoxicated rats, this seems to be merely part of the normal processes of aortic remodelling accompanying the rapid growth of this vessel, especially during the first 30–40 days after weaning. The aortic ruptures, in our view, may perhaps represent the result of some disturbances of the balance between that lysis and regeneration of elastic membranes which probably occur simultaneously during the normal growth and remodelling of the aorta. We do not subscribe to the view outlined by Taylor (1953), and subsequently entertained by Churchill et al. (1955), that intramural accumulations of MMP's result from lysis or degeneration of elastic membranes. Detailed reasons for these views will be presented elsewhere.

In support of this view we found that, in normally fed rats, the aortic diameter increases by more than 40 per cent. during the first 60 days after weaning. Thereafter the aortic growth is much slower, the diameter increasing by only 25–40 per cent. (of that at weaning) between 60 and 100 days after weaning. Nor was any increase in the total number of aortic elastic membranes detected during growth and ageing in the rat. This perhaps implies that previously present membranes are remodelled in some way (if only at the molecular level) and replaced on a larger scale (perhaps by interstitial additions) as the aortic diameter and length increase with age and growth. The diameter of the aorta in the experimental weanlings increased by only 12 per cent. during the first 60 days after weaning, indicating some alterations in the normal processes of aortic growth and remodelling. Kajee (personal communication), working in our laboratories, has measured aortic growth in 70 normal male and 70 normal female rats from the day of birth to 150 days of age. Some of his findings, abstracted in Text-figs. 2 and 3, not only confirm our original observations, but have extended them in showing that the maximal rate of aortic growth in length in our strain of rats occurs before the 22nd post-natal day, the rate of increment
decreasing rapidly after the 30th day. These findings of Kajee may account for the great susceptibility to aortic rupture, originally described by Ponseti & Shepard and subsequently confirmed by us, of rats younger than 25 days of age.

When first fed odoratus seeds; the rapidly progressive decrease in aortic susceptibility to odoratus toxin in rats of greater initial age may also perhaps be due to the smaller rate of aortic growth in slightly older animals. This observation suggests that the rate of aortic growth and therefore the rate of its associated
remodelling may in large measure determine whether or not rupture of this vessel will occur. Greater rates of growth are taken to imply speedier remodelling and therefore speedier lysis and resynthesis of structures—hence probably greater susceptibility to ruptures, should these two normally carefully integrated processes be disturbed or deranged in any way.

The above facts make understandable Ponseti & Shepard's (1954) and our own finding that the incidence of aortic ruptures decreases extremely suddenly in rats initially 28 days old as compared with rats 25 days and younger when first fed odoratus seeds. The variations in the incidence of aortic aneurysms described by other workers (Menzies & Mills, 1957) and the complete failure to produce such lesions recorded by Meyer & Vos (1956) may indeed yet be shown to be a function of the initial ages and therefore of the lesser rate of aortic growth in their experimental animals when they were first fed odoratus seeds or given the equally toxic nitriles. Thus, Menzies & Mills reported the initial weights of their rats to be 45–60 g, while Meyer & Vos stated their rats to be 33 days old (average initial weights of 70 g.). The former workers recorded only 9 instances of aortic haemorrhages, i.e. approximately 10 per cent. of rats, while the latter observers did not encounter a single instance of aortic rupture or haemorrhage in their 96 animals. All these findings are not surprising in view of Ponseti & Shepard's original report (later confirmed by us) of a profound drop in the incidence of aortic aneurysms from 75 per cent. of 50 rats which were less than 28 days old when first fed odoratus seeds, to 31 per cent. in initially 28-day-old rats. Ponseti & Shepard simply state, in a general way, that '... the incidence of dissecting aneurysms of the aorta was related to the age of the rat when the experimental diet was started'. Our own findings, however, lead us to suggest that the aspect of age which seems to be important in determining whether or not aortic aneurysms and other lesions will appear in odoratism (as well as their severity and speed of appearance) is the rate of aortic growth and remodelling during the early days or weeks of intoxication. Since this seems to be maximal in rats immediately after weaning, i.e. between 22 and 25 days old, we suggest that animals of this age should be used as standards—especially in experiments such as those which have previously been designed to determine the effects of various dietary supplements, or of hormones, on the incidence, speed of development, and course of these lesions (Strong, 1956; Selye & Bois, 1957). It is noteworthy in this regard, too, that Ponseti and Shepard reported the aortic lesions to supervene far more rapidly in younger than in older rats. In rats 22–23 days old, aortic lesions were noted as early as 12 days after the experimental diet was started; haemothorax was encountered between 34 and 56 days in 22-day-old rats as compared with 41–57 and 63–154 days in rats initially 23 and 28 days old respectively. Such observations apply primarily to the aortic lesions, but apparently not to those of the bones, since the latter seem to grow continuously in the rat.

The accumulation of MMP's in the aorta, throughout the 60-day experiments, while prominent in weanlings, is not obvious in animals which were adults when
first placed on the sweet-pea diets. Nor were excessive quantities of MMP's detectable in the aorta, even of initially weanling rats, after they had been on odoratus diets for 80 or more days. Such MMP's excesses may represent derangements in the regeneration of normal wear and tear injuries in aortae. The fact that this MMP's accumulation, in odoratus-fed rats, is always most marked towards the intima of the aorta, seems also to support our previous suggestion (Gillman, Hathorn, & Penn, 1957) that the rate of polysaccharide turnover in this zone of the aorta is perhaps more active than elsewhere in the vascular tree, thereby increasing the susceptibility to injuries of this part of the aorta.

Disturbances in reactivity of the connective tissues generally in intoxicated adults, or even in the older group of 'young' rats studied by us, was nevertheless evinced by the occurrence of highly cellular bone lesions, especially at sites of tendon attachments. Other evidence favouring our interpretation that the lesions in odoratism may represent the end results of derangements of fibrillogenesis (rather than fibrillo- or elasto-lysis) and of the remodelling necessitated by normal growth, are (a) the disturbances in fibre deposition in zones of fibroblastic proliferation associated with attempts at repair around sites of aortic injury, (b) the highly cellular, relatively afibrillar character of the exostoses on long bones, and (c) the profound disorganization of the normally integrated processes of endo- and perichondrial osteogenesis and of endosteal osteolysis both in adults and in weanlings.

Thus, our preliminary studies on the bones indicate aberrations in reticulin formation within the osseous tissue, which defect, in the organic matrix of the bones, may in part account for the ultimate deformities. This observation is in conformity with that recently reported by Menzies & Mills (1957). Profound alterations were also noted in the amount of PAS-positive material in both matrix and cartilage cells, and in the intensity and distribution of metachromasia of the matrix of epiphyseal cartilages. Also noteworthy were the striking increases in marrow mast-cells, especially in weanlings which had been on the odoratus diet for long periods (Gillman, 1957a).

Particularly impressive, too, especially in initially young rats fed odoratus seeds for long periods, was the gross disorganization in endosteal lysis of bone which, in healthy rats, is precisely integrated with periosteal neogenesis of bone. Thus, as shown in the Plate, figs. A–C, for the femur, two shafts were frequently noted in several long bones—the apparently older shaft within the bones being surrounded by new marrow, the latter in turn being enclosed within another and apparently newly deposited shaft. These changes, as well as the gross bony deformities (Plate, figs. D, E), seem to represent clear-cut proof of disorganization of remodelling processes—at least in the long bones—resulting from odoratus intoxication.

The herniae, reported by earlier workers (but not encountered in our rats) may also, perhaps, be similarly explained by derangements in fibre lysis and regeneration during remodelling of the abdominal wall.
COMMENT

Our studies of the aortic and osseous reactions in rats fed *L. odoratus* seeds seem to substantiate our previous contention that the integrity of the collagen-like ‘cores’ of the vascular elastic membranes (VEM’s), and possibly also of fibres in osseous matrix, is intimately dependent on their apparently spatially related and persistent reticulin and polysaccharide metabolism. The association of damage to VEM’s with distinct alterations in the reticulin and mucopolysaccharide contents, around and between the aortic VEM’s, confirms our previous findings in this regard (Gillman, Hathorn, & Penn, 1957). In the latter communication it was shown that single acute severe injuries to the media, of coronary arteries in particular, culminate, on healing, in fibrosis, tortuosity, and, with contracture of the medial scar tissue, in the ultimate stenosis of these vessels. Such repair of coronary arteries may, and we think does, predispose the now rigid, fibrosed vessels to subsequent injury of the intima and ultimately to fibrin deposits and even to thrombosis of their narrowed lumina—given certain prevailing metabolic conditions outlined elsewhere (Gillman, 1957c; Gillman & Naidoo, 1957, 1958; Gillman, Naidoo, & Hathorn, 1958).

It also seems possible, from our studies of odoratism, that in the aorta the most intimal of the medial elastic membranes seem to lyse first, as the aorta grows in diameter, such lysis of more intimaly situated membranes being associated with the simultaneous circumferential (adventitial) formation of new membranes as the aorta grows in diameter. Such remodelling of the aorta, seen in the rat, if it occurs also in man, may account for the finding of ‘abnormalities’ of the intimal ‘elastic’ tissue, in both the coronaries and the aorta, reported even at a very early age post-natally (Schornagel, 1956; Levene, 1956a, b). The frequently encountered ‘pseudo-elastic’ tissue in arteries, previously reported from this laboratory (Gillman et al., 1955) and recently confirmed for human coronary arteries by Levene (1956a, b), may also, perhaps, represent the late outcome of disturbances in the remodelling of VEM’s as the delicately integrated processes, responsible for such remodelling, becomes less nicely timed with advancing age or even following metabolic disturbances.

Once the mechanisms for alterations in arterial elastic membranes, and especially those attendant on the repair of injuries, are elucidated, it may then be a relatively simple matter to account for the localization of lipid or fibrin deposits in such sites of injury on the basis of chemically and histologically definable mechanisms detailed elsewhere (Gillman & Naidoo, 1957; Gillman, 1957c).

Comparison of vascular and osseous reactions indicate that the way in which the balance between these two processes—lysis and neogenesis of structures—is disturbed can apparently vary somewhat in different loci. Thus, both in arteries and in bones, excessive tissue may be formed. This is evident from the marked thickening of the aortic wall and, in bone, by exostoses at tendon insertions as
well as by overall thickening of bone shafts. In the aorta lysis may simultaneously be highly active at one stage of post-natal development—hence the ruptures or aneurysms. In the bones, however, osteolysis may be delayed or defective at some stage and certainly the normally close integration between endosteal osteolysis and periosteal neogenesis is upset—hence the remains of old shafts within much widened marrow cavities here depicted, and associated distortions of shafts and articulating surfaces, e.g. femoral head.

In view of the apparently widespread connective tissue derangements here disclosed, the rate of growth and remodelling of arteries and bones and their apparent dependence on connective tissue carbohydrate metabolism, it is anticipated that the application of toxic nitriles, together with the use of radioisotypes, will be invaluable in testing our interpretations. Such studies may also prove most useful in unravelling the mechanisms whereby amino acids are incorporated into the protein chains of fibres as well as the relation between polysaccharide metabolism and fibre synthesis, especially in connective tissue throughout the organism at different stages of growth and maturation.

SUMMARY

1. Evidence is presented in support of the view that lesions in vessels, bones, and perhaps in connective tissues generally, induced by feeding *L. odoratus* seeds to rats, may be attributable primarily to some disturbance in the neogenesis of fibres during the growth, regeneration, and/or repair of connective tissues. This failure seems to be at a stage after sulphation of those mucopolysaccharides which accumulate in connective tissue ground substance during fibre formation. It is suggested that the aortic ruptures may be the result of some derangement in the balance between lysis and regeneration of elastic membranes, which probably occur simultaneously during the normal growth and remodelling of the aorta. This interpretation differs from that provided by other workers who attribute aortic ruptures solely to elastolysis. Evidence from the study of lesions in bones and alterations in repair processes around the aorta in *L. odoratus*-fed adult and weanling rats supports the interpretations here presented. The frequency of 'double-shafts' as well as other deformities of long bones are taken as clear indications of disorganized remodelling of bones during growth.

2. These studies of the aortic reactions in rats fed *L. odoratus* seeds also substantiate our previous contention that the integrity of the collagen-like 'cores' of the VEM's is probably intimately dependent upon their 'sleeves' of reticulin and polysaccharides. The association of damage to the elastic membranes with distinct alterations in the reticulin and mucopolysaccharide contents immediately around and also between aortic VEM's confirm our previous findings in this regard.

3. There are indications, too, from histochemically detectable increases in circulating mucopolysaccharides, from the marked increases in osseous
mast-cells and from reported delay in blood-clotting, that widespread disturbances in polysaccharide metabolism prevail in odoratus-intoxicated rats.

4. Some possible implications of these findings for understanding the genesis of human arterial lesions are briefly discussed with special reference to a developmental approach to the aetiology and pathogenesis of these vascular reactions in terms of general features of connective tissue regeneration and repair.

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REFERENCES


**EXPLANATION OF PLATE**

FIGS. A, B, C. Longitudinal sections of upper ends of femora. A from control rat to compare with B and C which are serial sections of the same femur of an initially weanling rat after consuming *L. odoratus* seeds for 103 days. Note in B and C the distortions of the head and neck, the difference in endosteal trabeculae in neck and great trochanter, the delayed resorption of old neck bone within marrow (in B), the dual shaft and marrow cavity (in C), and the extremely thin periosteal bone in B and C as compared with A; all these being indicative of disturbed osteal modelling and growth.  3.
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Figs. D, E. Macroscopic appearances of several lower limb bones of two, initially weanling, rats when 198 days old. D: control; E: fed L. odoratus seeds for 174 days. Shows in intoxicated rat gross distortions of femoral, tibial, and fibular shafts and especially their great widths (compare also with figs. B and C), exostoses at muscle insertions, and disturbed proportions, e.g. of femoral head, neck, trochanters, and condyles. × 2.

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