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THE EFFECTS OF DIHYDROTACHYSTEROL AND FERRIC DEXTRAN UPON THE PERIODONTIUM IN THE RAT

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Summary—The sequence of changes that occur in the periodontium as a result of chronic dihydrotachysterol (DHT) administration in rats was studied. The influence of ferric dextran on the above changes was also evaluated.

The earliest changes noted in the periodontal tissues after initiation of DHT feeding include the presence of numerous, minute basophilic-staining granules throughout the connective tissue of the periodontal membrane and in the marrow spaces of the alveolar bone. These granules, which apparently are small foci of calcification, act as centres for further calcification throughout the entire periodontium. Progressive changes include degeneration of the periodontal ligament and gross apposition of osteo-cementum and new sclerotic type bone, ankylosis of the roots, and calcification of the transeptal fibres at their insertion on the teeth. The utilization of ferric hydroxide and dextran intraperitoneally every fifth day did not appear to influence these changes significantly.

CALCIPHYLAXIS has been defined as a system of induced systemic hypersensitivity which can result in selective calcification of various organs. It is produced in animals by pretreatment with vitamin D derivatives such as DHT (the "sensitizer") followed after a time interval ("critical period") by an eliciting agent ("challenger"). Since the publication of SEYLE's monograph on calciphylaxis (1962) several reports have appeared in the literature which have been concerned with the dental manifestations of this phenomenon (RATCLIFF and ITOKAZU, 1964b; MOSKOW, BADEN and ZENGO, 1965; GLICKMAN, SELYE and SMULOW, 1965). Preliminary studies have dealt with the effects of dihydrotachysterol (DHT), a so-called "sensitizing" agent, on the teeth, the periodontium and the soft tissue structures of the oral cavity (MOSKOW and BADEN, 1964; BADEN and MOSKOW, 1964; RATCLIFF and ITOKAZU, 1964a).

When administered in low dosages for extended periods of time, DHT consistently produces a syndrome in rats which has been compared with the clinical picture of progeria. (SELYE and STREBEL, 1962). The animals develop muscular weakness, skin wrinkling, loss of hair and a peculiar curvature of the spine related to calcification of the intervertebral discs. There is widespread calcinosis of the blood vessels and generally a marked catabolic effect. The changes in the periodontal tissues that have been described include marked cementogenesis, sclerosis and endosteal proliferation of the alveolar bone, osteoid formation, fibrosis of the marrow spaces, calcification and degeneration of the periodontal ligament fibres and finally ankylosis (MOSKOW and BADEN, 1964). These systemic and oral changes produced by DHT administration have been reported to be severely compromised when challenging dosages of ferric dextran (Fedex) are given (RATCLIFF and ITOKAZU, 1964b; GLICKMAN *et al.* 1965).

Since prior studies described only the advanced changes produced in the periodontium by long term DHT feeding, this experiment was designed to study the sequence of changes as they occur on a week to week basis following administration of the drug. An additional objective of the experiment was to attempt to prevent the contemplated periodontal changes by inducing a calciphylactic reaction with ferric dextran.

MATERIALS AND METHODS

Forty female Sprague-Dawley rats of the Holzman strain weighing approximately 200 g were divided into three groups. Twenty animals received 50 μg of DHT in 0·5 ml of sesame oil by stomach tube daily. Representative animals of this group were sacrificed at weekly intervals beginning on the seventh day and continuing for a period of 50 days. Group 2, consisting of ten rats, received similar dosages of DHT daily. In addition however, they were given intraperitoneal injections of ferric dextran every 5 days starting on the fifth day after the initiation of DHT feeding. The ferric dextran was administered in dosages which were equivalent to 50 mg of iron in 1 ml of water. Rats of this group were kept on this regimen for a period totalling 50 days. The third group of ten rats acted as controls and received 0·5 ml of sesame oil by stomach tube daily for a period of 50 days. All animals were fed stock purina laboratory chow and water *ad libitum* for the duration of the experiment. The rats were weighed and examined daily and any observable clinical changes were noted. All surviving animals were sacrificed with ether on the fiftieth day of the experiment and their jaws were fixed in a 10% buffered formalin solution for subsequent decalcification and sectioning. The staining techniques employed were haematoxylin and eosin, PAS, PAS with diastase digestion, prussian blue and Verhoeff's silver stain.

RESULTS

Shortly after initiation of the DHT feeding, the animals of both experimental groups began to lose weight and this tendency continued unabated for the duration of the experiment. The control animals, on the other hand, demonstrated progressive weight gain and appeared extremely well-nourished during the 50-day experimental period. Within 1 week, the animals of both experimental groups began to appear listless and weak. Their coats became shaggy and ruffled, they consumed less food and water, and had a tendency toward diarrhoea. Continued DHT administration resulted in a marked spinal kyphosis in several animals (Fig. 1). The only clinically evident oral change was the progressive spreading of the incisor teeth. (Fig. 2). After 9 days of DHT ingestion, two animals expired and subsequent deaths occurred during the course of the 50-day experiment. Rats receiving DHT plus ferric dextran did not begin to succumb to the effects of the drugs until the sixteenth day of the study and a greater percentage of animals in this group was alive at the termination of the experiment. All surviving animals of both the DHT and ferric dextran groups appeared moribund at the end of the 50 days.

Contrary to the findings reported by RATCLIFF and ITOKAZU (1964b), and GLICKMAN *et al.* (1965), the results of the present study indicate no major differences in the periodontal changes observed in both experimental groups. The following histopathologic changes in the periodontal tissues, therefore, represent the findings of both the DHT group and the DHT and ferric dextran group:

At 1 week following the initiation of DHT administration, numerous small basophilic-staining granules were noted throughout the connective tissue of the periodontal membrane. (Fig. 4) These granules, which probably represent minute foci of calcification, were attracted mainly to the fibre attachments on the bone and tooth sides, but could also be noted within the periodontal ligament and in the transeptal fibre region. On occasion, the small granules were noted within macrophages. The granules tended to accumulate around dilated capillaries and adhered closely to the perivascular connective tissue sheath. They became closely packed around the endothelial cells and eventually formed a rigid sleeve around the small vessels. Minute foci of calcification could also be noted within the lumen of the capillaries and could be seen extending out of the vessels. The granules stained negatively for iron, were PAS positive and were resistant to diastase digestion. A homogenous amorphous round or oval type of granule (Fig. 4) and a linear type could be distinguished (Fig. 5). At the apex of the tooth, heavy basophilic-staining projections were observed. The projections reflected the orientation of the periodontal ligament fibres which were calcified at their insertion on the cementum and alveolar bone. The alveolar bone proper of the 7-day experimental specimens also stained strongly basophilic, and in addition, small areas of osteoid formation were present in the periodontal membrane in the region of the bifurcation and at the crest of the alveolus. The new bone resembled pseudo-cartilage and lacunae containing many entrapped osteocytes were observed.

Within 2 weeks following initiation of DHT administration, the appositional surfaces of the alveolar bone became grossly accentuated and thickened (Fig. 6). Active osteoid formation was noted on the surface of the alveolar bone proper resulting in narrowing of the periodontal membrane space (Fig. 7). Focal areas of osteoid formation were also present within the periodontal ligament and in the region of the transeptal fibres. These tended to localize around accumulations of granules which apparently act as centres of calcification. Calcification of the periodontal fibres at their attachment on cementum became more pronounced in the 2-week specimens, particularly in the cervical and apical regions (Fig. 8). This pattern of calcification has been described in a previous report, (MOSKOW and BADEN, 1964), and has been referred to as the "sunburst" effect. At this stage in the experiment, the fibres of the periodontal ligament progressively lost their normal pattern of orientation and degenerative and oedematous changes were commonly noted. In the bone marrow, the capillaries appeared widely dilated and accumulations of the granules previously described were seen. In a few specimens, small areas of ankylosis could be found in the interradicular regions (Fig. 9).

Three weeks after initiation of the experiment, the many small granules which were scattered throughout the periodontium, progressively disappeared and were replaced by localized and well-circumscribed foci of metastatic calcification. The lamina dura was increasingly thickened and stained strongly basophilic. Similarly, there was marked apposition of highly calcified bone in the endosteal spaces. Rapid apposition of osteoid and cementoid occurred at this stage in the experiment, and in many areas, bridged the space formerly occupied by the periodontal ligament (Figs. 10 and 11).

In the 4- and 5-week experimental specimens, accentuation of the changes already described were noted. In many specimens, large segments of the periodontal ligament were obliterated by active osteoid formation. In the bone, decreased haematopoietic activity was seen and progressive fibrosis of the marrow spaces was observed. The "sunburst effect" induced by calcification of the periodontal membrane fibres at their insertion was extremely intense and involved a great segment of the transeptal fibre apparatus. In several 5-week specimens, calcification of the media of the small arterioles and venules in the periodontal membrane and gingivae could be noted (Figs. 12 and 13).

Animals surviving the 50 days of the experiment demonstrated a periodontal membrane space which was extremely narrow or totally absent as a result of rapid apposition of bone and cementum (Fig. 14). New bone deposited on the alveolar crest was also extensive and produced the effect of pushing the transeptal fibres upward. Bone apposition also was seen on the periosteal surfaces particularly towards the crest of the alveolus producing an exaggerated distortion of the buccal and lingual plates (Fig. 15). The new bone was unlike normal bone in several respects; it was extremely dense and sclerotic in nature and stained heavily basophilic with haematoxylin and eosin. In some areas, the lacunae were devoid of osteocytes. Cementum, although actively deposited about the entire root, was most marked in the apical portion of the root, demonstrating the classical drumstick pattern seen in human teeth with hypercementosis (Fig. 16). Structurally, the rapidly depositing cementum was quite different from normal cementum. The natural orientation of cemental layers was absent and the cementocytes were extremely irregular in arrangement. The cementum at the apical portion of the tooth was extremely basophilic in staining and demonstrated a granular character under the microscope.

The periodontal ligament in the 50-day specimens was extremely narrow, and the connective tissue fibres were sparse and fibrillar in character. The fibroblasts were shrunken and small and their nuclei pyknotic. The remaining fibres were loosely arranged and lacked specific orientation. Small islands of osteoid and bone were noted within the periodontal membrane. These bony spicules enlarged by apposition of osteoid but occasionally osteoclastic resorption was seen.

DISCUSSION

Dihydrotachysterol and the related vitamin D compounds apparently have in common the ability to mobilize calcium, thus predisposing the animal to metastatic calcification. The mechanism by which this phenomenon occurs is still obscure. The action of the drug is presumably direct and not mediated through the parathyroid gland, as the reaction does not appear to be adversely effected in parathyroidectomized animals.

The earliest changes noted in the periodontal tissues following DHT administration were the appearance of minute amorphous and linear granules in the connective tissue of the periodontal ligament and in between the transeptal fibres. These granules are apparently foci of calcification, which act to induce further apposition of calcified tissue throughout the periodontium. Within 3 weeks after the initiation of DHT feeding, these centres of calcification disappeared, and were replaced by more discrete and larger areas of calcification.

While ferric dextran tends somewhat to slow up the severe changes in the periodontium induced by long term DHT administration, the final changes in both experimental groups were ultimately the same. In a few animals of the Fedex group some evidence of suppression of the pathologic changes could be seen, but these could easily be attributed to individual variation in response to the drugs, rather than a particular prophylactic effect. It must be pointed out, however, that the ferric dextran group did not begin to demonstrate the classical DHT intoxication syndrome until slightly later than the DHT group and fewer animals succumbed to the effects of the drugs.

Both RATCLIFF and ITOKAZU (1964b) and GLICKMAN *et al.* (1965) have indicated that ferric dextran exerts a protective effect against the changes produced in the periodontal structures by DHT. Similar dosages, time intervals, and modes of administration of the iron compound were employed in the experiment reported herein. Despite the relatively similar findings in the periodontal tissues of the DHT experimental group, our results do not substantiate the finding of prevention of the chronic DHT syndrome by intraperitoneal injections of ferric dextran every fifth day.

The question has been raised as to whether the forces of occlusion act as a challenging agent thereby preventing or minimizing the effect of the iron compound. (GLICKMAN *et al.*, 1965). The earliest and most severe changes that occur in the periodontal tissues after DHT feeding are seen in the bifurcation area, a region which is most vulnerable to forces placed on the teeth.

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Résumé—Les effets de l'administration chronique de dihydrotachysterol (DHT) sur le parodonte de rats ont été étudiés, de même que l'effet de dextrane ferrique.

Les effets les plus précoces sur les tissue parodontaux, au début de l'administration de DHT, consistent en la présence de nombreuses petites granulations basophiles, disséminées dans le tissu conjonctif ligamentaire et dans les espaces médullaires de l'os alvéolaire. Ces granulations, qui semblent être de petits centres de calcification, agissent comme des noyaux de calcification ultérieure dans tout le parodonte. On abserve ensuite progressivement des phénomènes de dégénérescence du ligament, une apposition grossière d'ostéocément et d'os scléreux nouvellement formé, ainsi qu'une ankylose radiculaire et une calcification des fibres transseptales au niveau de leurs insertions aux dents. L'emploi d'hydroxyde ferrique et de dextrane par voie intrapéritonéale tous les cinq jours, ne semble pas influencer ces modifications de façon significative.

Zusammenfassung—Die Veränderungen wurden untersucht, die im Periodontium nach chronischer Anwendung von Dihydrotachysterol (DHT) bei Ratten zustande kommt. Ausserdem wurden der Einfluss von Eisen (III)—Dextran auf diese Veränderungen geprüft.

Die ersten im periodontalen Gewebe festgestellten Veränderungen nach Beginn des DHT-Zusatzes betreffen die Gegenwart von zahlteichen, kleinen, sich basophil anfärbenden Granula im Bindegewebe der periodontalen Membran und in den Markräumen des Alveolarknochens. Diese Granula, scheinbar keline Verkalkungsherde, wirken als Ansatzpunkte für weitere Verkalkung im gesamten Periodontium. Fortschreitende Veränderungen betreffen die Degeneration des periodontalen Ligaments und massive Anlagerung von Osteozement und neuem Knocken sklerotischen Typs, Ankylose der Wurzeln und Verkalkung der transseptalen Fasern an ihrem Ansatz an den Zähnen. Die intraperitoneale Anwendung von Eisen (III)-Hydroxyd und Dextran jeden fünften Tag schienen diese Veränderungen nicht nachweisbar zu beeinflussen.

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PLATE 1

FIG. 1. Marked kyphosis of spine and skin wrinkling induced by administration of low dosages of DHT for 50 days.

FIG. 2. Spreading of the incisor teeth in chronic DHT overdosage.

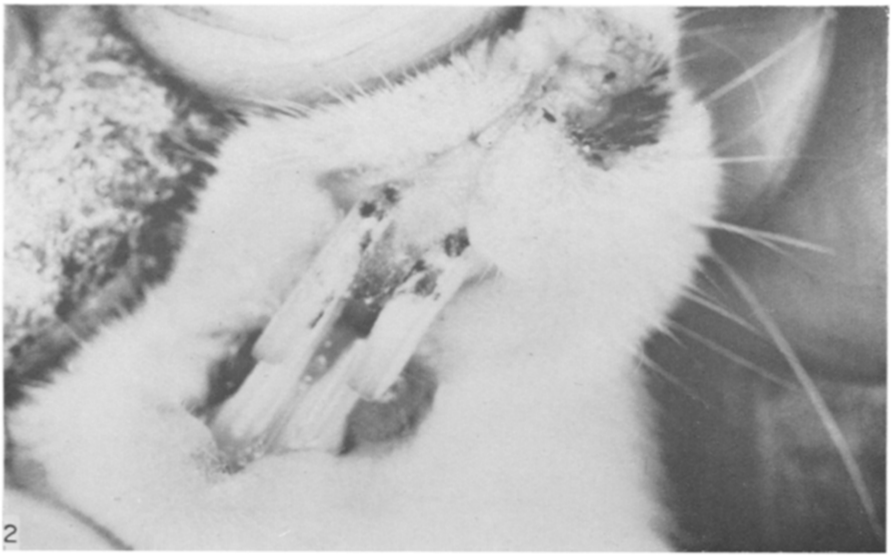
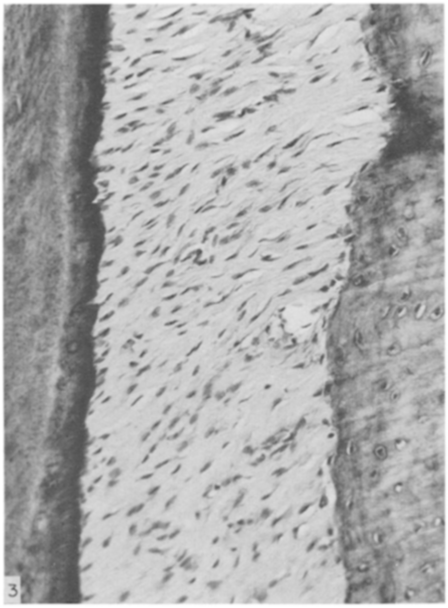
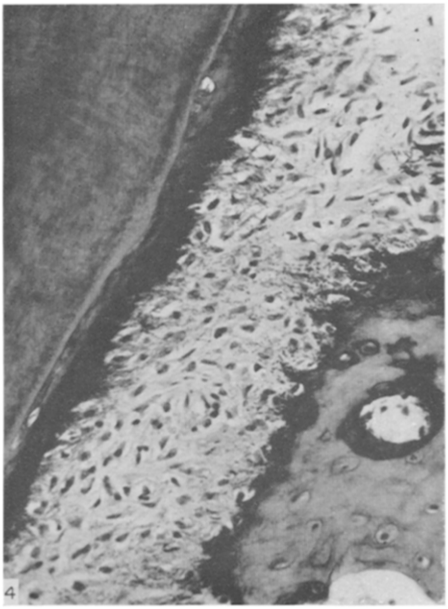
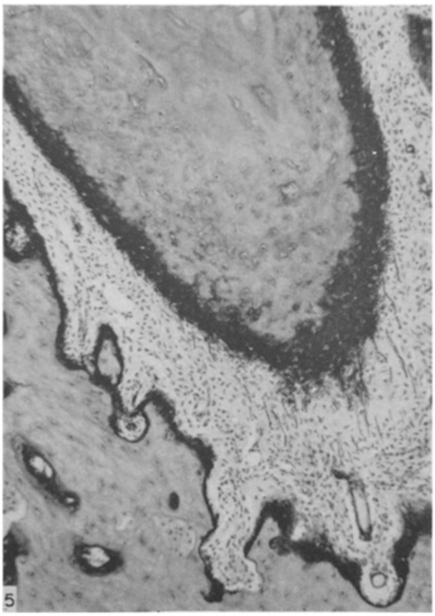


PLATE I







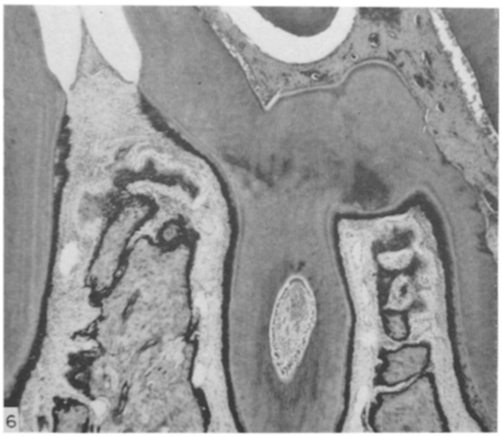


Plate 2

PLATE 2

FIG. 3. Periodontium of control rat. The functional orientation of the fibres of the periodontal membrane can be seen. Haematoxylin and eosin. ×180.

FIG. 4. Minute basophilic staining oval granules in periodontal membrane of 7-day experimental rat. (DHT). Degenerative changes can already be noted. Haematoxylin and eosin. ×250.

FIG. 5. Multiple linear-type granules in apical region of 17-day experimental animal (DHT). Note the granular character of the newly depositing cementum. Haematoxylin and eosin. ×110.

FIG. 6. Extensive apposition of new bone in crestal and interradicular area of 17-day experimental rat (DHT). Degenerative changes are present in the periodontal ligament. Haematoxylin and eosin. ×35.

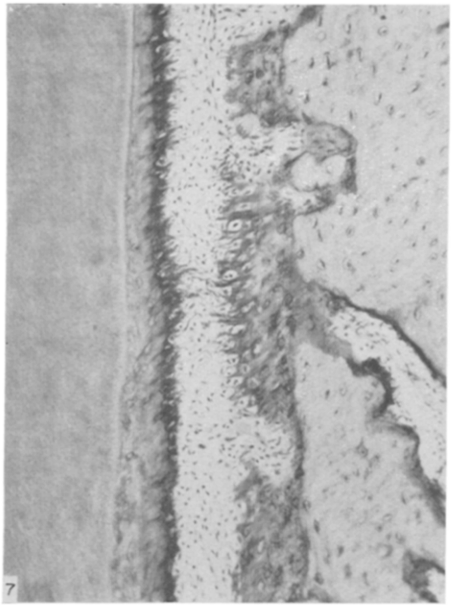
PLATE 3

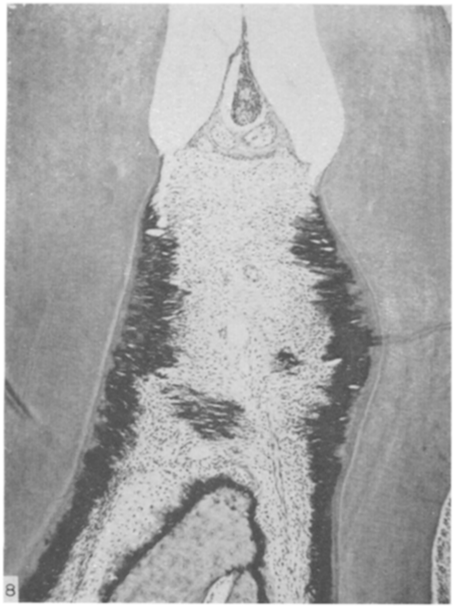
FIG. 7. Active osteoid formation obliterating the periodontal membrane space of 18-day animal (DHT). Note the calcification of the Sharpeys fibres. Haematoxylin and eosin. ×125.

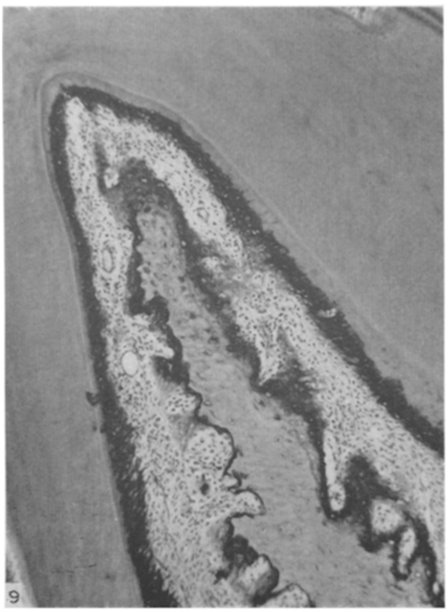
FIG. 8. "Sunburst" effect produced by calcification of the transeptal fibres at their insertion on the teeth. Focal areas of calcification are also seen. (16-day DHT rat). Haematoxylin and eosin. ×70.

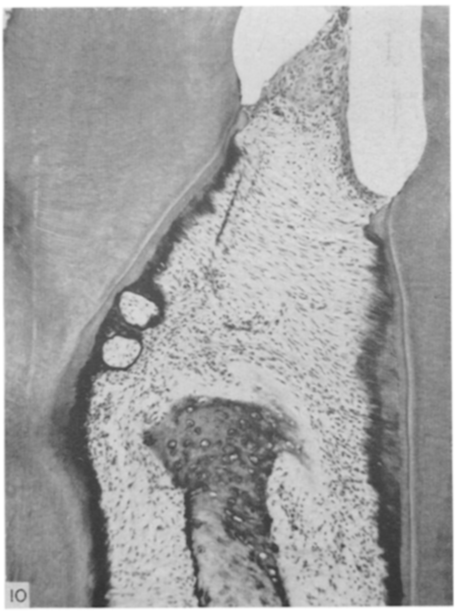
FIG. 9. Areas of ankylosis in bifurcation region of 16-day experimental rat (DHT). Haematoxylin and eosin. ×90.

FIG. 10. Knob-like apposition of new bone on alveolar crest of 23-day animal (DHT). Calcification of transeptal fibre insertions can be seen. Ring-like calcifications are probably calcified vessel walls. Haematoxylin and eosin. ×100.









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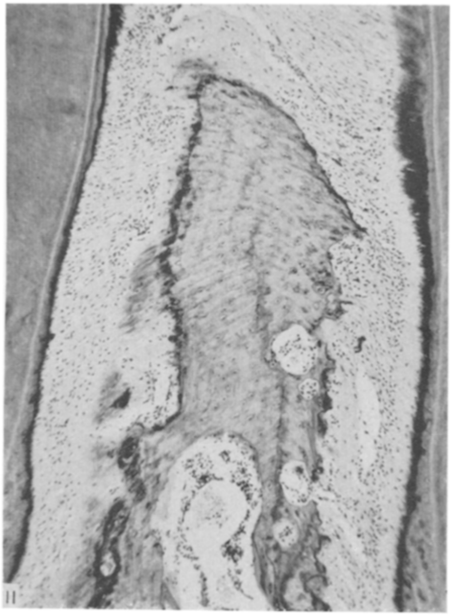
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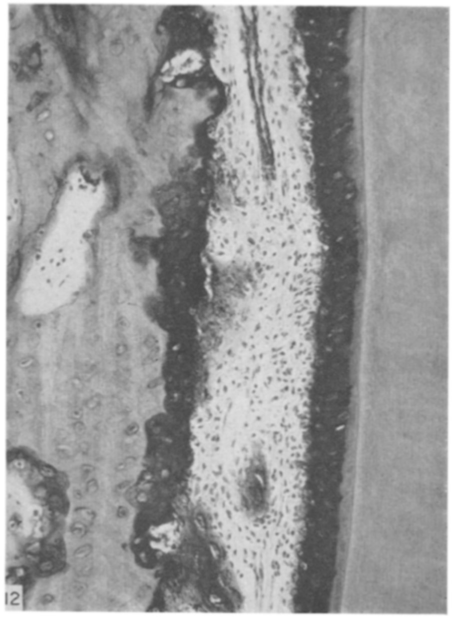
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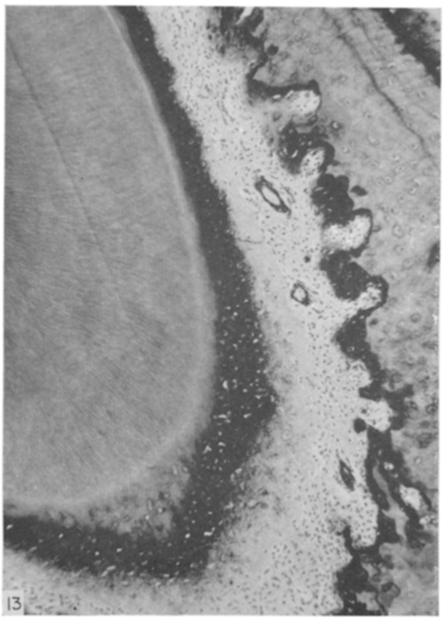
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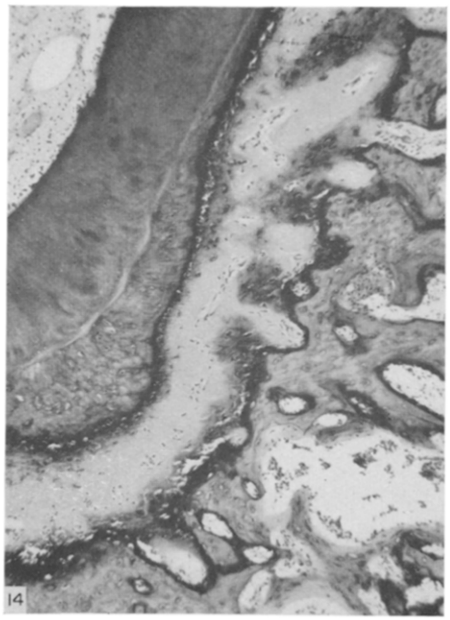
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PLATE 4

FIG. 11. Rapid deposition of new bone and cementum narrowing the periodontal space. Crestal apposition of osteoid can also be noted. (21-day experimental rat. DHT plus ferric dextran). Haematoxylin and eosin. ×75.

FIG. 12. Small islands of new bone in the periodontal ligament in 37-day DHT animal. Medical calcification of a small venule can be seen. Haematoxylin and eosin. ×160.

FIG. 13. Apical aspect of 38 day experimental animal (DHT plus ferric dextran) showing proliferation of new cementum. Numerous small blood vessels with calcified walls are seen. Haematoxylin and eosin. ×100.

FIG. 14. Complete ankylosis of root seen in 50-day animal (DHT plus ferric dextran). Haematoxylin and eosin. ×85.

PLATE 5

FIG. 15. Bulbous distortion of alveolar plate induced by rapid bone apposition on periosteal surface in 37-day experimental rat (DHT). Haematoxylin and eosin. ×85.

FIG. 16. Marked deposition of cementum about apices of molar roots. Note the sclerotic appearance of the alveolar bone. 41-day experimental animal. (DHT plus ferric dextran). Haematoxylin and eosin. ×30.

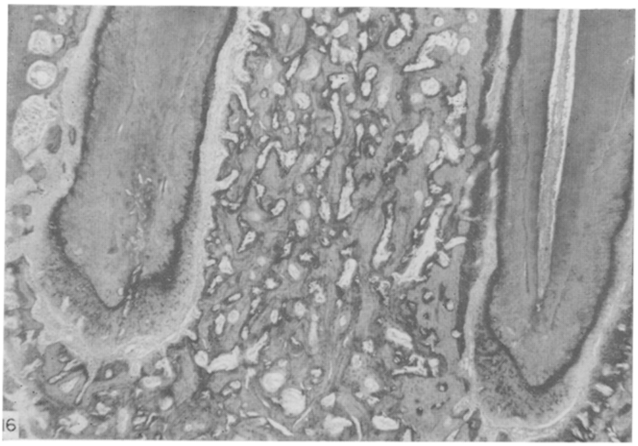

PLATE 5