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The Role of Mucopolysaccharides in the Protection of Cartilage Cells Against Immune Reactions [and Discussion]

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INFLUENCE OF THE PERICELLULAR ENVIRONMENT ON THE CELLS

The role of mucopolysaccharides in the protection of cartilage cells against immune reactions

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[Plates 6–9]

Problems related to rheumatoid arthritis have been investigated by a group at Cambridge using the organ culture technique. Since auto-allergic reactions may be concerned in the chronicity of the disease, the effects of reactive complement-sufficient antisera (AS + C′) on embryonic and post-foetal cartilage were examined.

The cartilaginous limb bone rudiments enlarged to several times their original volume in control medium, but in the presence of AS + C′ they gradually disintegrated, owing to the breakdown of the cartilage matrix; only the superficial cells of the enveloping soft connective tissue were killed, however. Provided breakdown had not advanced too far, the effects of AS + C′ were reversible.

It was not clear how AS + C′ produced these changes, since cartilage matrix is impermeable to molecules as large as the immunoglobulins. To find whether there was a difference in permeability between embryonic and post-foetal cartilage, similar experiments were made on the articular cartilage of young pigs. AS + C′ had no effect on pure articular cartilage, and it was shown immunohistochemically that IgG did not penetrate beyond the most superficial layer of cartilage. When, however, the explant was associated with soft connective tissue either as invading marrow or as an adjacent explant of synovium, the cartilage matrix was depleted of proteoglycan; IgG antibodies then entered the cartilage and reacted with the chondrocytes. After a lapse of 8–10 days, collagen also began to break down. If the degradation of collagen was not too extensive, the changes were reversible.

Pure cartilage was depleted of proteoglycan by trypsinization and then cultivated in AS + C′. All the chondrocytes reacted with the IgG antibodies. The peripheral cells were killed, but those in the interior survived and rapidly secreted pericellular capsules rich in proteoglycan, which shielded them from further contact with antibodies. In other experiments, pure cartilage was associated with a synovial explant and cultivated in AS + C′ for 10 days; this caused severe depletion of the matrix. The synovial tissue was then removed and the isolated cartilage cultured for a further 10 days in either AS + C′ or control medium. If mainly proteoglycan had been lost during the primary culture period, breakdown did not continue in AS + C′, and sometimes a little new matrix was regenerated, though less than in control medium; if, however, the collagen had been extensively degraded, breakdown continued even in control medium.

It is suggested that in both the embryonic and post-foetal cartilage, degradation of the cartilage matrix was due to the enzymatic activity of the associated soft connective tissue which caused a loss first of proteoglycan, which enabled antibodies to reach the chondrocytes, and then of collagen. The possible relevance of these results to the pathogenesis of rheumatoid arthritis is discussed.

Introduction

My colleagues and I were introduced to the protective action of proteoglycans, of which the major components are mucopolysaccharides, quite by chance, in the course of some experiments designed to shed light on certain aspects of rheumatoid arthritis. In this disease the synovium (the membrane lining the cavity of the joint) becomes grossly inflamed, heavily infiltrated with lymphocytes and plasma cells and gradually invades and replaces the articular cartilage; eventually the two bones of the joint may fuse. In the study of rheumatoid arthritis, three main questions must be anwered: (1) how is the arthritis initiated? (2) why does it become chronic? (3) by what mechanism is the cartilage destroyed? Up to the present we have been concerned chiefly with the third and easiest question: by what mechanism is the cartilage destroyed?

There are a number of hypotheses about the pathogenesis of rheumatoid arthritis. One of these is as follows. Immune complexes become localized in the synovium; these may be formed in the general vascular system and then deposited in the synovium, or they may be elaborated from antibody reacting with antigen (possibly microbial) already present in the synovium. In either case, such complexes would have an irritant effect on the joint (type III reaction of Coombs & Gell's classification of allergic reactivity; Gell & Coombs 1968) and cause breakdown products to be released from the tissues; these products might induce an auto-allergic reaction that was responsible for the chronicity of the disease. The work reviewed in this paper was undertaken with the object of discovering whether cartilage would in fact break down when exposed to a reactive complement-sufficient antiserum, and if so by what process this would be achieved.

Throughout the research we used an organ culture system (Fell & Dingle 1963; Fell & Barratt 1973) similar to that already described in this symposium by Sylvia Fitton-Jackson. The object of the organ culture technique is to maintain tissues in a differentiated, functional state resembling as closely as possible that of their prototypes in the body. Such cultures are being increasingly used for studying the direct effect of biologically active agents on a target tissue, and provide detailed information which could not be obtained *in vivo*. A large group of people have been concerned in the work to be reported here. It began in 1965 in collaboration with Leonard Weiss (Fell & Weiss 1965). We prepared an antiserum in rabbits against minced foetal mouse tissues, added 15% of this unheated antiserum to the chemically defined medium BGJ (Biggers, Gwatkin & Heyner 1961) and studied its effect on the isolated limb bones of foetal mice near term, during 6–8 days' cultivation; for our controls we used either the same serum heated for 1 h at 56 °C (to destroy the complement), or unheated normal serum. In spite of their crudity the experiments gave an interesting result. The unheated antiserum (which also contained complement) caused resorption of bone and loss of metachromatic material from the terminal cartilage.

The effect of complement-sufficient antiserum on chick limb-bone rudiments

(*a*) *Morphological effects*

It was decided to extend the initial experiments of Fell & Weiss (1965) by investigating the biochemical as well as the morphological effects of complement-sufficient antiserum (AS + C′). At this point, Dr (now Professor) R. R. A. Coombs, F.R.S., joined us as a collaborator. Foetal

mouse bones were inconveniently small for biochemical purposes, so instead we used the much larger skeletal rudiments from chick embryos. In preparing the bones for explantation, I was careful to preserve the periosteal and perichondrial tissue intact, as naked cartilage and bone became very abnormal in culture. I mention this technical point because, as will be seen later, in the light of recent work, we believe that it may have had an important bearing on our results. We explanted limb bone rudiments from 7–13 day embryos, and the frontal and mandibular bones from the older (12–13 day) chicks. The marrow cavity had not yet been excavated in the 7–8 day long-bones which were entirely cartilaginous, but was present at 12–13 days. We used rabbit anti-Forssman antiserum and antiserum against fowl erythrocytes, both chosen as reagents that cross-react with the membrane of most cells of the chick, in conjunction with rabbit serum complement; the two antisera gave similar results. The control medium usually contained antiserum + heat-inactivated complement. The sera were added to the chemically defined medium BGJ modified by the addition of 5 mg sodium acetate and 5 mg ascorbic acid per 100 ml (BGJ 4).

During 8 days' cultivation in control medium, the 7–8 day rudiments enlarged to several times their original size and continued to develop (figure 2), but in AS + C′ the cartilage matrix underwent extensive degradation (figures 1 and 3) and sometimes largely disappeared (figures 7 and 8); the periosteal bone (*b*) also was partly resorbed (figure 8) (Fell, Coombs & Dingle 1966). Only a thin superficial layer of tissue was killed by cytolysis (figure 7) and elsewhere the cells were viable. There was one interesting exception to this; in the explants of the very thin frontal bones, virtually all the cells were killed by exposure to AS + C′ and there was no resorption of bone. Although the chondrocytes and osteoblasts in the limb bones and the osteoblasts of the mandibular bones survived under the influence of AS + C′ they lost their characteristic appearance and underwent a fibroblastic transformation (figures 3, 7 and 8); the hypertrophic chondrocytes of the shaft (figure 4), which normally do not divide, lost their vacuoles, their cytoplasm became very basophilic and many of the cells underwent mitosis (figure 3).

(*b*) *Recovery*

If the explanted cartilage and bone were transferred to normal medium, the changes induced by AS + C′ were largely reversed at the histological level (Fell *et al.* 1966; Fell, Dingle, Coombs & Lachmann 1968); the chondrocytes and osteoblasts resumed their normal appearance and secreted new matrix (cf. figures 5 and 6). If disintegration of the shaft in the cartilaginous 7–8 day rudiments had advanced too far, only the terminal cartilage regenerated new matrix and the shaft disappeared, except for a few remnants of periosteal bone; the gross anatomy of the 7–8 day rudiments became grossly distorted during regeneration (figure 6). New cartilage was usually formed in the cavities of the periosteal bone and in the periosteum itself during the regeneration of the 12–13 day bones.

(*c*) *Biochemical changes*

The next step was to investigate the biochemical changes that accompanied the morphological effects of AS + C′. This biochemical aspect of the work was undertaken by John Dingle and his group (Dingle, Fell & Coombs 1967). Most of the experiments were made on paired rudiments (femur and tibia) from 7–8 day chick embryos; one member of each pair was treated with AS + C′ and the other which served as a control, was cultivated in normal heat-inactivated rabbit serum. Analysis of the explants at the end of the experiment, showed that

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rudiments grown in AS + C′ contained about a third of the hexosamine and hydroxyproline (a component of collagen) of paired controls cultivated in AS + heat inactivated complement. On a per cell (DNA) basis, the synthesis of these compounds was not inhibited, however, but in the presence of AS + C′ proportionately more was released into the medium than in the controls. This breakdown of the intercellular material was accompanied by the much increased synthesis and release of the lysosomal protease, cathepsin D. The enzymology of this work has already been described and discussed in this symposium by John Dingle, so will not be considered further here.

It was possible to inhibit the breakdown of the matrix in the 7–8 day rudiments in two ways. Hydrocortisone (0.1 μg/ml of medium) diminished the release of both hexosamine and cathepsin D from the explants, and so partly protected the tissue from the action of AS + C′. This hormone is known to stabilize lysosomal membranes (de Duve, Wattiaux & Wibo 1961; Weissman & Dingle 1961) and thus prevent the liberation of their hydrolases. The release of

Description of plate 6

Except where otherwise indicated, the photographs were taken by Mr R. C. Green (Department of Pathology, University of Cambridge). The author is indebted to the editors of the following journals for permission to reproduce figures: *International Archives of Allergy and applied Immunology* (figures 18 and 19) and the *Annals of the rheumatic Diseases* (figures 30, 31, 34, 35 and 36).

Figure 1. Explant of 7½ day chick femur after 8 days' cultivation in anti-Forssman antiserum (AS + C′). Metachromatic material has almost disappeared from the cartilage matrix. (Toluidine blue.)

Figure 2. Femur from the same embryo as that in figure 1 after 8 days' cultivation in control medium (AS + heat-inactivated C′); same magnification as figure 1. Note the relatively large size and intense metachromasia of this explant as compared with its sister treated with AS + C′. (Toluidine blue.)

Figure 3. Section of the shaft of the explant in figure 1. Many of the capsules contain two chondrocytes and one cell (m.) is in telophase. The cells are smaller, more irregular in shape and more basophilic than the hypertrophic chondrocytes of the control (cf. figure 4) and the intercellular partitions much narrower. (Harris's haematoxylin and chromotrope.)

Figure 4. Section of the shaft of the control explant in figure 2 showing typical hypertrophic chondrocytes and broad partitions of matrix. Same magnification as figure 3. (Harris's haematoxylin and chromotrope.)

Figure 5. Tibia from 8-day embryo after 4 days' cultivation in complement-sufficient antiserum against fowl erythrocytes (AFE + C′). The cartilage of the shaft has begun to break down and the tarsal end is detached (a common phenomenon in explants exposed to reactive complement-sufficient antiserum). Metachromasia has almost disappeared. (Toluidine blue.)

Figure 6. Tibia from the same embryo as that in figure 5, cultivated for 4 days in AFE + C′, then for a further 10 days in heat-inactivated normal rabbit serum (NRS). Same magnification as figure 5. Note regeneration of cartilage but anatomical distortion. The middle section of the shaft continued to break down in NRS. (Toluidine blue.)

Figure 7. Another section of the explant shown in figure 5, the region of breakdown and the fibroblastic transformation of the chondrocytes are seen. The superficial cells of the connective tissue envelope are necrotic (ne.). (Azan.)

Figure 8. Tibia from 7-day chick embryo cultivated for 6 days in AFE + C′; same magnification as figure 7, the matrix of the shaft has broken down completely and the hypertrophic chondrocytes have assumed a fibroblastic form. The periosteal bone (b.) has almost disappeared in places. (Azan.)

Figure 9. Section of part of a normal condylar ridge from the metacarpal of a young pig. Note invading marrow (i.m.), subchondral bone (b.) and weakly metachromatic transitional cartilage (i.c.). (Toluidine blue.)

Figure 10. Explant of pure articular cartilage from a pig metacarpal, after 16 days' cultivation in complement-sufficient antiserum against pig erythrocytes (AS + C′); same magnification as figure 9. The cartilage shows no effect. (Toluidine blue.)

Figure 11. Sister explant to that in figure 10, after 16 days' cultivation in NRS; same magnification as figure 9. Some new cartilage (n.c.) has been formed beneath the original fragment (never produced in AS + C′) but otherwise the explant appears similar to that in figure 10. (Toluidine blue.)

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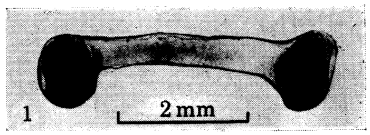

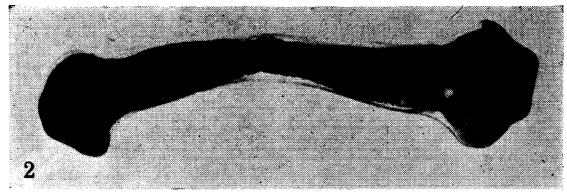

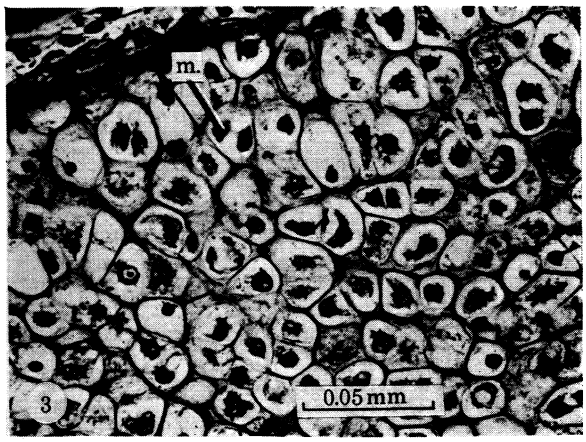

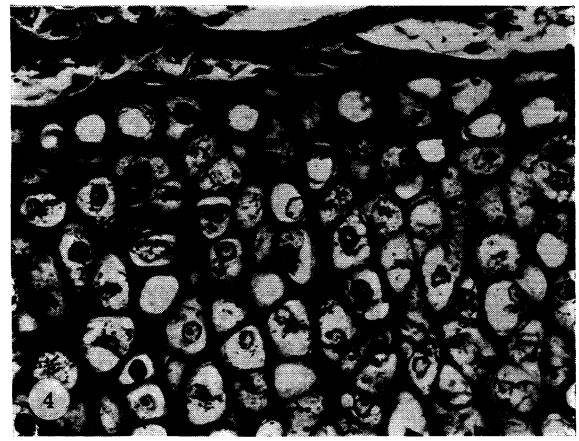

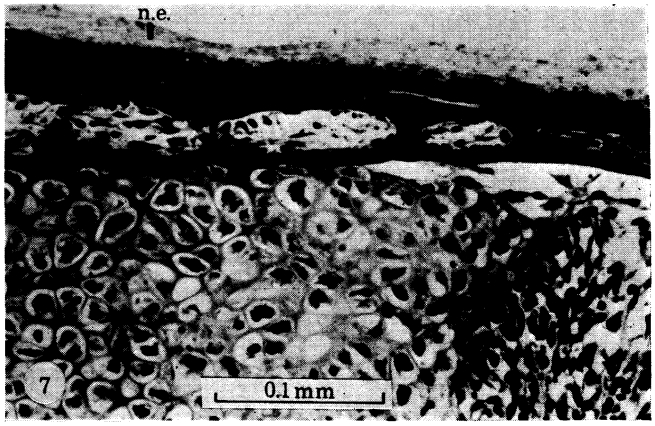

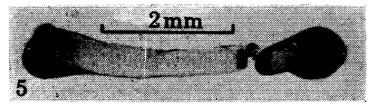

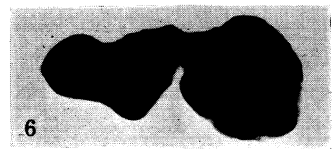

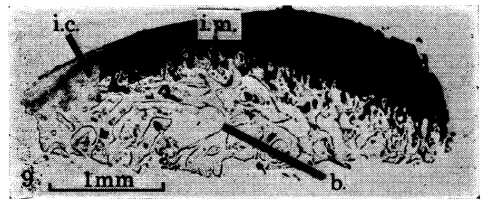

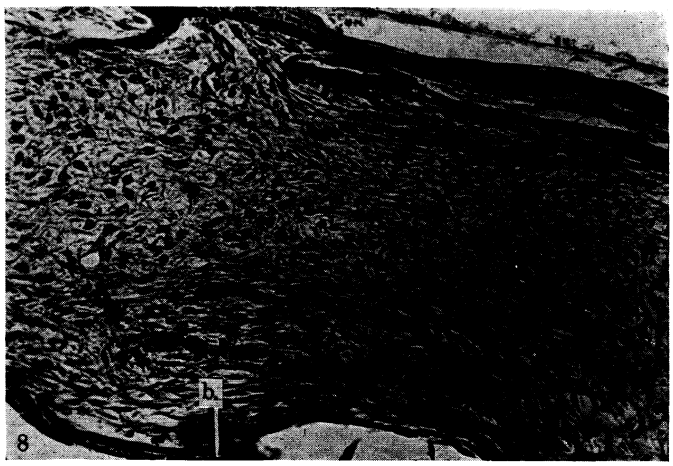

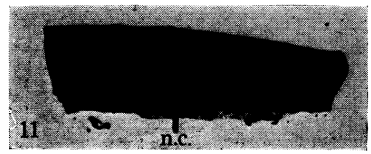

FIGURES 1–11. For description see opposite.

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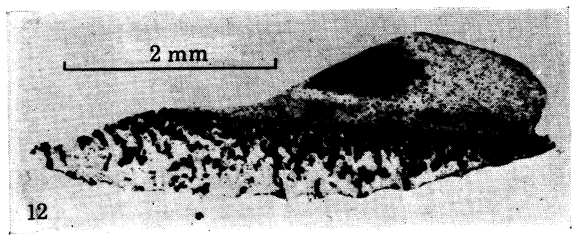

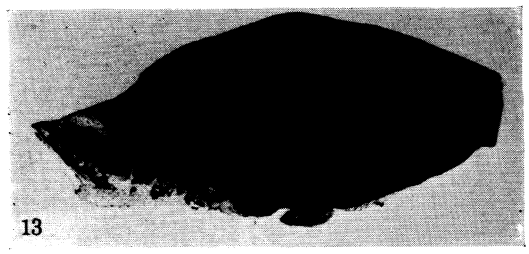

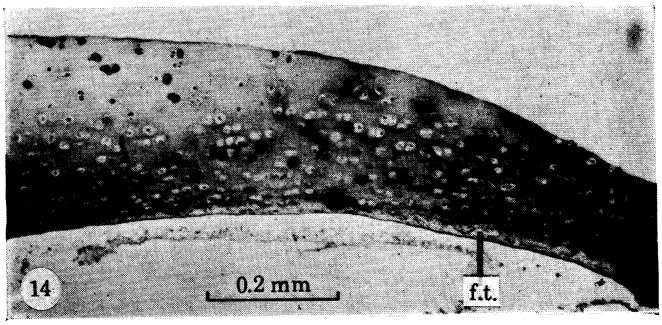

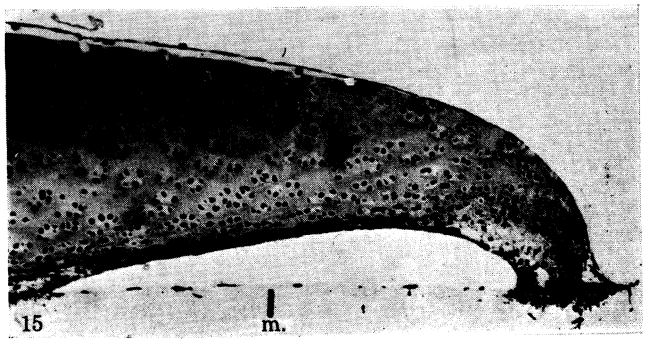

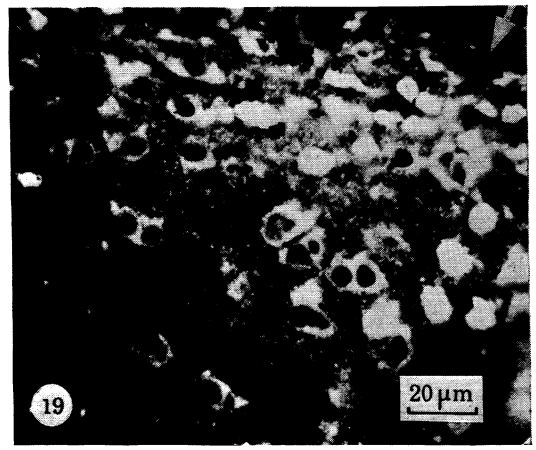

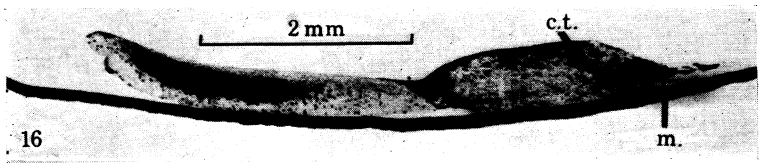

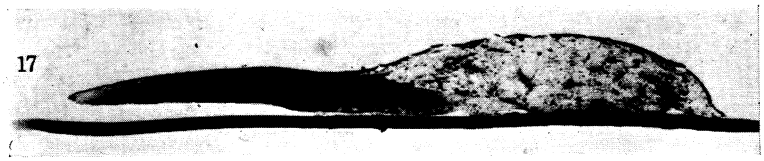

FIGURES 12–19. For description see opposite.

hexosamine and hydroxyproline by antisera was also largely prevented by ε-aminohexanoic acid, an inhibitor of lysosomal protease.

(*d*) *Immunology*

Our immunological colleagues thought that we ought to have more detailed immunological data about the action of AS + C′ on embryonic (7–8 day) chick limb bones (Lachmann, Coombs, Fell & Dingle 1969). The results of this study showed that the breakdown of the cartilagenous bone rudiments in response to AS + C′ required the involvement of both antibody and complement, that the antibody might be either IgM or IgG and that antiserum directed against serum proteins of the chicken was ineffective. Serum complement alone caused some depletion of the matrix, but much less than AS + C′; its effect was possibly due to naturally occurring antibodies reactive with chicken cells, present in the serum used as the source of complement. The serum of rabbits lacking the C′ 6 factor of complement was inactive when associated with either IgG or IgM antibody, and complement-sufficient normal serum could be inactivated by absorption with antigen–antibody aggregates or with zymozan which inactivates complement.

The explants were also examined by immunofluorescent methods. To demonstrate the presence of antigens reactive with anti-fowl red cell serum, frozen sections of the limb bone rudiments were treated first with the IgG fraction of the rabbit anti-fowl erythrocyte serum, then washed and stained with fluorescein-conjugated (goat) anti-rabbit IgG, or else treated with guinea-pig complement and stained with fluorescein-conjugated anti-guinea pig C′ 3 (one of the components of complement); the two methods gave similar results. In the unaffected and moderately affected rudiments, the chondrocytes and osteocytes reacted strongly for fowl erythrocyte antigens, but less intensely in the severely affected rudiments.

Description of plate 7

Figure 12. Explant of pig articular cartilage with the invasion zone, after 14 days' cultivation in AS + C′. The cartilage matrix is severely depleted and only a small patch of metachromasia remains in the non calcified cartilage above the invasion zone. The calcified cartilage of the invasion zone never loses its metachromasia. (Toluidine blue.)

Figure 13. Pig articular cartilage with invasion zone, cultivated for 14 days in NRS. Sister explant to that in figure 12, photographed at the same magnification. The matrix is intensely metachromatic. (Toluidine blue.)

Figure 14. Pig articular cartilage without the invasion zone but including some fibrous transitional cartilage (see figure 9), cultivated for 10 days in AS + C′. The transitional cartilage is readily penetrated by IgG antibody; most of the chondrocytes are dead. Fibrous tissue (much degraded), f.t. (Harris's haematoxylin and chromotrope.)

Figure 15. Pig articular cartilage including fibrous transitional cartilage; cultivated in NRS for 10 days. Sister explant to that in figure 14; same magnification. There is no necrosis; where the fibro-cartilage touches the millipore membrane (m.) on which the tissue rests, cells are invading the membrane. (Harris's haematoxylin and chromotrope.)

Figure 16. Pig articular cartilage without invasion zone associated with a piece of capsular tissue (c.t.), after 10 days in AS + C′. There is severe depletion of the cartilage matrix. m.: millipore. (Toluidine blue.)

Figure 17. Articular cartilage and capsular tissue after 10 days in NRS. Sister explant to that in figure 16; same magnification. There is considerable depletion of the matrix but less than in figure 16. (Toluidine blue.)

Figure 18. Pig articular cartilage without invasion zone, cultivated for 10 days in AS + C′. Section stained with fluorescein-labelled (pig) antibody to rabbit IgG and photographed on a dark-ground fluorescence microscope. Only the articular surface (arrow) and the chondrocytes immediately beneath it react for IgG, showing that the antibody cannot penetrate normal cartilage matrix. (Photograph by A. R. Poole; reproduced from Poole, Barratt & Fell 1973.)

Figure 19. Pig articular cartilage with invasion zone, cultivated for 10 days in AS + C′. Section stained and photographed as for figure 18. IgG antibody has penetrated throughout the depleted cartilage and reacted with the chondrocytes. Articular surface: arrow. (Photograph by A. R. Poole; reproduced from Poole *et al.* 1973.)

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The distribution in the explants of bound antibody derived from the medium during cultivation was then examined immunohistochemically. In the unaffected explants, the surrounding connective tissue reacted strongly, but in the cartilage only the peripheral chondrocytes were faintly stained. There was more IgG binding in the interior of the moderately affected explants but less than in the connective tissue; the most intense reaction was in the superficial layer of dead cells. There was IgG binding throughout the most severely affected rudiment and the peripheral slough of necrotic material, where still present, stained extremely brightly.

At about this time (1969), I gave an account of all this work at an international conference. In the discussion after my paper, a member of the audience said that it was difficult to see how antibodies could affect cartilage *in vivo*, because the matrix was impermeable to such large molecules as IgG. I did not know this at the time but Maroudas (1970) and others have shown that substances with a molecular mass above that of haemaglobin (68000) are excluded by cartilage matrix; on these grounds antibodies which have a molecular mass of not less than 140000 would certainly be unable to enter cartilage. I was very puzzled, because the immunohistochemical findings that I have just mentioned showed clearly that as the effect of AS + C′ progressed, IgG molecules penetrated the matrix and reacted with the cells.

The effect of complement-sufficient antiserum on post-foetal (pig) articular cartilage

The weak point of our early work was that it was done entirely on embryonic tissues, and the composition of embryonic cartilage matrix differs from that of post-foetal cartilage; for one thing it contains proportionately far less mature collagen. Thus it seemed possible that it might be more permeable to large molecules than post-foetal articular cartilage. We therefore decided to abandon embryonic material and turn our attention to post-foetal cartilage. We chose pigs' trotters as our source of tissue, because they can be easily obtained from a local slaughter-house and thanks to the courtesy of the owner, they cost nothing. Moreover, in response to infection with *Erysipelothrix rhusiopathiae* the pig develops an arthritis which histologically, closely resembles that of rheumatoid arthritis (Collins & Goldie 1940); this suggested that pig tissue might be favourable material in which to simulate arthritic changes in culture. These experiments were made in collaboration with Michael Barratt.

(*a*) *Morphological effects*

The animals were pork or bacon pigs weighing between 70 and 160 kg. Explants, usually 5–6 mm in length, were cut from the condylar ridges at the proximal ends of the 3rd and 4th metacarpals. The culture technique (Fell & Dingle 1963; Fell & Barratt 1973) was essentially the same as that used for the earlier experiments on chick rudiments. The culture medium consisted of BGJ5 which contained a higher concentration of ascorbic acid (15 mg/100 ml) than the BGJ4 used previously, to which was added a total of 15% rabbit serum. The antiserum was prepared in rabbits against pig erythrocytes, and was shown by immunofluorescence to cross-react with pig chondrocytes, osteoblasts and cells of the invading marrow (see p. 333). For most of the experiments 5% antiserum + 10% rabbit serum complement (AS + C′) was given to the experimental explants and usually heat-inactivated normal rabbit serum (NRS) to the controls, though other control media were also tested. The cartilage was placed on the grid in the culture dish with the articular surface upwards.

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Our first experiment was a great disappointment. We cultivated cartilage sliced from the top of the condylar ridges, in AS + C′ or (controls) NRS for 10 days. When we examined the explants histologically, we could see no difference between the treated explants and their controls. This looked very much as if there was indeed a difference either in permeability or reactivity between the embryonic and post-embryonic cartilage. Nevertheless we persevered, and to our surprise we began to get some very variable results; in some explants there was obvious depletion and even breakdown of cartilage matrix in response to AS + C′, in others there was no obvious effect. Histological study of the cartilage, however, soon gave us the explanation of these anomalies; whether we obtained an effect or not, depended on how and where we had excised the explant from the metacarpals (figure 9) at the beginning of the experiment. If we had cut deeply enough to include some of the invading marrow (to be termed 'invasion zone'), metachromasia partially or wholly disappeared from the matrix, indicating a loss of proteoglycan. Alternatively, if we had cut above the level of the marrow but had included some of the very fibrous, weakly metachromatic transitional cartilage that links the articular cartilage with the periosteum and periosteal bone, we found necrosis in and immediately below the fibrous region and usually some depletion of the matrix in adjacent viable cartilage.

So we proceeded to study in detail (Fell & Barratt 1973) the response to AS + C′ of these three types of explant: (1) pure cartilage sliced from the top of the middle condylar ridge, and above the level of the invasion zone; (2) cartilage from the same region but cut at a deeper level so as to include part of the invasion zone; (3) cartilage without the invasion zone but incorporating some of the more laterally placed, very fibrous transitional cartilage. To obtain a valid comparison between experimental and control cartilage in culture, it was important to prepare pairs of comparable explants (sister explants); the members of each pair were taken from the same pig, from the same articular region, and were carefully matched for size and, in series (2), for the amount of invasion zone present. Small explants were avoided, as they do not thrive in culture and are apt to give spurious results.

(1) Pure cartilage devoid of soft connective tissue or transitional cartilage. The results of our first experiment were confirmed. After 10 days' treatment with AS + C′ the explants were indistinguishable from their controls in NRS (Fell & Barratt 1973); even after 16 days (cf. figures 10 and 11) the only obvious difference was that new cartilage was often formed beneath the original explant in the controls but never in the presence of AS + C′ (Dingle, Horsfield, Fell & Barratt 1975).

(2) Cartilage + invasion zone. After exposure to AS + C′ these explants gave a very different histological picture from that of the previous series (Fell & Barratt 1973). By the tenth or fourteenth day the matrix in large areas and sometimes throughout the non-calcified cartilage had ceased to stain metachromatically with toluidine blue (cf. figures 12 and 13), indicating depletion of proteoglycan, but most of the cartilage still stained normally with van Gieson's stain, implying that the collagen was largely intact (Dingle *et al.* 1975). It was interesting that the region that is metabolically the most active *in vivo*, namely, that immediately above the invasion zone where normally proliferation and early maturation of the cartilage cells take place, was the first to break down when the cartilage was exposed to AS + C′. Staining reactions indicated that not only proteoglycan but collagen also had been degraded. Curious changes had also taken place in the chondrocytes; this was particularly clear in explants from the older pigs (Fell, Barratt, Poole, Welland & Green, unpublished). These cells had become

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irregular in shape, were often dividing actively and the cytoplasm had become very basophilic; they had come to resemble fibroblasts rather than chondrocytes (figure 23), and were very similar to the transformed hypertrophic chondrocytes seen in the embryonic chick limb-bones treated with AS + C′ (figures 7 and 8). These cellular and intercellular effects spread to the overlying articular cartilage and after 20 days the matrix had disappeared in all or most of the explant, leaving a mass of apparently healthy, often actively proliferating cells on top of the calcified invasion zone. In the absence of complement, AS had no detectable effect, but serum complement alone caused appreciable depletion and resorption of the cartilage, though much less than when antiserum was present. This effect of the complement was not diminished by absorbing the serum with pig erythrocytes before use, but this does not necessarily exclude the possibility that antibodies reactive with pig cells were present in the rabbit serum complement. A narrow band of depletion often appeared just above the invasion zone of the controls in NRS.

(3) Articular cartilage associated with transitional cartilage. When part of the laterally placed intermediate cartilage (figure 9), which links the articular cartilage with the periosteum and periosteal bone, was incorporated in the explant, the cells of the very fibrous weakly metachromatic cartilage were killed (cf. figures 14 and 15); the cells next to this region survived and sometimes appeared to initiate a general breakdown of the surrounding matrix.

(4) Explants of cartilage without the invasion zone associated with synovial tissue. We found that breakdown could be induced in pure articular cartilage devoid of either soft connective tissue or intermediate cartilage, by combining the cartilage with a piece of synovium and capsular tissue. Even in NRS there was loss of metachromasia from the matrix near or in contact with the synovial explant (figure 17); this effect was usually exaggerated by cultivation in AS + C′ (figure 16) and in this medium sometimes involved the breakdown of collagen as well as proteoglycan.

(*b*) *Biochemical changes*

Recently Peter Horsfield, in John Dingle's department at the Strangeways Laboratory, investigated the breakdown of proteoglycan and collagen, the two main polymers of cartilage matrix, in explants without and with the invasion zone (Dingle *et al.* 1975). In the absence of the invading marrow, AS + C′ had no effect on the release into the medium of either proteoglycan on hydroxyproline, a breakdown product of collagen. When the invasion zone was present, however, analysis showed that during the first 10 days the explant lost 80 % of its proteoglycan, more than double that shed by the controls; there was a time-lag of 8–10 days, however, before collagen began to disintegrate as indicated by the release of hydroxyproline into the medium.

It is thought that the breakdown of the cartilage matrix was effected by proteases, including both lysosomal cathepsins and collagenase, released from the adjacent connective tissue. Dingle *et al.* (1975) suggested that the proteoglycan may protect the collagen from enzymatic digestion, and that consequently collagenase cannot act until the proteoglycan has been removed. This hypothesis receives some support from recent work by Lotke & Granda (1972). These authors subjected human cartilage to various types of enzymatic digestion and then investigated its permeability to compounds of different molecular size. They found that cathepsin alone increased permeability to γ globulin (molecular mass: 160000); this effect was no greater when the cartilage was treated first with collagenase and then with cathepsin,

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but if the order was reversed, and the cartilage exposed first to cathepsin and then to collagenase, the permeability was the highest recorded in their experiments.

(*c*) *Immunohistochemical observations*

Before we could attempt to interpret the observations described above, it was necessary first to determine the capacity of the cells and intercellular material of the explants before and after cultivation, to bind rabbit IgG antibodies; secondly we had to discover whether during cultivation the explants took up antibody immunoglobulin (IgG) from the AS + C′ in the culture medium, and if so where it was localized in the tissue. Dr. A. R. Poole of the Strangeways Laboratory joined forces with us and investigated this question by immunohistochemistry (Poole *et al.* 1973; Fell *et al.* unpublished).

To investigate the capacity of the explants to bind IgG antibodies to pig erythrocytes (Fell *et al.*, unpublished), frozen sections of cartilage + invasion zone were cut and fixed briefly in formaldehyde in phosphate-buffered saline; they were then treated with either AS or (controls) NRS, washed and stained with the fluorescein-labelled IgG fraction of (pig) antiserum against rabbit IgG. The reaction of antibody IgG was indicated by a green fluorescence when the specimen was examined by fluorescence dark-ground microscopy. In sections of normal cartilage + invasion zone examined *ex vivo*, the chondrocytes, osteocytes and invading marrow reacted very feebly with NRS but intensely with AS; the cartilage matrix was unstained. Sections of explants cultivated in NRS for 10 days presented a similar picture to that of the normal cartilage. After 10 days in AS + C′, the chondrocytes also reacted intensely for bound antibody IgG with the exception of those that had undergone the fibroblastic transformation described above; these cells showed very little or no green fluorescence. There was no detectable reaction of antibody with the matrix.

Other experiments were made to study the distribution of rabbit IgG antibodies derived from the medium, in explants cultivated in the presence of AS + C′. Frozen sections prepared as before, were stained with the fluorescein-labelled IgG fraction of (pig) antiserum against rabbit IgG and examined by fluorescence dark-ground microscopy. After 10 days' cultivation in AS + C′, no rabbit IgG could be demonstrated in pure articular cartilage devoid of soft connective tissue, except in a very thin layer beneath the articular and cut surfaces (figure 18) (Poole *et al.* 1973); thus the intact matrix had completely protected all but a few superficial chondrocytes from contact with antibody IgG. On the other hand, in explants with the invasion zone, an intense reaction appeared in the invading marrow and also on the surfaces of the cells and in the matrix wherever the latter had been depleted of proteoglycan (figures 19 and 20); some of the more superficial cells which had been killed by AS + C′ stained brilliantly throughout. There was one interesting exception, however (Fell *et al.*, unpublished); the fibroblast-like chondrocytes in the severely affected region above the invasion zone gave very little fluorescence, although the disintegrating matrix around them reacted moderately (figure 21). This loss of reactivity with rabbit antiserum to pig erythrocytes during culture may explain the surprising fact that these transformed cells, though no longer protected by normal matrix, appeared healthy and often multiplied quite actively. This is a very new observation (Fell *et al.*, unpublished) and cannot be safely interpreted until the phenomenon has been investigated further.

Fibrous transitional cartilage was readily penetrated by IgG and the dead cells mentioned above fluoresced brilliantly (Poole *et al.* 1973); there was a distinct but less intense reaction in the intercellular material and also in both cells and matrix in neighbouring depleted but

viable cartilage. In the affronted explants, the cells of the synovial and capsular tissue fluoresced strongly, and the chondrocytes and matrix of the depleted part of the cartilage explant also reacted.

In all the above systems, controls were grown in NRS. A weak reaction was seen in the cavities of the invasion zone in explants + invading marrow, in the fibrous intermediate cartilage and in the depleted region of the affronted cartilage.

4. Recovery

The changes induced by AS + C′ in cartilage + invasion zone were to some extent reversible if the explant was transferred to NRS for a further period of cultivation (Fell *et al.*, unpublished) (cf. figures 22 and 24). The tissue's capacity for regeneration largely depended on the extent to which the matrix had been broken down in AS + C′. As described above, the region that reacted most rapidly and in which the matrix suffered the most severe degradation, was the original zone of proliferation and maturation of chondrocytes situated immediately above the level of the invading marrow (figure 23). Rather surprisingly this was also the region in which regeneration (figure 25) proceeded most actively. After only 2 days in NRS the fibroblast-like chondrocytes, though still irregular in shape, had begun to secrete metachromatic material; by the fourth day the transformed cells had not only resumed their normal form but, as shown by immunohistochemical examination after 10 days in NRS, they had also regained their

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Description of plate 8

Figure 20. Pig articular cartilage with invasion zone, after 10 days in AS + C′; section stained and photographed as for figure 18. Nests of chondrocytes with strongly fluorescent surfaces are seen, indicating that the cells have reacted with the antibody. (Photograph by A. R. Poole.)

Figure 21. Same preparation as that shown in figure 20, but photographed at a deeper level just above the invasion zone. Same magnification as figure 20. The cells are undergoing fibroblastic transformation (cf. figure 23) and as indicated by their very weak fluorescence, have largely lost their capacity to react with the IgG antibodies of the medium. (Photograph by A. R. Poole.)

Figure 22. Pig articular cartilage with the invasion zone, after 10 days in AS + C′. The non calcified cartilage is severely depleted as indicated by loss of metachromasia, and the cells immediately above the invasion zone are beginning to undergo fibroblastic transformation; f.t.: region where transformation has taken place. (Toluidine blue.)

Figure 23. Another section of the explant in figure 22, showing the fibroblastic transformation of the chondrocytes. The matrix in this region has largely disintegrated. (Celestine blue, Carrazi's haematoxylin and van Gieson's stain.)

Figure 24. Sister explant to that shown in figures 22, 23, after 10 days in AS + C′ followed by a recovery period of 10 days in NRS. Same magnification as figure 22. During the recovery period, there has been extensive regeneration of metachromatic material and the formation of new cartilage (n.c.). (Toluidine blue.)

Figure 25. Another section of the explant in figure 24; same magnification as figure 23. The fibroblastic-like chondrocytes just above the invasion zone (cf. figure 23) have resumed their normal appearance and formed new matrix. (Celestine blue, Carrazi's haematoxylin and van Gieson's stain.)

Figure 26. Pig articular cartilage with invasion zone, cultivated in AS + C′ for 14 days. The original non calcified cartilage is greatly reduced in amount and the matrix severely degraded. (Toluidine blue.)

Figure 27. Sister explant to that in figure 26, after 14 days in AS + C′ followed by a 10-day recovery period in NRS. Same magnification as figure 26. The original non calcified cartilage has disappeared completely but new cartilage (n.c.) has been formed in the cavities of the invasion zone. (Toluidine blue.)

Figure 28. Same section as in figure 27, showing the new cartilage (n.c.) in the cavities of the invasion zone. Colourless regions: dead bone (d.b.).

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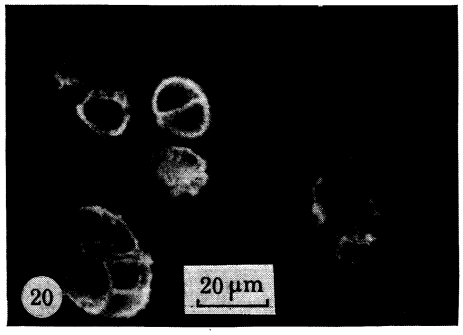

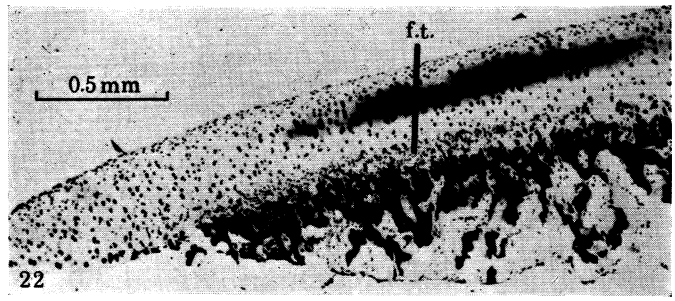

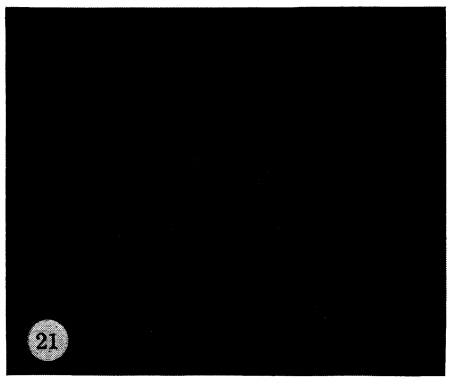

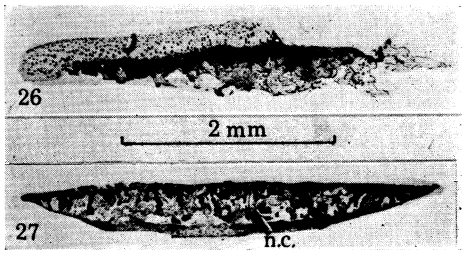

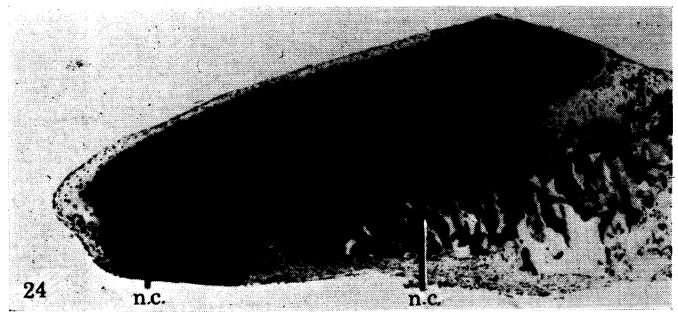

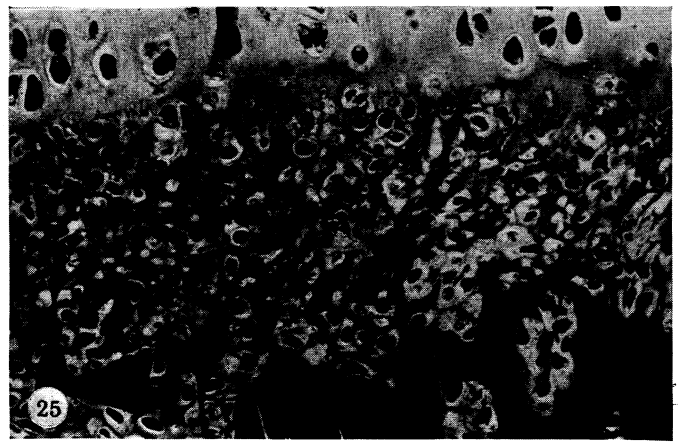

FIGURES 20–28. For description see opposite.

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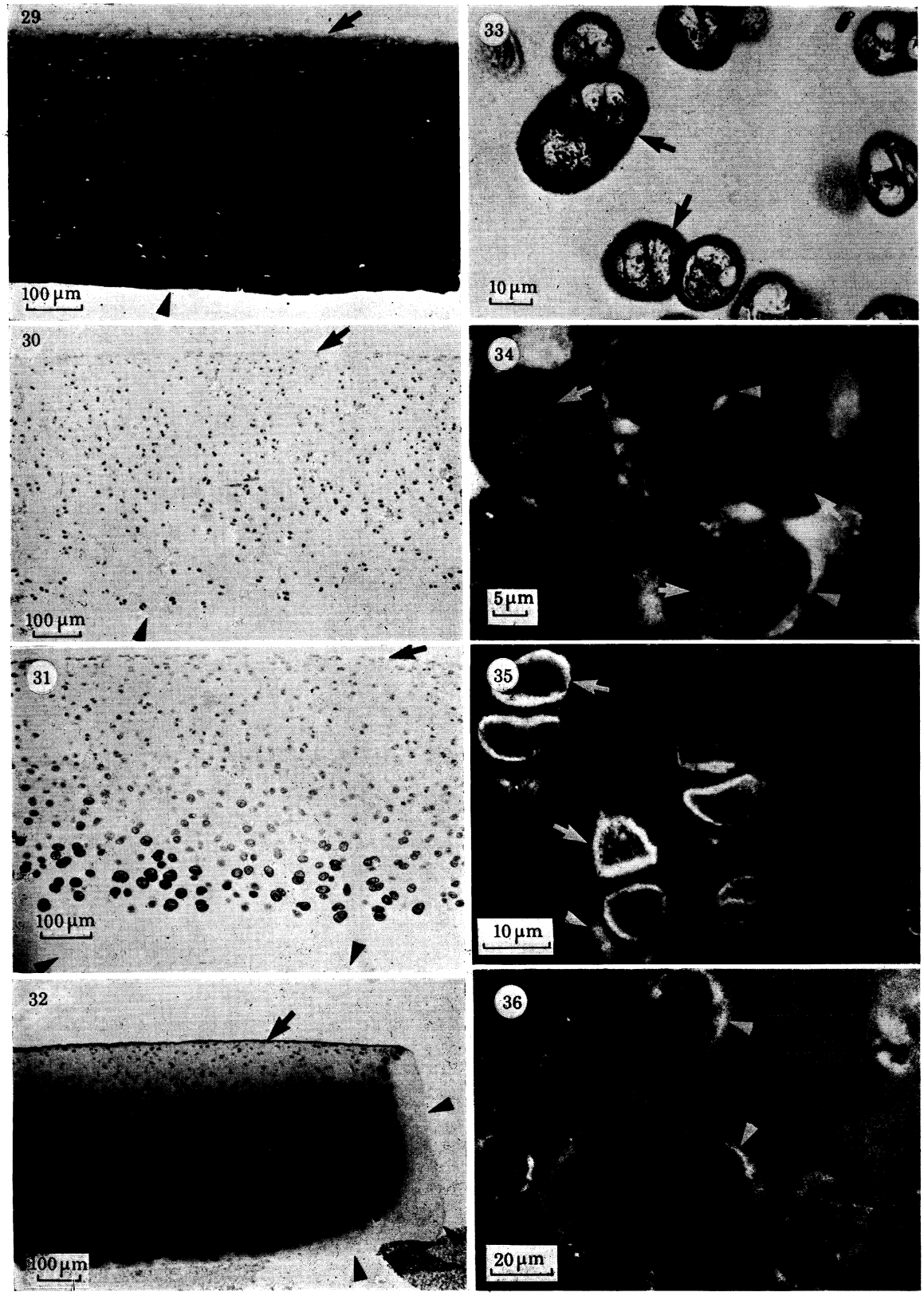

FIGURES 29–36. For description see opposite.

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capacity to fix IgG antibodies reactive with chondrocytes. In the less affected cartilage above this region, where the collagen was still largely intact although the proteoglycan was greatly depleted, the chondrocytes formed first pericellular, metachromatic capsules and then in some explants, fresh intercapsular material (figure 24). Many of the cavities in the invasion zone became filled with either new cartilage or less differentiated, non metachromatic chondroid tissue. On the other hand if the degradation of collagen had spread to the overlying articular cartilage (figure 26), the explant continued to break down after transfer to NRS and new cartilage was not regenerated above the calcified invasion zone (figure 27); nevertheless both cartilage and chondroid tissue (figure 28) were sometimes formed in the cavities of this zone and may have been partly derived from chondrocytes immigrating from the disintegrating cartilage overhead.

The role of the chondrocytes in the breakdown of pig articular cartilage in antiserum and complement

(*a*) *The effect of trypsinization on the subsequent response of articular cartilage to AS+C′*

We wondered whether the chondrocytes in matrix depleted of proteoglycan, once they had reacted with complement-sufficient antibodies, would continue to degrade their intercellular material. Sheila Millroy in our department investigated this problem in collaboration with A. R. Poole of the Strangeways Laboratory (Millroy & Poole 1975).

Fragments of pure cartilage devoid of soft connective tissue were incubated in a solution

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Description of plate 9

Note. All the figures in this plate are from the work of Millroy & Poole (1975).

Figure 29. Section of freshly isolated normal cartilage without the invasion zone; arrow indicates articular surface. (Toluidine blue.)

Figure 30. Similar fragment to that in figure 29, after 1 h incubation in trypsin. The metachromatic material has been completely removed. Arrow: articular surface; arrowhead: cut surface. (Toluidine blue.)

Figure 31. Explant of trypsinized cartilage after 2 days in AS+C′. The lower part of the explant is necrotic and almost invisible; arrowheads indicate lower (cut) surface; arrow: articular surface. Elsewhere the chondrocytes have secreted metachromatic pericellular capsules. (Toluidine blue.)

Figure 32. Trypsinized cartilage after 10 days in AS+C′. Except in the necrotic region (surface indicated by arrowheads) the matrix is largely restored. Arrow: articular surface. (Toluidine blue.)

Figure 33. Trypsinized cartilage after 4 days in AS+C′, showing the metachromatic capsules around the chondrocytes. (Toluidine blue.)

Figure 34. Section of trypsinized explant after 4 days in AS+C′, stained by the same immunofluorescent method as the preparation shown in figure 18. There is intense diffuse and particulate fluorescence (arrows) at the surfaces of the chondrocytes where the cells had reacted with the antibodies at the beginning of the experiment. The pericellular capsules are unstained, but there is intense fluorescence at their outer margins (arrowheads), indicating that the metachromatic capsules were protecting the cells from further exposure to AS+C′.

Figure 35. Another field in the same preparation as that in figure 34, here the pericellular capsules are poorly developed, and the antibody has reacted intensely with the surfaces of the cells (arrows). Reaction outside a developing capsule: arrowhead.

Figure 36. Trypsinized explant cultivated in NRS for 2 days, and then in AS+C′ for a further 2 days. Section stained as for figure 18. The pericellular capsules formed during the 2 days in NRS have almost completely excluded the antibody from the enclosed chondrocytes, but intense reactivity (arrowheads) is seen outside the capsules where the antibody has accumulated.

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of 0.25 % trypsin in BGJ5 for 1 h, thoroughly washed and explanted in BGJ5 containing 10 % AS + 10% C′ (the effect of lower concentrations of AS was also investigated). Histological examination of the trypsinized explants showed that the metachromatic material had been completely removed (cf. figures 29 and 30) except in a small central area in some fragments. The effect of maintaining the depleted explants in AS + C′ was different from what we had expected. When the explants were examined after 2 days' cultivation in 10 % AS + 10 % C′, a broad peripheral zone of necrosis was seen, but elsewhere the cells were not only viable but also had secreted pericellular metachromatic capsules (figures 31 and 33) similar to those in trypsinized explants cultivated in NRS. After a few more days intercapsular material also had appeared, and the explants cultured in AS + C′ were only distinguishable from their controls in NRS by the necrotic peripheral zone which never recovered its metachromasia (figure 32).

Millroy & Poole then investigated the distribution of rabbit IgG by the immunohistochemical method mentioned above. They found that within 2 days antibody IgG had spread throughout the explant and reacted with all the chondrocytes including those in the interior (figure 35); that these inner cells had received a sub-lethal dose was probably due to a diminished penetration of IgG from the periphery to the centre of the cartilage. The newly formed metachromatic capsules became impermeable to IgG; this was demonstrated by their failure to react with fluorescein-labelled antiserum to rabbit IgG, in contrast to the surrounding depleted matrix (cf. figures 33 and 34). The permeability of the pericellular capsules was tested further by an experiment in which the trypsinized explants were first grown for 2 days in control medium, by which time all the chondrocytes were encapsulated, and then transferred to AS + C′ for a further 2 days. IgG entered the intercapsular matrix but its advance on the cells was arrested at the outer margins of the capsules so that the chondrocytes remained unstained (figure 36). It was clear from these results, that provided the chondrocytes were not killed on first contact with AS + C′, they rapidly shielded themselves from further attack by secreting a pericellular capsule rich in proteoglycan and impenetrable to IgG.

Thus reaction with a sub-lethal dose of AS + C′ was not in itself sufficient to induce the chondrocytes to continue the breakdown of their matrix or to prevent them from rapidly regenerating proteoglycan. It was most unlikely that trypsinization caused any appreciable degradation of collagen (cf. Harris, Di Bona & Krane 1970), but collagen by itself appears to have no protective action. It will be seen that the chondrocytes of the trypsinized explants were only exposed to AS + C′ for a very brief period because, as stated above, once they had formed their metachromatic pericellular capsules they were safe from further exposure to AS + C′.

(*b*) *The effect of AS + C′ on cartilage depleted by contact with capsular tissue, and then cultivated in isolation*

Depletion of the matrix by a single treatment with trypsin followed by cultivation in medium containing AS + C′ (10 % AS + 10 % C′), as described in the preceding section, created a very different situation from that in which proteoglycan was degraded by the slow, continuous action of soft connective tissue in explants cultivated in the presence of AS + C′ (5 % AS + 10 % C′). Under the latter conditions the condrocytes (with a few scattered exceptions) were prevented from forming protective metachromatic capsules like those so rapidly secreted after trypsinization, and thus were exposed to AS + C′ for long periods (10–20 days). Michael Barratt (1975) undertook experiments to find whether cartilage depleted in this gradual way

would continue to break down in AS + C′ after removal of the soft connective tissue. To isolate cartilage from its contact with soft connective tissue was technically possible in cultures of affronted explants of pure cartilage and synovium (see p. 332).

Barratt prepared pairs of carefully matched cartilage fragments removed from the middle condyles of the metacarpals above the level of the invading marrow. Equivalent pairs of synovial explants, consisting of villi and a small piece of fibrous capsule, were also obtained. The cartilage was laid articular surface upwards on a piece of Millipore filter (8 μm pore size) resting on the grid in the culture dish. The synovial explant was placed on top of the cartilage, and affronted explants were thus made. The pairs of affronted explants were cultivated in AS + C′ for a 'primary' period of 10 days; one of each pair was then fixed for histological examination; from the other the synovial tissue was removed and the isolated cartilage was cultivated for a 'secondary' 10-day culture period either again in AS + C′ or in NRS.

The results depended on the degree of breakdown that the cartilage had undergone by the end of the primary culture period. If only proteoglycan but not collagen had been seriously depleted, breakdown did not progress during a secondary culture period in AS + C′, and sometimes there was even a slight regeneration of metachromatic material usually in the form of pericellular capsules around some of the chondrocytes. The regeneration of matrix, however, was much less than in explants transferred to NRS for their secondary culture period, showing that AS + C′ had partially inhibited the restoration of intercellular material, probably by inhibition of synthesis. When not only had proteoglycan been lost during the primary culture period, but collagen also had begun to disintegrate, as indicated by the weak coloration of the matrix with van Gieson's stain, the chondrocytes assumed the fibroblastic form described above and immunohistochemical examination showed that their surfaces had almost lost their capacity to react with rabbit IgG; having reached this stage of breakdown, the isolated cartilage continued to disintegrate whether the secondary culture period was passed in AS + C′ or NRS.

In the light of these observations, it seems probable that until the breakdown of the matrix reached its final stage when the degradation of collagen began, the chondrocytes played a negligible part in the process. That the chondrocytes synthesize an enzyme, probably lysosomal, that is capable of digesting proteoglycan, was shown by experiments on the effects of vitamin A (retinol) on pure articular cartilage (pig) in organ culture (Dingle *et al.* 1975). In medium containing added retinol, about 80 % of the proteoglycan was lost from the matrix during 14 days' cultivation, but in the absence of soft connective tissue (invading marrow) the degradation of collagen was negligible; this result implied that the chondrocytes produce little or no collagenase. Contrary to what was originally thought (Barratt 1973), this later result, which was obtained on material from older animals, indicates that normal cartilage matrix was permeable to retinol which could thus affect the chondrocytes directly, probably by activation of their lysosomal system. Our immunohistochemical findings have demonstrated that, unlike retinol, IgG could not enter cartilage matrix until its proteoglycan had been depleted under the influence of soft connective tissue. Thus most of the proteoglycan had already gone by the time IgG reached the chondrocytes; since these cells seemed to have little if any collagenolytic activity, they were not equipped to continue the degradation of the matrix, which now consisted almost entirely of collagen, during the secondary culture period in AS + C′.

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(c) *The significance of the fibroblastic transformation of chondrocytes*

The puzzling feature of these experiments and also of those on the capacity of cartilage + invasion zone to recover when transferred from AS + C′ to NRS, is the behaviour of the chondrocytes during the final stage of breakdown when degradation of the collagen, begun under the influence of the soft connective tissue (invading marrow or affronted synovial explant), had spread throughout most of the non calcified cartilage. There are two major questions, to neither of which do we have an answer. First, how did the chondrocytes after degradation of the collagen had begun, contrive to side-step the toxic action of AS + C′ by undergoing a fibroblastic transformation and losing their reactivity with rabbit IgG antibodies? Secondly, why did the breakdown of the matrix then continue even in NRS until virtually no intercellular material remained? Perhaps the collagen, once it was partially degraded by the exogenous enzymes of the soft connective tissue, was rendered susceptible to the chondrocytes' proteases, but it is idle to speculate until more information about these phenomena is available. It should be pointed out that the fibroblastic transformation of chondrocytes is not confined to explants exposed to AS + C′ but also appears in cartilage (with invasion zone) breaking down under the influence of retinol (Barratt 1973) and even in the small depleted areas sometimes present in the controls in NRS (Fell & Barratt 1973). Experiments will be made to find whether the fibroblast-like chondrocytes in the retinol-treated explants also fail to react with the IgG antibodies of AS, or whether this loss of antigenicity occurs only in the presence of AS + C′.

Interpretation of results

(a) *The sequence of events in embryonic and post-foetal cartilage during exposure to AS + C′*

The embryonic chick cartilage and the articular cartilage of the young pigs appeared to respond to reactive, complement-sufficient antisera in essentially the same way. On the other hand the importance of soft connective tissue in the breakdown of the matrix was far more easily determined in the pig cartilage which could be cultivated either with or without association with this tissue, than in the chick rudiments which were always enclosed in an envelope of soft connective tissue. Thus it was shown that the intact matrix of isolated pig cartilage was virtually impervious to antibodies even after 10 days' exposure to AS + C′, but that once the matrix had lost its proteoglycan through the agency of adjacent soft connective tissue, it no longer excluded antibodies.

It is suggested that the sequence of events in both the embryonic and post-foetal cartilage during cultivation in the presence of reactive complement-sufficient antibodies is as follows, but it should be emphasized that other factors, at present unknown, may well be involved. Initially AS + C′ reacts with the cells of the soft connective tissue associated with the cartilage, kills those with which it comes in immediate contact and stimulates the rest to secrete proteases, including lysosomal cathepsins and collagenase(s). This enzymatic activity depletes the cartilage matrix of proteoglycan, thus leaving the chondrocytes defenceless against the action of AS + C′ and at the same time probably rendering the collagen susceptible to attack by collagenase (Dingle *et al.* 1975); eventually the tissue may completely disintegrate. The significance of the fibroblastic transformation of the chondrocytes during the final stages of breakdown remains obscure.

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(b) The possible relevance of the results to the pathology of rheumatoid arthritis

In the active stage of rheumatoid arthritis, inflamed synovium (pannus) begins to invade the cartilage at the margin of the articular surface and then advances centripetally (Kulka 1959; Harris *et al.* 1970). Damage to the cartilage can be seen for some distance ahead of the invading pannus. Before it is eroded the cartilage softens (Chaplin 1971), the matrix loses its metachromasia (Gardner 1972) and a band of depleted collagen fibres appears between the pannus and the normal cartilage, suggesting extracellular degradation (Harris *et al.* 1970). Both normal and rheumatoid synovium have been shown to secrete collagenase (for review, see Evanson 1971). These changes in the cartilage matrix in rheumatoid arthritis are very similar to those observed in the explants treated with AS + C′ and especially to the breakdown of pure cartilage associated with synovial tissue. In both, proteoglycan and collagen are lost from the cartilage matrix under the influence of soft connective tissue, though the explants lack the inflammatory cells that so heavily infiltrate the rheumatoid synovium and no doubt play an important part in the breakdown of the articular cartilage in this disease.

There is evidence that in chronic antigen-induced arthritis in the rabbit knee-joint, the cartilage is permeable to antibodies and complement. Thus Cooke, Hurd, Ziff & Jasin (1972) using immunofluorescent methods, demonstrated IgG antibodies, the complement component C′3 as well as the inducing antigens (bovine serum albumin or egg elbumin) in the superficial regions of the articular cartilage and meniscus. In a preliminary note, Cooke *et al.* (1972) have reported the presence of IgG, IgM and C′ in the articular cartilage and menisci of rheumatoid arthritic patients, but whether the immunoglobulins represent autoantibodies is not known. It is interesting that in rheumatoid arthritis the chondrocytes are often necrotic for some distance in front of the pannus (Kulka 1959). Although this necrosis could be due to various causes, it is tempting to wonder whether the chondrocytes, rendered defenceless by the loss of proteoglycan from their matrix, are killed by toxic materials of large molecular size, such as antibodies and complement, diffusing into the depleted cartilage from the pannus.

In recent years our knowledge of the factors concerned in the breakdown of articular cartilage in rheumatoid arthritis has advanced considerably, but we are still ignorant of what initiates the disease and of whether the production of autoantibodies is in fact responsible for its chronicity.

As suggested by Bascish & Wyburn (1947), it is probable that the proteoglycans of the matrix are also largely responsible for the well known capacity of cartilage to survive for long periods as an allograft. The importance of the matrix in this connexion has been emphasized by Heyner (1969) and more recently by Langer & Gross (1974) who conclude that immunologically, intact matrix acts 'as both an afferent and efferent block'.

It is clear from our experiments that the chondrocytes owe much to the proteoglycan of their matrix and to their own ability rapidly to regenerate this component even in the face of an adverse immunological environment; under these conditions the enemy that may destroy the cells' defences is the soft connective tissue with which normally cartilage is always associated.

The author is greatly indebted to the Medical Research Council for a personal grant and to the Nuffield Rheumatism Committee for funds to cover assistance and research expenses. In preparing this manuscript for press, she has received valuable criticism and advice from Professor R. R. A. Coombs, F.R.S., Dr J. T. Dingle and Dr A. R. Poole. She wishes to thank Mr R. Green for expert technical help and for taking most of the photographs.

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Discussion

Alice Maroudas (*Biomechanics Unit, Department of Mechanical Engineering, Imperial College of Science and Technology, London SW*7 2*AZ*). With reference to the question of the penetration of IgG into adult human articular cartilage, I would like to mention some points arising from our studies on the permeability of articular cartilage to large solutes (Maroudas 1970; Snowden & Maroudas, in preparation).

Our recent results obtained with ^{125}I labelled IgG, kindly prepared for us by Dr Alan Barrett from the Strangeways Laboratory, give partition coefficients varying from about 0.001 for a cartilage specimen with glycosaminoglycan content corresponding to a fixed charge density of 0.2 mmol/g of wet tissue, to about 0.1 for a fixed charge of 0.05 mmol/g. The same sort of results were obtained when [^{125}I]serum albumin was used. This great variation in the partition coefficient with the glycosaminoglycan content implies that there will be enormous differences in the penetration of IgG, depending on (*a*) whether one is dealing with the superficial or the deep zones of cartilage, (*b*) whether the cartilage comes from a joint like the femoral head, with a high overall fixed charge density or from joints like the knee or the ankle, with a much lower fixed charge density and (*c*) whether the cartilage is entirely intact or whether it shows fibrillation.

Our quantitative results are entirely in agreement with the observations of Dame Honor Fell.

Reference

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A. H. Rahi (*Institute of Ophthalmology, Judd St., London, W.C.* 1). Dame Honor has suggested that the formation of a mucopolysaccharide coat around cartilage cells incubated in immune sera is a protective phenomenon and the antibodies do not play a direct role in such a change. I would have thought that this phenomenon is also antibody dependent. The superficial cartilage cells being killed in the presence of high antibody concentration whereas the deeper cells showing a stimulatory effect because of antibodies present in a non-toxic dose. Receiving increasing attention today is a stimulatory effect produced by antibody (+complement), i.e. mitosis, lysosomal activation and differentiation rather than destructive lesion. The stimulatory effect of LATS (an IgG) on the thyroid is a well recognized phenomenon.

Dame Honor Fell, F.R.S. I do not think that this is an antibody dependent phenomenon, because the formation of the pericellular capsules is even more pronounced in trypsinized explants returned to control medium.

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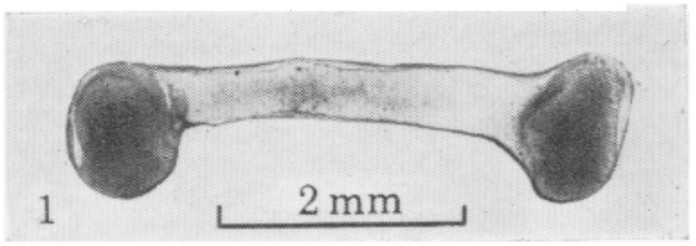

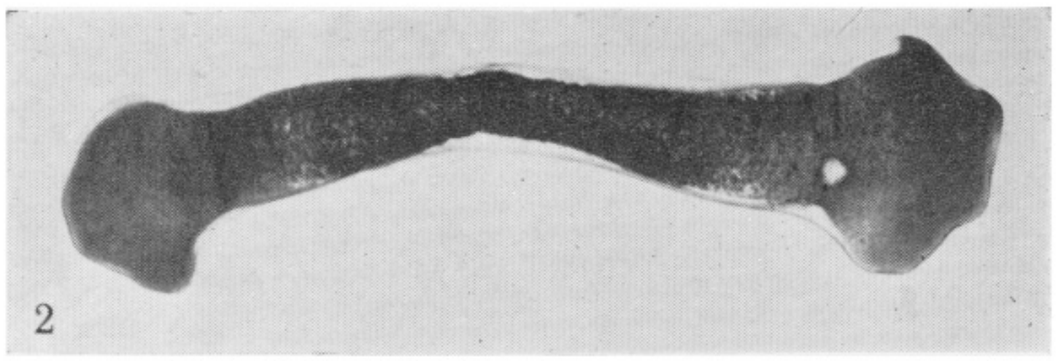

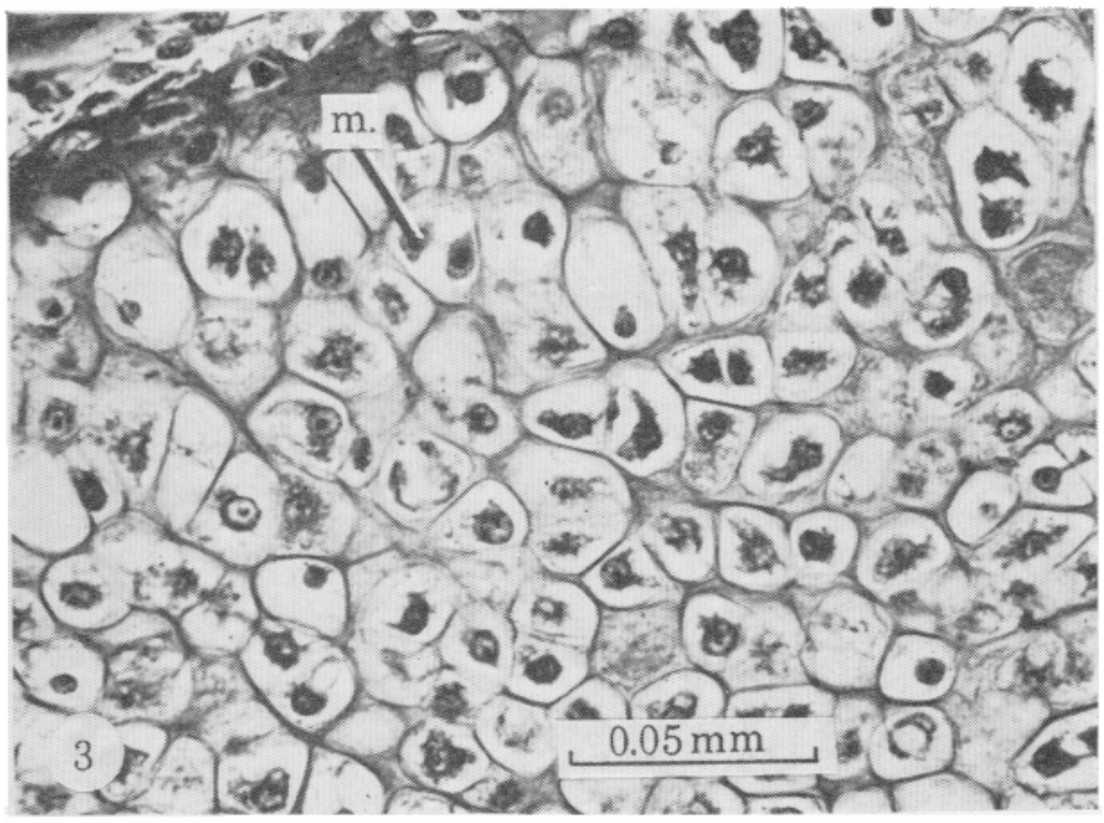

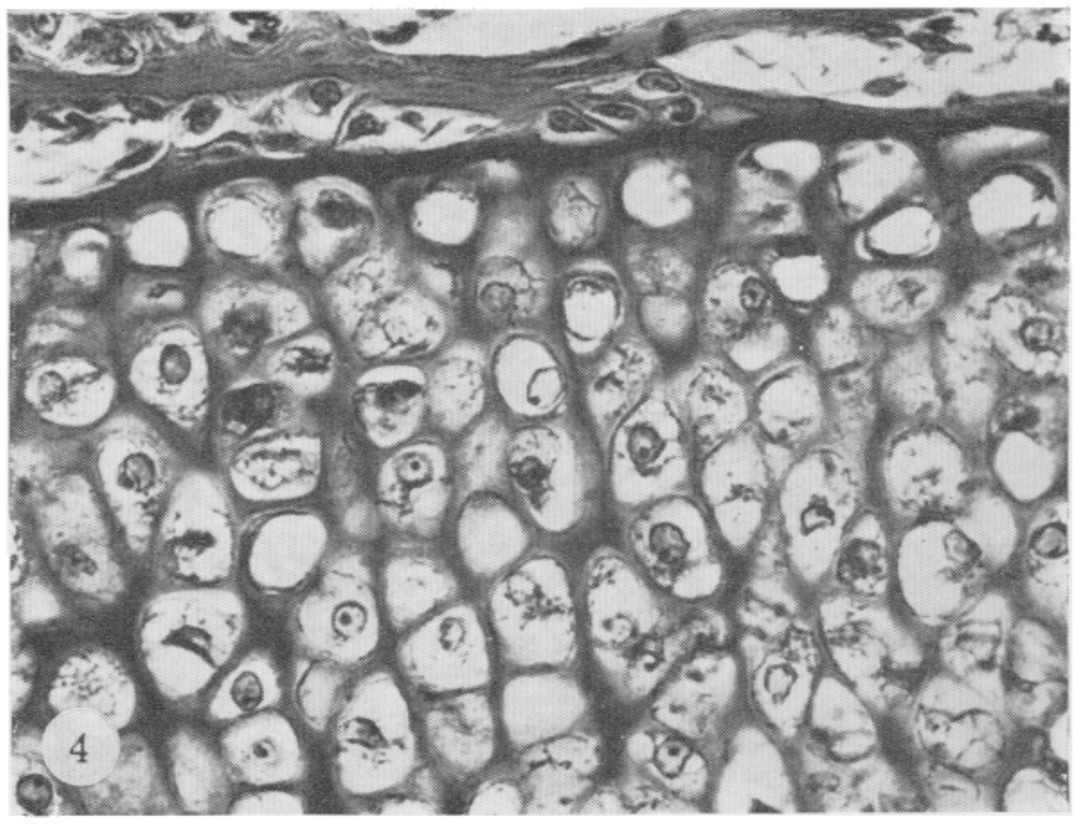

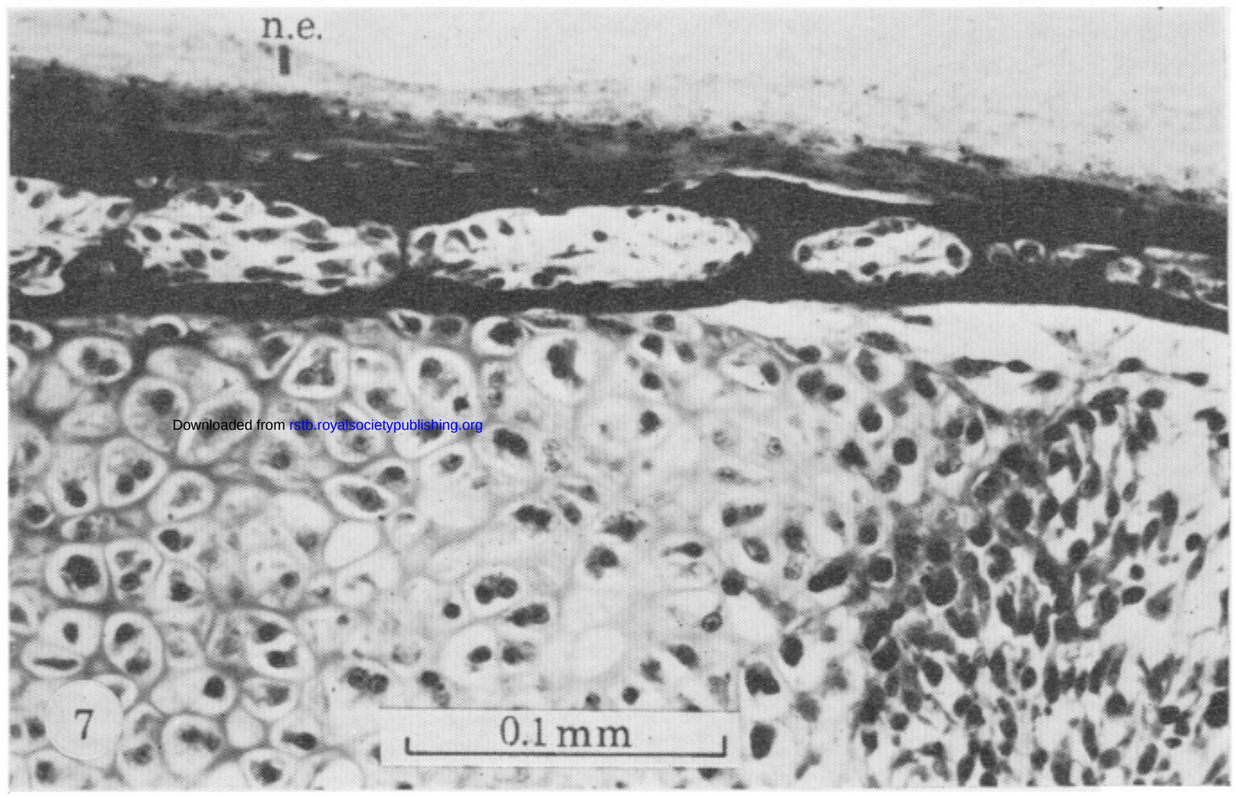

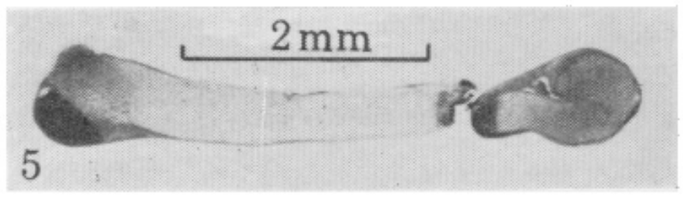

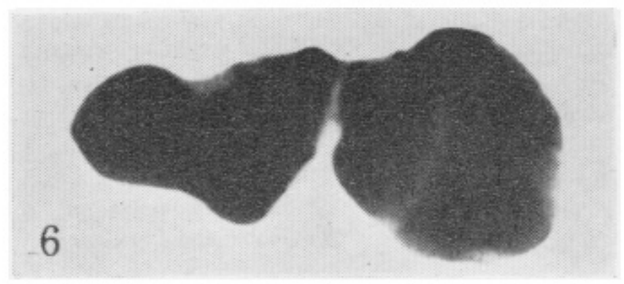

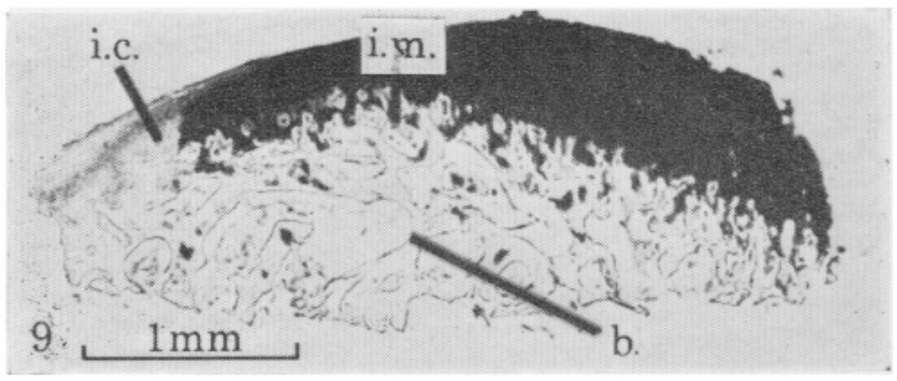

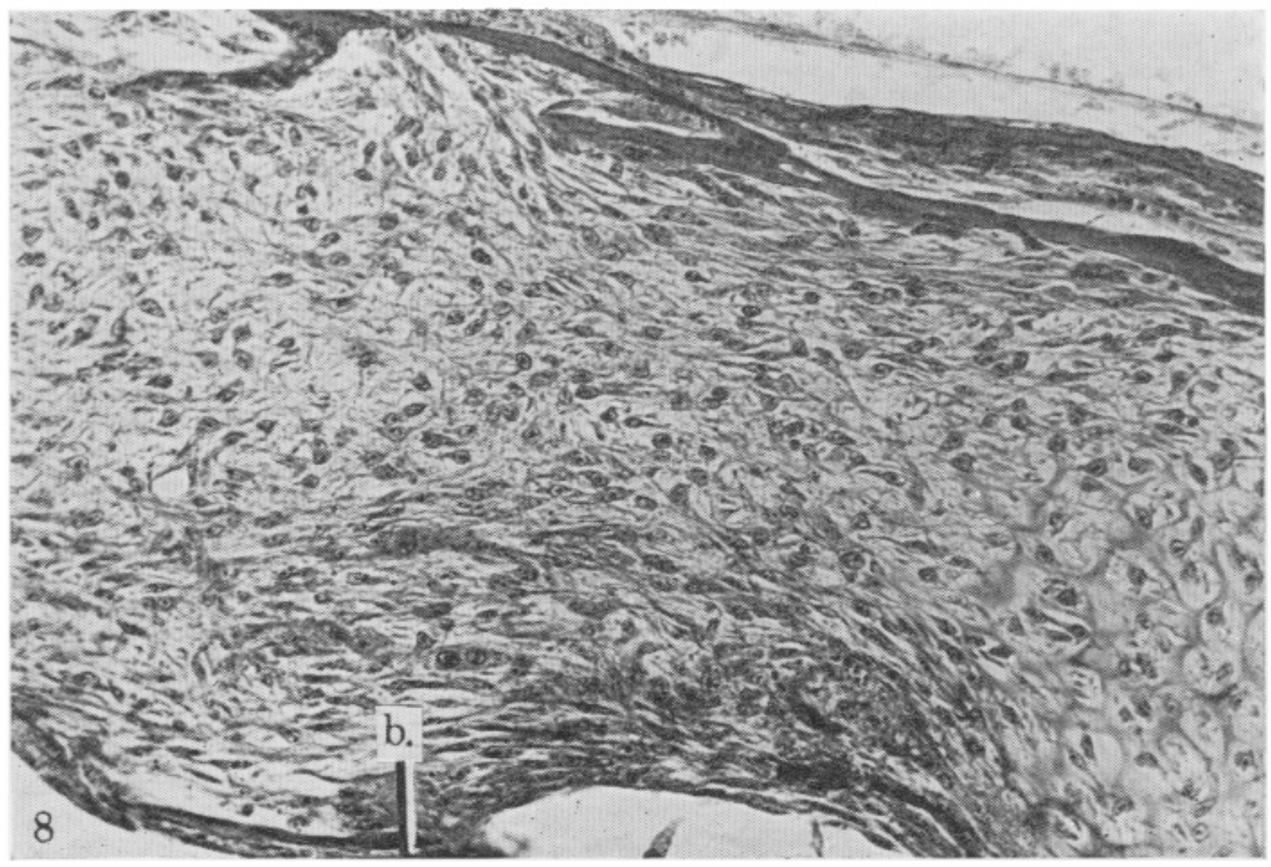

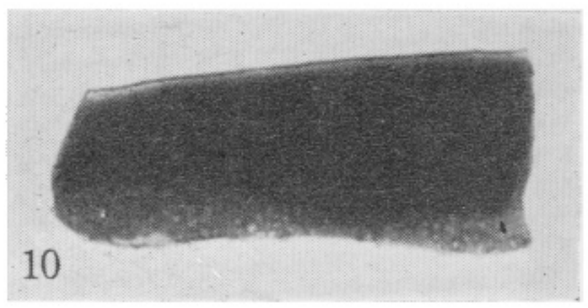

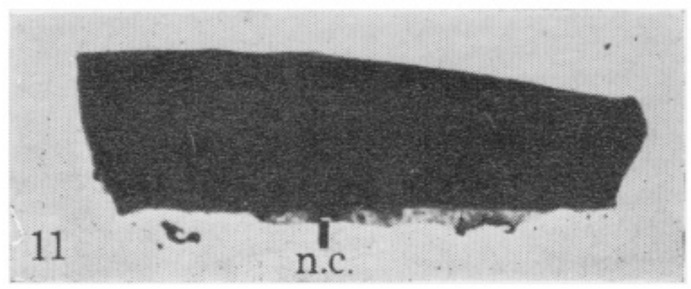

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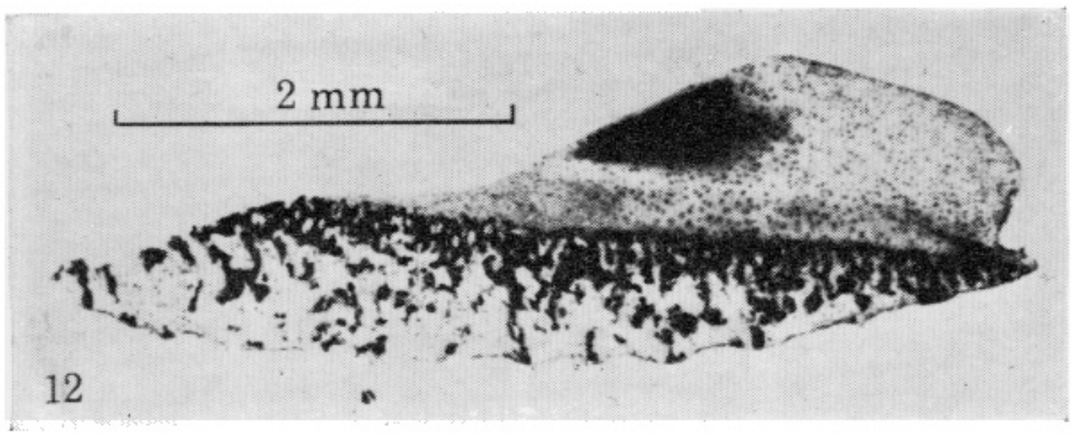
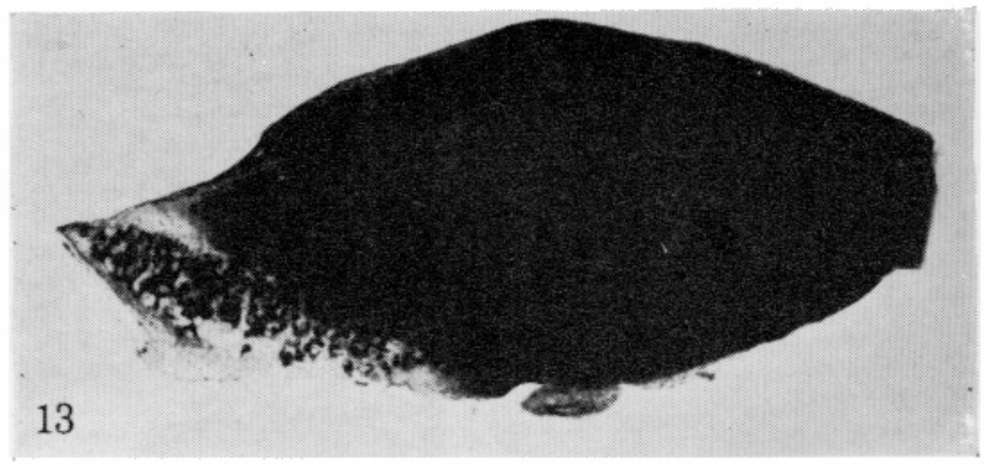
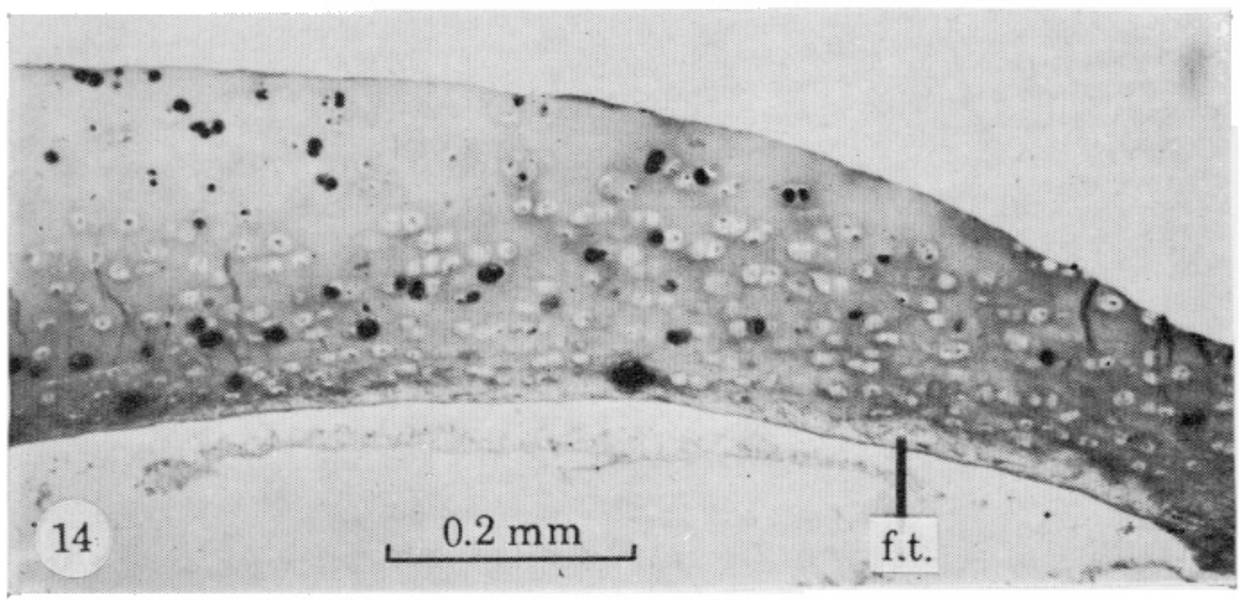
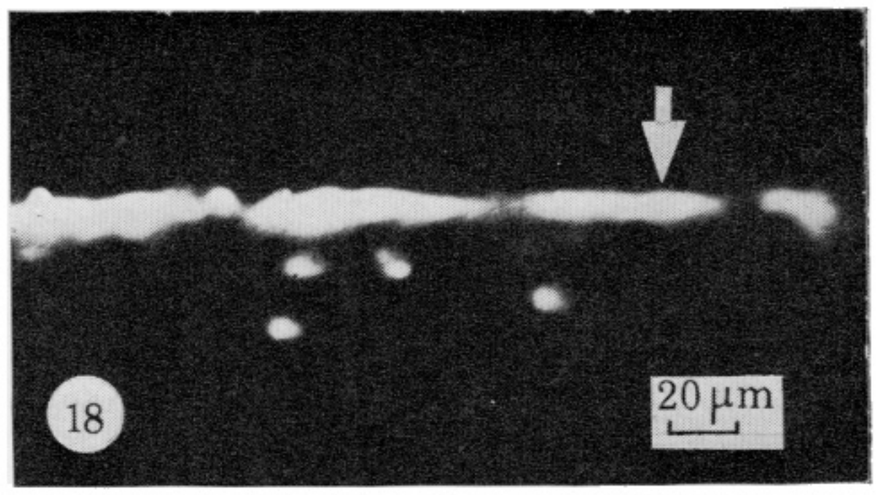
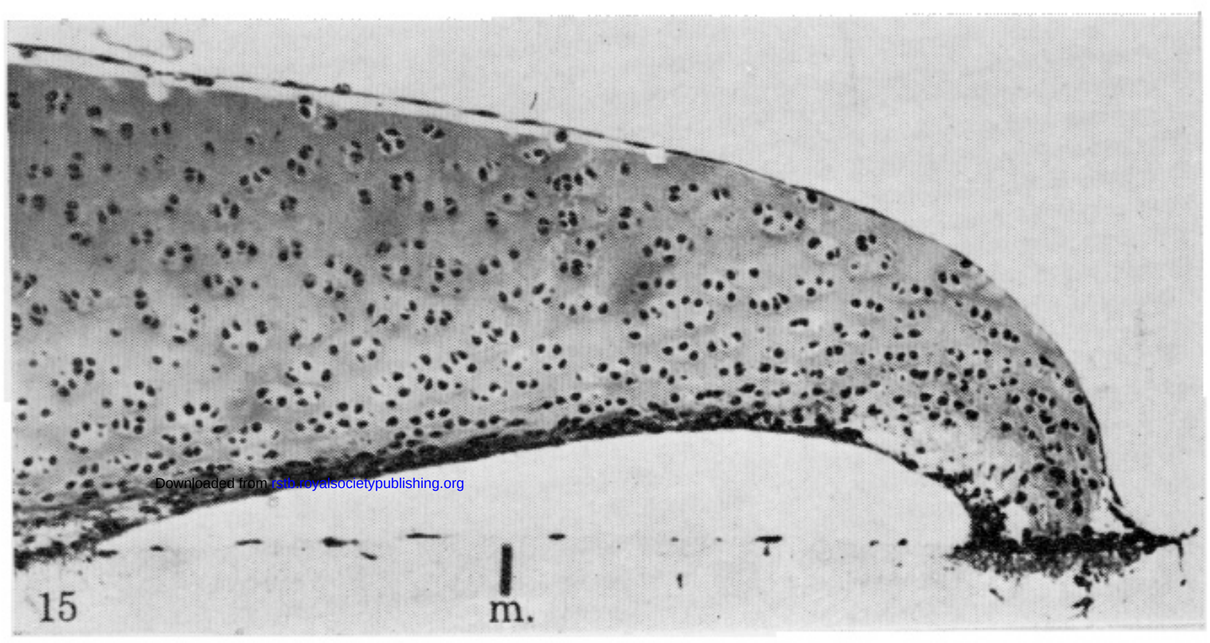
FIGURES 1–11. For description see opposite.

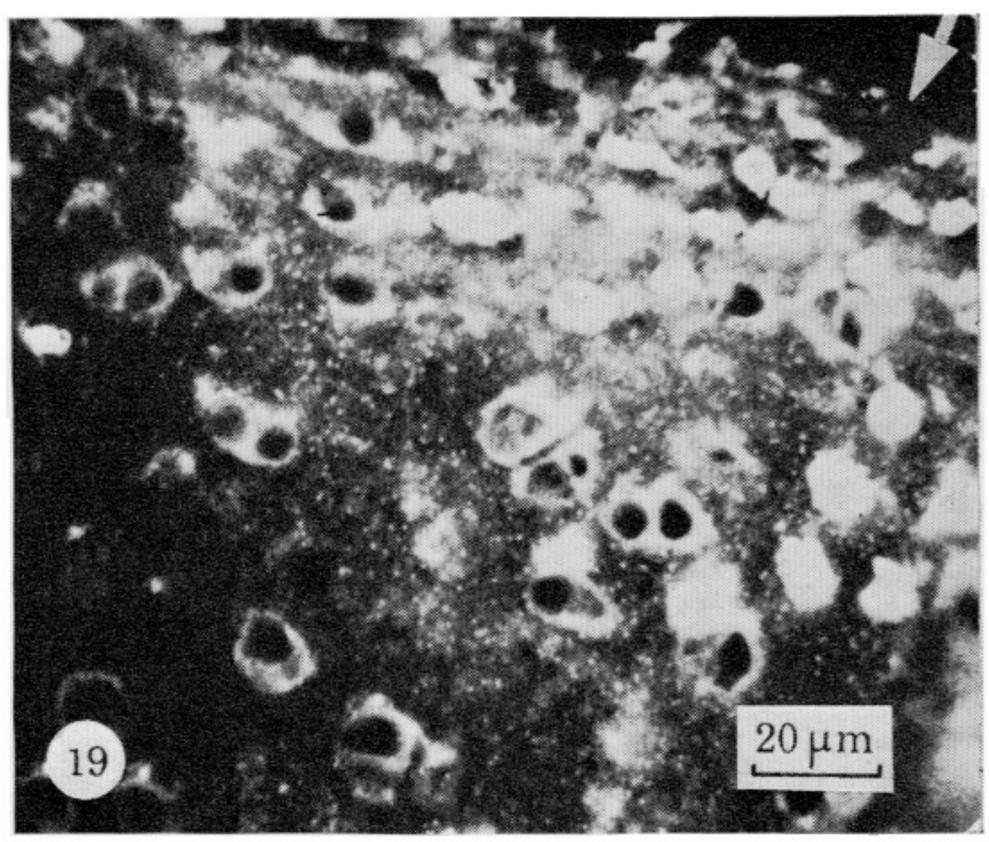


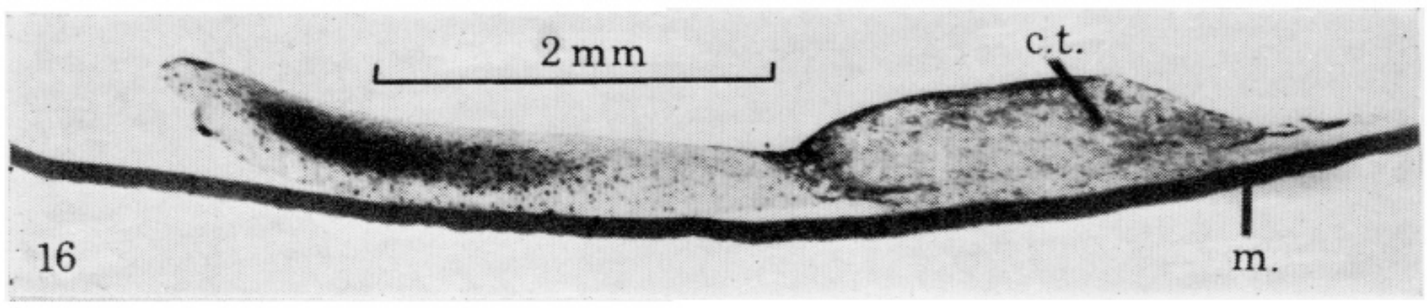


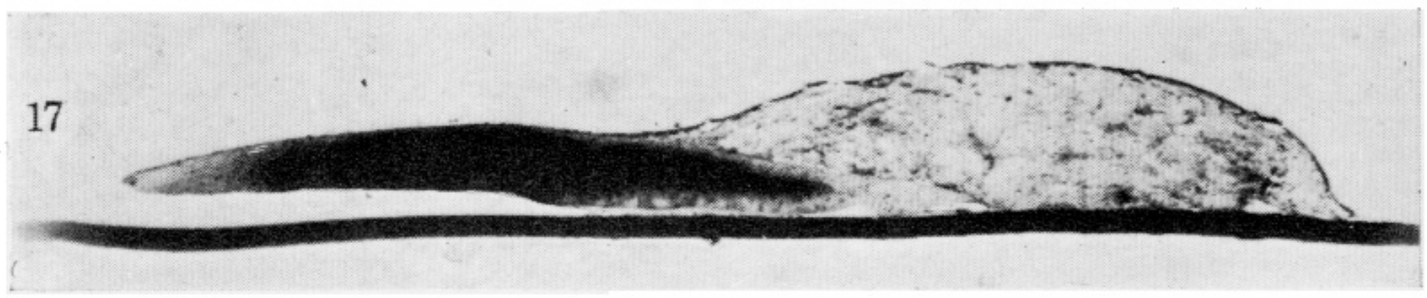




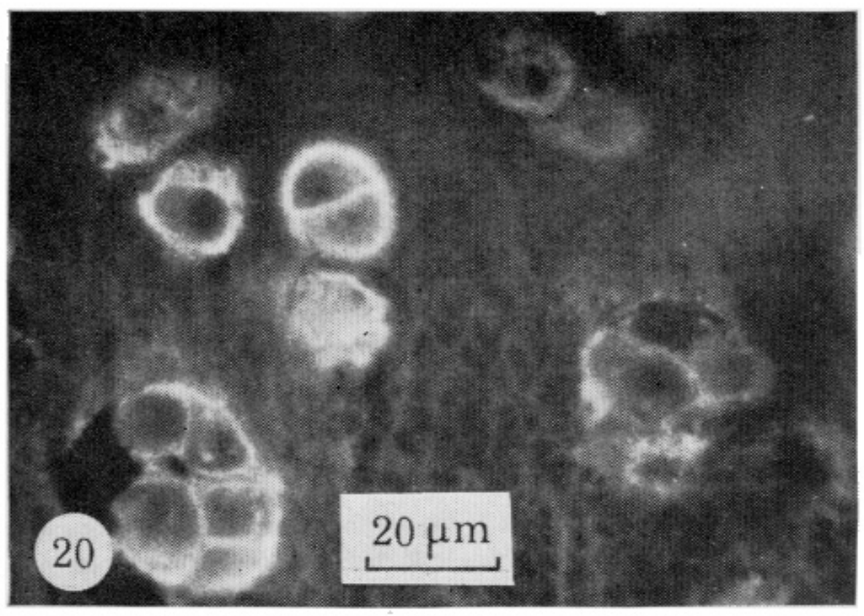


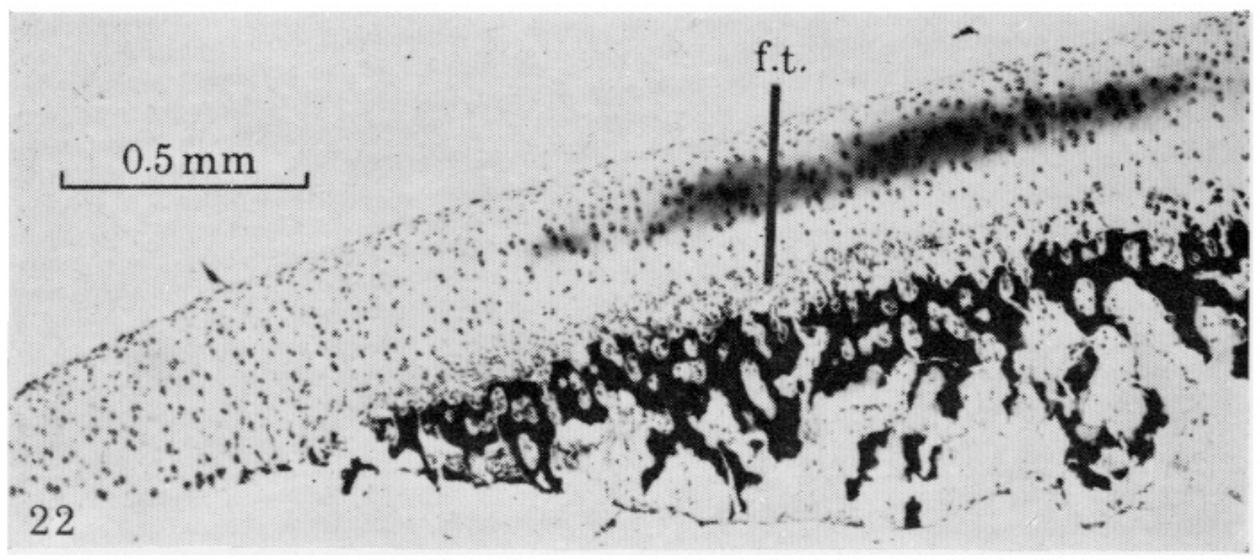


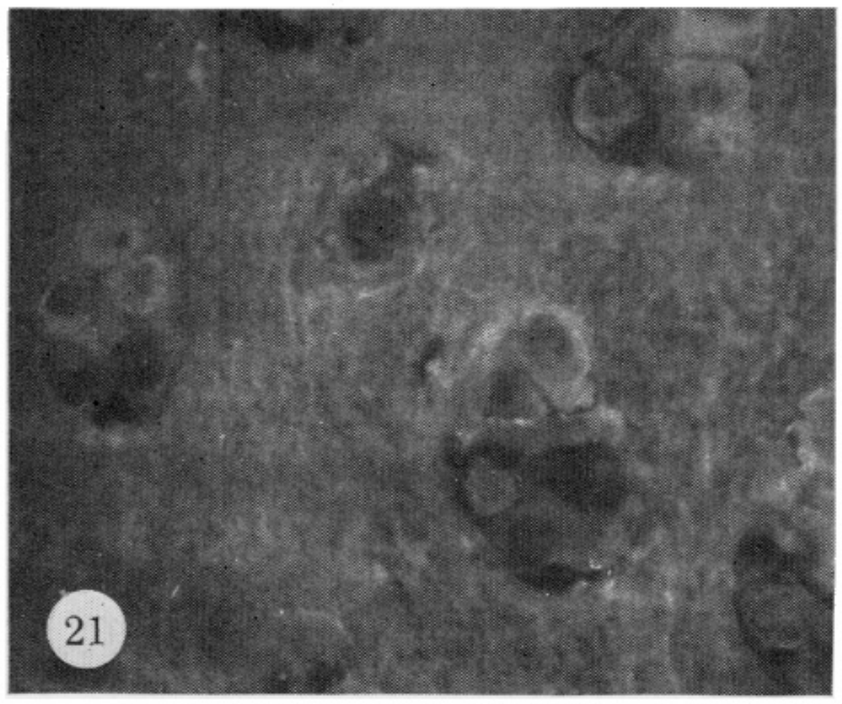


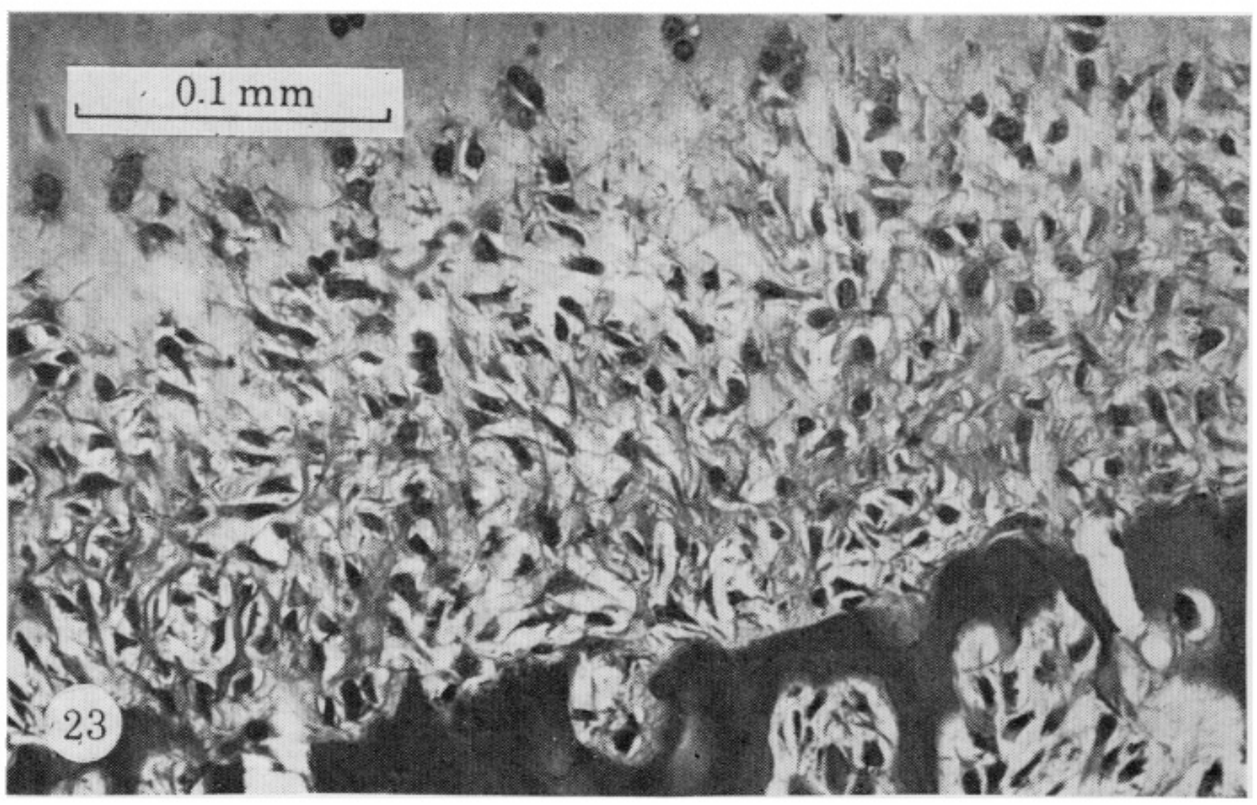


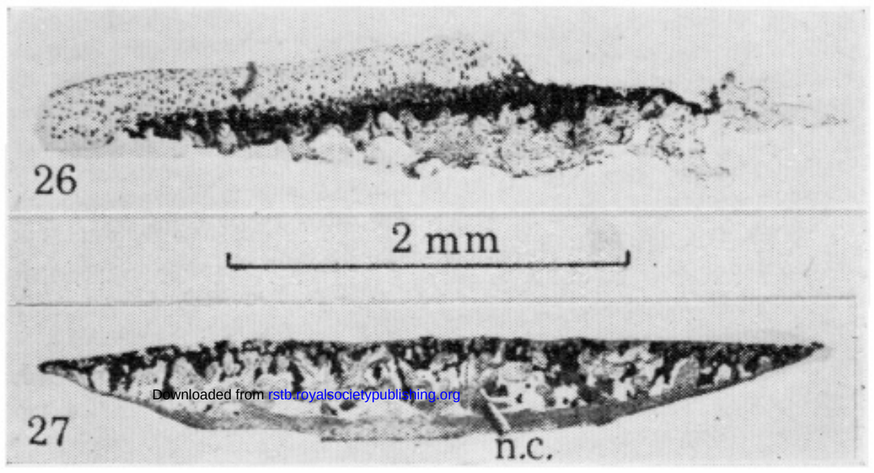
FIGURES 12–19. For description see opposite.

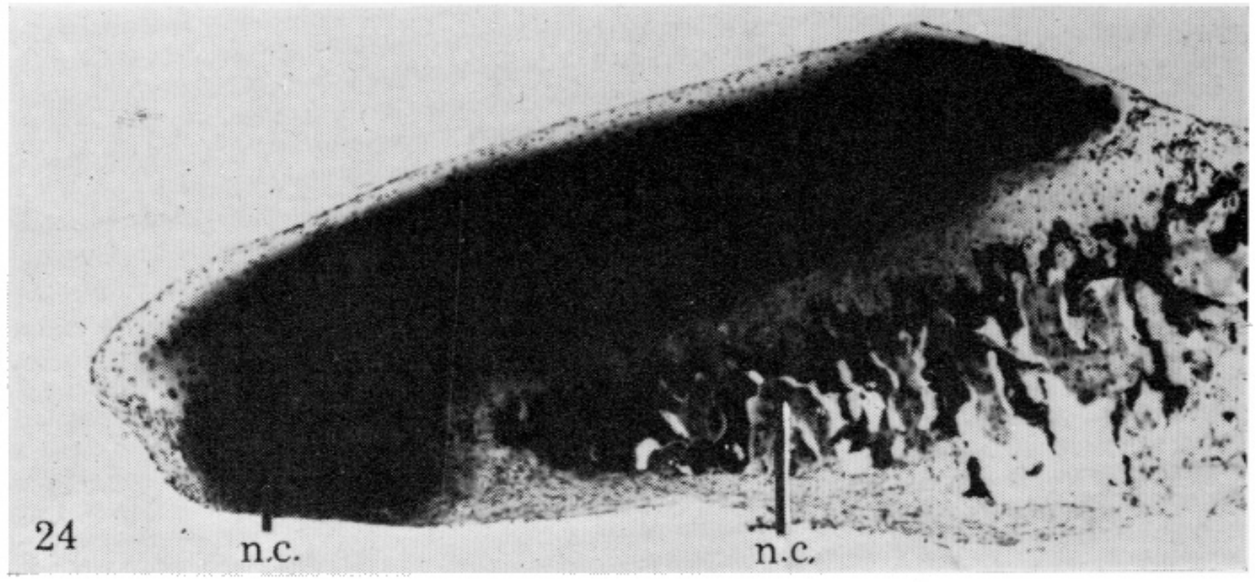


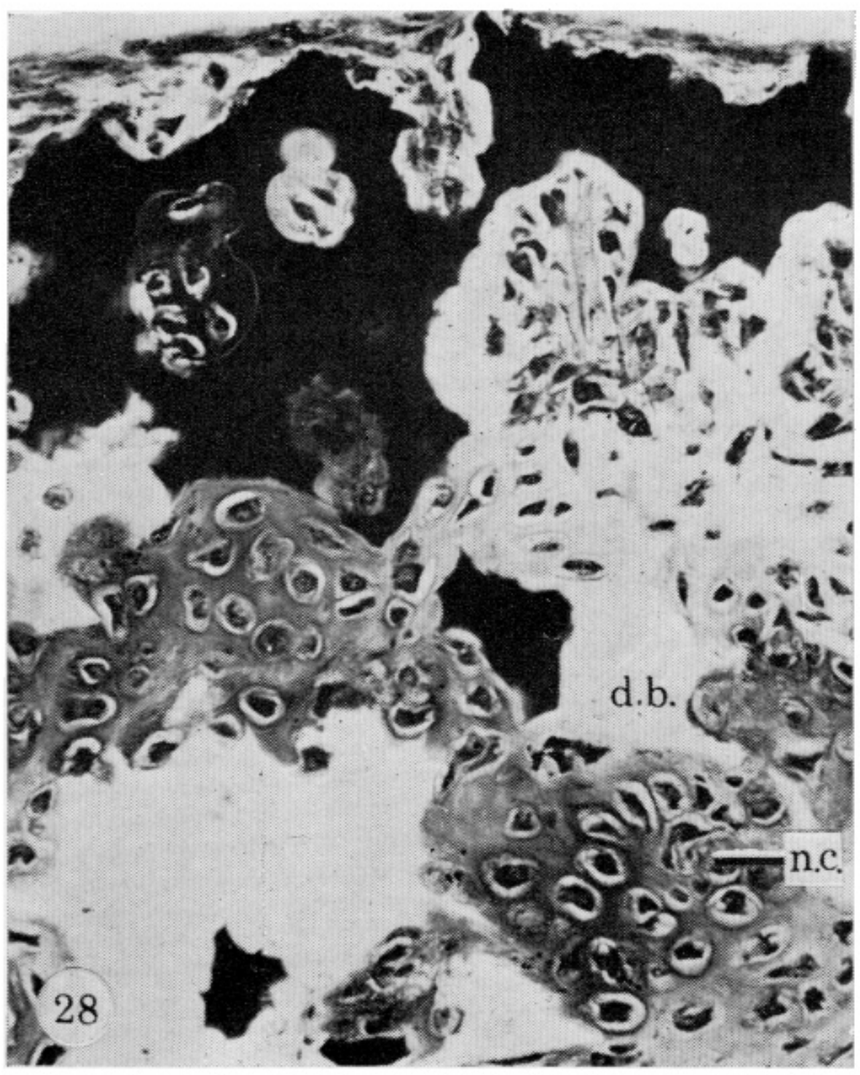


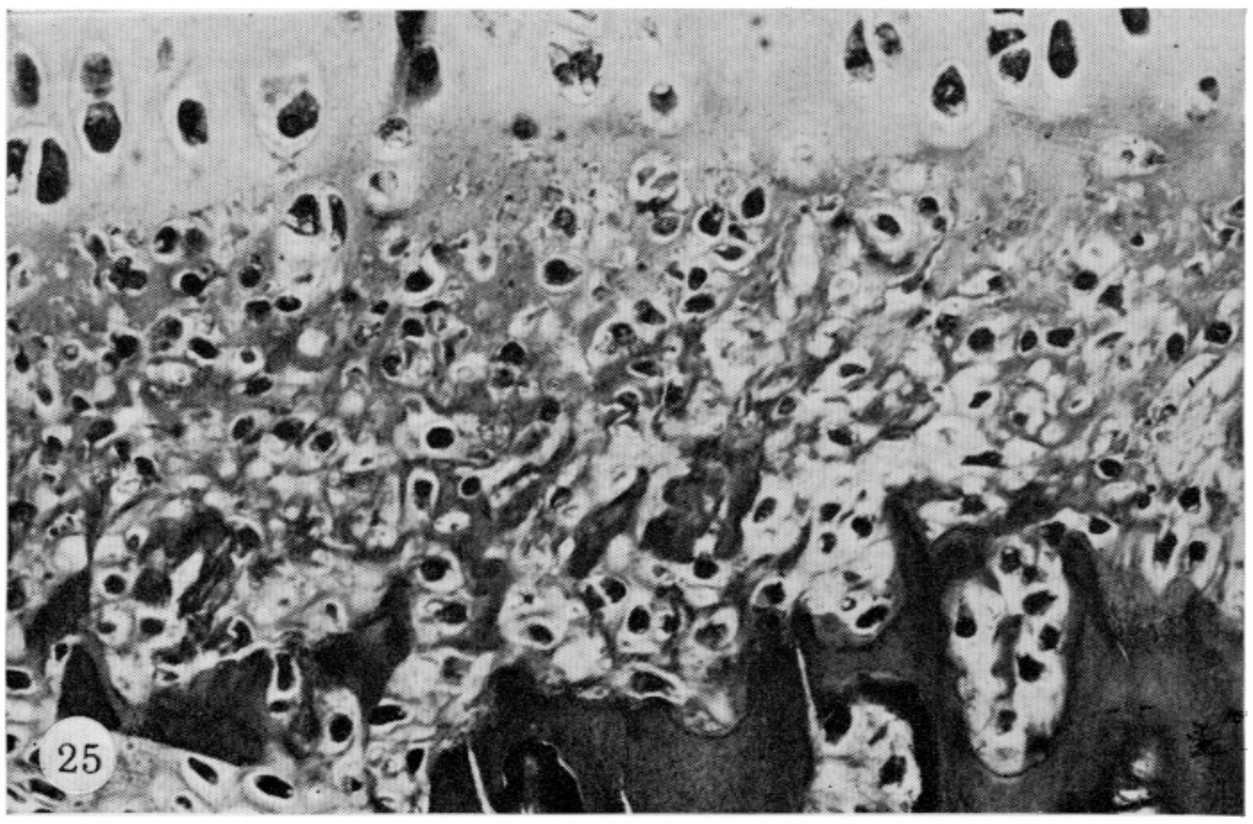




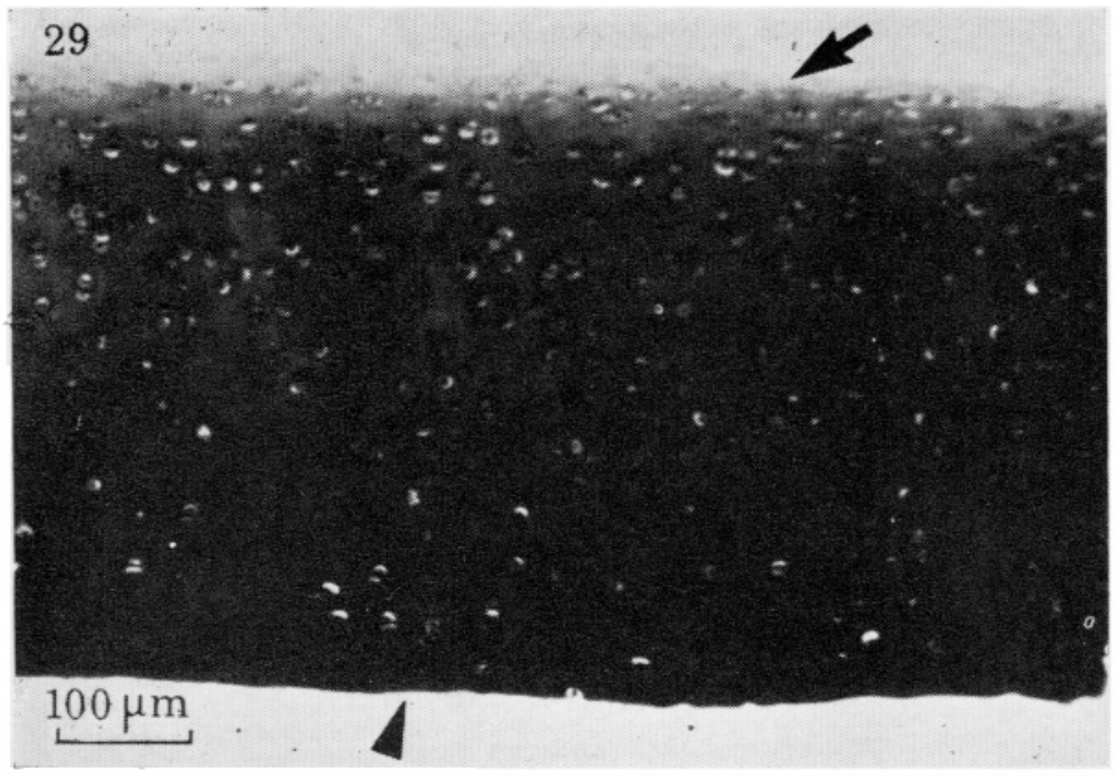


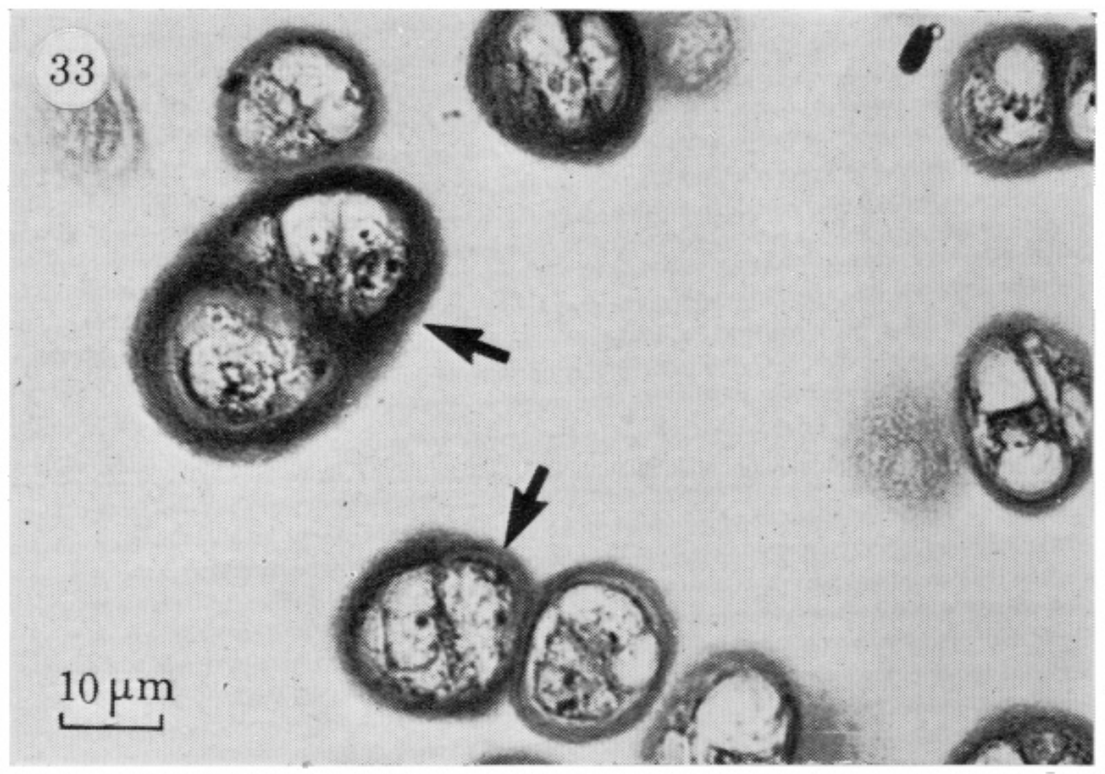


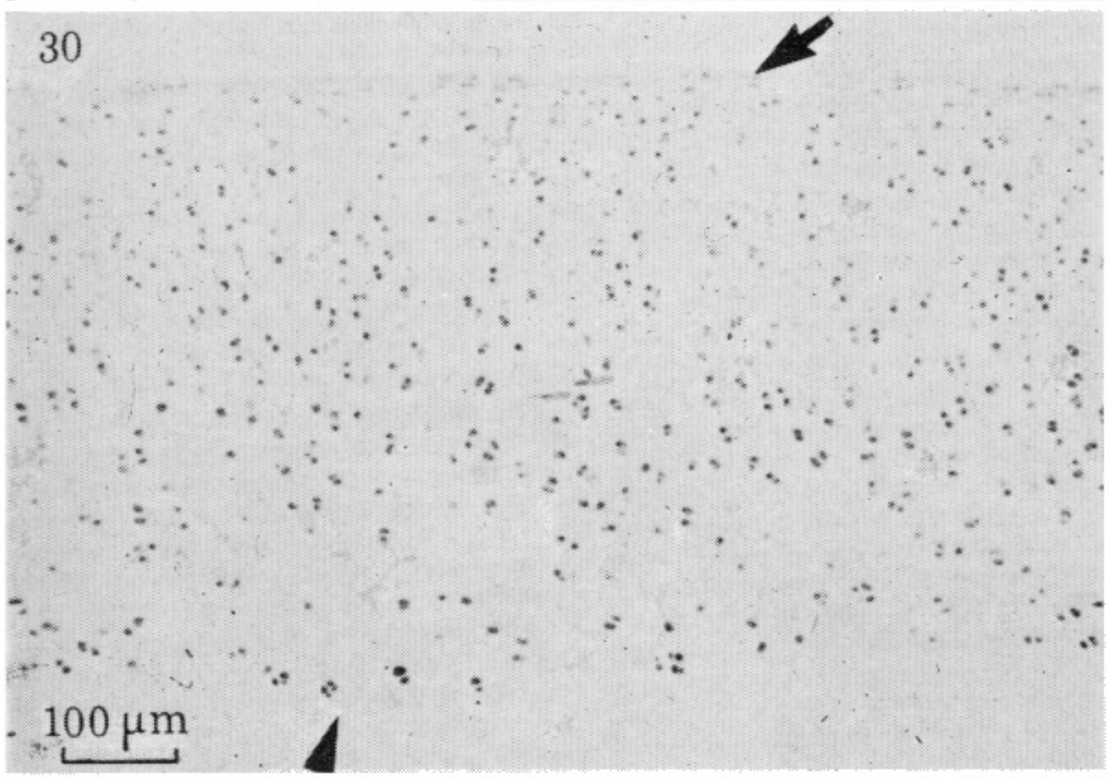


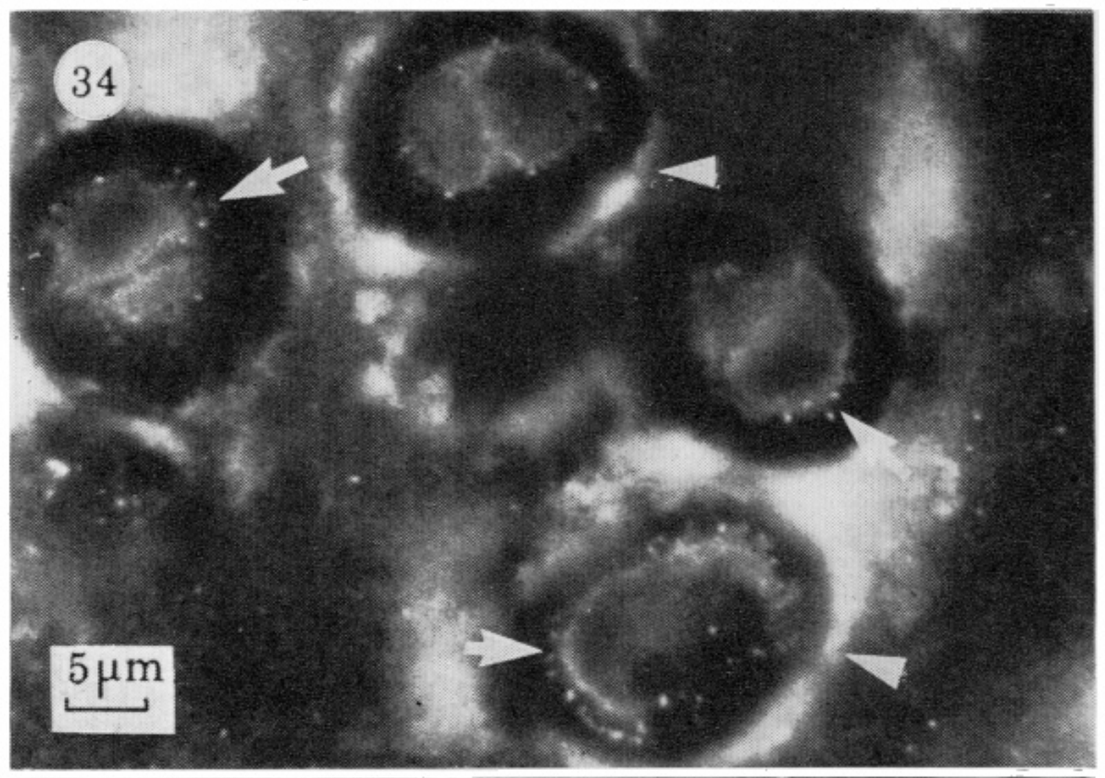


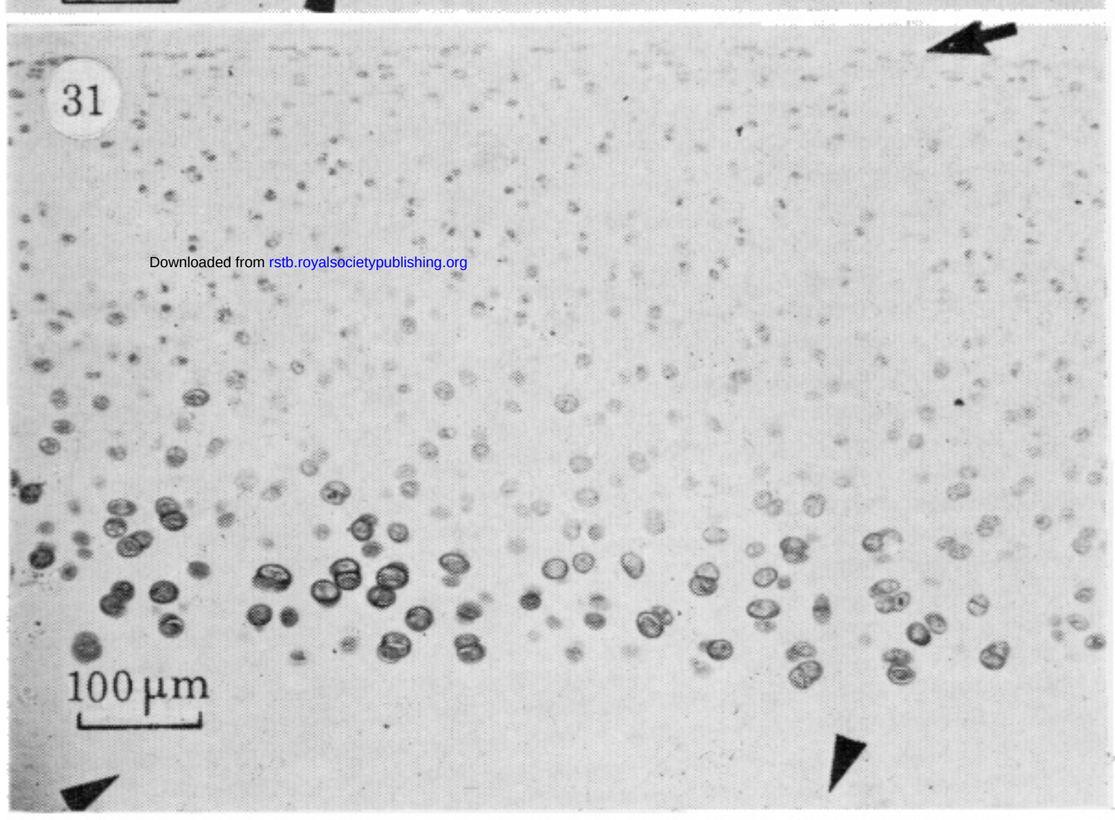
FIGURES 20–28. For description see opposite.

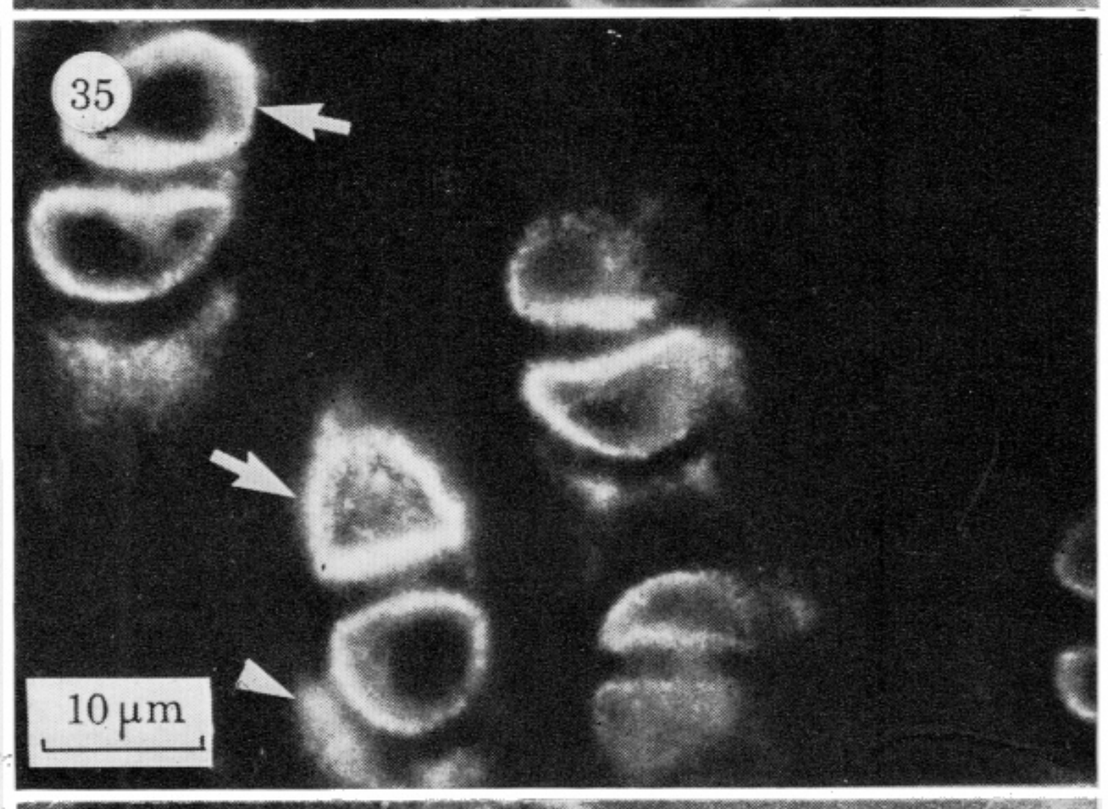


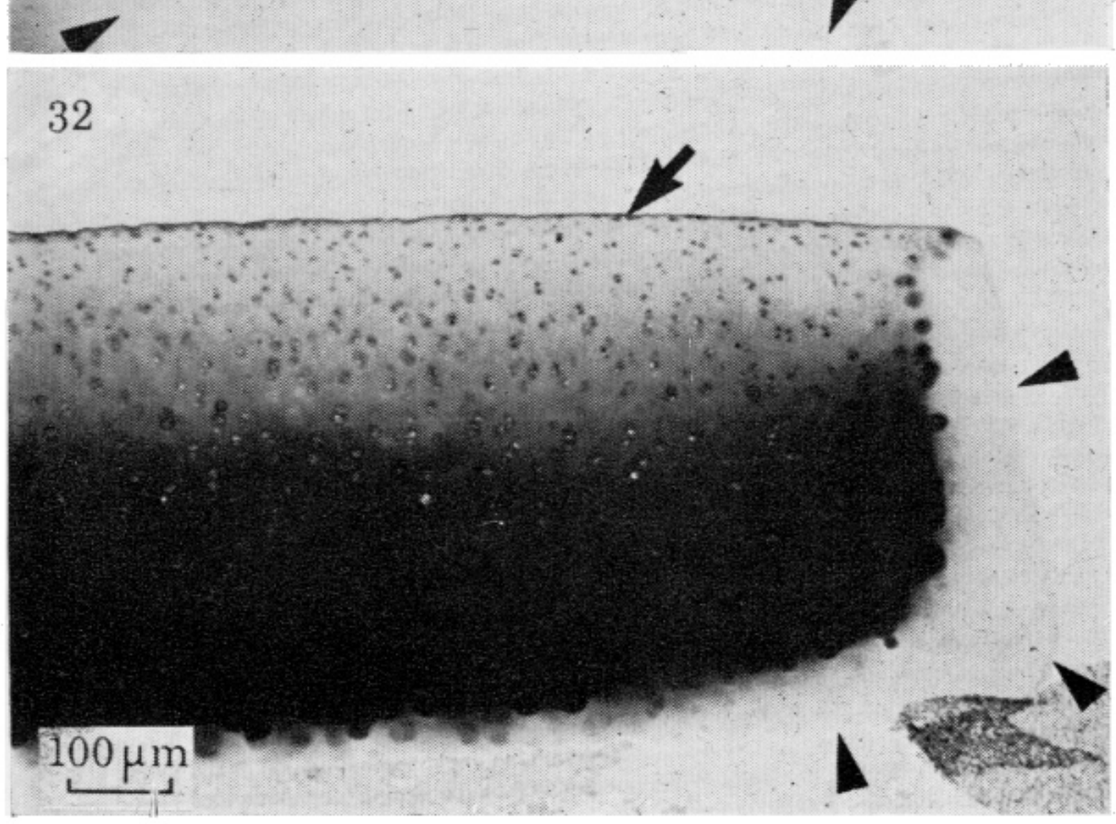


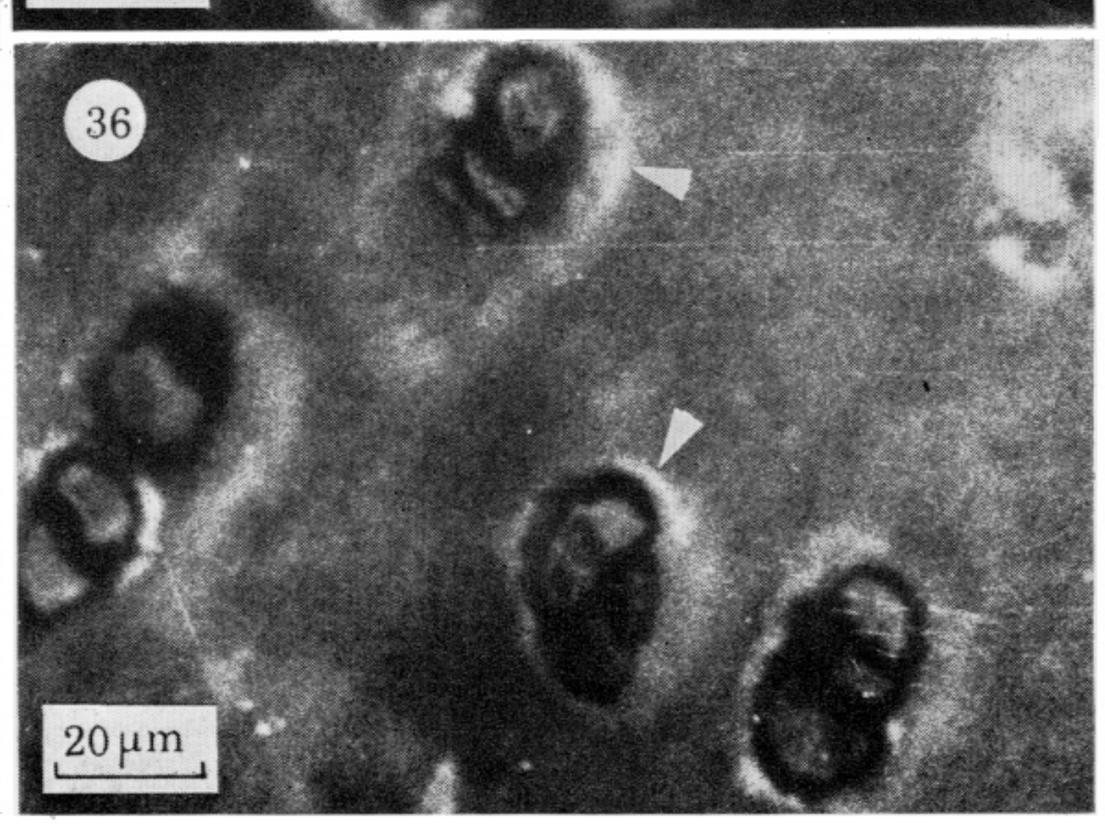












FIGURES 29–36. For description see opposite.