# The Arthritogenic Effect of Indole, Skatole and Other Tryptophan Metabolites in Rabbits

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ABNORMALLY HIGH CONCENTRATIONS of certain metabolites of tryptophan have been found in the urine of a large percentage of patients with rheumatoid arthritis. Forbes and Neale [1] reported that the urine from patients with rheumatoid arthritis contained high levels of volatile indoles. Forbes *et al* [2] found that the indoluria diminished with clinical improvement and then disappeared with recovery. Since these findings suggested that indole might be pathogenetically related to the disease, Forbes and Neale [3] injected indole and skatole into the joints of rabbits and indeed produced a chronic arthritis.

Subsequently, a number of studies in patients with rheumatoid arthritis have demonstrated abnormalities in the kynurenine pathway of tryptophan metabolism, which is the major route of tryptophan breakdown in man. The indole ring of tryptophan is broken by tryptophan pyrrolase, a hepatic enzyme, to form N-formyl kynurenine, which is then degraded via kynurenine and 3-hydroxykynurenine to 3-hydroxyanthranilic acid and ultimately to nicotinic acid (Text-fig 1). McMillan,[4] Spiera,[5,6] Spiera and Christian,[7] Bett,[8] Pinals,[9] and others [10-12] have shown that urine from rheumatoid patients contained excessive levels of one or more of tryptophan metabolites from the kynurenine pathway (kynurenine, 3-hydroxykynurenine and 3-hydroxyanthranilic acid).

Whether or not the abnormal tryptophan metabolism plays any part in the pathogenesis of the disease is, however, unknown. It was felt that, before one could justifiably attribute the lesions in rheumatoid arthritis to excessive levels of any of the tryptophan metabolites, it was essential to determine which of these compounds, if any, were capable of initiating lesions in joints. Also, to our knowledge, there has been no recorded description of the morphologic changes in the joints following the injections of kynurenine metabolites. The objects of this investigation were (1) to determine which metabolites from the kynurenine pathway are arthritogenic and to study in detail the morphologic changes in the

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rabbit joints caused by these metabolites, and (2) to repeat and to extend the initial study of Forbes and Neale [3] by documenting the sequence of events in the development of indole and skatole induced arthritis. Our preliminary data indicated that the arthritogenic effect of the two metabolically related compounds might depend upon their lipophilic activity. Subsequent study in vitro and in vivo of 27 aryl compounds [18] supported this hypothesis. The significance of these findings in regard to the possible mechanism involved is discussed.

## Materials and Methods

Young male New Zealand white rabbits, weighing between 1.0 and 1.5 kg, were used in the study. They were kept in individual cages, fed Purina laboratory chow, and had free access to water.

The test compounds included indole, skatole, tryptophan, kynurenine, kynurenic acid, anthranilic acid, xanthurenic acid, nicotinic acid and nicotinamide, as well as oxindole, isatin and indican, 3-hydroxykynurenine and 3-hydroxyanthranilic acid.

Various amounts of each compound were prepared either in aqueous 50% propylene glycol or in 0.9% NaCl solution depending on their solubility. Saline solutions of acidic compounds were neutralized with NaOH. One ml of the sterile test solution was injected aseptically into the joint cavity of one hind knee. For control purposes, either aqueous 50% propylene glycol or 0.9% NaCl solution was injected into the other knee joint.

The rabbits were sacrificed by cervical fracture and were immediately autopsied. The hind legs were removed and examined grossly and microscopically. Tissues from various visceral organs and from all injected joints and para-articular structures were fixed in 10% neutral formalin and bone specimens were decalcified in Decal.* Tissues were imbedded in paraffin, 5 $\mu$ sections were cut and stained with hematoxylin and eosin (H&E), and, whenever indicated, with Masson's trichrome, Putt's and periodic acid-Schiff (PAS) stains.

*Experiment 1.* Five rabbits were injected with a single dose of indole (0.26 mmol) prepared in 1.0 ml of aqueous 50% propylene glycol, and another 5 were injected with a similar preparation of skatole. The rabbits were sacrificed 1, 2, 3, 4, and 7 days after the injection.

*Experiment 2.* Six rabbits were injected with one of the following amounts of indole: 0.04, 0.08, 0.13, 0.17, 0.21 and 0.26 mmol respectively. Another 6 rabbits were treated similarly with skatole. The injections were repeated once a week for 6 weeks. All animals were sacrificed 8 weeks after the initial injection.

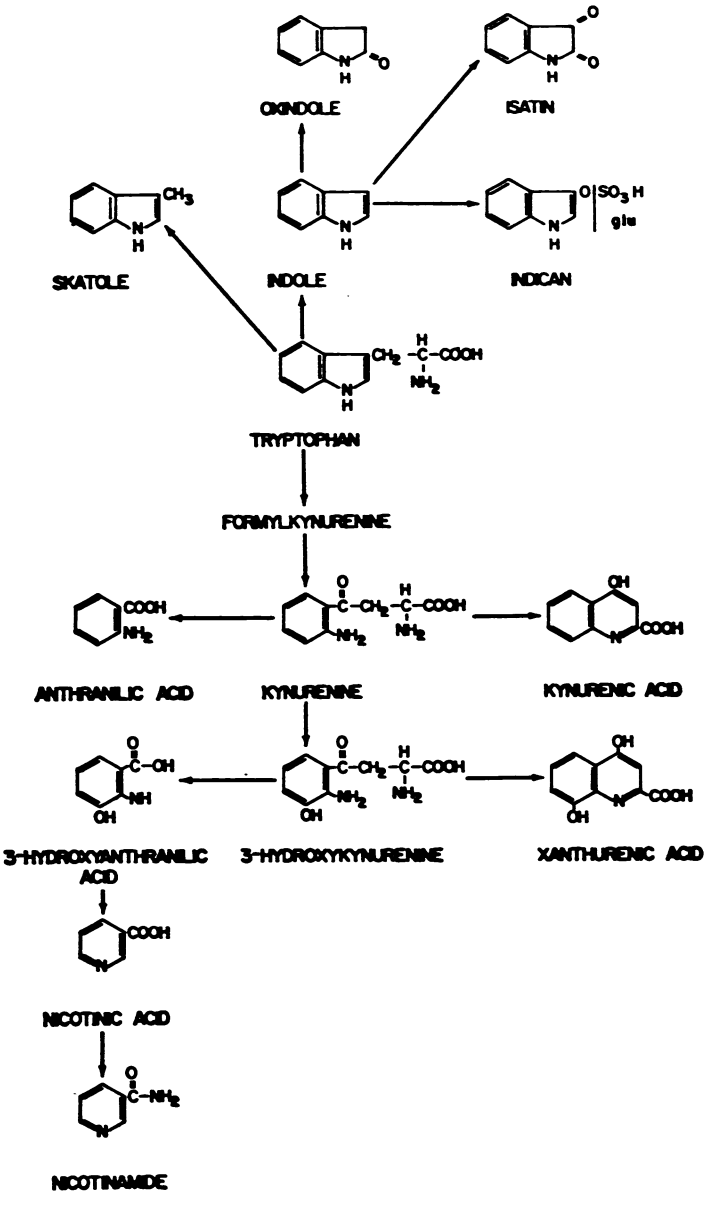
*Experiment 3.* Fourteen rabbits were injected with 0.1 mmol of one of 14 test compounds (Text-fig 1). The injections were repeated once a week for 6 weeks. All animals were sacrificed 8 weeks after the initial injection.

*Experiment 4.* The skin on the back of one rabbit was shaved and 0.2 mmol of indole in aqueous 50% propylene glycol was injected intracutaneously. Intracutaneous injection of solvent served as a control.

## Results

All control knee joints injected with 1.0 ml of aqueous 50% propylene glycol or 0.9% NaCl were grossly and microscopically normal. Examina-

* Decal Chemical Corporation, Pomona, New York.




TEXT-FIG 1. Diagram of tryptophan metabolism via the kynurenine and indole pathways.

tion of the heart, liver and kidneys of the experimental animals failed to reveal any pathologic changes indicative of a systemic effect of the test compounds.

**Experiment 1: Acute arthritis produced by indole and skatole**

*Indole.* In rabbits sacrificed 24 hours after the injection an acute inflammatory reaction was already well established. The synovial membrane was congested and edematous, and small numbers of neutrophils were scattered along the synovial surface. Synovial fluid was slightly increased in volume and contained a few neutrophils.

Two days after the injection, the congestion and edema had increased; synovitis was more pronounced. Synovial lining cells showed mild hypertrophy and focal proliferation. The cellular infiltrate was more intense and consisted of a mixture of neutrophils and some mononuclear cells.

Three days after injection, there was a more diffuse proliferation of synovial cells with scattered clumps of fibrinous material on the surface. The joint cavity contained masses of eosinophilic fibrillar material mixed with neutrophils and a few desquamated synovial cells.

Four days after the injection, the subsynovial fat was partially replaced by proliferating fibroblasts. The synovial lining cells appeared elongated and palisaded at right angles to the surface (Fig. 1). Mixed neutrophilic and mononuclear cell infiltration extended from the synovial membrane to involve the connective tissues around the tendon sheaths and bursae. Focal myositis was found in a few areas.

Seven days post injection, the synovial lining cells were hypertrophied and hyperplastic (Fig. 2). The synovial membrane was infiltrated with numerous mononuclear cells (lymphocytes, plasma cells and macrophages) but only sparse polymorphonuclear leukocytes were seen (Fig. 3). Subsynovial fat was almost totally replaced by granulation tissue containing numerous inflammatory cells. The articular capsule showed mild diffuse fibrous thickening, but the mobility of the joint was not impaired.

*Skatole.* The changes observed with skatole were similar to those produced by indole, with only minor differences. The acute inflammatory reaction was more severe and was still present on the seventh day. The synovial congestion was particularly intense close to the margins of the cartilage of the patellar facet of femur (circulus vasculosus). Both the articular cartilage and the underlying subchondral bone were normal. The articular capsule showed diffuse fibrosis which was more severe than in the joints injected with indole. The fibroblastic proliferation was more marked on the lateral aspects of the knees than elsewhere around the joint.

The sequential pathologic changes during the first week after a single intra-articular injection of indole and skatole indicated that both compounds were arthritogenic and that they produced an acute inflamma-

tion which originated in the synovial tissue. The earliest manifestations were hypertrophy and proliferation of the synovial lining cells, and congestion and edema of the synovial membrane, followed by a neutrophilic infiltration and fibrinous exudation. The acute inflammatory response subsequently changed from predominantly neutrophilic to mononuclear cell type and at the same time proliferation of the surrounding connective tissue appeared.

#### Experiment 2: Chronic arthritis produced by indole and skatole

The severity of chronic arthritis resulting from repeated injections of indole and skatole (6 times over a period of 8 weeks) was closely related to the amount of the compound injected. The mildest lesions were produced by injections of 0.08 mmol of indole and 0.04 mmol of skatole. In these animals, passive joint movement was not restricted. The articular capsule was slightly thickened on both sides of the joint. The cartilage of the patellar facet of the femur showed either slight focal marginal erosion or hyperplasia. The synovial membrane was infiltrated with small numbers of lymphocytes and macrophages and the subsynovial fat had been partially replaced by fibroblasts. A few mononuclear cells and some desquamated synovial lining cells were present in the synovial fluid.

Injections of 0.13 and 0.17 mmol of indole, or 0.08 and 0.13 mmol of skatole produced moderately severe chronic arthritis. Here, the passive joint movements were slightly to moderately impaired; extension of the joints was limited to about 60 degrees. The joints were enlarged and slightly deformed. The articular segments of both the femur and tibia were somewhat widened and the distal end of the femur was slightly angulated and protruded dorsally. The articular capsule showed moderate fibrosis and thickening, particularly at its lateral attachments. The articular cartilage showed small focal erosions. The articular cavity was somewhat diminished; hyperplastic synovial villi projected into the joint cavity. There was a diffuse cell infiltration in the synovial and subsynovial tissues extending along the tendon sheaths and around the bursae. The cellular infiltrate was composed of moderate numbers of lymphocytes, plasma cells and macrophages with occasional multinucleated giant cells. Most of the latter cells contained phagocytosed small, irregular fragments of cartilage. Pannus extended from the marginal synovial reflections for variable distances over the articular cartilage, or between the cartilage and underlying bone, undermining or replacing portions of the cartilage. In some areas the granulation tissue extended deeply into the subchondral bone and spread out into the subjacent marrow spaces.

Increasing the amount of the injected indole to 0.26/mmol produced knee joints that were moderately enlarged and deformed. Dorsal bowing and remodeling of the distal end of the femur were prominent. The joint mobility was almost totally lost. There was marked fibrous thickening of the capsule and massive fibrous tissue proliferation in the lateral and posterior regions of the capsule (Fig 4 and 5). The articular cartilage was extensively replaced by pannus which merged with granulation tissue in the marrow spaces of the underlying bone (Fig. 6). Fibroblasts invaded some tendons and caused adhesions between them and the adjacent connective tissue. Large, hyperemic synovial villi composed of collagenous connective tissue projected into the joint cavity. Focal hyperplasia of synovial lining cells was associated with subjacent proliferation of granulation tissue and an intense mononuclear cell infiltration.

The injections of an equivalent amount of skatole resulted in markedly enlarged and deformed joints. The distal end of the femur was club-shaped, and was easily fractured during manipulation of the joint. The joint cavity was almost totally obliterated by granulation tissue. This, together with massive periarticular fibrosis resulted in fibrous ankylosis with total loss of mobility. The subchondral bone, in addition to foci of penetrating granulation tissue, showed focal osteoclastic bone resorption in some areas, and deposition of new bone in others (Fig. 7). The remaining portions of subchondral bone were osteoporotic. These joints exhibited the most severe inflammatory reaction. The predominant cells were lymphocytes, plasma cells and macrophages.

Figures 8–11 show the gross appearance of a control (normal) joint as contrasted with mild, moderate, and severe arthritic changes.

#### Experiment 3

Repeated injections of tryptophan, kynurenine, 3-hydroxykynurenine, kynurenic acid, anthranilic acid, xanthurenic acid, 3-hydroxyanthranilic acid, nicotinic acid, nicotinamide, oxindole, isatin and indican into rabbit knee joints did not produce any pathologic changes.

#### Experiment 4

Intracutaneous injection of 0.2/mmol of indole did not produce any pathological changes.

## Discussion

The experiments described above clearly indicate that kynurenine, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid as well as other

kynurenine metabolites do not produce any pathologic changes when injected into the joint cavities of rabbits. The high urinary levels of these compounds found in rheumatoid patients are probably not related to the pathogenesis of human joint disease.

Injections of indole and skatole on the other hand, do cause acute and chronic arthritis in the rabbit. Many morphologic features of this experimental arthritis are the same as those seen in the joints of human arthritics. These include hypertrophy and hyperplasia of synovial lining cells, infiltration of the synovium by polymorphonuclear leukocytes followed by lymphocytes, plasma cells and macrophages, pannus formation, and erosion and destruction of the articular cartilage. Whether or not indole is involved in human arthritis is not known. It is tempting to speculate that rheumatoid arthritis could be triggered by a metabolic defect and later perpetuated by an autoimmune process.[14] Since indole and skatole were arthritogenic in rabbits, it is conceivable that these substances might initiate the inflammatory process in the joints of man when present in sufficiently high concentrations. Certain intestinal bacteria containing tryptophanase convert tryptophan to indole and skatole, which are then excreted by the bowel and kidney. Some indole is metabolized in the liver and excreted in the urine as indican. It is possible that an increased formation of indole in the intestinal tract, or a decreased rate of its detoxification by the liver, or both, may result in an escape of indole into the general circulation. A beneficial effect of a low-tryptophan diet in rheumatoid patients has been reported by Houpt *et al.*[15]

It was interesting to observe that intracutaneous injection of 0.2 mmol of indole into the rabbit did not induce any local inflammatory reaction or fibrosis, suggesting perhaps that indoles may have a specific effect on synovial lining cells. In contrast, however, Verne and Kubikowski [16] found that in tissue culture indoles stimulate fibroblastic proliferation.

In order to understand what possible mechanism may be involved in the arthritic effect of indole and skatole, we examined the physicochemical properties of these and other related aromatic compounds and attempted to correlate these properties with the pathogenicity of the compounds. The results of this study are reported in detail elsewhere.[13] In brief, we injected into the joints of rabbits, in addition to indole and skatole, 25 other related aromatic compounds. Of all tryptophan metabolites tested the most severe injury was caused by skatole, which also showed a relatively high degree of lipophilic activity. On the other hand, the metabolites from kynurenine pathway and the breakdown

products of indole in the liver, all of which failed to induce any articular changes, were not greatly lipophilic. An approximate gaussian distribution between the logarithm of the partition coefficient (ratio of *n* octanol solubility to aqueous solubility) of the test compounds and the extent of joint injury was observed. In other words, the degree of joint damage produced was closely related to the lipophilic activity of the compound.

The data suggested that a multistep partitioning process may be involved in the arthritic response to the injected compounds—ie, a compound moves across more than one membrane before it binds at the site of action. Thus, a lipophilic compound may interact with lipid molecules present in the membranes of cells and of their organelles, possibly interfering in their metabolic processes or causing complete disruption. The disruption of cell membranes by indole and skatole has been demonstrated in vitro by Rogers [17] by their hemolysing effect upon erythrocytes.

Weissmann *et al* [18] have recently shown that leukocytic lysosomal contents are capable of inducing arthritis. The experimental arthritis produced by indole and skatole closely resembles the lesions induced by intra-articular injections of streptolysin S which labilizes lysosomes.[19] The possible role of lysosomes in the production of arthritis in our experimental model has to be considered.

Our observations suggest that synovial cells are probably the primary site of action of indolic substances. Hypertrophy and proliferation of these cells associated with edema and a mild neutrophilic infiltration were the earliest findings. Erosion of the articular cartilage was not seen until several weeks later. In-vitro experiments failed to reveal any chondrolytic activity of indole (incubation of the fragments of articular cartilage with high concentration of indole for over 5 weeks). These findings suggest that agents other than indoles are responsible for the erosion of the cartilage; these may be lysosomal substances released by the initial injury to synovial cells, or derived from leukocytic or phagocytic cells.

Finally the strikingly rapid proliferation of articular connective tissue deserves attention. Massive capsular and periarticular fibrosis present in almost every case appeared to be out of proportion to the degree of preceding inflammation and, therefore, cannot be attributed in its entirety to the reparative process. The excessive fibroblastic proliferation might have been stimulated by direct action of indoles upon connective tissues [16] or indirectly through release of lysosomal material.

We, therefore, postulate that the arthritogenic effect of indolic sub-

stances depends upon their lipophilic activity, which facilitates their diffusion into the cells by interaction with lipid components of the membranes. Resulting alterations of the cellular metabolic processes and/or disruption of the membranes and release of lysosomes may be responsible for both the proliferative and degenerative changes found in experimental arthritis. The synovial lining cells appear to be the primary target of indole activity, although other cell types may also be involved.

## Summary

Intra-articular injections of several metabolites of tryptophan from its kynurenine pathway (kynurenine, 3-hydroxykynurenine, kynurenic acid, anthranilic acid, xanthurenic acid, 3-hydroxyanthranilic acid, nicotinic acid and nicotinamide) as well as oxidation products of indole [20] (oxindole, isatin and indican) failed to produce any pathologic changes in rabbits. It is unlikely, therefore, that these compounds are significant in the pathogenesis of human arthritis. The lipophilic activity of these compounds is very low.

Injections of indole and skatole into joints of rabbits induced anatomic lesions similar to those of rheumatoid arthritis. The pathogenicity of these compounds correlated with their high lipophilic activity. It is suggested that these agents interact with the lipid components of the membranes of cells and their organelles and possibly interfere with cellular metabolic processes and/or release the lysosomal contents from the cells. Lysosomes may then be ultimately responsible for the inflammatory reactions in experimental arthritis.

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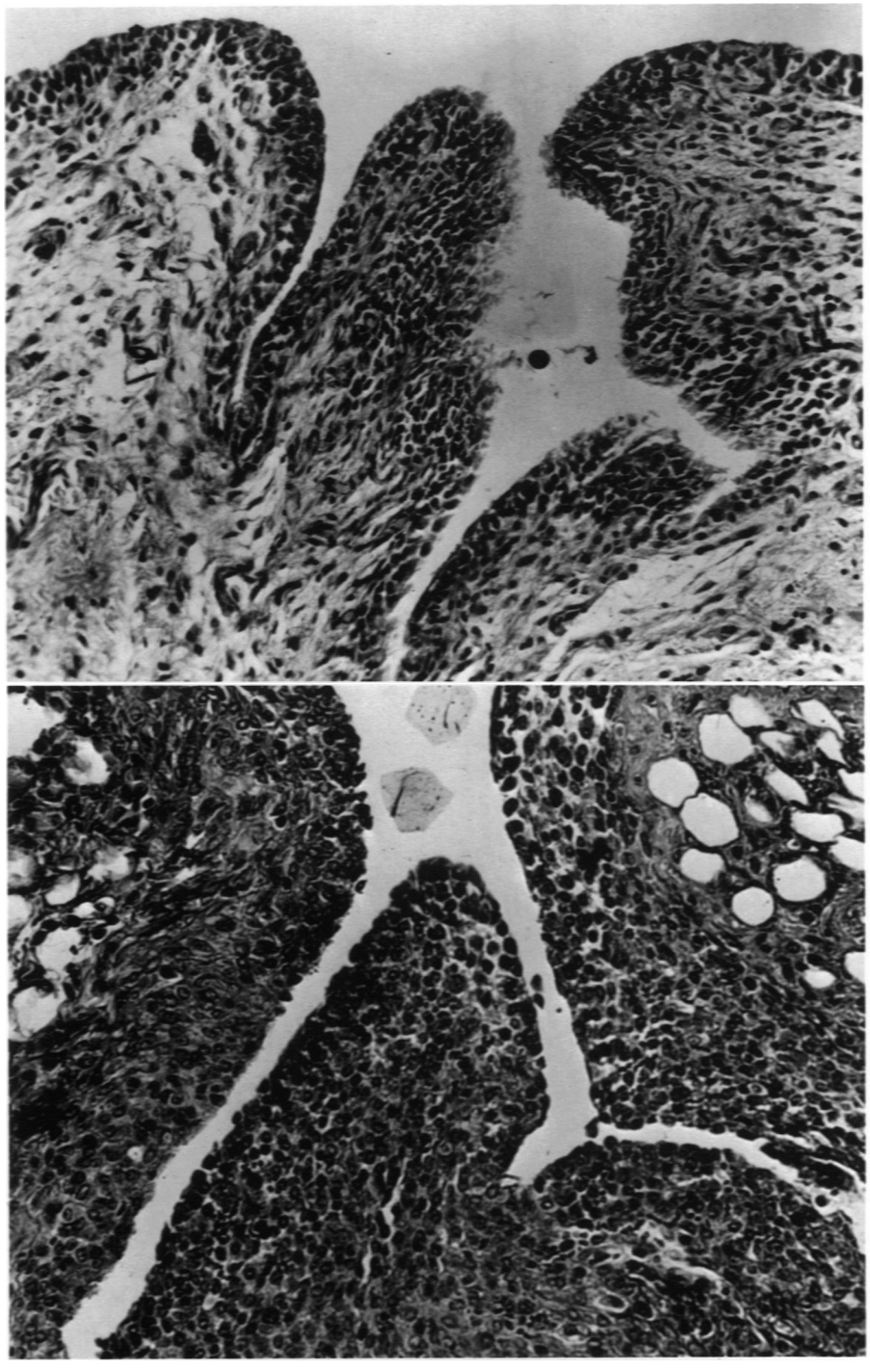

**Fig 1.** Synovium and subjacent tissue of the rabbit knee joint 4 days after injection of indole. Synovial lining cells have proliferated, are swollen, and show a tendency to palisading. Subsynovial fat is partially replaced by fibrous tissue. Mild mononuclear cell infiltration present. H&E. × 160.

**Fig 2.** Synovium of knee joint 7 days post injection of indole. Marked proliferation of synovial cells and mild mononuclear cell infiltration. H&E. × 200.

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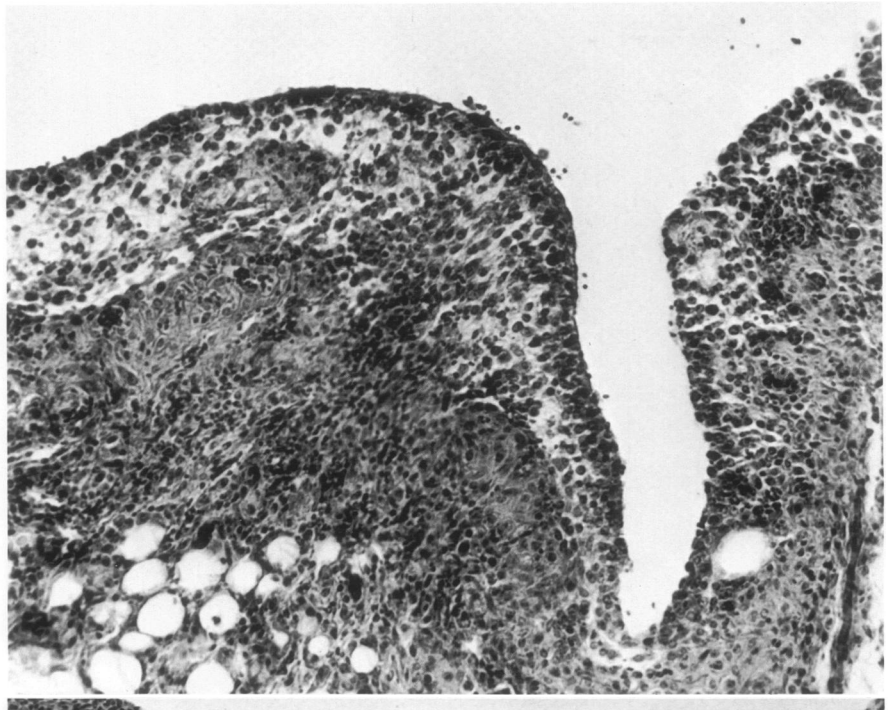

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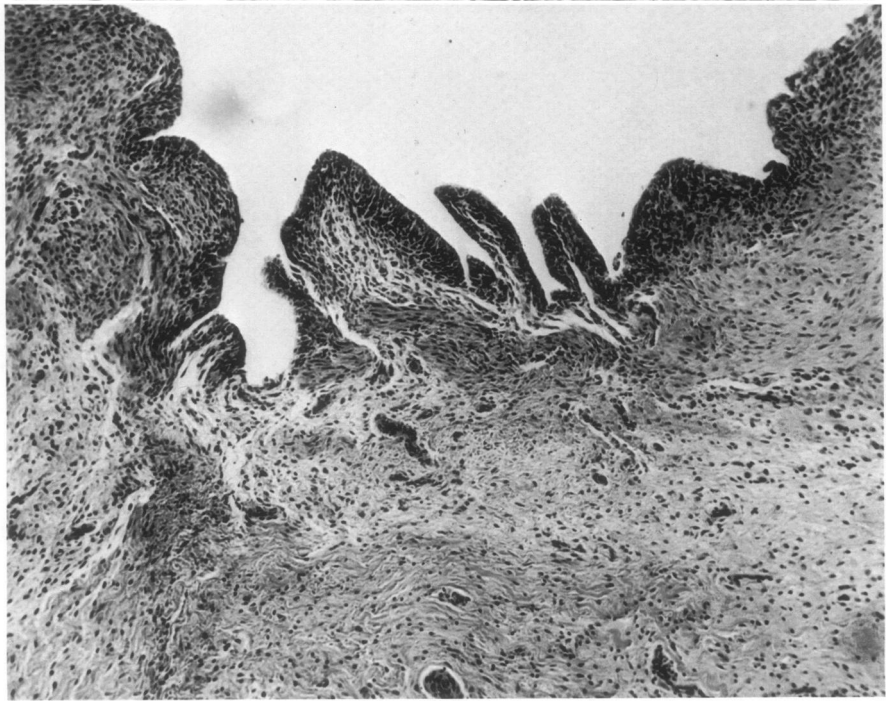

**Fig 3.** Same age as Fig 2. Synovial cell proliferation, subsynovial fibrosis and diffuse mononuclear cell infiltration. H&E. × 160.

**Fig 4.** Extensive replacement of subsynovial fat by fibrous connective tissue and focal synovial cell proliferation. H&E. × 63.

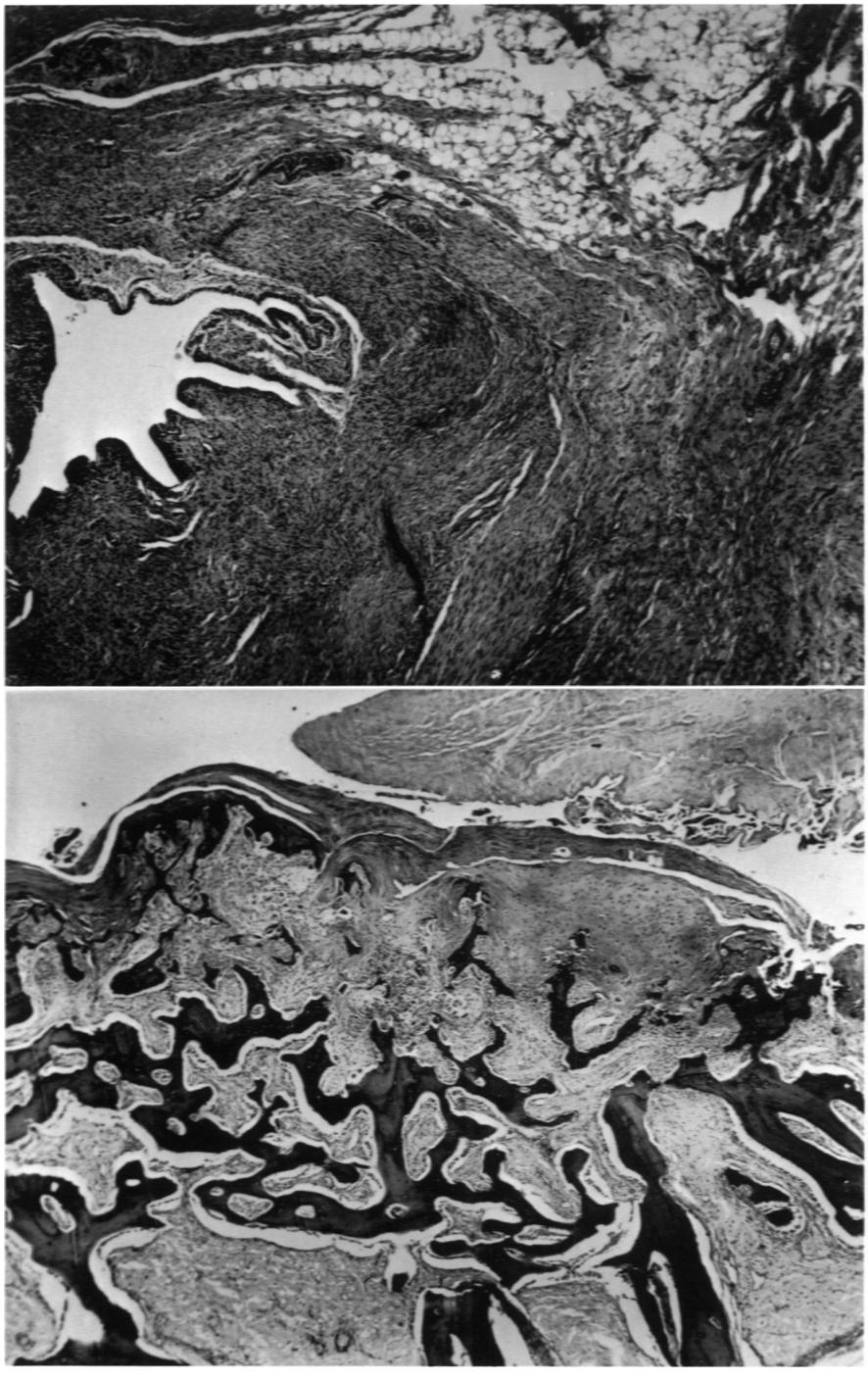

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**Fig 5.** Section across a joint recess showing marked thickening of synovium due to abundant dense fibrous tissue. H&E. × 50.

**Fig 6.** Partial destruction of articular cartilage of the head of tibia by ingrowing pannus. The fibrous tissue containing small number of lymphocytes undermines the remaining cartilage and spreads throughout the subjacent marrow spaces. In the subarticular region the bony trabeculae have almost entirely disappeared. H&E. × 10.

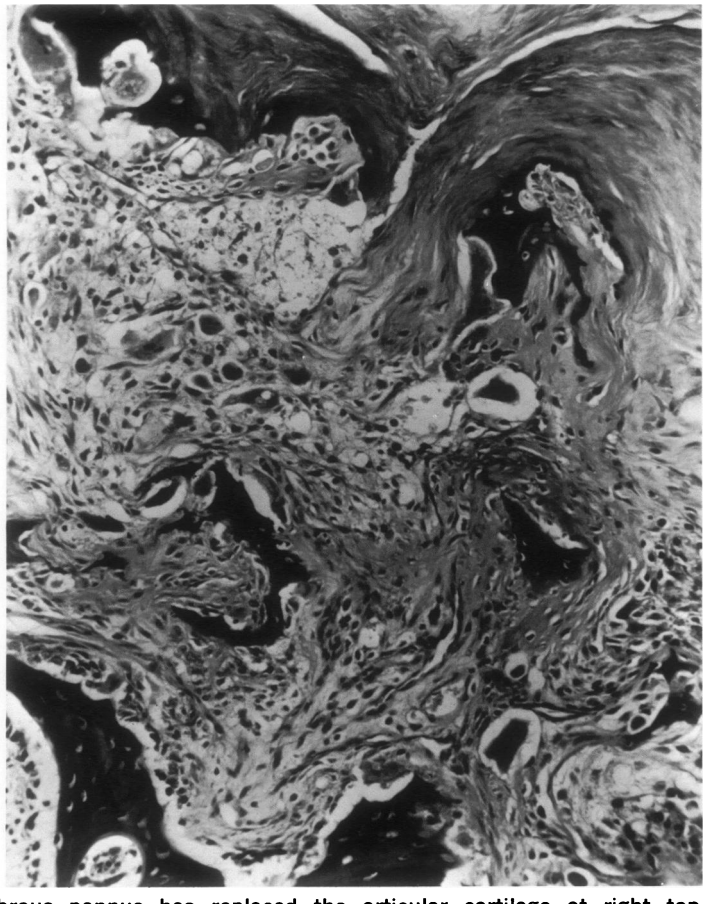

**Fig 7.** Fibrous pannus has replaced the articular cartilage at right top and has infiltrated subchondral bone. Note osteoclastic bone resorption and new bone formation. H&E. × 200.

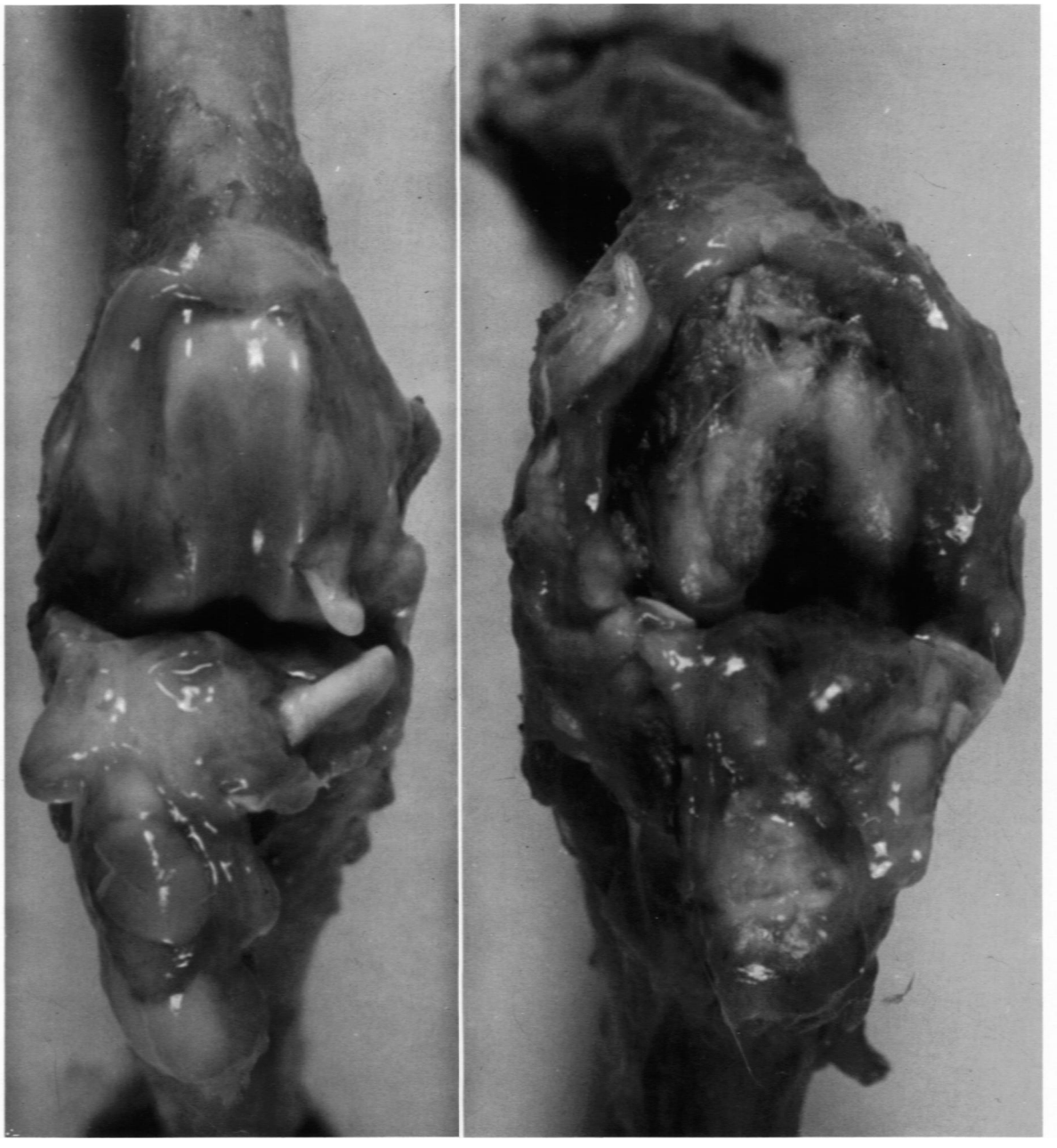

Fig 8. Control rabbit knee joint (normal) injected with aqueous 50% propylene glycol solution. Joint has been opened anteriorly; patella and patellar ligament have been reflected downward for better view of the joint cavity. × 3.

Fig 9. Large areas of erosion and focal hyperplasia of articular cartilage are present on the patellar facet of femur. Synovial membrane is congested, edematous, and articular capsule is thickened. × 3.

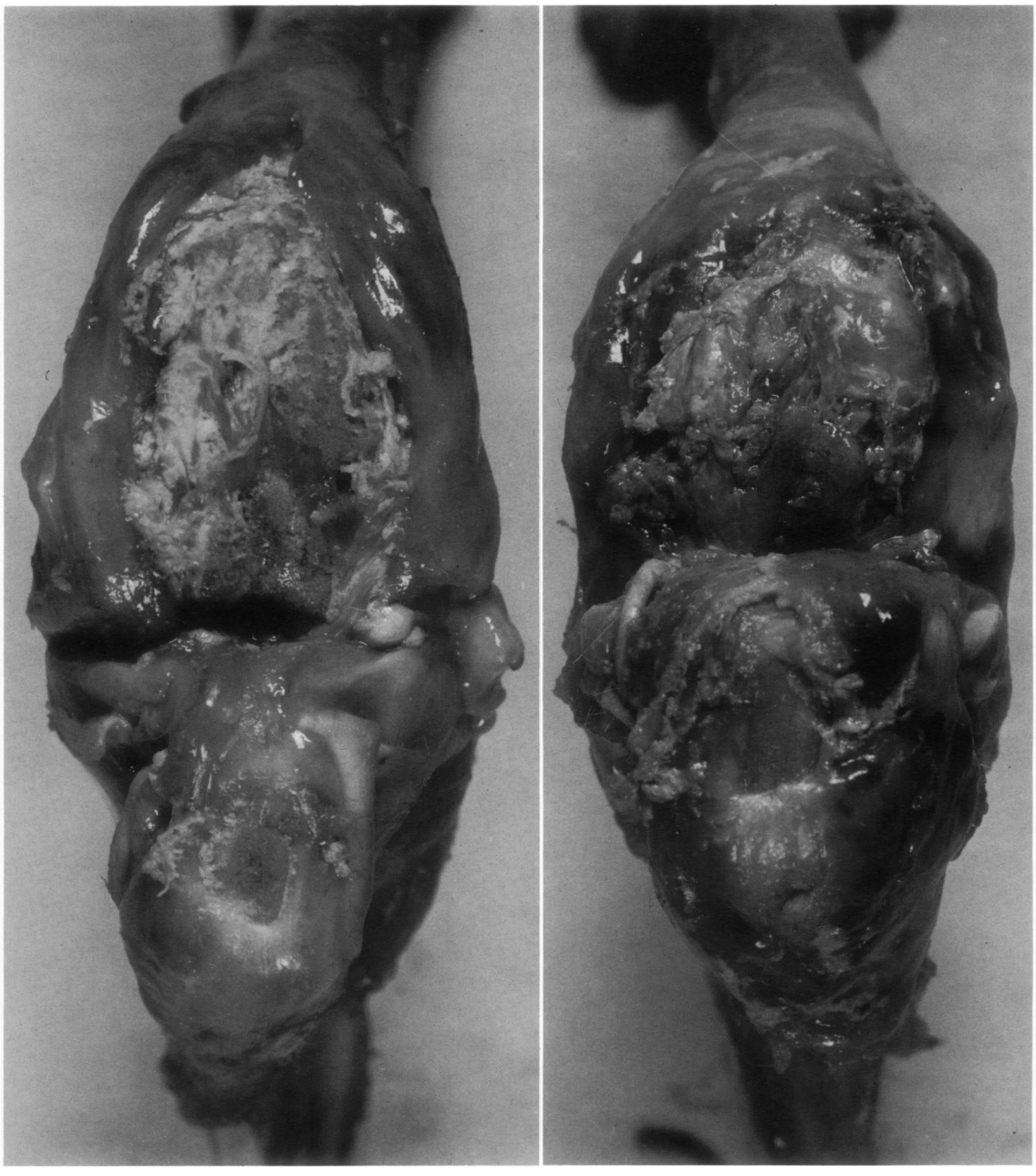

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**Fig 10.** Subtotal destruction of articular cartilage of joint. Synovial membrane is irregular, shaggy and inflamed. Articular capsule is markedly thickened. Joint cavity is partially obliterated. × 3.

**Fig 11.** Articular cartilage is totally destroyed, and joint cavity obliterated by fibrous tissue resulting in ankylosis. × 3.