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EXPERIMENTAL DISC LESION IN RABBITS

The Effect of Repeated ACTH Administration

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During the past thirty-five years, many works have been dedicated to the surgical and conservative treatment of acute disc lesions.

All kinds of conservative methods from rest and plaster jacket to electrotherapy, radiotherapy, leg or pelvic traction, spine manipulation, ACTH and cortisone, largactil (ampliactil), flaxedil, and many other drugs, have been tried and are still in use Haimovici 1959).

As a matter of fact, we are ignorant of whether any of the various conservative means exert any direct influence on the histologic evolution of a disc lesion, either in accelerating or delaying its healing.

In order to shed some light on this problem, it was decided to study the effect of repeated ACTH administration on the evolution of experimentally induced disc lesions.

In the course of this experiment, we observed that disc lesions in the animals used, with or without ACTH administration, revealed many striking peculiarities in the healing process which did not correspond to previous descriptions and therefore warrant this detailed report.

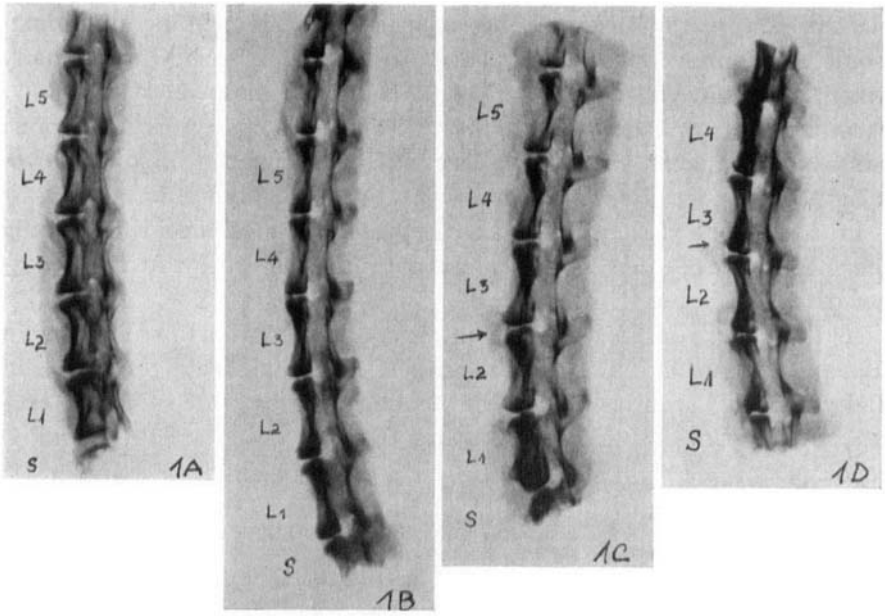
MATERIAL AND METHODS

The material first consisted of a group of eleven rabbits, weighing 1½–2 kg each. The experiments were later repeated on a larger group of twenty-five rabbits of similar size. Three animals of the first group and one of the second group died during the experiment, three of them under anesthesia and one of unknown cause ten days after operation.

Under general anesthesia with ether and sterile conditions, the anterior aspect of the spine was exposed through a left lateral longitudinal abdominal subperitoneal approach. Transverse incisions of 1½–2 cm were performed through the anterior aspect of the three lower lumbar intervertebral discs. The incisions were

carried through the entire thickness of the annulus fibrosus until the nucleus pulposus was seen emerging at the surface. The wound was closed in layers and the animals were left to recover. ACTH PROLONGAL (Cortrofina Z-ORGANON) intramuscular injections of 10 units were administered to half of both lots of rabbits beginning the third day after operation. The injections were repeated every second day during the first month and every third day during the second month.

Half of both lots of rabbits served as control and did not receive ACTH after operation. Both ACTH-treated and control animals were divided in four groups according to time of sacrifice after the operation: 13, 26, 39, and 55 days. Four groups of eight animals, four ACTH-treated and four control, were studied. The total amount of ACTH received by each rabbit was: 50 U by day 13, 110 U by day 26, 140 U by day 39, and 200 U by day 55. After sacrifice the lumbar spine was removed and examined macroscopically and X-ray pictures were also taken. The spine fragment was kept in formalin for at least ten days, then decalcified and embedded in paraffin. Microscopic sections were cut and colored with hematoxilin with specific coloration for the fibrotic and elastic tissues.



Figures 1A to 1D: X-ray pictures of lumbar spines removed from rabbits. Lesions of the intervertebral discs have been previously induced. Figure 1A: Fragment of the lumbar spine with thirteen-day old lesion. Normal aspect. Figure 1B: Twenty-six-day old disc lesion. Normal aspect. Figure 1C: Thirty-nine-day old disc lesion. Small anterior osteophytes between L2–L3. Numerotation L1 first vertebra beginning from the sacrum. Figure 1D: Fifty-five-day old disc lesion. Small osteophytes particularly between L2–L3.

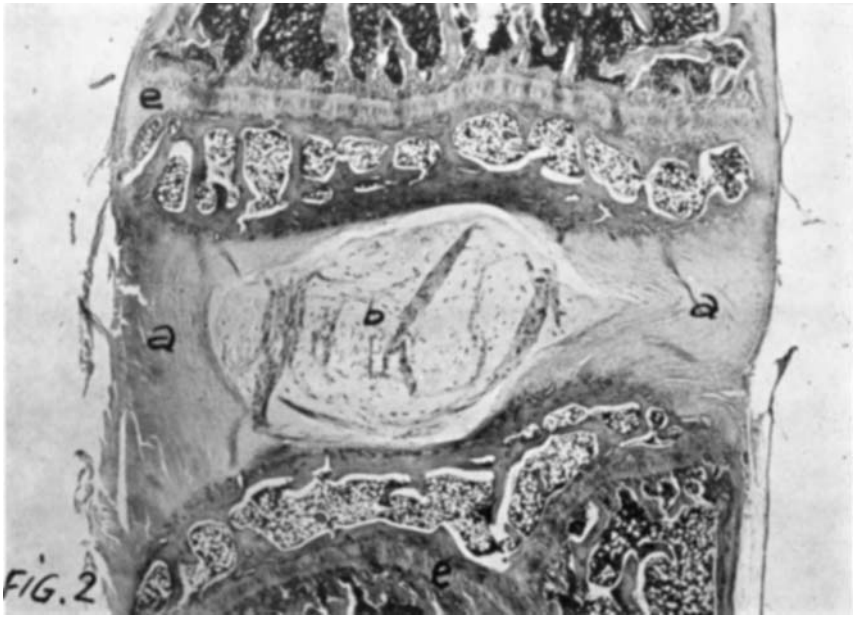


Figure 2. Normal aspect of intervertebral disc in rabbit. a: annuplus fibrosus; b: nucleus pulposus; e: epiphyseal cartilage plates.

RESULTS

Macroscopical examination of the lumbar spine revealed scars at the place of incisions, more or less indurated in consistency and adherant to the prevertebral muscles. These scars were small but visible, even in the thirteen-day specimens. The mobility of the lumbar spine appeared generally unchanged in all specimens. In fact, upon removal, the lumbar spines inversed spontaneously from the usual kyphotic into a lordotic curve, demonstrating that the anterior lesions and ensuing scars did not significantly restrain the flexion-extension.

The X-ray pictures of the removed spines (Figures 1 A to D) did not show any remarkable narrowing of the intervertebral spaces. Small anterior osteophytes could be seen on the X-ray pictures of the thirty-nine and fifty-five day specimens (Figures 1 C and 1 D) from both the control and ACTH-injected animals.

Microscopical examination: From four to six sections were prepared from every disc and a total of about 400 sections from 96 discs were examined. The lesion in the annulus fibrosus was visible in the early and later sacrificed animals with more or less the same characteristics.

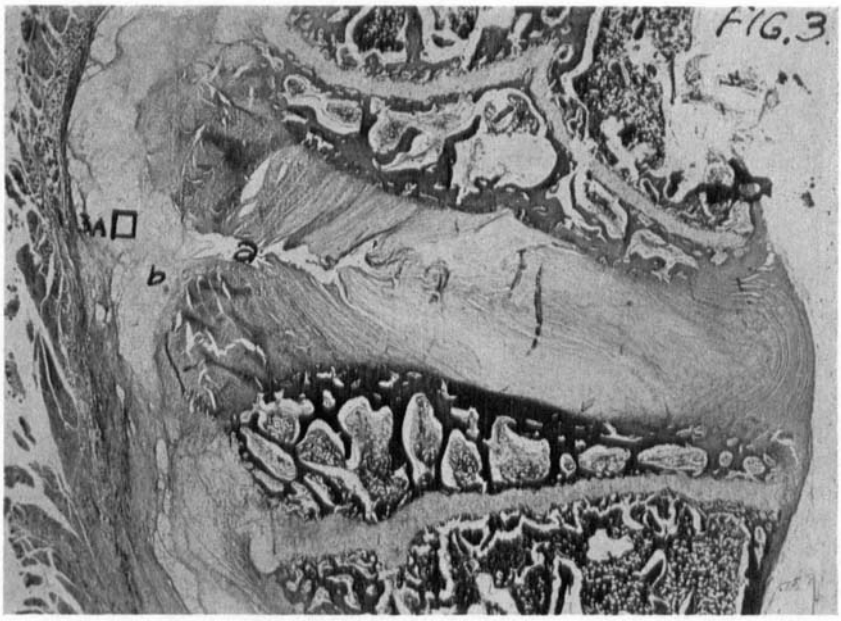


Figure 3. Intervertebral disc lesion, thirteen days old, of control rabbit. a: traject through annulus fibrosus; b: herniated cartilage blocking the lesion of the annulus fibrosus.

The lesion resembled a traject formed by the cut lips of the annulus fibrosus, connecting the cavity of the nucleus pulposus with the surface of the disc where herniated nucleus pulposus tissue could be observed (Figure 3). The described traject, usually narrow and sometimes slightly enlarged, was empty or contained fragments of nucleus pulposus. The lesion was practically blocked in front by a thicker or thinner layer of cartilaginous tissue resulting from the herniated nucleus pulposus. This latter tissue is known in normal disc to consist of a small number of large cartilaginous cells; however, after herniating outside the disc cavity, its appearance was characterized by the presence of many small cartilaginous cells intruding into the cartilaginous fundamental tissue. The explanation of this newly acquired appearance seemed to be that the young large cartilaginous cells of the nucleus, while herniating, react in two ways to the new conditions of pressure and nutrition through multiplication and degeneration. Whereas the small cells are the result of multiplication, the degenerated cells are represented by areas with many vacuolated cells (Figure 3 A).

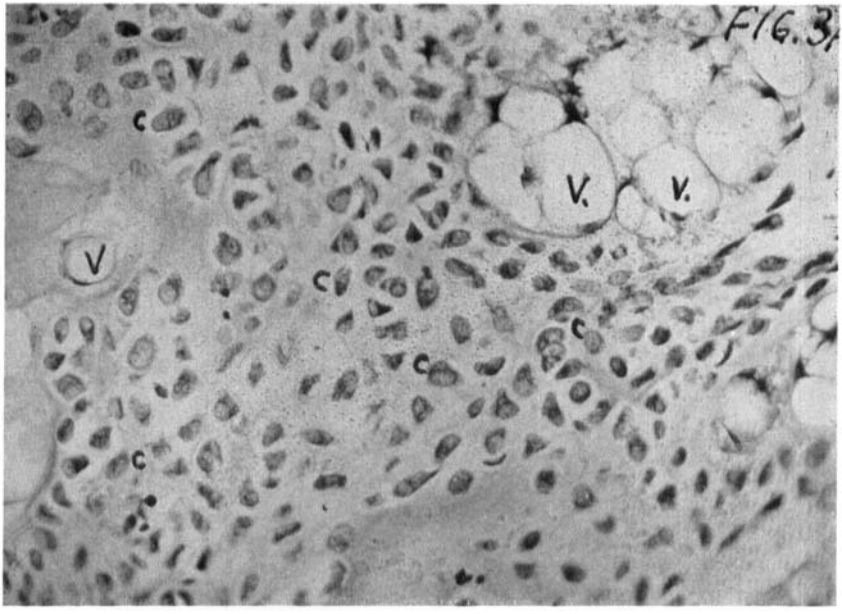


Figure 3A. Higher magnitude (260 ×) of fragment from the herniated cartilage of previous picture; c: small cartilage cells; v: vacuolated cells interpreted as degenerating cartilaginous cells from the nucleus pulposus.

The cartilaginous tissue covering the defect of the annulus fibrosus did not show any tendency to grow in thickness, even after fifty-five days. However, proximal and caudal growth, corresponding to the lines of cleavage of the overlying tissues and directed toward the upper and lower epiphyseal cartilages, is proved by the continuity established between the latter tissues and the herniated cartilage (Figures 5 A and 7 A).

The proliferation of the herniated cartilage derived from the nucleus pulposus was usually accompanied by a proliferative tendency of the anterior ends of the related epiphyseal cartilages (Figures 3, 4, 5, 7 A).

This process was clearly seen in the twenty-six day specimens.

Islands of enchondral ossification were frequently seen on the thirty-nine day and even on the twenty-six day lesions. Ossification was noted at the level of the related epiphyseal cartilage ends, also as smaller or greater islands dispersed through the herniated cartilage (Figure 5 and 5 A).

Newly formed vessels could be observed in thirty-nine-day lesions and even earlier. Localized and dispersed through the herniated

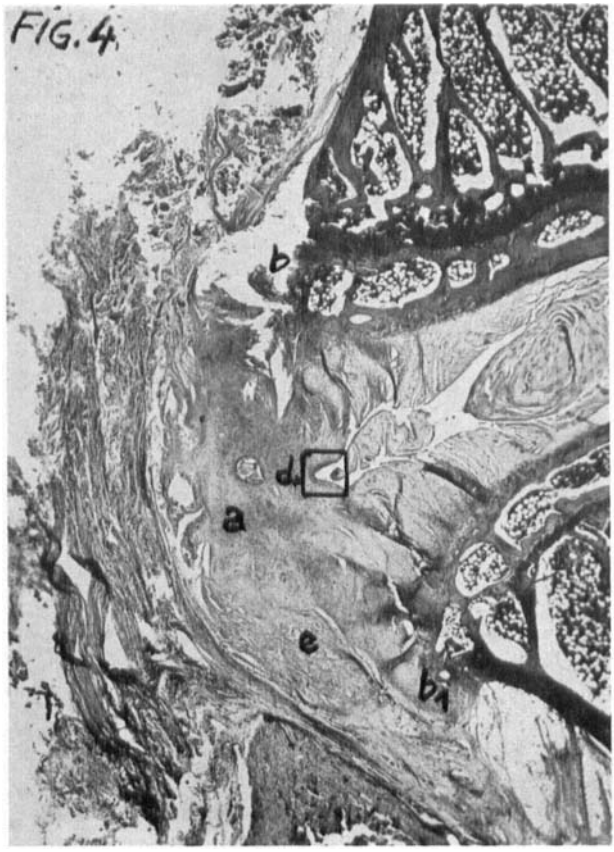


Figure 4. Intervertebral disc lesion twenty-six days after operation, control rabbit. Continuity is established between the herniated cartilage (a) and the anterior ends of the related epiphyseal cartilages (b, b1); e: blood vessels.

cartilage, they increased in number in the areas of enchondral ossification (Figure 7 A).

In most of the microscopic sections these vessels, originating from the external structures, penetrated into the herniated cartilage at the level of the anterior ends of the related epiphyseal cartilage.

Fibrotic tissue was very poorly represented in all the specimens examined. Usually the thirty-nine-day specimens showed a thick herniated cartilage covering the lesion of the annulus and establishing more or less continuity with the related epiphyseal cartilage ends (Figure 5).

However, in a smaller number of specimens from thirty-nine-day

Figure 4A: Higher magnitude (205 ×) of fragment (d) in Figure 4. Enchondral ossification of the herniated cartilage blocking the lesion of the annulus fibrosus.

disc lesions, the persistence of the nucleus pulposus could be observed as a large protruding tissue filling the opening in the annulus fibrosus and bursting outside without undergoing chondroid transformation after herniation, at least in the central zone (Figures 6 and 6 A).

This means that there are two posibilities in the evolution of the disc lesions, depending on the persistence of part of the nucleus pulposus or its entire chondroid transformation.

DISCUSSION

Experimental disc lesions in animals have been studied and reported by Filippi (1935) in rabbits, Key & Ford (1948) and Bercovici & Paraschivesco (1968) in dogs, and Lindblom (1952) in rat tails.

Bercovici & Paraschivesco produced disc degeneration by intradiscal injection of hyaluronidase (1958).

Lindblom fixed rat tails in the shape of "U" for various periods of time (1952) and succeeded in reproducing disc degeneration.

Key & Ford (1948) used a posterior approach in dogs; in four

Figure 5. Intervertebral disc lesion, thirty-nine-days old, from ACTH-injected rabbit showing enchondral ossification (c) of the herniated cartilage (a) and continuity established between the epiphyseal cartilage (b) and the herniated cartilage (a).

intervertebral lumbar discs they inflicted lesions of various type and degree by puncture, incision, excision, and excision followed by vigorous curettage. However, they only described the results of disc excision followed by vigorous curettage, which was the central problem of thier work. At the beginning of our experiment, we tried to use the posterior approach to the intervertebral disc in rabbits, the same approach Key & Ford have used in dogs. However, the procedure proved very traumatic, as it was necessary to remove most of the bone tissue due to the overlying laminae. We therefore decided to reach the intervertebral disc through the anterior abdominal approach, which proved easy and less traumatic, and the animals recovered shortly after operation. At this time we were not aware of the work of Filippi (1935) who had used a method in rabbits similar to ours and had produced similar lesions; however, he described the healing process following the sequence: lesion, granulation tissue, fibrosis, restitution; fibrocartilaginous regeneration of the annulus beginning within twenty days and completed after forty days, impaired mobility of the related vertebra, and final *restitution ad integrum.*

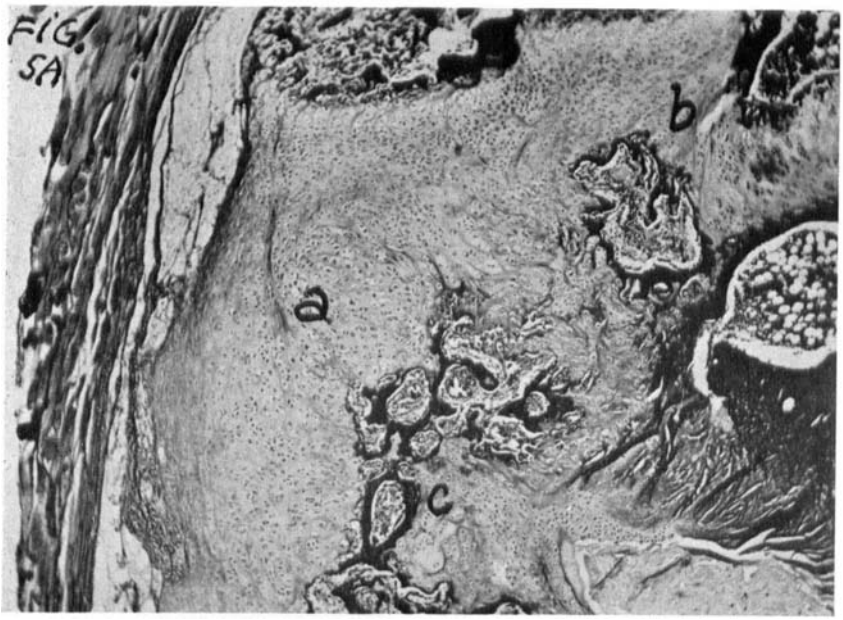


Figure 5A. Same lesion, higher magnification of the quadrant. The islands of ossified herniated cartilage and the continuity between the latter and the epiphyseal cartilage are more clearly shown (b).

We did not observe any sign of a fibrocartilaginous regeneration of the annulus in our experiment. The cut ends of the annulus always appeared inert, without blood vessels, and did not manifest any regenerative tendency following the lesion. Such false impressions of regeneration or *restitution ad integrum* of the annulus were found when looking at microscopical specimens cut some distance from the lesion which therefore did not include the lesion.

In our experiment, the healing of the disc lesion proved to be a process involving especially on one side, the herniated cartilaginous tissue deriving from the nucleus pulposus and, on the other side, the related ends of the epiphyseal cartilage. In the first stage, the defect in the annulus is covered by the herniated nucleus which undergoes a chondroid transformation. The traject of the lesion is clearly visible. This aspect was seen on the thirteen-day lesions. Around twenty-six days the related ends of the epiphyseal cartilages begin to establish continuity with the herniated cartilage. Signs of enchondral ossification are more evident in the thirty-nine day lesions. The enchondral ossification could be remarked as smaller or greater islands localized

Figure 6. Unusual appearance of thirty-nine-day old intervertebral disc lesion of ACTH-injected rabbit. The large protrusion of the nucleus pulposus, surrounded on its upper and lower sides by previously herniated cartilage, gives the appearance of a fresh re-herniation.

in the central part of the herniated cartilage or near the related epiphyseal cartilage ends.

The animals which received small and even higher doses of ACTH did not present any considerable change of the described aspect and evolution of the disc lesions. Previous studies concerning the action of ACTH/cortisone on articular processes were concentrated mostly upon rheumatoid effects. Philip Hench (1951) described reduction of the cellular reaction with decreased numbers of plasma cells and lymphocytes, reduction or absence of deposition of fibrin, and lessened edema following ACTH/cortisone administration. Coste, Piguet, Delbare & Bourel (1952) stated that the longitudinal growth of collagen fibers by apposition of structures is inhibited by cortisone. R. H. Ramsay & Key (1955), on experimental talc arthritis in rabbits, found the vascular dilatation element of inflammation quite diminished, and there were fewer infiltrating phagocytes following cortisone. No influence was observed on elastic fibers.

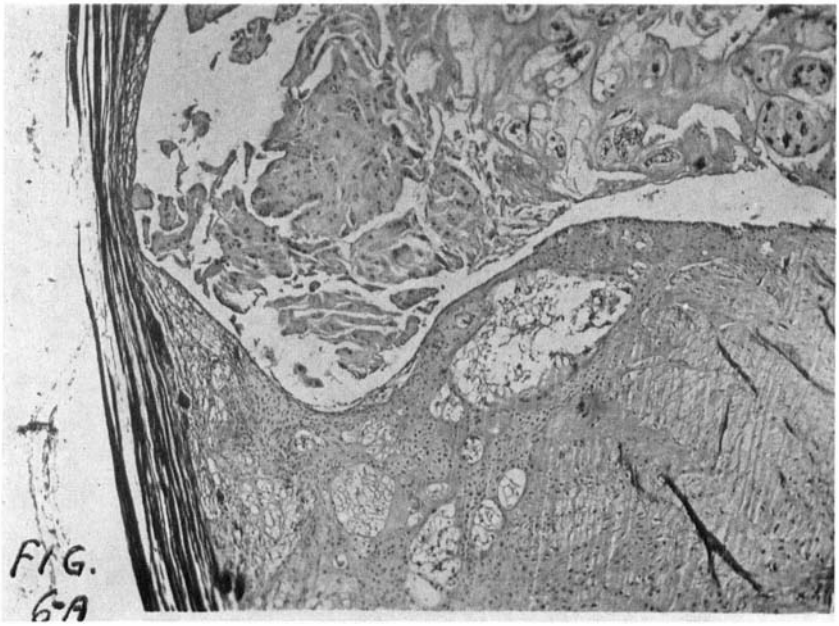
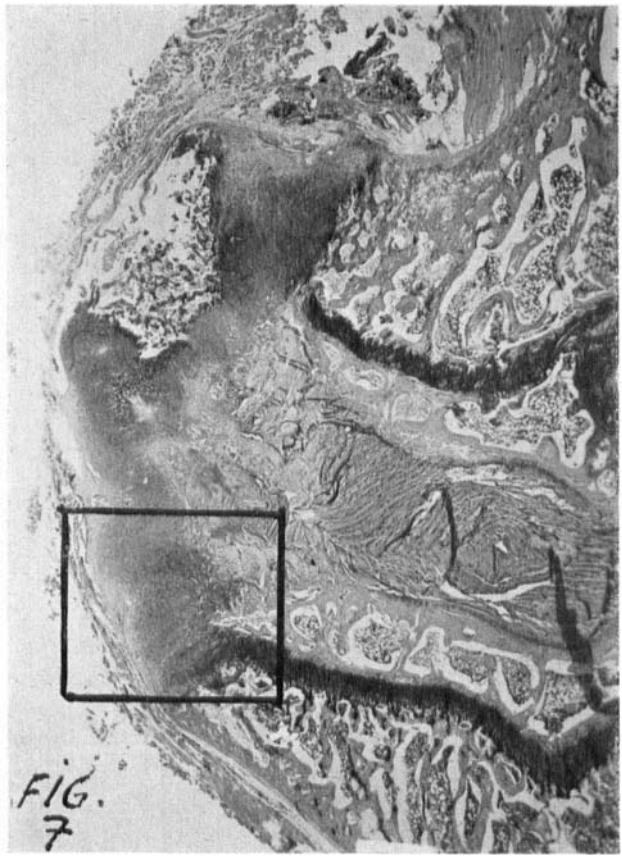


Figure 6A. Same lesion seen with higher magnification.

Generally the vascular structure reflecting inflammatory reactions of an articulation appears in the sinovial tissue. The absence of sinovial tissue reduces the intervertebral disc to an avascular unit until advanced degeneration allows the penetration of small blood vessels into the annulus.

Since disc lesion is reduced to avascular and low metabolic cartilaginous tissue, intramuscularly administred ACTH could not reach high enough concentrations at this place to influence the evolution of the disc lesions. In order to reach a high local concentration of hydrocortisone, Feffer (1956) used direct intradiscal administration and obtained a definite clinical improvement in a considerable number of patients with disc lesions. This action of hydrocortisone was explained as polymerization of the connective tissue polysacharides. Further experiments are needed for a better understanding of the histologic changes following intradiscal hydrocortisone administration. The observations in this experiment are valuable in understanding similar processes in humans. Structurally the main difference between the intervertebral disc of the rabbit and the human (Coventry



Figures 7 and 7A. Fifty-five-day old intervertebral disc lesion from ACTH-treated rabbit, showing enchondral ossification and the continuity of the herniated cartilage and the related epiphyseal cartilages.

et al. 1945), lies in the absence of the cartilage plates and reduced viscosity of the nucleus pulposus in rabbit (Figures 2, 8, and 9).

In the rabbit the epiphyseal cartilages, whose anterior and posterior ends are bent toward the intervertebral disc, take part in the processes following lesion of the disc.

The standing posture with the resulting increased pressure and strain over the disc, peculiar to the human, is nullified in the prone position. Therefore we can conceive that the process of disc lesion in the human at rest follows a similar course as described in our experiment.

Many anterior disc lesions, such as we induced in rabbits, may be

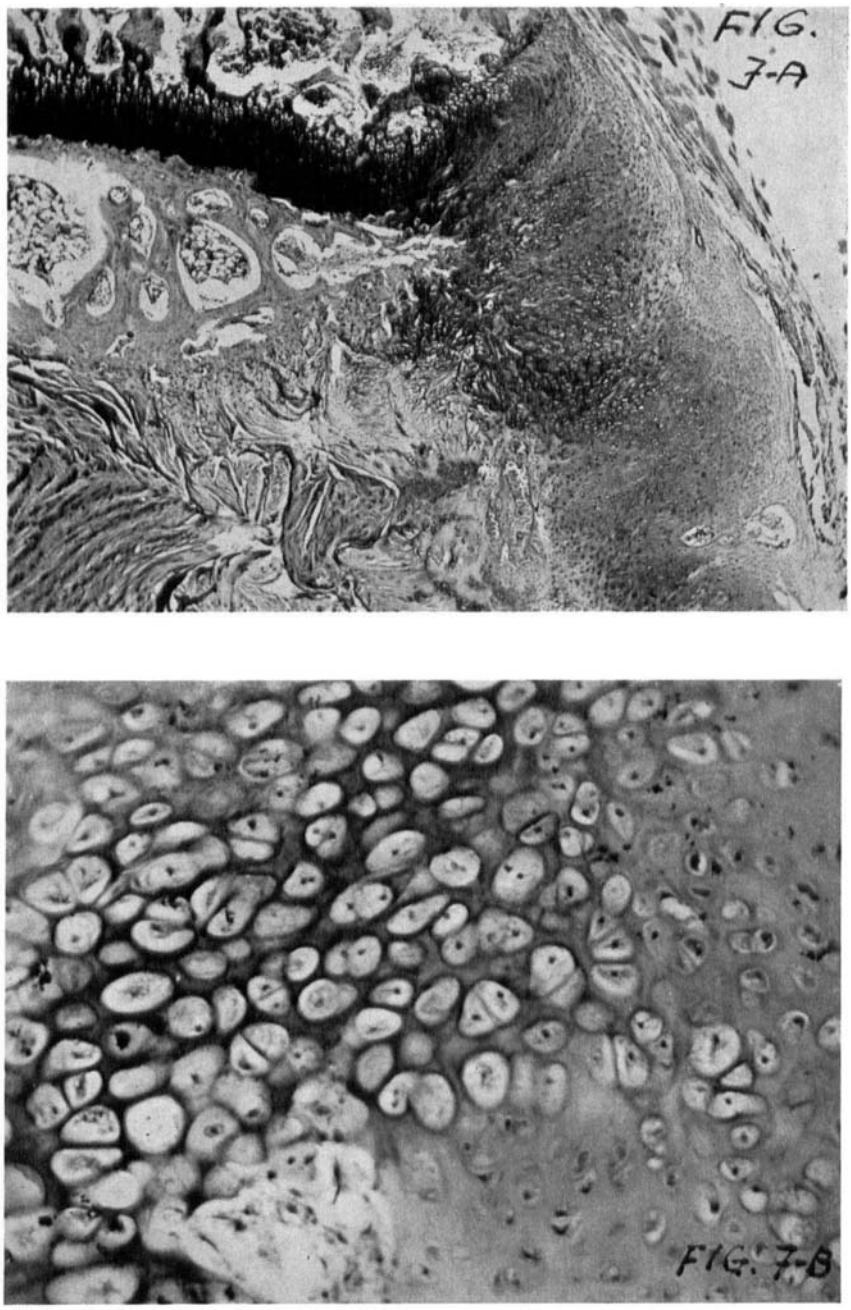


Figure 7B. Same lesion at higher magnification, showing the process of calcification and enchondral ossification of the herniated cartilage.

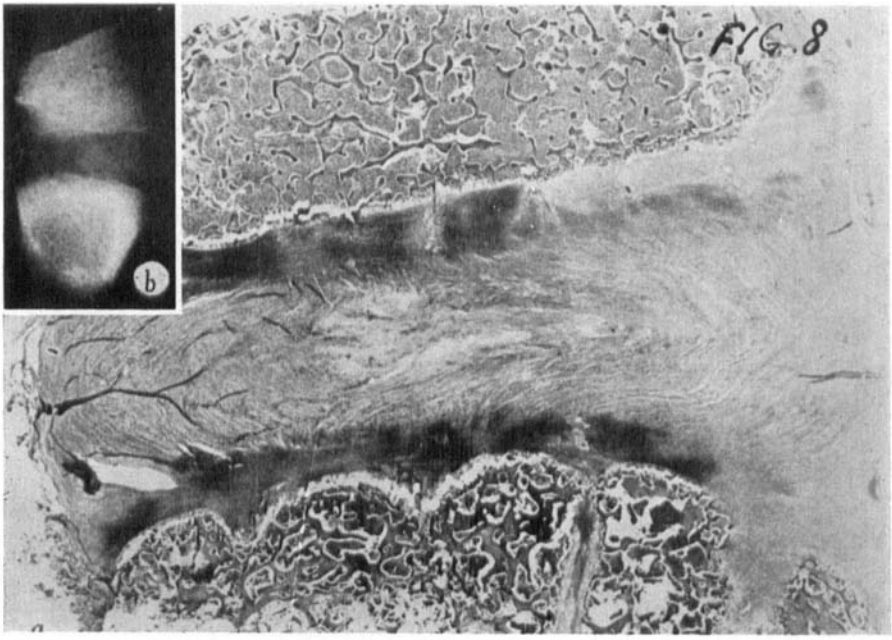


Figure 8. Normal intervertebral disc from young human, showing particularly the cartilage plates.

observed among the low-back pain patients and were verified by typical long-lasting changes in the X-rays.

The scars following disc lesions observed in our experiment were hard in consistency and adherent to the prevertebral muscles. Although of reduced clinical significance when the lesions are anteriorly placed, it is easy to understand the serious neurologic compression pattern arising when the lesion develops posteriorly in the narrow spinal canal with the nearby nerve structures.

It is generally accepted that in most cases of acute disc conditions, benign lesions may be found varying from a swollen disc with intact annulus—a practically reversible process—to a small partial annulus rupture healing through granulation tissue and fibrous scar when advanced degenerative changes of the disc are present at the time when the lesion occurs (Hirsch & Schajowicz 1952).

Complete rupture of the annulus fibrosus, considered to be a rare occurrence, was found by Feffer (1956) in fifteen of sixteen surgically explored cases in a material of fifty-five well-diagnosed acute disc lesion cases.

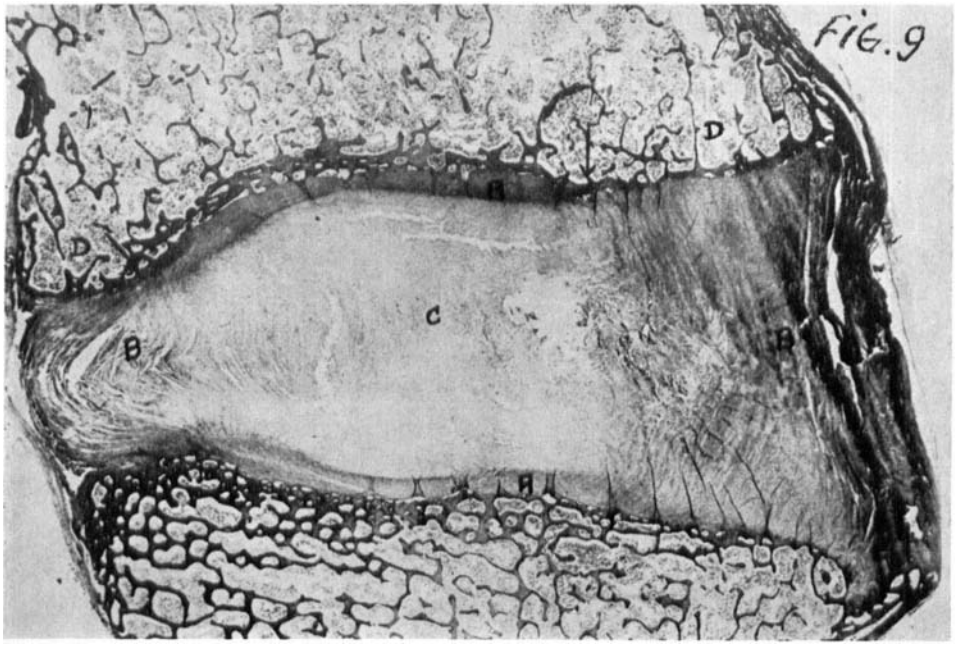


Figure 9. Normal intervertebral disc of older human. (Reproduced from Coventry, Ghormley & Kernohan (1945b) J. Bone Jt Surg. 27-A, 233–247).

In these fifteen cases he found complete rupture of the annulus fibrosus with free cartilage either in the canal or beneath the posterior longitudinal ligament. This means that up to 27 per cent of the cases, resistant to conservative treatment and selected for surgical exploration, may present a complete rupture of the annulus similar to the processes induced and studied in rabbits.

SUMMARY

1. Lesions of the lumbar discs were produced and studied in rabbits.

2. ACTH was administered to sixteen rabbits, and another group of sixteen rabbits with similar lumbar disc lesions served as control. The animals were sacrificed at intervals of 13, 26, 39, and 55 days after operation. The total amount of ACTH received by each rabbit varied from 50 U to 200 U.

3. No change of the lumbar spine mobility following the disc lesions was observed during the experiment.

4. Small anterior osteophytes were revealed in the X-ray pictures

of the lumbar spines from the thirty-nine day sacrificed control and ACTH-injected animals.

5. Healing of the disc lesions does not progress through granulation tissue-fibrosis-restitution *ad integrum*, as previously described by Filippi, but only represents a process involving cartilaginous and osteocartilaginous tissues. In the first stage, the lesion of the annulus fibrosus is covered by the herniated nucleus pulposus reaching the aspect of a proliferating cartilage; in the second stage, the related epiphyseal cartilage ends manifest a proliferative tendency toward the herniated cartilage, which may lead to establishment of continuity between both tissues; a process of enchondral ossificatin at the level of the herniated cartilage characterizess the third stage.

6. Repeated administration of ACTH did not lead to any visible change of the appearance and evolution of the disc lesions. It is supposed that ACTH, intramuscularly administered, could not penetrate and reach high enough concentrations at the level of the avascular intervertebral disc to interfere significantly with the evolution of the disc lesions. Such a condition may be corrected by direct intradiscal administration of hydrocortisone.

7. Up to 27 per cent of well-diagnosed acute disc lesions, resistant to conservative treatment and selected for operation, may present a complete rupture of the annulus whose healing process may reproduce the features described in rabbit.

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