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IN VITRO DIFFUSION OF DYE THROUGH THE END-PLATES AND THE ANNULUS FIBROSUS OF HUMAN LUMBAR INTER-VERTEBRAL DISCS

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The nutritional pathways into the nucleus pulposus of human intervertebral discs have aroused interest since histological examination demonstrated the lack of a direct vascular supply after the age of 25 years (Böhmgig 1930, Schmorl 1930, Übermuth 1930, Coventry, Ghormley & Kernohan 1945, Hirsch & Schajowicz 1952). It has been postulated that diffusion through the end-plates is the main nutritional route, a postulate confirmed in the adolescent rabbit by the finding that this structure is permeable (Brodin 1955, Hansen 1959). These authors suggested that the annulus fibrosus might also be a nutritional route, but of minor significance. On the other hand, recent studies suggest that the nutrition of adult (but not adolescent) articular cartilage depends entirely or almost entirely on the transsynovial route, since the calcified zone of the cartilage constitutes a virtually impermeable barrier (Collins 1949, Mankin 1963, Stockwell & Barnett 1964, Maroudas 1968, Maroudas, Bullough, Swanson & Freeman 1968, McKibbin & Holdsworth 1968). It thus seemed possible that the end-plates of the discs, where a calcified zone is present (Coventry, Ghormley & Kernohan 1945, Eckert & Decker 1957), might also be impermeable and that hence no nutritional route might be open through them. Although the bone/cartilage interface or the calcified zone of the cartilage appears to present an impermeable barrier to the passage of nutrients from blood vessels in the bone to the cartilage, this barrier is not always present. Thus in animals and man, before skeletal maturity, vessels are in con-

tact with cartilage at the endochondral ossification front, and the bone cartilage interface is permeable in adolescent cadaver specimens in man (Maroudas, Bullough, Swanson & Freeman 1968). In adult man localized communications exist between the medullary cavity and the articular cartilage in certain joint surfaces. They are present, for example, in the femoral head but not in the acetabulum (Greenwald & Haynes 1969) and not (by inference) in the femoral condyles (Maroudas, Bullough, Swanson & Freeman 1968). The nutritional significance of these communications is uncertain.

Keyes & Compere (1932) and Coventry et al. (1945) thought that degenerative changes of the disc were responsible for back pain. It is perhaps fair to summarize the present view by saying that disc degeneration is thought to be capable of producing back pain and sciatica, although the exact mechanism of pain production and the precise relationship between the morbid anatomy and the symptomatology are uncertain. Disc degeneration is characterized histologically by loss of tissue in the nucleus, increasing thickness of the collagen fibres, and the occurrence of fissures both in the centre and in the periphery of the disc. Insufficient diffusion into the disc has been said to account for premature disc degeneration (Böhmig 1930, Schmorl 1930, Übermuth 1930).

In a study of the pH of discs of patients operated upon for lumbar rhizopathy, a marked decrease of pH was noted in some discs (Nachemson 1969). These cases also showed an abundance of connective tissue scarring around the nerve roots. In other subjects in the same study, the pH of both normal and prolapsed discs varied between 6.5 and 7.4, with a lower value for those discs showing degenerative changes on ordinary radiographs. A number of mechanisms could have caused this increase in hydrogen ion concentration, but a separate study demonstrated that the main factor was probably an increased concentration of lactic acid, the concentration of which was found to be directly correlated with the hydrogen ion concentration of the nucleus (Diamant et al. 1968). Lactic acid is always present when intermediate carbohydrate metabolism is proceeding under anaerobic conditions, and thus it might be inferred that oxygenation of and hence diffusion into the acidotic discs was impaired.

In view of these findings, the present study was performed (1) to examine diffusion into the nucleus through the end-plates and the annulus fibrosus, and (2) to test the possibility that in some cases one or the other of these routes was blocked.

Table 1. *The investigated material.*

Spine no.	Age	Sex	Main cause of death	No. of days before postmortem (+2°C)	No. of days between postmortem and test (-20°C)	Discs tested	Macroscopic exam. of deg. Grade 0-3
1	83	♀	Circul. insuff.	2	14	L3	3
			Cardiosclerosis			L4	3
2	75	♀	Cardiosclerosis	1	1	L3	2
			Cerebral hemorrh.			L4	2
3	74	♀	Myocardial infarct	3	2	L2	3
			Pancreatitis			L3	3
4	69	♀		1	5	L3	3
						L4	3
5	68	♀	Pulmonary embolism	7	4	L3	3
			Malign. brain tumor			L4	3
6	64	♀		1	3	L3	3
						L4	3
7	62	♀	Skull fractures	2	21	L3	3
						L4	3
8	53	♂	Circul. insuff.	3	0	L3	1
			Cardiosclerosis			L4	3
9	52	♀	Ca. recti	1	1	L3	2
						L4	3
10	47	♂	Cerebral contusion	2	2	L3	2
						L4	2

Table 1. *The investigated material. (cont.)*

Spine no.	Age	Sex	Main cause of death	No. of days before postmortem (+2°C)	No. of days between postmortem and test (-20°C)	Discs tested	Macroscopic exam. of deg. (Grade 0-3)
11	44	♂	Ca. ventriculi	2	7	L3	2
12	34	♂	Hepatic rupture	1	8	L4	2
						L3	2
						L4	3
13	33	♀	Intrapleural hemorrhage	1	3	L3	1
						L2	1
14	24	♀	Drug intoxic.	3	3	L3	1
						L4	1
15	22	♂	Ethyl intoxic.	4	4	L3	1
						L4	1
16	18	♂	Skull fractures	3	1	L3	1
						L4	1
17	11	♀	Pulmonary embolism	3	2	L3	0
						L4	0
18	5	♀	Cerebral hemorrhage	4	4	L3	0
						L4	0
19	0	♂	Pneumonia	3	0	L4	0
						L5	0

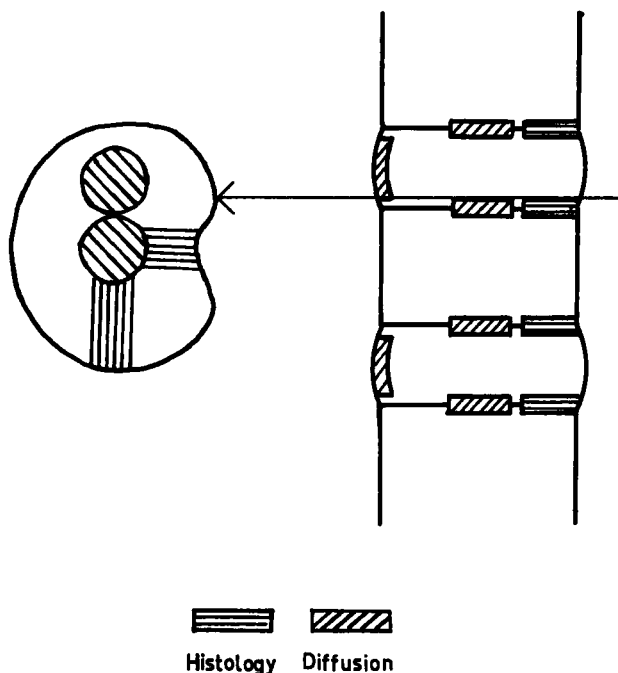


Figure 1. Drawing showing the sites from which specimens were taken for diffusion and histological studies.

MATERIAL AND METHODS

This study was designed to investigate qualitatively the diffusion of the dye disulphine blue V through the annulus fibrosus and the upper and lower end-plates of human cadaver lumbar discs. This dye was chosen because it diffuses readily through articular cartilage, without showing specific interactions with it.

The discs between the third and fourth (the L_3 disc) and the fourth and fifth (the L_4 disc) lumbar vertebrae from post-mortem specimens were used. A total of 38 discs (76 end-plates) from 19 individuals, representing at least four different discs from each decade, were studied (Table 1). The material was collected immediately after post-mortem and stored at -20°C . As seen from Table 1, the number of days between death and post-mortem examination varied between 1 and 7 (mean 2 days).

Lateral and frontal radiographs were taken of the spines to exclude pathological changes in the vertebral bodies and also to obtain a rough estimate of the state of the discs. Since the discs were also examined macroscopically (a more reliable method of assessing their condition according to Friberg & Hirsch 1950), the evaluation of the radiographs has not been included in Table 1. The spines were then cleansed of muscles and ligaments and the discs L_3 and L_4 were removed through cuts in the adjacent vertebral bodies.

The anterior portion of the annulus fibrosus was then removed and the disc

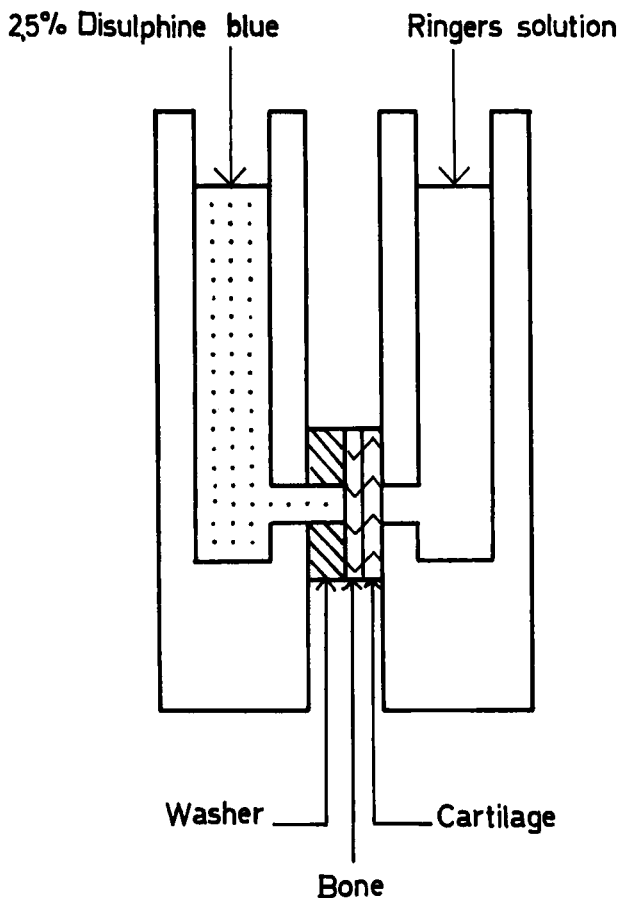


Figure 2. Drawing showing the construction of the diffusion cell.

was sectioned horizontally through its centre and photographed. On the cut disc, the degree of degeneration was evaluated on a 0-3 scale according to the criteria used earlier by among others Friberg & Hirsch (1950), Nachemson (1960) and Galante (1967).

Sections were prepared for diffusion by removing the soft material of the nucleus pulposus down to the cartilaginous end-plate, taking care to avoid damage of the cartilage itself. Circular specimens with a diameter of 10 mm were then cut with a special tool from the middle and lateral portions of the upper and lower end-plates (Figure 1). The vertebral part was ground with sandpaper almost down to the subchondral bone plate. The final thickness of the specimen varied from 3 to 4 mm.

The annulus fibrosus was sliced vertically to produce a specimen whose thickness varied from 2 to 3 mm.

The prepared specimens were placed in the specially constructed diffusion cham-

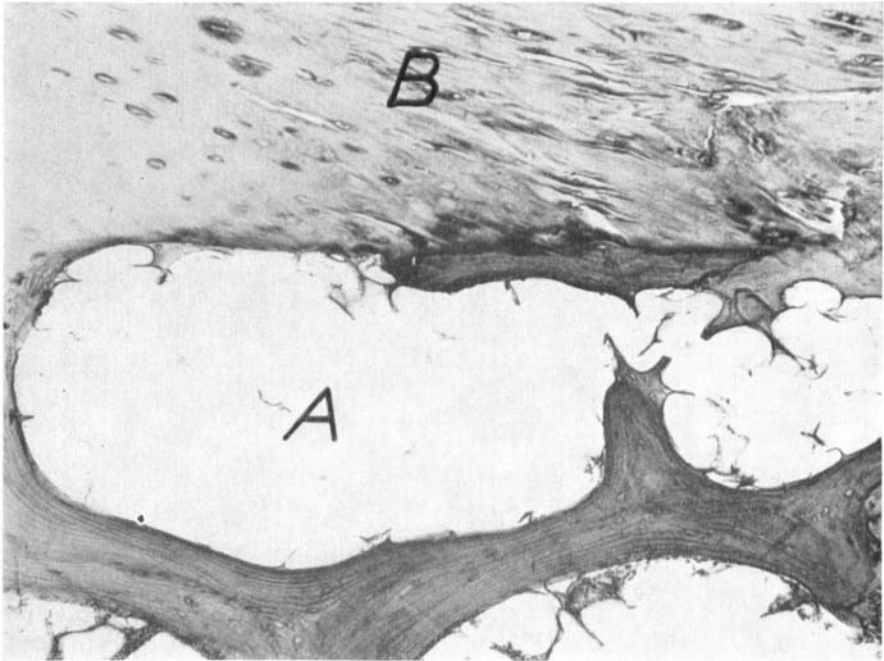


Figure 3 a. Lateral portion of vertebra with wide ampulla-like contact between medullary cavity (A) and end-plate cartilage (B). Case 5. Age 68.

ber described by Maroudas et al. (1969). In order to prevent leakage round the edges of the specimen, the specimen was fitted with rubber washers on the bony side before being clamped in the diffusion cell. One compartment of the diffusion cell was filled with 10 ml of Ringer's solution, and 10 ml of 2 % disulphine blue dye in Ringer's solution was added to the other compartment (Figure 2). The appearance of the dye was noted in the first compartment as it diffused through the specimen. The area of the specimen exposed to the dye was 35 mm² (Figure 2). The dye solution was always placed in contact with the vertebral side of the end-plate. The diffusion experiments lasted 24 hours and were conducted at +4°C. If at the end of this time no dye was seen to have penetrated the specimen, the experiment was continued for a further 24 hours.

Since this study was designed to be purely qualitative, no attempt was made to measure the amount of dye which penetrated the preparations. The results were regarded as positive if dye traversed the specimen so as to be visible in the Ringer's solution on the other side of the diffusion chamber, and as negative if no dye penetrated after 48 hours.

After completion of the diffusion experiment, the end-plates were sectioned for naked eye inspection and for histological examination. For the latter, the specimens were fixed in 10 % formaldehyde solution and then decalcified in a mixture of equal volumes of concentrated formic acid and 7 % sodium formate solution. Each decalcified specimen was embedded in paraffin and 3-4 sections approximately 10 μ

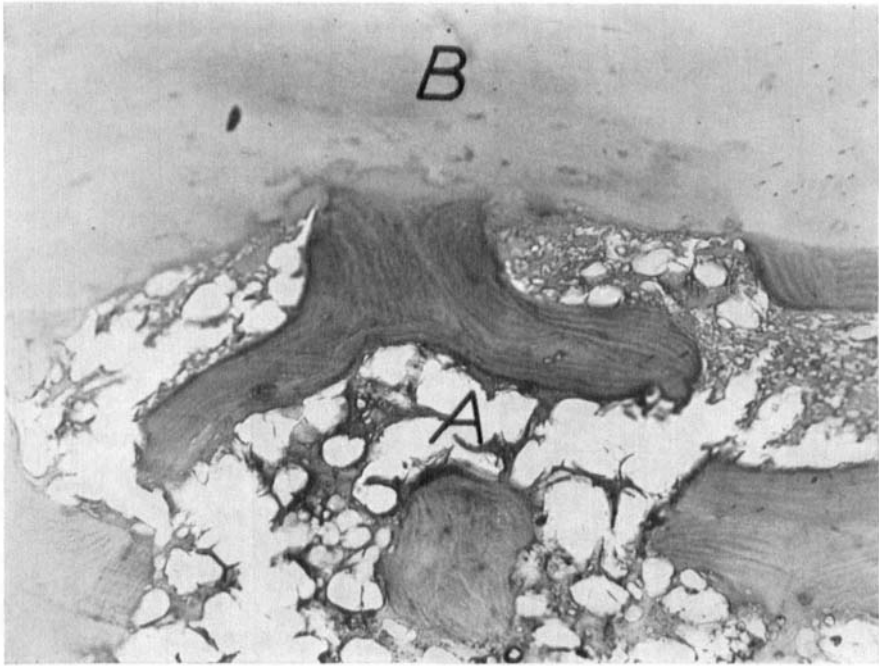


Figure 3 b. Central portion of vertebra with wide ampulla-like contact between medullary cavity (A) and end-plate cartilage (B). Case 12. Age 34.

thick were cut from it on every second millimeter on a standard microtome. In this fashion a representative histological sample of the total specimen could be obtained.

In every lateral specimen the histological appearance of the end-plate was judged from a section immediately adjacent to the specimen prepared for diffusion (Figure 1). In addition, 10 of the central specimens subjected to diffusion experiments were themselves subsequently prepared for histological examination. In all, 72 end-plates from 18 individuals were examined histologically. Due to technical difficulties, 6 end-plates were omitted from this part of the study (cases 4, 12, 14).

The histological status of the end-plates was grouped on a 0 to 3 scale (Lewin 1964) as follows:

Grade 0: Hyaline end-plate cartilage without any degenerative changes. Smooth subchondral bone plates.

Grade 1: Minimal changes in the cartilage, such as cell proliferation and/or paucity of cells with or without minor cartilage necrosis.

Grade 2: Necrosis of cartilage and the beginning of connective tissue in-growth from the subchondral bone which shows sclerosis and/or atrophy.

Grade 3: Cartilage completely destroyed in many areas where it is replaced by connective tissue. Advanced subchondral remodeling.

Special note was made of the existence of the contact between the end-plate cartilage and the medullary cavity as demonstrated in Figure 3.

The histological investigation was performed without knowledge of the results of the diffusion experiments.

RESULTS

Overall results

The overall results are presented in Table 2. It can be seen that in almost all cases the central portion of the end-plates was permeable. Thus the upper central portion was permeable in 33 of 38 studied end-plates of the L₃ and L₄ discs. In the central part of their lower end-plates, permeability was seen in 33 of 37 examined. The annulus fibrosus was also usually permeable: 27 specimens were permeable, only 5 were impermeable. In contrast, the lateral part of the end-plate was usually impermeable. Thus the upper as well as the lower lateral portion of 36 end-plates of the L₃ and L₄ discs was impermeable in 27 specimens.

The measurements of permeability on the annulus are particularly exposed to the criticism that the exact thickness of the specimen was not measured. The apparently impermeable specimens may therefore have been the thickest, so that the dye did not have time to traverse the whole specimen. Since inspection of the impermeable specimens revealed the presence of dye in the annulus, and since the annulus does not contain any structure comparable to the calcified zone of cartilage which might arrest the movement of dye within the thickness of the structure, eventual penetration of the annulus even in the apparently impermeable specimens would seem to have been inevitable. In contrast, in the impermeable end-plate specimens, the dye was found to be sharply held up at the calcified zone.*

Histological studies

The following general observations should first be stressed. The microscopical picture of the bony end-plate and the hyaline cartilage plate fits well into the earlier observations by Böhmig (1930), Schajowicz (1938), and Coventry, Ghormley & Kernohan (1945), up to age 25–30 years. The same holds true regarding the picture of degenerative changes in all ages. In the histological appearance of the bony end-plate after age 30, however, we found that earlier investigations have not taken into consideration the existence of communications between the

* We could not reproduce a photograph here because black and white did not show the features we described and a colour reproduction was too expensive.

Table 2. Results of diffusion

Spine no.	Age years	Disc	Upper central	End-plate		Lower lateral
				Upper lateral	Lower central	
1	83	L3	+	—	—	—
		L4	+	—	—	—
2	75	L3	+	—	+	—
		L4	+	—	+	—
3	74	L2	+	—	+	—
		L3	+	—	+	+
4	69	L3	+	—	+	—
		L4	+	—	+	—
5	68	L3	+	—	+	+
		L4	+	+	+	+
6	64	L3	+	+	+	—
		L4	+	—	+	+
7	62	L3	+	+	—	—
		L4	—	—	not perf.	—
8	53	L3	+	+	+	+
		L4	+	—	+	—
9	52	L3	+	+	+	+
		L4	—	—	—	—
10	47	L3	+	—	+	—
		L4	—	+	+	—
11	44	L3	+	+	+	—
		L4	+	+	+	—
12	34	L3	+	—	+	—
		L4	+	+	+	+
13	33	L3	—	—	+	—
		L2	—	—	+	—
14	24	L3	+	—	+	—
		L4	+	—	+	—
15	22	L3	+	—	+	—
		L4	+	—	+	—
16	18	L3	+	—	+	—
		L4	+	—	+	—

experiments and anatomic studies.

Annulus ventral part	Macrosc. exam. of disc deg. Grade 0-3	Microsc. exam. of deg. Grade 0-3	Vascular buds observed			
			Upper central	Upper lateral	Lower central	Lower lateral
—	3	1-3	×	—	×	—
not perf.	3	1-3	×	—	×	—
—	2	0-2	×	—	×	—
—	2	0-2	×	—	×	—
+	3	0-2	×	—	×	—
+	3	0-2	×	—	×	+
+	3	Not available				
+	3	Not available				
+	3	2-3	×	—	×	+
+	3	2-3	×	+	×	+
+	3	1-3	×	+	×	—
+	3	0-3	×	—	×	+
+	3	1-3	×	+	×	—
+	3	1-3	×	—	×	—
—	1	1-2	×	+	×	+
—	3	1-2	×	—	×	—
+	2	0	×	+	×	+
not perf.	3	0	×	—	×	—
+	2	0	×	+	×	—
+	2	0	×	+	×	—
not perf.	2	0	×	+	×	—
not perf.	2	0	×	+	×	—
+	2	1-3	+	×	+	×
+	3	1-3	+	×	+	×
+	1	1	×	—	×	—
+	1	1	×	—	×	—
+	1	1	+	×	+	×
+	1	1	+	×	+	×
+	1	0	×	—	×	—
+	1	0	×	—	×	—
+	1	0	+	—	+	—
+	1	0	+	—	+	—

Table 2. Results of diffusion experiments

Spine no.	Age years	Disc	Upper central	End-plate		Lower lateral
				Upper lateral	Lower central	
17	11	L3	+	—	+	+
		L4	+	—	+	+
18	5	L3	+	—	+	—
		L4	+	—	+	—
19	0	L4	+	not perf.	+	not perf.
		L5	+	not perf.	+	not perf.

× = not examined histologically.

end-plate cartilage and the medullary cavity of the vertebral body (Figure 3). Such communications between the medullary cavity and the articular cartilage are known to exist in synovial joints of man (Greenwald & Haynes 1969).

Permeability vs histological appearance of the end-plate

The histological appearance of the end-plate sections on the 0–3 scale showed no correlation with diffusion through the annulus nor through the two areas of the end-plate (Table 2). In this connection it should be pointed out that in cases with high grades of end-plate degeneration, numerous scattered areas showed little or no degenerative changes.

Permeability vs the age of the specimen

No correlation was observed between the permeability and the age of the specimen, except in the central portion of the end-plate where an apparent decrease in permeability with advancing age could be attributed to the appearance of significant macroscopic degeneration in the older age groups (see *Permeability vs macroscopic disc degeneration*).

Permeability vs macroscopic disc degeneration

A correlation was sought between the degree of degeneration as assessed by the naked eye appearance of the disc and the permeability of the three groups of specimens. A significant positive correlation was found between discs with grades 2 and 3 degeneration and impermeability of

and anatomical studies (cont.).

Annulus ventral part	Macrosc. exam. of disc deg. Grade 0-3	Microsc. exam. of deg. Grade 0-3	Vascular buds observed			
			Upper central	Upper lateral	Lower central	Lower lateral
++	0	0	+	—	+	+
++	0	0	+	—	+	+
+	0	0	×	—	×	—
+	0	0	×	—	×	—
not perf.	0	0	+	×	+	×
not perf.	0	0	+	×	+	×

the central portion of the end-plate. Thus 9 specimens were impermeable, whereas only 5 were expected to be so assuming an equal distribution between permeable and impermeable specimens vs degree of disc degeneration. No correlation was found between degeneration and permeability of the lateral portion of the end-plate, but of 8 end-plates with impermeability both of the lateral and central portion, 6 were found to belong to discs with grade 2 or 3 degeneration. From the hypothesis of equal distribution of specimens with impermeability between discs with grade 0 or 1 and discs with grade 2 or 3 degeneration, only 2.5 were expected. Thus a significant positive correlation exists between disc degeneration of grade 2 or 3 and impermeability of the end-plate.

Between disc degeneration and permeability of the annulus fibrosus no correlation was found. There were only five impermeable annulus specimens. This is probably due to an experimental artifact, since it has been argued that even the apparently impermeable specimens of the annulus would have eventually allowed dye penetration.

Permeability vs the presence of contacts between the end-plate cartilage and the narrow cavity

Histologically, 8 totally impermeable end-plates showed an absence of contacts between the end-plate cartilage and the medullary cavity of the underlying bone, and these plates were markedly thicker and more dense than were end-plates in which such contacts were present. The histology of the lateral portion of the end-plate was studied in 60

specimens (Table 2). In all 16 cases where diffusion occurred through the lateral part of the end-plate, there were marrow contacts with the cartilage in the histological sections. In contrast, the remaining 44 lateral end-plate specimens showed no diffusion and no such contacts. In the 20 specimens of the central portion of the end-plate which were examined histologically, contacts between marrow spaces and end-plate cartilage were present. All these 20 specimens were permeable.

Thus specimens possessing such contacts were invariably permeable, and specimens lacking these contacts were invariably impermeable.

DISCUSSION

The method used on this study to examine the permeability of the end-plates of the lumbar discs and of the annulus obviously does not closely simulate the physiological situation. It does, however, have the advantage over more physiological methods that the permeability of defined areas of these tissues can be examined and that human material can be used. The method departs from a physiological situation in two ways: the tissue is dead and has been subjected to storage, and the transport of dye through the specimen examined depends entirely upon diffusion.

The effect upon permeability of death and subsequent storage has been examined by Maroudas et al. (1969), who concluded that no significant change from the situation obtaining in life could be attributed to death itself, to storage for a short time at $+4^{\circ}\text{C}$, nor to storage for longer periods at -20°C . It would thus seem reasonable to suppose that the permeability of the tissues examined in this experiment was similar to their permeability in life.

No quantitative conclusions can be drawn from this study since the thickness of the specimens in the diffusion chambers could not be accurately controlled. Thus if a specimen was shown to be permeable, it could be concluded that a nutritional route through the specimen in question was potentially open in life, but no conclusion could be drawn as to the extent to which the nutrition of the disc might have depended upon the route in question. On the other hand, if a specimen of the end-plate, but not of the annulus, was demonstrated to be impermeable, it could be concluded that no physiologically significant nutrition could have occurred through that specimen. Impermeability in this experimental situation implies that the tissue itself was impermeable and that it contained no pores (in life containing, for example, blood ves-

sels) through which the material might have "leaked". Thus transport through the material in life could not have occurred. The impermeable specimens were examined for no longer than 48 hours, and it might have been the case that, had they been examined for longer, some dye would have penetrated the apparently impermeable specimens. Penetration after this length of time would imply such a reduced permeability that from the nutritional standpoint it seems reasonable to regard the specimen as having been impermeable. Our observation that the dye was sharply held up at the calcified zone implies that there was a block to permeability at this level in the end-plate specimens and therefore suggests that even if the specimens had been left for a longer period of time, no dye would have penetrated them. On the other hand we believe, for reasons advanced above (see Results), that apparently impermeable specimens of the annulus would have allowed dye penetration if given sufficient time.

To summarize, we therefore feel that this method can be used to determine which routes are nutritionally insignificant but that it cannot be used to provide a quantitative comparison of the relative significance of the various potentially available routes.

We have found that two routes appear to be potentially available, that through the centre of the end-plate and that through the annulus. The peripheral portion of the end-plate is nearly always impermeable and would therefore seem to be of no practical functional significance as a nutritional route. For reasons outlined above, we are unable to say whether the annulus or the central portion of the end-plate represents the more important nutritional route in life.

It might be thought that the demonstration of permeability of the central portion of the end-plate contradicted the earlier observations of Maroudas et al. (1969) that the bone/cartilage interface was relatively impermeable and hence unimportant as a source of cartilage nutrition. This apparent contradiction is resolved by our finding that the end-plate was permeable only when marrow spaces in direct contact with the cartilage could be seen histologically. Such a route of diffusion has been shown to exist, for instance, in the rabbit knee-joint (Ekholm 1951). "Vascular buds" or marrow space contacts with the cartilage are probably absent or very few in number in the bone/cartilage interface in adult synovial joints. Furthermore, articular cartilage has a thicker calcified layer than has the normal or degenerative end-plate (Lewin 1964, and the present study). Thus, if the permeability of the central portion of the end-plate depends entirely upon these marrow space con-

tacts with cartilage, and if the calcified zone is impermeable, our observation upon the end-plates and the observation of Maroudas et al. (1969) upon synovial joints are perfectly compatible. We thus conclude that the nutritional route to the nucleus through the end-plates depends upon these contacts or "vascular buds". This conclusion is similar to that reached by Greenwald & Haynes (1969) who showed that dye penetration from bone to cartilage in the human femoral head also only occurred via such vascular contacts.

Our observations, although compatible with the widely held view that the intervertebral disc is nourished by diffusion through its end-plates, demonstrate that usually only the central portion of the end-plate can function in this way since the lateral portion is generally impermeable. Our observations further suggest that nutrients may reach the periphery of the disc by diffusion through the annulus fibrosus. Thus it appears that the disc may depend upon two nutritional routes: (a) diffusion through the central portion of the end-plate from wide ampulla-like contacts between the medullary cavity of the bone and overlying cartilage (the "vascular buds"), and (b) diffusion through the annulus fibrosus from the surrounding vessels.

The central portions of a small number of end-plates were found to be impermeable. A statistically significant correlation was found between impermeability of the central portion of the end-plate and the presence of Grade 2 or 3 degeneration as assessed by the macroscopic appearance of the disc. The association between impermeability of the end-plate and the presence of marked degeneration of the disc was further strengthened by the finding that all the totally impermeable end-plates were found in discs showing Grades 2 or 3 degeneration as assessed by their macroscopical appearance. The question arises: is the association between impermeability of the end-plate and degeneration of the disc one of cause and effect? Our observations would suggest that if these factors are causally connected it is not because degeneration invariably causes impermeability, since many markedly degenerate discs had permeable end-plates. It might be argued that degeneration only results in impermeability after the passage of time and hence that in this study only a few degenerated discs had had the opportunity to develop impermeable end-plates. Although this possibility cannot be excluded, it seems to us more likely that degeneration does not cause impermeability of the end-plate, since the histological appearance of the end-plate in the degenerated discs, particularly the finding of penetration of the end-plate by vascularised connective tissue, suggests that

if anything, the development of degeneration in the disc may render an originally impermeable end-plate permeable. Thus if there is a causal link between these two factors, it seems likely to be due to the fact that impermeability of the end-plate causes disc degeneration. If this is so, impermeability must be only one of several causes of disc degeneration, since many degenerate discs are not impermeable. It is interesting to note that Greenwald & Haynes (1969) also noted a disappearance of vascular buds and consequently a reduction in permeability, with the development of degenerative changes in the human femoral head.

Thus our results suggest that, whereas the normal disc is dependent nutritionally upon the annulus fibrosus and the central portion of the vertebral body end-plate, certain end-plates are deficient in marrow space contacts with the end-plate cartilage and, as a consequence, are impermeable. It may be that the result of impermeability is an inadequate supply of nutrients to the disc which in turn may cause the fall in pH and increase in lactic acid concentration observed in certain degenerated discs (Diamant et al. 1968, Nachemson 1969). If this is the case, the absence of marrow space contacts with the cartilage of the central portion of the end-plate may be a cause of disc degeneration.

SUMMARY

Previous *in vivo* and *in vitro* experiments, using dye and radioactive glucose and sulphate, have demonstrated that the calcified zone of articular cartilage in normal adults is for practical purposes impermeable. Such impermeability, should it occur in the intervertebral disc, might be relevant to premature disc degeneration and would throw doubt on the importance of the osseous route for nutrition of the disc.

The present investigation has demonstrated that there is a statistically significant difference in permeability between the lateral portion of the end-plate, on the one hand, and the central portion of the end-plate and annulus fibrosus on the other, the latter two usually being permeable, the former impermeable.

Histological examination revealed that permeability of the end-plate was always associated with the presence of "vascular buds" (vascular projections making wide ampulla-like contacts between the marrow space of the vertebral body and the hyaline cartilage of the disc's end-plate). Thus this study suggests that two nutritional routes are open for the intervertebral disc: (1) diffusion through the central portion of the end-plate from these marrow space cartilage contacts and (2) dif-

fusion through the annulus fibrosus from the surrounding vessels. Our observations do not allow us to comment on the relative importance of these two routes in life; all we can say is that they are both potentially available.

Of 9 impermeable central portions of 75 end-plates tested, 7 were from segments with degenerated disc (Grades 2-3). Impermeability of both the central and lateral portions of 71 end-plates studied was seen in 8 end-plates. Of these 6 were from segments with degenerated discs.

Thus a statistically significant association was observed between impermeability of the end-plate and the presence of macroscopic disc degeneration of Grades 2 and 3. The pathological significance of this association is discussed, and it is suggested that the absence of marrow space contacts with the cartilage in certain end-plates results in impermeability of the end-plate, which in turn may interfere with disc nutrition and be a cause of disc degeneration.

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