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Factors involved in the nutrition of the human lumbar intervertebral disc: cellularity and diffusion of glucose *in vitro*

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INTRODUCTION

The nutrition of the intervertebral disc has long been a topic of interest (Ubermuth, 1929; Bohmig, 1930; Schmorl, 1931; Coventry, Ghormley & Kernohan, 1945; Hirsch & Schajowicz, 1952). The lumbar discs, for example, are the largest avascular structures in the body and it is suggested that poor nutrition may account for premature disc degeneration.

In the adolescent rabbit Brodin (1955) has shown experimentally that the cartilage–bone interface is permeable to nutrients and he suggests that this is the main nutritional route, although solutes may also be supplied to a limited extent through the anulus fibrosus.

A qualitative *in vitro* investigation of the adult human intervertebral disc (Nachemson, Lewin, Maroudas & Freeman, 1970) has revealed that the end plates are indeed partly permeable to solutes, this being associated with the presence of vascular contacts between the marrow spaces of the vertebral body and the hyaline cartilage of the end plate; the central portion of the end plate is usually more permeable than the peripheral parts. The anulus is always permeable. However, it was not possible to determine the relative importance of the different routes and, in particular, whether or not all parts of the disc can obtain sufficient nutrients by diffusion alone.

The present study was undertaken to attempt to answer these questions by investigating quantitatively the diffusion of glucose both through the anulus fibrosus and across the bone–cartilage interface. Determinations of the cellularity of the disc have been carried out in order to estimate the glycolytic requirements of the disc, extrapolating from the glycolysis data for articular cartilage and the relative cell densities of the two tissues. From information obtained from these two independent investigations it should be possible to calculate the theoretical balance between the metabolic requirements of the disc and the transport of glucose into the disc.

Apart from their relevance to the problem of the nutrition of intervertebral discs, both the diffusion and the cellularity studies are of intrinsic interest. Data are available on the cellularity of articular cartilages of varying thicknesses from a wide range of

species and joints (Stockwell, 1971). Although more heterogeneous in structure than articular cartilage, the lower lumbar intervertebral discs are very thick and of large diameter. Hence there should be an interesting comparison with articular cartilage where an inverse relationship appears to exist between tissue thickness and cellularity. The diffusivity of small, uncharged solutes in articular cartilage is approximately one-third of that in water and is practically independent of fixed charge density (Maroudas, 1970): similar behaviour might be expected in the anulus and experimental verification is of basic interest.

MATERIALS AND METHODS

Histological studies

Normal lumbar intervertebral discs (L4–L5) for histology were obtained at post-mortem from three subjects aged 15, 18 and 56 years. Each disc was removed together with the contiguous parts of the vertebral bodies above and below the disc. Blocks were fixed in phosphate buffered (pH 7·2) 10% formalin for 2–7 days, before decalcification in Bensley's formic–citrate (equal volumes of 50% formic acid and 20% sodium citrate). On completion of decalcification (usually 7 days), most of the bone was trimmed away and each disc was bisected in the median sagittal plane. After dehydration and double embedding in celloidin and wax, one half of each disc was sectioned in the horizontal plane midway between the two vertebral bodies, while the other half was sectioned parallel and adjacent to the median sagittal plane of bisection. After median sagittal (vertical) sectioning, this block was trimmed and reorientated on the microtome chuck to enable coronal vertical sections to be obtained. Sections were cut at 8 μm, dewaxed, stained with haematoxylin and eosin and mounted in Depex.

Cellularity studies were carried out according to the procedure of Stockwell (1971), using an eyepiece squared graticule and a microscope magnification of $\times 100$. In vertical sections, nuclei and parts of nuclei were counted in rectangular areas of tissue about $\frac{2}{3}$ mm wide extending across the full thickness of the disc between the two bone–cartilage interfaces. One area passed through the middle of the nucleus pulposus and the other was placed halfway between the periphery of the disc and the outer part of the nucleus pulposus, hence passing through the anulus fibrosus only (Fig. 1). Within each rectangle, counting was made in successive narrow strips lying parallel to the bone–cartilage interface. Hence it was possible to estimate the distribution of cells within the disc. In the horizontal sections, rectangles (also $\frac{2}{3}$ mm wide) were placed either in the coronal plane or in an anteroposterior direction, the medial border of the latter area abutting on the median sagittal plane. This procedure was carried out on at least two sections from each block; about 5000 nuclei were counted in each specimen. Measurements of the dimensions of the disc were made both macroscopically and in association with the cell-counting procedure. From these data, crude estimates of cells per unit volume of tissue could be made. Since more than one successive histological section may pass through the same cell nucleus, a correction factor (Abercrombie, 1946) was applied, involving both section thickness and mean diameter of cell nuclei. These were measured according to methods used previously (Stockwell, 1971). No allowance was made for possible shrinkage during

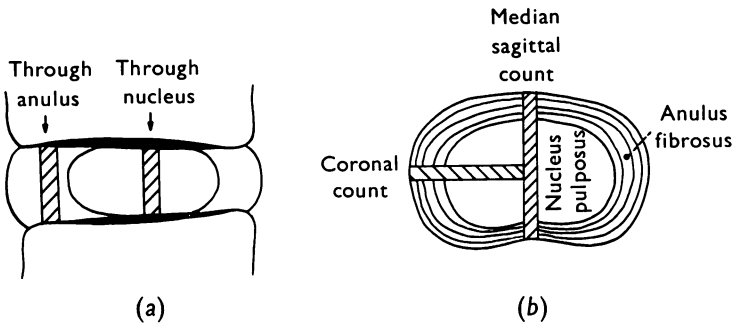


Fig. 1. Diagram showing positions of areas of disc for cell counting: (*a*) median sagittal (vertical) section, (*b*) horizontal section.

tissue preparation. Hence the corrected cell density values presented here refer to the number of cells per mm^3 of fixed tissue on the slide.

Estimates were also made of the degree of contact of the vertebral bone marrow spaces with disc tissue. Only those spaces not covered by bone and hence in direct contact with calcified (recognized by the presence of a basophilic 'tide-mark') or uncalcified hyaline cartilage were included. Using an eyepiece graticule, both the length of the bone–cartilage interface visible in the section and the portions of that length occupied by marrow space contacts were measured, and hence the fraction of interface occupied by these contacts could be estimated.

Diffusion studies

Lumbar intervertebral discs (L4–L5) obtained at post-mortem from ten individuals ranging in age from 18 to 80 years were used. Two kinds of specimens were used in diffusion experiments:

(i) sections from end plates incorporating the bone–cartilage interface;

(ii) sections from the anulus fibrosus.

The former specimens were taken from both the central and the peripheral portion of the upper and lower end plates. The total thickness of these specimens was about 1 mm, of which about 0·5 mm was cartilage and 0·5 mm bone. After measuring the rate of transfer through the bone–cartilage specimen, the cartilage and the calcified zone were scraped away, so that the permeability of the bone itself could be tested. The anulus fibrosus was sliced on a freezing microtome into 500 μm slices. Both horizontal and vertical slices from the anulus fibrosus were tested.

The specimens were clamped in a diffusion chamber as previously described (Maroudas, Bullough, Swanson & Freeman, 1968; Nachemson *et al.* 1970), with one modification. When specimens of the anulus were being tested, they were held between rigid sheets of gauze to stop them from swelling. The diffusion cell was placed in a 37 °C water bath and 2 ml of Ringer's solution, containing 0·1 % glucose (equivalent to the serum content), were placed in each compartment of the cell. One compartment also contained the radioactive tracer [^{14}C]glucose (specific activity 20 mCi/mole). Samples of the originally non-radioactive solution were withdrawn at hourly

intervals for counting and 2 ml of fresh Ringer's was introduced each time. When the same counts (within 5 % of one another) were obtained 2–3 times (indicating that a steady state had been certainly achieved) the experiment was stopped. 'Hot' solution was counted at the beginning and end of each run. From the data obtained in the above experiments it was possible to calculate the permeability coefficient of the slice from the formula:

$$\bar{P} = \frac{N_C \times x \times V}{N_H \times A \times t},$$

where N_C = counts per ml of initially 'cold' solution, N_H = counts per ml of 'hot' solution, V = volume of cold solution in ml, x = thickness of specimen, A = cross sectional area in cm^2 of specimen exposed to solutions, and t = time in seconds. In order to calculate the diffusion coefficient D from the value of P the following formula was used:

$$\bar{D} = \bar{P}(\bar{C}/C),$$

where $\bar{D}$ = diffusivity in the tissue in cm^2/sec, $\bar{P}$ = permeability coefficient in cm^2/sec, and $\bar{C}/C$ = distribution coefficient.

The distribution coefficient $\bar{C}/C$ was determined for each specimen by allowing the latter to equilibrate in glucose–Ringer solution containing [^{14}C]glucose and then desorbing it into 2 ml of 'cold' solution. By comparing the radioactivity in the desorbate with that in the original solution the distribution coefficient could be readily calculated.

In the case of the specimens incorporating the bone–cartilage interface, the resistance to diffusion offered by the cancellous bone was subtracted from the total resistance and the permeability for the combination deep hyaline cartilage + calcified zone of the end plate calculated. The resistance offered by the cancellous bone was very small compared with the total resistance.

RESULTS

Histological studies

Contact with blood vessels

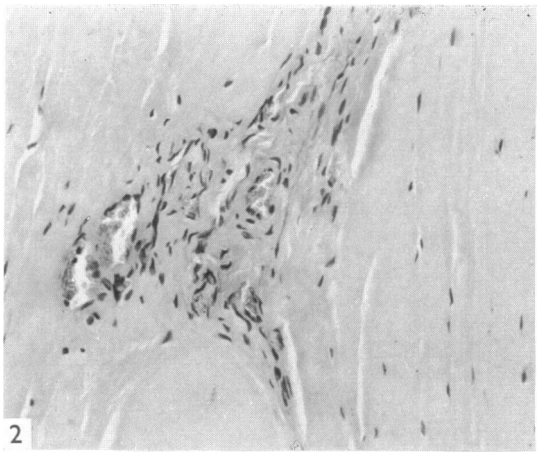
The disc tissue was avascular. Blood vessels were seen only at the margin of the disc or in the marrow spaces of the vertebrae. Arterioles and capillaries (Figs. 2, 3) at the outer aspect of the anulus were numerous in all three specimens and, as seen in the horizontal section, equally distributed around the circumference. There was a

Fig. 2. Blood vessels in peripheral part of anulus fibrosus; 15 year old, vertical section. H and E, ×210.

Fig. 3. Blood vessel at margin (right) of anulus fibrosus; 56 year old, horizontal section. H and E, ×210.

Fig. 4. Bone–cartilage interface. Large marrow space projecting into uncalcified cartilage (top); 18 year old. H and E, ×130.

Fig. 5. Bone–cartilage interface. Several canal-like marrow spaces, two of which abut on to uncalcified cartilage; 18 year old. H and E, ×130.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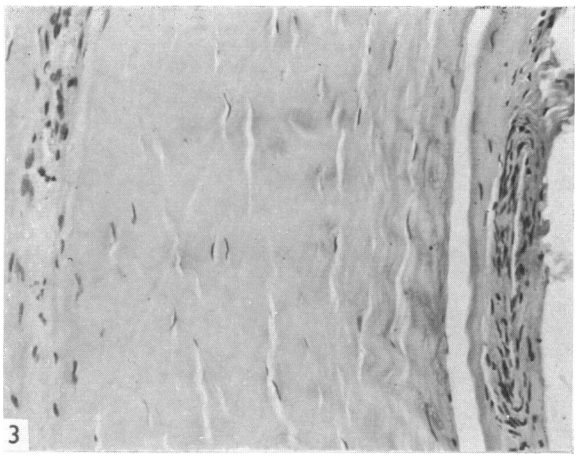
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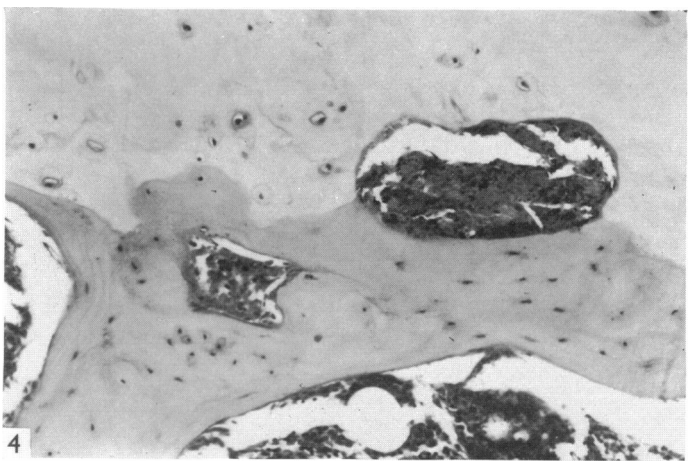
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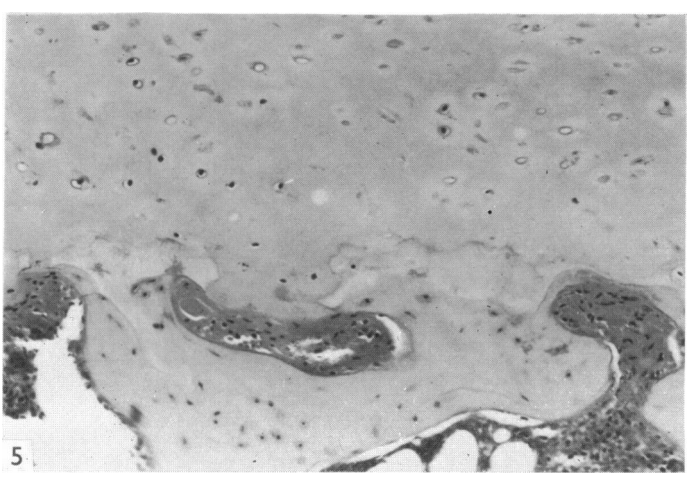
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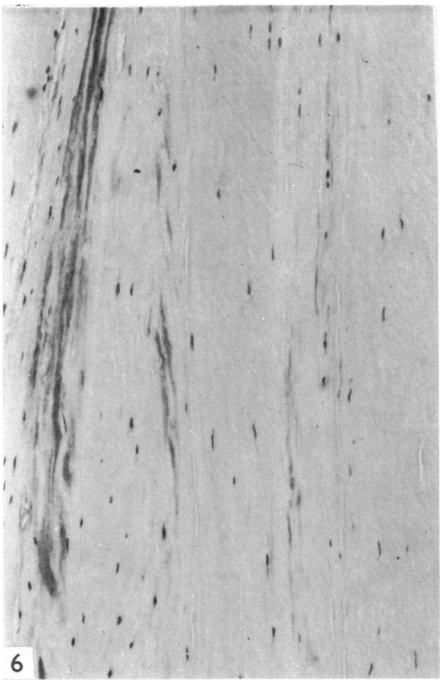

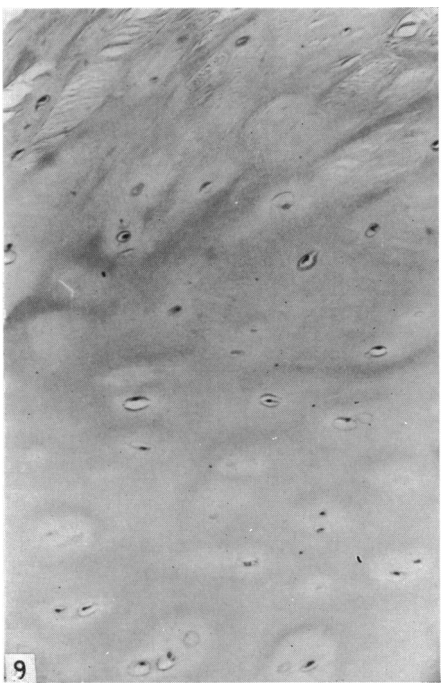

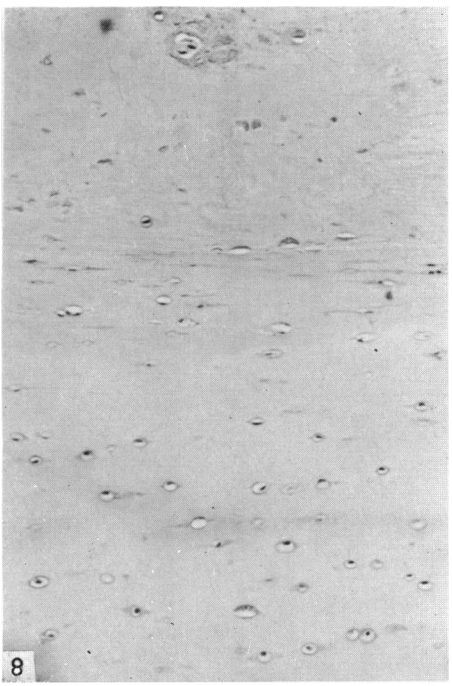

Fig. 6. Fibrous lamellae of peripheral anulus fibrosus; compare matrix, form of nuclei and cellularity with Fig. 7. 18 year old, horizontal section. H and E, × 130.

Fig. 7. Typical field in nucleus pulposus. Note sparse cells. 18 year old, horizontal section. H and E, × 130.

Fig. 8. Junction of cartilage end plate (below) and anulus fibrosus; 18 year old. H and E, × 130.

Fig. 9. Junction of hyaline cartilage end plate (below) and nucleus pulposus; 18 year old. Note change of cellularity. H and E, × 130.

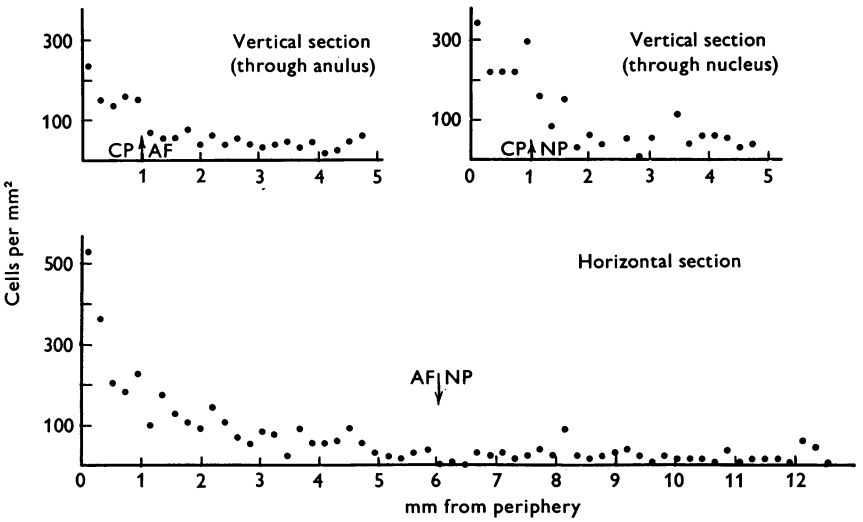

Fig. 10. Distribution of cells in intervertebral disc (18 year old). CP, cartilage end plate; AF, anulus fibrosus; NP, nucleus pulposus. Arrows indicate transitions.

tendency for the smaller of the vessels to run horizontally around the disc. It was impracticable to quantify the vascularity of the disc margin.

The vertebral marrow vessels were studied in vertical sections. Again, it was difficult to quantify the vessels. Instead contacts between marrow spaces and disc tissue were measured. The proportion of the bone–cartilage interface occupied by marrow spaces was considerable (7–10%), but tended to be smaller in amount near the periphery as compared with the central region of the interface. In addition to 'direct' marrow contacts, a further and larger proportion (20–30%) of the bone–cartilage interface overlaid marrow spaces, separated from disc cartilage by only a thin layer of bone, often less than 25 μm thick.

Marrow contacts were both wide ampullary and narrow canalicular in character (Figs. 4, 5). In either case they frequently penetrated through the calcified cartilage to lie in, or abut on, the uncalcified tissue. No differences between the upper and lower bone–cartilage interfaces were noted, either in the type or amount of contact.

Cellularity

In the hyaline cartilage end plates chondrocytes were typically rounded as in other cartilages. In the anulus fibrosus the cells were elongated, with long cigar-shaped nuclei (Fig. 6), in general aligned with their long axes parallel to the direction of the fibrous lamellae. In the nucleus pulposus (Fig. 7), the cells varied in shape, usually having a spheroidal nucleus, and occurring either singly or in groups of six or more. Typical areas of the three parts of the disc could be distinguished easily by the characteristics of the cells and matrix. Clearly defined transitions occurred between hyaline cartilage end plate and nucleus pulposus (Fig. 9) and, rather less obviously, between end plate and anulus fibrosus (Fig. 8). However, the anulus fibrosus was not clearly demarcated from the nucleus pulposus. Hence, the boundary

Table 1. *Zonal variation in cellularity of human intervertebral disc*

	Cell density ($\times 10^{-3}$)/mm³			
	15 years	18 years	56 years	Average
Cartilage end plate	12·6	15·4	17·1	15·0
Anulus fibrosus	6·9	8·4	11·6	9·0
Nucleus pulposus	3·3	4·3	4·7	4·0

Table 2. *Dimensions and cellularity of the human lumbar intervertebral disc*

	15 years		18 years		56 years	
	Width (mm)	Cell density ($\times 10^{-3}$)/ mm³	Width (mm)	Cell density ($\times 10^{-3}$)/ mm³	Width (mm)	Cell density ($\times 10^{-3}$)/ mm³
Vertical section	12·4	4·7	8·8	8·0	10·0	5·45
Horizontal section (coronal × med. sagittal)	44·3 × 42·8	4·65	54·9 × 30·4	4·95	49·5 × 31·9	7·15
Average cell density	—	4·68	—	6·48	—	6·30

Table 3. *Permeability of human intervertebral disc to glucose*

			Bone–cartilage interface and deep cartilage	
	Anulus	Deep cartilage	Central region	Peripheral region
No. of specimens tested	20	10	10	10
Permeability coefficient, $\bar{P}$ (cm²/sec)	$1{\cdot}7 \pm 0{\cdot}15 \times 10^{-6}$	$1{\cdot}6 \pm 0{\cdot}20 \times 10^{-6}$	$0{\cdot}58 \pm 0{\cdot}21 \times 10^{-6}$	$0{\cdot}18 \pm 0{\cdot}03 \times 10^{-6}$
Molar distribution coefficient, $\bar{C}/C$	$0{\cdot}68 \pm 0{\cdot}05$	$0{\cdot}66 \pm 0{\cdot}03$	—	—
Diffusion coefficient, $\bar{D}$ (cm²/sec)	$2{\cdot}5 \times 10^{-6}$	$2{\cdot}43 \pm 10^{-6}$	—	—
Fractional interfacial area available for diffusion	—	—	0·36	0·11

between these two parts was taken as the mid-point between the innermost recognizable lamella of the anulus and the outermost zone of typical pulposus tissue.

The cellularity of the intervertebral disc was low compared with most tissues. The distribution of the cell population was not homogeneous. Cells were most numerous in the cartilage end plates (Fig. 9), especially close to the vertebral bone, and in the outer parts of the anulus fibrosus (Fig. 6). In the nucleus pulposus cells were sparse (Fig. 7) although there was some local variation because of cell grouping. Thus in the disc as a whole there was a decline in cellularity on passing either from the cartilage end plate or from peripheral parts of the anulus fibrosus, inwards towards the nucleus pulposus (Fig. 10). In the anulus fibrosus most of the diminution in cell

density was found in the peripheral 2–3 mm. The cell density of the nucleus pulposus remained at a low plateau. As may be seen from Table 1, the mean cell density of the cartilage end plate was nearly four times, and that of the anulus fibrosus about twice, that of the nucleus pulposus.

The mean cell density for the whole disc was calculated from the mean values obtained from the vertical and horizontal cell counts (Table 2). On this basis the average cell density of the disc was 5800 cells/mm^3.

Diffusion studies

The values of the permeability, distribution and diffusion coefficients are given in Table 3. The permeability of the end plate specimens was considerably lower than that of the anulus fibrosus ($1{\cdot}7 \times 10^{-6}$ cm^2/sec). However, these specimens incorporating the bone–cartilage interface contained a relatively impermeable zone (perforated at the marrow contacts) as well as the deep cartilage of the end plate. When the low permeability of the calcified zone was taken into account, it was found that the coefficient of the deep cartilage ($1{\cdot}6 \times 10^{-6}$ cm^2/sec) was very nearly the same as that of the anulus. This was also true for the distribution and diffusion coefficients of the two localities.

The end plate specimens varied in their permeability according to the anatomical site at which they were sampled. Thus the permeability coefficient was significantly lower in the periphery than in the central regions. By comparing the permeability of the end plate specimen with that of the cartilage it was possible to obtain a value for the proportion of the bone–cartilage interface occupied by spaces for marrow vascular contact (the fractional interfacial contact area – Table 3).

DISCUSSION

The quantitative results obtained from the histological material must be regarded as approximate only. Counting cells in histological sections is a relatively inexact procedure, although probably as useful as DNA estimation in tissues with low cellularities. Moreover, only three specimens have been examined. However, the results indicate at least the order of magnitude of the cellularity and its variation within the human intervertebral disc and also the degree of vascular contact of the vertebral bone marrow with the cartilage of the disc. Qualitative observations on other human specimens and animal material confirm the present quantitative findings as regards cell distribution and marrow contacts.

The mean cell density of whole disc tissue, about 6000 cells/mm^3, is much less than that of articular cartilage, which is about 14000–15000 cells/mm^3 in the human knee joint (Stockwell, 1971). The disc is of considerable size; for example, its thickness alone is approximately four to five times that of knee-joint cartilage. Hence its low cellularity is not unexpected in view of the inverse relationship between cellularity and tissue thickness, demonstrated in articular cartilage.

The cells of the different parts of the intervertebral disc vary in their form and probably in their function. Nevertheless, when quantifying the cell population with the object of gaining insight into the overall metabolic requirements of the disc, it is necessary as a first approximation to assume that all the cells are equal in this

respect. Some justification exists for this assumption: for example, even liver and kidney cells have approximately the same glycolytic rate as chondrocytes (Bywaters, 1937). Apart from such evidence, however, it is probable also that most, if not all, cells in the adult disc are of the same fundamental mesenchymally derived type (Keyes & Compere, 1932). Notochordal cells of the nucleus pulposus are thought not to persist into adult life (Peacock, 1952), except as necrotic remnants (Meachim & Cornah, 1970), but even these cells of endodermal origin exhibit similar respiratory enzyme systems with activity similar to those of end plate chondrocytes (Wolfe, Putschar & Vickery, 1965). Such differences as exist in the various regions may well be due to modulation, i.e. environmentally induced (Young, 1963), by mechanical and nutritional factors.

The variation of cell density with distance from the periphery of the anulus fibrosus is reminiscent of the similar diminution which occurs in articular cartilage relative to the articular surface (Stockwell, 1971), and in horse nasal cartilage (Galjaard, 1962) and human costal cartilage (Stockwell, 1966) relative to the perichondrium. In the two last-named cartilages the perichondrial vessels are virtually the only source of nutrition and there is good evidence that the synovial fluid nourishes at least a major part of articular cartilage (Maroudas *et al.* 1968). Hence the distribution of cells in the periphery of the anulus fibrosus is in itself circumstantial evidence that there is a major source of nutrition at the margin of the disc. Similar arguments hold for the cartilage end plate. The more rapid fall in cell density in this region is compatible with a less plentiful supply of nourishment.

Contact between the marrow spaces of the vertebral bone and the intervertebral disc cartilage appears to be considerable. Earlier studies have shown that diffusion through the end plate occurs in the immature (Brodin, 1955) and that, in the adult, such diffusion is correlated with the presence of marrow contacts (Nachemson *et al.* 1970). In adult human synovial joints, such contacts through the bone–cartilage interface have been demonstrated in the femoral head (Greenwald & Haynes, 1969), but in the case of the joints of the thoracic vertebral arches the subchondral bone forms a continuous plate (Enneking & Harrington, 1969). It is probable that the proportion of the bone–cartilage interface occupied by marrow contacts is less in sub-articular regions than in the intervertebral disc. It must also be emphasized that a high proportion of the contacts in the disc penetrated through the rather thin calcified layer of the cartilage end plate.

Earlier studies (Maroudas *et al.* 1968) of permeability carried out on necropsy material have shown that the results may be validly applied to the tissue *in vivo*. In articular cartilage, the experimental determination of permeability and diffusion coefficients has proved to be of considerable importance in understanding both the nutrition of the tissue and its mechanical function. In the present study, the mean values of the diffusion coefficient for glucose of the anulus fibrosus and deep end plate cartilage (2·5 cm^2/sec and 2·4 cm^2/sec respectively, at 37 °C) are very close to that of human articular cartilage (2·7 cm^2/sec at 37 °C, calculated on the basis of an average value of 2·15 cm^2/sec obtained at 25 °C by Maroudas, 1973). As far as the cartilaginous elements of the disc are concerned, therefore, there is only a slight difference between the two tissues as regards the diffusion of small solutes.

The relatively low permeability of the bone–cartilage interface is consistent with

Table 4. *Articular cartilage glycolysis* (*Bywaters*, 1937; *Rosenthal* et al. 1941) *in relation to intervertebral disc metabolism*

Source of articular cartilage	Glycolytic rate (Q_G) (mm^3/mg/hour)	Cell density ($\times 10^{-3}$)/mm^3	Cell density ratio: articular cartilage/disc	Calculated Q_G for disc	m-moles of glucose/cell/hour $qx10^{11}$
Human	0·15	43	6·7	0·022	1·9
Horse	0·21	58	9·1	0·023	2·0
Cow					
Young adult	0·44	47	7·3	0·06	5·2
Aged	0·26	34	5·3	0·05	4·3
Rabbit					
Meniscus	0·34	110	17·2	0·02	1·7
Adult femoral condyle	0·64	128	20·0	0·032	2·7

the limited number of blood vessels penetrating the bone and calcified cartilage. Permeability is significantly lower in the peripheral than in the central zone and this is consistent both with the present findings relating to blood vessel contact and with earlier results (Nachemson *et al.* 1970).

Depth of penetration of glucose into the disc by diffusion

By utilizing data for the diffusion and glycolytic rates of the tissue and by making a limited number of assumptions, it should be possible to calculate how much of the intervertebral disc is adequately nourished by diffusion.

There have been no studies of the glycolytic rate of the disc. Hence only indirect estimates can be made, derived from a knowledge of the glycolytic rate of articular cartilage and the ratio of cell densities in the disc and in articular cartilage. This assumes that the glycolytic rate per cell is similar in the two structures: the few published data for cartilaginous tissues (Bywaters, 1937; Rosenthal, Bowie & Wagoner, 1941) show a remarkable uniformity in this respect, indeed there is little disparity between cartilage and epithelial tissues. The results obtained by adopting this procedure are set out in Table 4: two estimates of the glycolytic rate for the disc ($1{\cdot}9 \times 10^{-11}$ mM glucose/cell/hour from Bywaters (1937), using data for human cartilage, and $5{\cdot}2 \times 10^{-11}$ mM glucose/cell/hour from Rosenthal *et al.* (1941), using data for young adult bovine cartilage) can be derived from the lowest and highest values for glycolytic rate in cartilage.

The derivation of the formulae from Fick's law for the special conditions of the intervertebral disc (taking into account the varying cell distribution) are shown in the Appendix. This also gives for one specimen (18 years) the calculated distances of glucose penetration into the disc from the various sources of nutrition for both low and high estimation of glycolytic rate.

The calculated distances are summarized graphically in Fig. 11. If the low value for glycolytic rate is used, it follows that the whole of the anulus fibrosus is adequately supplied with glucose by the peripheral vessels, since the transition between anulus

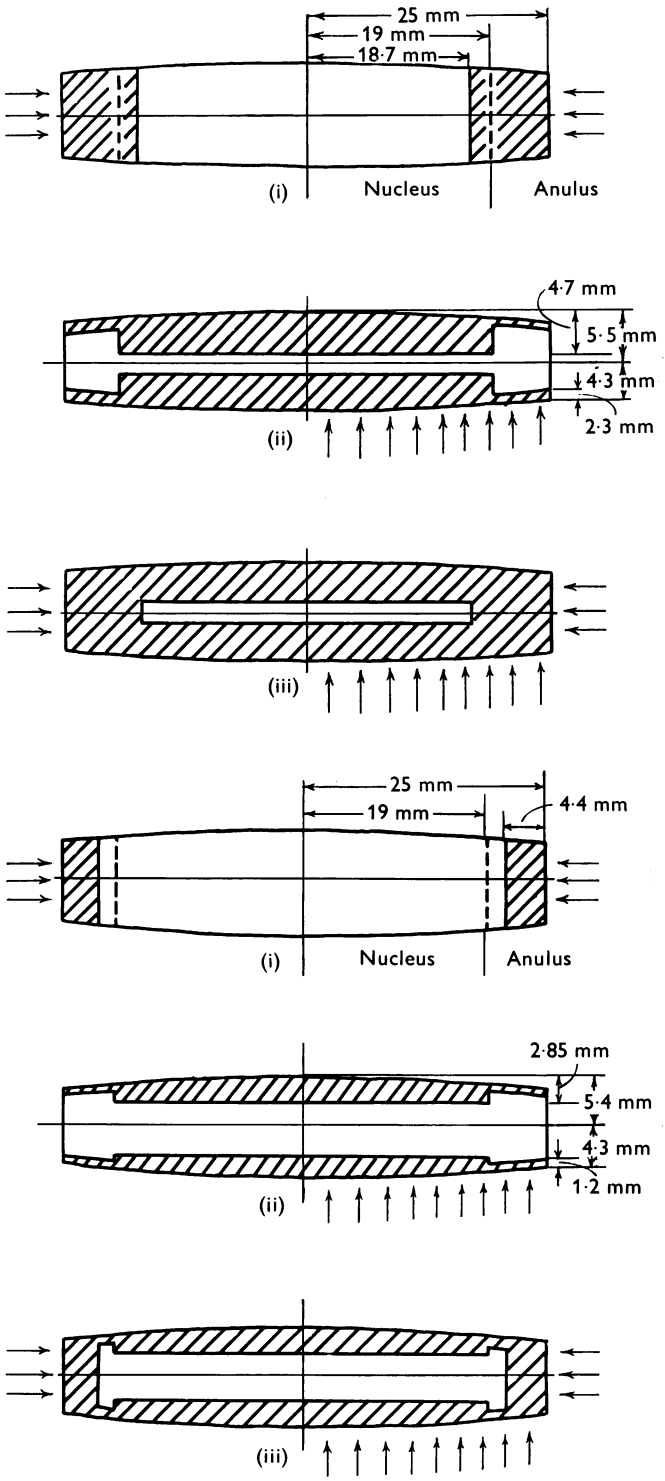

Fig. 11 (*a*). Schematic representation of glucose penetration into the disc where $q = 1{\cdot}9 \times 10^{-11}$ m-moles/cell/hour (coronal section): (i) radial diffusion, (ii) diffusion through end plates, (iii) overall diffusion. (*b*) Schematic representation of glucose penetration into the disc where $q = 5{\cdot}2 \times 10^{-11}$ m-moles/cell/hour (coronal section): (i) radial diffusion, (ii) diffusion through end plates, (iii) overall diffusion.

fibrosus and nucleus pulposus occurs approximately 6 mm from the periphery of the disc (Fig. 10). However, if the high glycolytic rate is used, then the results imply that one-third of the anulus is provided with nutrients from the bone end plate only and this is inadequate. In either case there remains a portion of the nucleus pulposus which apparently would be deprived of glucose, the rest being adequately provided either from the bone end plate or from the peripheral vessels.

Since at the present time it is not possible to say with certainty what the glycolytic rate actually is for the human disc, or whether or not it is concentration dependent, one cannot assess with confidence exactly what proportion of the disc is adequately supplied with nutrients. The importance of these results rests not so much in the absolute distances calculated but in emphasizing that nutritional conditions in the intervertebral disc are precarious compared with other avascular structures. Thus, it is clear that whilst articular cartilage has something like a 50% excess of supply over demand, assuming synovial fluid is the sole source of nutrients, in the intervertebral disc there is only just a balance when both routes are considered and even if the most optimistic conditions are assumed. The pH of discs is low (Nachemson, 1969): this has been correlated with an increased concentration of lactic acid (Diamont, Karlsson & Nachemson, 1968). The latter could have been due to insufficient oxygenation of the disc, in turn caused by inadequate diffusion.

In conclusion, it is interesting to note the importance of cell distribution, which is a maximum both at the periphery of the anulus and at the cartilage end plate interface. If one considered the same disc as above (Fig. 10) with the same overall cell density but a *uniform* cell distribution, the depth of penetration from the bone end plate would be 4·1 instead of 4·7 mm whilst the radial penetration would be only 5·0 instead of 6·3 mm (assuming low glycolytic rates). Thus the cell distribution actually observed appears well adapted to the nutrient supply.

SUMMARY

Post-mortem specimens of the human lumbar (L4–L5) intervertebral disc have been studied histologically and physico-chemically.

Blood vessels were found only at the margin of the anulus fibrosus and in the vertebral marrow spaces. Contact between disc tissue and marrow spaces occupied about 10% of the bone–cartilage interface.

The disc was most cellular at the periphery of the anulus fibrosus and in the hyaline cartilage next to the vertebral bone. Cellularity declined towards the nucleus pulposus where it achieved a low constant value. The cell density of the disc as a whole was about 6000 cells/mm^3.

For glucose, the diffusion coefficient of the anulus fibrosus and hyaline cartilage end plate was 2·5 cm^2/sec and 2·4 cm^2/sec respectively at 37 °C, comparable to that of cartilage elsewhere. The permeability of the bone–cartilage interface was low, particularly in the peripheral part.

Calculations, based on the present findings and derived values for glucose utilization in disc tissue, indicate that nutritional conditions in the intervertebral disc are more critical than, for example, in articular cartilage.

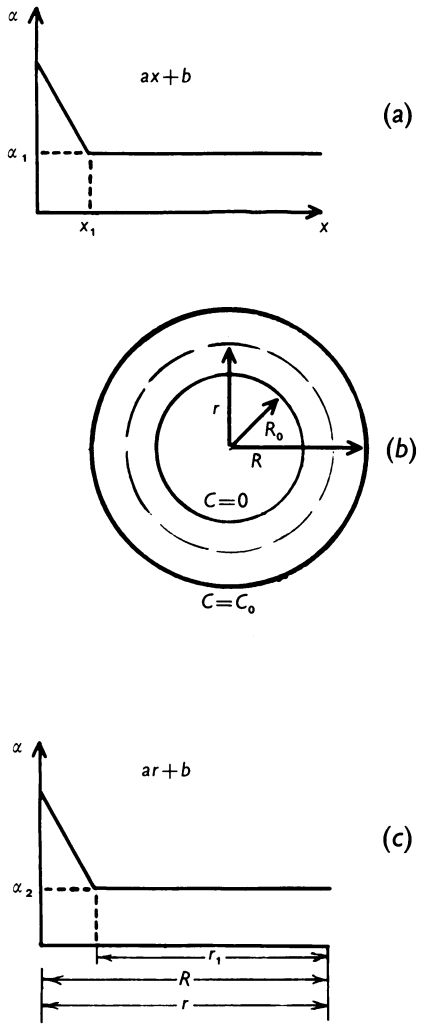

Fig. 12 (*a*). Schematic representation of cell density variation from end plate to centre of disc, with symbols defined as used in the text and the derivation of equation (2). (*b*) Schematic representation of disc as cylinder, with the definition of symbols used in the derivation of equation (3). (*c*) Schematic representation of cell density variation from the periphery of the anulus to the nucleus.

APPENDIX

Calculations are based on a number of simplifying assumptions:

(i) The disc is considered as a cylinder of radius R.

(ii) All cells in the disc have a uniform constant glycolytic rate.

(iii) The diffusion into the disc from the bone–cartilage interface and from the margins of the anulus fibrosus are considered independently of each other.

Penetration from the end plates

The starting differential equation is derived from Fick's law of diffusion into a flat plate:

$$-\bar{D}\frac{d^2C}{dx^2} = q\alpha, \tag{1}$$

where $\bar{D}$ = diffusion coefficient in the disc, C = glucose concentration in the disc, α = cell density at any point in the disc, x = vertical distance from the end plate, q = glycolytic rate per cell.

The manner in which the cell density varies as a function of x is clearly seen from Fig. 10 and is shown schematically in Fig. 12(*a*). This type of variation can be approximated mathematically in the following manner:

$$x \geqslant x_1, \quad 0 < x < x_1, \quad \alpha = \alpha_1 = \text{constant}, \quad \alpha = ax+b,$$

where the constants x_1, a and b have to be determined experimentally for each case.

Let d be the value of x at which glucose concentration becomes equal to zero, i.e. at $x = d$, $C = 0$.

The other boundary conditions are at:

$$x = 0, \quad C = C_0 \quad \text{and} \quad \bar{D}\frac{dC}{dx} = Na_T,$$

where Na_T = total glucose flux. But

$$Na_T = \frac{q \times \text{total no. of cells}}{\text{cross sectional area}}$$

$$= q\left[\frac{\alpha x_1^2}{2} + bx_1 + \alpha_1(d-x_1)\right]. \tag{1A}$$

Using these boundary conditions, equation (1) can be integrated to give:

$$d^2 = \frac{1}{\alpha_1 q}\left[-\frac{2ax_1^3}{3} - (b-\alpha_1)x_1^2 + 2\bar{D}\bar{C}_0\right], \tag{2}$$

where $\bar{C}_0$ is the concentration of glucose in the disc at $x = 0$.

The calculation of d was carried out using the values of α_1, x_1, a and b obtained from the results in Fig. 10:

$$\begin{aligned} \alpha_1 &= 2{\cdot}5 \times 10^7 \quad \text{at} \quad x = 0, \\ x_1 &= 0{\cdot}084 \text{ cm}, \\ a &= -25 \times 10^7 \quad \text{(from slope of the graph)}, \\ b &= 25 \times 10^6. \end{aligned}$$

For the bone–cartilage interface, the effective diffusion coefficient, $\bar{D}$, was found to be 0·8 cm^2/sec and for deep cartilage $\bar{C}_0/C_0 = 0{\cdot}7$. Thus $\bar{C}_0 = 3{\cdot}9 \times 10^{-3}$ m-moles/cm^3 (since for blood C_0 = 5·6 m-moles/litre). If all the above values are substituted

into equation (2), then, using the lowest value of glycolytic rate, $q = 1{\cdot}9 \times 10^{-11}$ mM glucose/cell/hour, $d = 4{\cdot}7$ mm, i.e. the penetration length is 4·7 mm from the bone–cartilage interface. When the high value of glycolytic rate is used, $q = 5 \times 10^{-11}$ mM glucose/cell/hour, $d = 2{\cdot}85$ mm.

These values apply to diffusion through the end plates in the central region of the disc. In the peripheral part of the end plates the effective diffusion coefficient is lower (see Table 3). Hence, in the peripheral region, the penetration distance, d, for glucose works out to be 2·3 mm for $q = 1{\cdot}9 \times 10^{-11}$ and 1·2 mm for $q = 5 \times 10^{-11}$ mM glucose/cell/hour.

Penetration from periphery of anulus

In order to calculate the distance glucose is likely to penetrate from the periphery of the anulus fibrosus the following procedure is used.

The disc is considered as a cylinder of radius R (see Fig. 12 *b*). The basic diffusion equation in this case is

$$-D\frac{d}{dr}\left(r\frac{dC}{dr}\right) = q\alpha, \tag{3}$$

where r = radial distance from centre of disc.

If the concentration of glucose is nil, i.e. $C = 0$ at $r = R_0$, where $R_0 > 0$, and if the cell density, α, varies with r so that:

$$r_1 \geqslant r \geqslant 0, \quad \alpha = \alpha_2 = \text{constant}$$

and

$$R \geqslant r \geqslant r_1, \quad \alpha = ar + b$$

(see Fig. 12 *c*), it can be shown, using analogous boundary conditions to those employed for diffusion from the end plates, that equation (3) can be integrated to give

$$\frac{\bar{D}C_0}{q} = \frac{a}{3}\left\{\frac{R^3}{3} - r_1^3\left(\frac{1}{3} + \ln\frac{R}{r_1}\right)\right\} + \frac{b}{2}\left\{\frac{R^2}{2} - r_1^2\left(\frac{1}{2} + \ln\frac{R}{r_1}\right)\right\}$$
$$+ \frac{\alpha_2}{2}\left\{r_1^2\left(\frac{1}{2} + \ln\frac{R_1}{r_1}\right) - R_0^2\left(\frac{1}{2} + \ln\frac{R}{R_0}\right)\right\}. \tag{4}$$

The calculation of the radial penetration distance, $R - R_0$, was carried out using the values of α_2, r_1, a, b and R obtained from the results plotted in Fig. 10 and the dimensions of the disc given in Table 2 for the 18 year old specimen.

Thus, for the coronal section,

$$R = 2{\cdot}5 \text{ cm}, \quad R - r_1 = 0{\cdot}15 \text{ cm}, \quad a = -30 \times 10^7, \quad b = 4{\cdot}6 \times 10^7.$$

In this particular case α_2 does not remain entirely constant, but is lower near the centre of the disc. If, as a first approximation, an average value of α_2 is taken over the whole section, i.e. for the range $2{\cdot}35 \geqslant r \geqslant 0$, this value works out to be $6{\cdot}5 \times 10^6$ cells/cm^3. When the latter is substituted into equation (4), and using the low glycolytic rate ($q = 1{\cdot}9 \times 10^{-11}$ mM glucose/cell/hour) the distance of penetration, $R - R_0$, is found to be 7·3 mm. If one determines the value of α_2 over the more realistic range, i.e. for $2{\cdot}35 \geqslant r \geqslant 1{\cdot}8$, then $\alpha_2 = 8{\cdot}5 \times 10^6$ cells/cm^2. When the latter value of α_2 is used, $R - R_0 = 6{\cdot}3$ mm.

For the sagittal section,

$$R = 1{\cdot}6 \text{ cm}, \quad \alpha_2 = 4{\cdot}6 \times 10^6 \text{ cells/cm}^3, \quad R - r_1 = 0{\cdot}25 \text{ cm},$$
$$a = -23 \times 10^7, \quad b = 3{\cdot}5 \times 10^7.$$

In this plane, using the above figures, $(R - R_0)$ works out to be 9·0 mm. If the high value for glycolytic rate is used ($q = 5 \times 10^{-11}$ mM glucose/cell/hour), the penetration distance of glucose from the peripheral vessels extends to 4·4 mm.

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