Multifunctional mesenchymal cells resembling smooth muscle cells in ganglia of the wrist

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The term ganglion was coined by Hippocrates to designate a knot of tissue containing mucinous fluid (Soren, 1966). The aetiology and pathogenesis of these cystic swellings which occur in the region of joints and tendon sheaths is as yet unresolved.

On the basis of light microscope studies (Carp and Stout, 1928; De Orsay, Mecray, and Ferguson, 1937; King, 1932; Soren, 1966), ganglia have come to be regarded as degenerative lesions occurring in or adjacent to the joint capsule, and older theories that they represent herniations of synovia, or neoplasms, have been more or less discarded. The wall of this cystic lesion appears to be composed of fibrous tissue, and two recent electron microscope studies, one in the Japanese (Arai, 1967) and the other in the German (Cotta and Becker, 1969) literature, support the idea that this lesion is composed of mesenchymal cells showing fibroblastic and synovial type of differentiation. However, we have recently studied five examples of ganglia arising in the region of the wrist joint and have found that the predominant cell resembles smooth muscle cells, and not fibroblasts or synovial cells as suggested by other authors.

A point unresolved by light microscopy is whether ganglion cavities have a cellular lining. The mucinous content and the glistening appearance of the cavity wall certainly suggest that the cavity may be lined by cells, and some authors have stated that the cavities are in fact so lined. Thus Soren (1966) states, 'Fibrocytes also lie along the inner walls of the cavities and form an incomplete lining which resembles the synovium of a joint capsule'. Contrary to this is the widely held view eloquently epitomized by Carp and Stout (1928) who state, 'There is often a dense, smooth, shiny, white membrane lining the lumen. This is simply a condensation of the fibrous tissue of the wall and has no special lining cells'. Recently published studies on the

ultrastructure of ganglia (Arai, 1967; Cotta and Becker, 1969) have again reiterated the idea that a synovial type of differentiation occurs in ganglia and that in parts at least the cavity is lined by such cells. As will be shown later, our findings and interpretations are different from this. In this paper we wish to record the structure of the ganglion wall, lining, and contents as revealed by the electron microscope. The ganglion contents have not been hitherto examined in this fashion but it seemed to us that by examining the contents one might be able to distinguish between material produced as a result of tissue necrosis from a secretion produced by lining cells.

Material and Methods

Ganglion tissue was collected immediately after surgical removal. The bulk of the tissue was fixed in 10 per cent. formalin for paraffin embedding and routine light microscopy, after selected pieces of the wall were taken for electron microscopy. These pieces were then cut into small thin strips and mounted on pieces of filter paper with the surface of the cavity facing upwards so as to facilitate handling and orientation of the specimens. Some of these strips were fixed in 2 per cent. osmic acid in cacodylate buffer for 2 hours, and others were fixed in 2 per cent. glutaraldehyde in cacodylate buffer for 2 hours, and post-fixed in 2 per cent. osmium for 1 hour.

For the purpose of this study the ganglion contents were also taken for electron microscopy and attempts were made to fix them either in osmium or glutaraldehyde as described above. Successful collection was achieved in only two instances, where the ganglion content had a jelly-like consistency and glutaraldehyde fixation was employed. Attempts at fixation in 2 per cent. osmium resulted in dissolution and disintegration of the material beyond recovery.

The fixed tissues and ganglion contents were then dehydrated in increasing concentrations of ethanol, cleared in propylene oxide and embedded in Epon. Semi-thin and ultra-thin sections were cut with Porter-Blum

microtomes and diamond knives. The former were stained with Richardson's stain (Richardson, Jarett, and Finke, 1960) and examined with the light microscope, the latter were stained with uranium acetate and lead hydroxide and examined with a Zeiss EM 9S electron microscope.

Results

CLINICAL NOTES AND GROSS APPEARANCE OF THE LESION

This study is based on ganglia occurring in the region of the wrist joints of five patients. In each instance they appeared to arise from the fibrous capsule of the joint, but there was no communication between the interior of the ganglion and the joint cavity. The ages of the patients ranged from 11 to 32 years and the size of the ganglion from 1.5 to 5 cm. in long diameter. All the ganglia were loculated, showing two to five locules on the bisected surface. In four cases the contents were mucinous but this varied in consistency from a viscid fluid to a soft jelly. The fifth ganglion had suffered trauma prior to removal and its contents were blood stained.

LIGHT MICROSCOPY

The light microscope appearance of this common lesion as revealed in routine haematoxylin and eosin stained sections is well known. Study of such sections and semi-thin sections from Epon embedded material showed that the wall of this lesion is composed largely of collagen fibres with some elongated cells dispersed among them. Small foci of myxomatous degeneration, occasional histiocytic

infiltration, and thick-walled vessels with slit-like lumina were often observed.

Major parts of the ganglion cavities did not appear to be lined by cells, but in some areas elongated cells could be seen lying at the surface. In no instance could we find an appearance similar to that of normal synovial membrane, where the synovial cells form a fairly continuous layer one to four cells deep. Synovial membrane belonging to the wrist joint was, however, found in routine paraffin sections from three of the cases where the capsule of the joint had been excised in order to completely remove the ganglion. Failure to appreciate this could easily have led to the notion that some parts of the ganglion were lined by synovial cells. Sections from Epon embedded ganglion contents were unremarkable, they showed amorphous debris lying in a clear matrix.

ELECTRON MICROSCOPY

(A) Ganglion wall

Low-power views of the ganglion wall (Fig. 1) revealed that it was composed largely of elongated strap-like cells set in a fibrous and mucopolysaccharide matrix. The proportion of fibres to intra-fibrillary matrix and the density of cell population varied from site to site.

Cells showing many morphological variations were found in the ganglion wall, but in almost all instances they contained an abundance of very fine intracytoplasmic filaments. In favourable sections, focal densities or attachment sites of the type known to occur along myofilaments were seen

FIG. 2 Ganglion cells set in a fibrous matrix, showing an indented nucleus (N) and myofilaments with focal densities (arrows). $\times 8,000$.

FIG. 1 *Portion of ganglion wall. The surface facing the cavity is not included but it lay just beyond the top right corner of the picture. Numerous long strap-like cells are seen lying in a fibrillary matrix (M). The cytoplasmic matrix appears dense as it is packed with myofilaments which cannot be resolved at this magnification. However, focal*

densities can be detected quite frequently (arrows). Dilated cisternae of the rough endoplasmic reticulum (E) are seen in one cell, and another, placed closer to the surface than the rest, also shows numerous vacuoles (V) and a disorganization of internal architecture. $\times 5,400$.

FIG. 3 High-power view of a portion of a cell illustrated in Fig. 2, showing myofilaments and focal densities. $\times 32,000$.

FIG. 5 Cell from ganglion wall, showing dilated cisternae (C) of the rough endoplasmic reticulum and myofilaments (M). $\times 16,000$.

among them (Figs 2 and 3). Besides this, the occurrence of numerous micropinocytotic vesicles, markedly indented nuclei, and the frequent juxta-nuclear position of cell organelles relate these cells (Fig. 4) to smooth muscle cells seen in other sites. In a few of these cells, however, a small or large segment of the cell was occupied by rough endoplasmic reticulum with markedly distended cisternae (Fig. 5) and the myofilaments were displaced to the periphery. Occasionally numerous smooth-walled vesicles and Golgi complex-containing cells of the type one associates with the production of mucopolysaccharides (Ghadially and Roy, 1969; Godman and Lane, 1964; Roy and Ghadially, 1967) were also

found (Fig. 6). Such cells also contained myofilaments.

Another cell type seen on rare occasions appeared to be well endowed with organelles, particularly rough endoplasmic reticulum and micropinocytotic vesicles. This cell can be regarded as an active fibroblast. Cells containing single membrane-bound bodies with heterogeneous electron dense contents were sometimes seen around blood vessels. These cells are acceptable as phagolysosome-containing macrophages.

The matrix of the ganglion wall composed mainly of characteristically banded collagen fibres and fine fibrils set in a medium density interfibrillary matrix.

FIG. 4 Cell from ganglion wall, showing indented nucleus, juxtannuclear organelles, numerous micropinocytotic vesicles, and myofilaments with a suggestion of focal densities. $\times 31,500$.

FIG. 6 Cell from ganglion wall. The cytoplasmic matrix is packed with myofilaments (M). Golgi complex (G) and numerous smooth-walled vacuoles (V), some apparently about to open or opening into the matrix (arrows), are also seen. $\times 21,000$.

FIG. 7 Ganglion wall adjacent to ganglion cavity (C), showing cell fragments (F), cells with pyknotic nuclei (P), and a blood vessel with a slit-like lumen (B). $\times 7,000$.

The ratio of fibres to interfibrillary matrix was variable, some sites being better endowed with fibres than others. Occasionally elastic fibres were also encountered.

(B) Lining of ganglion cavities

Since only small samples of tissue can be examined by electron microscopy, an unequivocal statement regarding the proportion of ganglion wall lined or not lined by cells cannot be made. However, it is our impression that large sections of the ganglion wall are lined not by cells but by necrotic debris, and that when cells are found at the surface they appear degenerate rather than engaged in the production of a secretion. The crumbling wall of the ganglion immediately adjacent to a cavity is illustrated in Figs 7 and 8, and it can be observed that disintegration of both cells and collagenous fibres is occurring in this region.

An interesting situation is seen in Fig. 9, where the ganglion cavity is lined not by synovial cells

but by disintegrating cells resembling one of the variants of the smooth muscle cell types already described earlier in the wall of the ganglion. There is gross dilatation of the cisternae of the rough endoplasmic reticulum in these cells. Such cells contained an abundance of myofilaments and in many instances characteristic dense foci could be detected. In another case a row of cells with large vacuoles was seen lining the surface (Fig. 10). It is difficult to be certain whether these vacuoles represent secretory activity or are the product of cellular degeneration.

(C) Ganglion contents

The ganglion contents from the two cases studied were of almost identical appearance (Fig. 11). They were composed largely of an amorphous low density matrix within which were embedded small and large accumulations of fine fibrillary material and irregular osmophilic flakes of a membranous nature. Intermingled with the fine filamentous

FIG. 8 Ganglion wall adjacent to cavity (C) which is just included in this electronmicrograph, showing a disintegrating cell with pyknotic nucleus (P), cell remnants (D), and

fibrillary material (F), presumably derived from breakdown of collagen, as evidenced by some residual banding (arrows). $\times 20,000$.

FIG. 9 Cells with a dense cytoplasmic matrix, indicating the presence of myofilaments, are seen in the wall and on the surface facing a ganglion cavity (C). Focal densities

(arrow) can just be discerned. Well-marked dilatation of the cisternae of the rough endoplasmic reticulum is evident. $\times 8,000$.

material were recognizable bundles of disintegrating collagen fibres. At higher magnifications one could at times just discern the characteristic collagen banding.

Discussion

NATURE OF CELLS IN THE WALL OF GANGLION

Ganglia are composed of a mass of fibrous tissue and light microscopists have for long regarded the cells in this lesion as fibroblasts.

The nature of the cells involved in this process has been recently investigated by electron microscopy (Arai, 1967; Cotta and Becker, 1969), and the general conclusion reached was that these are mesenchymal cells showing a fibroblastic or synovial type of differentiation. It is interesting that in both these studies the authors observed an abundance of fine filamentous material in these cells, and they discussed at some length the possibility that these might be the precursors of the fibrils and fibres of the matrix. During an early stage of our study, before we had found focal densities among the

filaments which characterize them as myofilaments, we considered the possibility that this might be a degenerative change, for large accumulations of fine filamentous fibres are known to occur in chondrocytes with ageing and osteoarthritis (Barnett, Cochrane, and Palfrey, 1963; Meachim and Roy, 1967; Roy and Meachim, 1968) and in the synovial cells in a variety of chronic pathological states (Ghadially and Roy, 1969) such as chronic haemarthrosis (Roy and Ghadially, 1969), and rheumatoid arthritis (Ghadially and Roy, 1967). However, such filaments are usually thicker and more widely spaced and show a random or whorled arrangement, while in the ganglion cells we find very fine closely packed filaments with a parallel orientation. These features are in fact identical with myofilaments (Fawcett, 1966) seen in typical smooth muscle, a point further demonstrated by the occurrence of focal densities which do not occur with other varieties of intracytoplasmic filaments. It is also perhaps worth noting that a few intracytoplasmic filaments occur in a large variety of cells, probably all cells (Fawcett, 1967) including fibroblasts. Yet such filaments are usually very

FIG. 10 A cell containing numerous large vacuoles (V) is seen lying adjacent to a ganglion cavity (C). The cells in the wall are packed with intracytoplasmic filaments resembling myofilament, but no focal densities are seen. $\times 15,000$.

sparse and morphologically at least, distinct from the filaments in smooth muscle cells.

Thus, our studies clearly indicate that, except for a rare fibroblast and an occasional macrophage, the wall of the ganglion contains variants of only one type of cell, and that it resembles the smooth-muscle cell, rather than any other known variety of mesenchymal cell. One can further argue that, since this is virtually the only cell present, it must be responsible for the production of the various components of the matrix, namely collagen fibres, elastic fibres, and the mucopolysaccharide-containing ground substance of the interfibrillary matrix. Such a concept is not unacceptable at an ultrastructural level for we have seen that these myofilament-containing cells are at times also well endowed with rough endoplasmic reticulum, and in other instances with many smooth-walled vacuoles and Golgi complexes. Thus, by analogy with other mesenchymal cells such as fibroblasts or chondrocytes, one can speculate that the rough endoplasmic reticulum is involved in the production of tropocollagen for the production of fibres in the

matrix, while the Golgi and smooth-wall vacuoles synthesize and deliver the mucosubstances of the interfibrillary matrix.

Although this is an unexpected and unprecedented finding as far as ganglia are concerned, this situation is not totally without precedent, for in at least one other site, namely the arterial wall (Wissler, 1967), the smooth muscle cell is believed to be a multifunctional cell capable of producing not only myofilaments but also collagen fibres, elastic fibres, mucopolysaccharides of the interfibrillary matrix, and basement membrane. It is also capable of trapping serum lipoproteins and turning into a foam cell.

Indeed, there is a remarkable similarity between some of the morphological variations of the smooth-muscle cell or multifunctional mesenchymal cells we have depicted in this paper and those illustrated in some recent papers on atherosclerosis and ageing arteries (Flora, Dahl, and Nelson, 1967; Knieriem, 1967), and one is led to speculate whether ganglia arise from the walls of vessels in or around the capsule of joints rather than from the joint capsular

FIG. 11 Contents of ganglion cavity. Collections of fibrillary material (F) and some electron dense fragments (D) are seen lying in a medium density matrix (M). Disintegrating collagen fibres (C) are admixed with the fibrillary material. $\times 28,000$.

tissue itself. The absence of blood in the ganglion cavities (except in rare instances) perhaps argues against such a view. Further, no ultrastructural studies on the joint capsule have yet been carried out and it may well be that multifunctional mesenchymal cells occur in this site normally. On the other hand, the intermutability of mesenchymal cells is well known and one could propose that pre-existing mesenchymal cells may alter to form the type of cells we have seen, given a suitable stimulus.

The aetiological factors involved in the production of ganglia are not clearly established, but it has frequently been suggested that trauma, or repeated over-stretching of the joint capsule, may be responsible. The possibility that such a stimulus may provoke mesenchymal cells to proliferate and produce a contractile substance is appealing. In this respect it is also interesting to recall that many ganglia disappear when the tension within is relieved by bursting the ganglion by squeezing or a sharp blow.

LINING AND CONTENTS OF GANGLION

The results of our studies bear on some of the many controversial aspects of the nature of ganglion

contents and lining. Two questions which have been frequently asked are (Arai, 1967; Carp and Stout, 1928; Cotta and Becker, 1969; De Orsay, Mecray, and Ferguson, 1937; King, 1932):

(1) Is the ganglion cavity lined by cells and if so do they show a synovial differentiation?

(2) Are the mucinous ganglion contents the result of myxomatous degeneration of connective tissue or are they a secretion from lining cells?

The light microscope studies of Carp and Stout (1928) and the present study support the idea that the ganglion has no true or extensive cellular lining. However, one must concede that in some areas at least one can detect cells lying at the surface. The important point, however, is whether these cells can be regarded as analogous to synovial cells, and hence the secretors of the mucinous material in the cavity, or whether the occurrence of cells in this situation is fortuitous and dependent on no more than a process of degeneration and necrosis laying bare both cells and fibres as they disintegrate to form the ganglion contents. It seems to us that one can readily show that there is virtually no resemblance between the ganglion and synovial cells.

It is now well established that synovial intimal cells have a characteristic morphology which can be correlated with their functional activity (Ghadially and Roy, 1969). Two main types of synovial cells have invariably been seen in the synovial intima of all species examined to date:

- (1) Type A cells with well-developed Golgi and numerous large smooth-walled vacuoles, but little or no rough endoplasmic reticulum.
- (2) Type B cells well endowed with rough endoplasmic reticulum but with scanty Golgi complexes and mucopolysaccharide containing smooth-walled vacuoles.

Intermediate forms well endowed with both Golgi and rough endoplasmic reticulum are also encountered. Further, it is worth noting that the cytoplasmic matrix of synovial cells is electron lucent, and only on rare occasions are a few intracytoplasmic filaments detectable (Ghadially and Roy, 1969). In contrast to this smooth muscle cells are densely packed with filaments which are often difficult to resolve and give the cytoplasmic matrix a dense appearance (Fawcett, 1966).

Thus one may deduce that there is little resemblance between synovial cells and ganglion cavity lining cells, and the morphology of these cells clearly indicates that they are largely degenerating forms of the cells encountered in the ganglion wall.

We have already given reasons why these myofibril containing cells are best regarded as multifunctional mesenchymal cells. Thus, according to this concept, ganglion cells should be capable of producing a variety of substances including mucopolysaccharides. One can therefore argue that such cells lying at the surface may contribute to the mucinous ganglion contents. Such a contention is perhaps supported by appearances of the type depicted in Fig. 10 where large vacuole-containing cells are seen. However, it seems to us that this can be only a minor factor in the production of the mucoid ganglion contents, for there is overwhelming evidence supporting the thesis that the ganglion contents are the result of a degenerative change. These could be summarized as follows:

- (1) Much of the cavity wall is not lined by any cells.
- (2) When cells are found at the surface they show degenerative changes and a paucity of organelles, particularly Golgi complexes, which one would expect to find if these cells were engaged in the synthesis of mucosubstances.

- (3) The overall appearance of the ganglion wall adjacent to the cavity is one of degeneration and disintegration, affecting not only the cells but also the collagen fibres which show fraying and fragmentation as the surface is approached.

- (4) The ganglion contents are also in keeping with this idea, for cell debris, fibrillary material, and disintegrating collagen fibres occur in abundance.

Thus the notion that an actively secreting cellular membrane resembling synovial intima lines ganglion cavities is totally irreconcilable with our findings, and we uphold the idea that ganglion contents are largely though perhaps not entirely the result of tissue degeneration and necrosis.

Summary

Five ganglia removed at surgery were examined with the electron microscope. Most of the cells found in the wall of this lesion resembled smooth-muscle cells, for they contained numerous fine filaments similar to myofilaments among which focal densities could be frequently demonstrated. Variants of this cell type containing abundant rough endoplasmic reticulum or smooth-walled vacuoles and Golgi complexes were also found. Cells acceptable as fibroblasts and macrophages were seen only in rare instances. It is postulated that the situation existing here is analogous to that seen in the arterial wall, and that these cells in the wall of ganglia are multifunctional mesenchymal cells capable of producing not only myofilaments but also collagen fibres, elastic fibres, and the interfibrillary mucopolysaccharide matrix.

The lining and contents of ganglia were also examined by electron microscopy. It was found that much of the ganglion wall does not have any cellular lining, and that disintegrating collagen and necrotic debris lie at the surface. In segments where lining cells are found they appear to be degenerate rather than engaged in secretory activity. Thus the long-held notion that the ganglion cavity is lined by fibroblasts or synovial cells is not supported by our observations. Examination of the mucoid ganglion content also suggests that a degenerative rather than a secretory process is in operation, for abundant disintegrating collagen and fibrillary and cellular debris are seen lying in the mucoid matrix.

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