SYNOVIAL CELLS

A STUDY OF THE MORPHOLOGY AND AN EXAMINATION OF PROTEIN SYNTHESIS OF SYNOVIAL CELLS

BY

N. WILLIAMSON, K. JAMES, N. R. LING, AND L. P. HOLT

From the Rheumatism Research Wing, Department of Experimental Pathology, University of Birmingham

Because of the infrequency with which fluid is withdrawn from normal joints, most descriptions of the morphology of normal synovial fluid cells are based upon examination of the small quantity of fluid obtained either at autopsy (Ropes and Bauer, 1953) or from patients with non-articular disease following prolonged rest in bed (Coggeshall, Warren, and Bauer, 1940). The cells present have been reported to consist predominantly of monocytes and "clasmatocytes" with small numbers of lymphocytes and only occasional polymorphonuclear leucocytes. Certain other cells, regarded as the actual synovial cells and originating from the joint lining, were extremely few in number (Davies, 1964). Ropes and Bauer (1953) reported an average differential count for normal synovial fluid as:

monocytes 47·9 per cent.	polymorphonuclear leucocytes 6·5 per cent.
clasmatocytes 10·1 per cent.	unclassified phagocytes 4·9 per cent.
lymphocytes 24·6 per cent.	synovial cells 4·3 per cent.

A study of normal synovial membrane (Hamerman and Blum, 1959; Hamerman, Stephens, and Barland, 1961), using the tetrazolium technique for the demonstration of lactic dehydrogenase and DPNH diaphorase activities, not only demonstrated the distribution of the oxidative enzymes, but also revealed the morphological detail of the lining cells more clearly than had previously been seen with routine staining methods.

An extension of this study using the electron microscope to examine the fine structure of normal synovial membrane (Barland, Novikoff, and Hamerman, 1962) revealed the presence of two cell types in the lining membranes:

(i) Numerous type A cells containing many vacuoles, a prominent Golgi apparatus, and finger-like extensions of the cell membrane (filopodia).

(ii) Less frequent type B cells containing abundant ergastoplasm, less extensive Golgi apparatus, few vacuoles, and few filopodia.

It seems reasonable to expect that both these cell types are present in normal synovial fluid.

It is believed that the synovial cells produce the small protein fragment attached to hyaluronic acid (Sandson and Hamerman, 1962), but the synthesis of other specific proteins by the lining cells has not been reported.

Fraser and Catt (1961) and Fraser and McCall (1965) described a method of stripping off some of the lining cells from the synovia of knee joints at autopsy by the use of trypsin. It is possible to compare the properties of cells freed from the synovial membrane in this way with the cells occurring in normal and pathological synovial fluids.

In this and future communications we shall consider the morphological and biochemical features of lining cells obtained from knee joints and make comparisons with normal and pathological synovial fluids and synovial membrane. This paper will be concerned with the types of cells obtained, their superficial morphology, and changes occurring in culture. Evidence for the production of an α -globulin by these cells in culture will also be presented.

Material and Methods

Synovial Cell Suspensions obtained from Autopsy Material.—Synovial fluid was removed through a needle inserted beneath the patella. The joint was washed out with 100 ml. warm phosphate-buffered saline, and about 50 ml. warm 0·25 per cent. trypsin in phosphate-buffered saline introduced and left *in situ* for 30 min. during which time the distended synovial pouches were massaged and the solution frequently withdrawn into the syringe and returned to the joint. The opalescent solution was then withdrawn as completely as possible and transferred to a sterile bottle. The technique, which is essentially that of Fraser and Catt (1961) and Fraser and McCall (1965),

was not always successful. On many occasions the introduced fluid escaped from the joint space and could not be recovered by aspiration. Leakage occurred more frequently from joints of older persons and as the length of time *post mortem* increased. It could not be prevented by "cuffing" above and below the knee. Because of this "leakage" factor it was decided not to force fluid into the joint beyond the point where slight back-pressure was felt in the syringe. On some occasions a solution containing 0.1 per cent. collagenase as well as 0.25 per cent. trypsin was used; on these occasions a slightly better yield of cells was obtained. The cell suspensions, which frequently contained small pieces of tissue and fat globules, were centrifuged at 1,500 r.p.m. for 5 min. as soon as possible after harvesting, and then suspended in Eagle's medium containing 15 per cent. human or calf serum.

Preparation and Examination of Smears.—Smears were prepared directly from the cell deposits. Smears of normal synovial fluid cells obtained before trypsinization were prepared directly from the fluid or after centrifugation when the quantity of fluid obtained was sufficient. Smears of cells from pathological synovial fluids were prepared from the spun deposit. The majority of the smears obtained were air-dried, fixed in methanol, and stained with Jenner-Giemsa, periodic acid-Schiff, methyl green-pyronin, or toluidine blue. Other smears were left unfixed for the detection of lactic dehydrogenase using the method of Nachlas, Walker, and Seligman (1958). Ribonuclease was obtained from Koch Light Laboratories Ltd.

Cell Culture.—Suspensions of the cells in Eagle's medium containing 15 per cent. human or calf serum were transferred to "medical flat" bottles or petri dishes, gassed with 5 per cent. CO₂ in air and incubated at 37° C. Short-term cultures for histochemical and other tests were prepared in 1 cm. polystyrene petri dishes (Oxoid Ltd.) in the bottom of which had been placed a circular cover slip (1 cm. in diameter) specially cleaned for tissue culture. To remove the cover slip after culture without disturbing the cells the supernatant fluid was sucked off, a hole punched in the base of the petri dish with a red-hot needle and the cover-slip pushed up. On some occasions 5 per cent. chick embryo extract was incorporated into the medium. One of the cell lines which grew rapidly was transferred to the revolving Winchester apparatus designed by Wildy (1964). Cells were removed from the glass with 0.1 per cent. trypsin in 0.02 per cent. EDTA in M/180 phosphate in saline.

Examination of Cell Proteins.—Synovial cell cultures for protein investigation were grown in Eagle's medium containing 15 per cent. calf serum from the time of isolation. After removal of culture medium, the monolayer cultures were rinsed with phosphate buffered saline and the cells removed with EDTA-trypsin (20 min. at 37° C.). The cell suspension was centrifuged and the supernate discarded. A soluble extract of the pellet was prepared by suspending the cells in phosphate buffered

saline (pH 6.9, 0.06M containing 0.15M NaCl) and repeatedly freezing and thawing the suspension. The extract obtained was concentrated by ultra-filtration through $\frac{8}{32}$ in. dialysis tubing (Grant, Rowe, and Stanworth, 1958) to give 0.5 ml. of a product with a protein concentration of approximately 2 g./100 ml. Horizontal starch-gel electrophoresis of the protein was performed using the discontinuous buffer system described by Poulik (1957). The immuno-electrophoretic technique was similar to that of Grabar and Williams (1953) using 1 per cent. (w/v) barbitone buffered agar (pH 8.6, I=0.05). Gel diffusion precipitin analysis was also performed in the above agar. The antisera used were prepared in rabbits using as antigens whole human serum, isolated α_2 -macroglobulin, and IgM globulin. Antisera to the purified individual antigens were rendered monospecific by absorption (James, 1965a; Rowe 1962). Hela cell cultures were subjected to a similar procedure and the extract obtained was used as a control.

¹⁴C-lysine Incorporation Technique.—Synovial cells freshly obtained from joints at autopsy were mixed with 1/5th volume of human serum in order to neutralize the effect of the trypsin. The cells were then spun down and suspended in lysine-free Eagle's medium containing 0.5 μ c. ¹⁴C-lysine (Radiochemicals, Amersham). After 24 hours the medium and the suspended cells were removed. Cells attached to the glass were removed with trypsin and added to the cell suspensions after removal of trypsin. The cells were disintegrated by freeze-thawing and homogenization and the homogenate centrifuged at 10,000 r.p.m. for 30 min. The supernate was concentrated by dialysis against Carbowax and subjected to immuno-electrophoresis after the addition of carrier human serum. Two antisera were used. One was from an animal immunized with whole human serum and the other was an antiserum to the three immunoglobulins (IgG, IgA, and IgM). It was prepared by immunizing three separate rabbits with Cohn fraction II human γ -globulin, IgA separated from the serum of a case of IgA myeloma, and IgM from the serum of a case of macroglobulinaemia. The three antisera were then mixed to produce a composite antiserum. The washed and dried plate was clamped to Kodirex x-ray film, exposed for one week in the dark and developed with Kodak D19B for 6 min.

Synovial Membrane Specimens.—These were obtained by needle biopsy (Williamson and Holt, 1966) of *post mortem* joints. Frozen sections were prepared using the Frigistor. Paraffin sections were prepared from material fixed in cold ethanol as described by Sainte-Marie (1962). Sections were examined by the methods described for isolated cells.

Results

Synovial Cell Smears

A study of the smears obtained from knee joints at autopsy after the injection of trypsin revealed a cell population of predominantly two types (Type 1

Fig. 1.—Synovial cells (trypsin technique, *post mortem* joint). Type 1 cells predominate. Occasional Type 2 cells (arrows) are also seen. Stain: Jenner-Giemsa. $\times 380$.

Fig. 2.—High-power view of synovial cells. Azurophilic granules are seen in the Type 1 cells. The single Type 2 cell is easily distinguished by its long cytoplasmic process. Stain: Jenner-Giemsa. $\times 1,050$.

and Type 2). Of these types, Type 1 was far the most commonly seen cell (Fig. 1). When stained with Jenner-Giemsa the Type 1 cells were seen to be large mononuclear cells with pale blue-grey cytoplasm containing numerous azurophilic granules (Figs 2 and 3). These granules were usually arranged in a perinuclear pattern, although in some cells they appeared to be scattered throughout the cytoplasm. The oval shaped nucleus was found mainly in a central position and contained one or two prominent nucleoli. Occasionally, multinucleate forms were seen (Fig. 4, opposite). Much variation in shape and size of these cells was seen, and in many the irregular cytoplasmic membrane appeared to be concentrated to give a beaded appearance around the edge of the cell (Fig. 5, opposite). In some cells the nucleus was displaced to the edge of the cell and the very granular cytoplasm was distended with numerous vacuoles (Fig. 6, opposite).

The second type of cell (Type 2) had a large, eccentric, darkly-staining nucleus and deeply basophilic cytoplasm (Figs 2 and 3). One or two nucleoli were occasionally seen but these were not very prominent. The cytoplasm was often elongated to form a short process and the basophilia tended to fade near the tip.

Fig. 3.—A further group of synovial cells. This shows one Type 2 cell (arrow), two cells with the characteristics typical of monocytes (M→), and three Type 1 cells. Stain: Jenner-Giemsa. $\times 1,440$.

Fig. 4.—Type 1 cells. A multinucleate form is present. Stain: Jenner-Giemsa. $\times 945$.

Fig. 5.—Type 1 cell, showing cytoplasmic extensions, which often give a beaded appearance to the cell membrane. Stain: Jenner-Giemsa. $\times 2,000$.

Also seen was another mononuclear cell differing from the Type 1 cell in that it was smaller with an eccentric often indented nucleus, a smooth cell outline, and fewer cytoplasmic particles (Fig. 3). It had the appearance and characteristics of a monocyte. Occasional lymphocytes, polymorphonuclear leucocytes, and mast cells were also observed.

The average differential count in smears from five normal *post mortem* joints was as follows:

Type 1 cells 73 per cent., Type 2 cells 14 per cent., lymphocytes 6 per cent., monocytes 3 per cent., polymorphonuclear leucocytes 3 per cent., mast cells 1 per cent.

On staining with periodic acid-Schiff (PAS) stain, the cytoplasm of Type 1 cells was usually weakly positive. It was granular and appeared to be arranged in a similar way to the azurophilic granules described earlier. Type 2 cells were unstained by this technique (Table, overleaf).

Pyroninophilic staining, indicating the presence of ribonucleic acid (RNA), was observed in both cell types after staining with methyl green-pyronin. Type 1 cells showed a granular staining scattered

Fig. 6.—Type 1 variant, showing an eccentric nucleus and cytoplasm filled with granules. Stain: Jenner-Giemsa. $\times 1,200$.

TABLE
COMPARISON OF THE STAINING REACTIONS OF TYPE 1 AND TYPE 2 SYNOVIAL CELLS

Stain	Type 1 cell	Type 2 cell
1. Jenner-Giemsa	Cytoplasm: Grey-blue Numerous azurophilic granules Nucleus: Scattered chromatin Prominent nucleoli	Cytoplasm: Deeply basophilic fading towards tip of process No granules Nucleus: Darkly stained Nucleoli not usually seen
2. Methyl green-pyronin	Cytoplasm: Faint granular pyroninophilia Nucleus: Two or more prominent nucleoli	Cytoplasm: Deep pyroninophilic staining fading near tip Nucleus: Nucleoli difficult to distinguish
3. Periodic acid-Schiff	Granular cytoplasmic staining	Unstained
4. Toluidine blue	Occasional metachromatic granules	Unstained
5. For lactic dehydrogenase	Moderate staining Several short irregular cytoplasmic processes	Heavily-stained cytoplasm A single long cytoplasmic process

throughout the cytoplasm. Nucleolar staining was also marked in these cells. Type 2 cells gave intense, general cytoplasmic staining, strongest around the nucleus and fading towards the tip of the cytoplasmic process. Nucleoli were not so easy to distinguish. All pyroninophilic staining was abolished by pre-treatment with ribonuclease.

The majority of cells failed to stain metachromatically with toluidine blue. Occasional Type 1 cells did, however, appear to contain faintly metachromatic granules. The mast cells present gave strong metachromatic staining.

In unfixed smears stained to demonstrate lactic dehydrogenase, long cytoplasmic processes were seen in both Type 1 and Type 2 (Fig. 7). However, considerable variation occurred, some cells having numerous, long branching processes and others very short processes or none at all. This staining tech-

nique gave better morphological detail than was seen in fixed smears stained by Jenner-Giemsa stain. Comparable results were obtained when fresh frozen sections of normal synovium were stained to demonstrate lactic dehydrogenase. Similar long cytoplasmic processes were seen (Fig. 8, opposite) and when sections were cut tangentially across the surface of the membrane it was possible to observe the orderly arrangement of these cytoplasmic processes (Fig. 9, opposite).

This arrangement of the cells was not very easily seen in paraffin sections stained routinely. Paraffin sections of synovium stained by the methyl green-pyronin technique demonstrated pyroninophilic staining in most of the lining cells. The majority of the cells showed a fine granular staining while occasionally other cells gave a deep red staining of their cytoplasm.

Fig. 7.—Type 1 cell with cytoplasmic processes and a typical Type 2 cell (arrow). Stain: for lactic dehydrogenase. $\times 1,134$.

Fig. 8.—Section of normal synovium, showing long cytoplasmic processes of lining cells. Stain: for lactic dehydrogenase. $\times 756$.

Fig. 9.—Section of synovium. This section was cut tangentially across the surface of the membrane. Note orderly appearance of cytoplasmic processes. Stain: for lactic dehydrogenase. $\times 300$.

Examination of smears of normal synovial fluid cells taken before the introduction of trypsin demonstrated a cell population which was virtually identical with that seen after the addition of trypsin. Far fewer cells were available for examination in these preparations so that differential counts were not very reliable, but the impression gained was that the distribution of cells was about the same as in smears made after trypsin treatment except for a slight increase in lymphocytes and the absence of mast cells.

Over a hundred smears of synovial fluid cells from several different pathological conditions were examined. Considerable differences were found in these smears, but in almost all it was possible to demonstrate cells similar in structure to the Type 1 and Type 2 cells seen in the normal fluid smears.

Synovial Cell Culture

Variable proportions of the cells obtained by trypsinization of joints were viable, as judged by the numbers spread out on the glass after overnight incubation. The proportion depended mainly upon the interval after death which elapsed before the cells were obtained and also apparently upon the age of the patient. Leaving the cells for as short a time as possible in the trypsin also improved the yield of viable cells. The best results were obtained when cells were harvested within 6 hours of death, although a considerable number of viable cells were still present as long as 12 hours after death.

The morphology of cells stained after some days in culture was similar to that of the cells described by Fraser and co-workers (1961, 1965). Variations in

Fig. 10.—Synovial cells (after 48 hrs' culture), showing marked variation in morphology. Stain: Jenner-Giemsa. $\times 380$.

Fig. 11.—Synovial cells (after 48 hrs' culture), stained to demonstrate lactic dehydrogenase. Numerous delicate processes are seen. Stain: for lactic dehydrogenase. $\times 480$.

shape and size of cells were noted: some showed numerous cytoplasmic processes; others were rounded with large eccentric nuclei, prominent nucleoli, and basophilic cytoplasm; still others were spindle-shaped (Figs 10 and 11). It was impossible to say whether one original cell type or more was responsible for these appearances.

Protein Synthesized by the Cultured Cells

Although there was usually no difficulty in keeping the cells alive and spread over the glass for periods of a week or longer, it was more difficult to produce an

established culture of dividing cells. On some occasions a contaminant appeared after about one week but usually the cultures remained sterile. Even in sterile cultures the cells did not proliferate or did so only for a short period. The large number of cells required for protein analysis were obtained from only one cell preparation. These cells were cultured for the first few weeks in medium containing 5 per cent. chick embryo extract and 15 per cent. calf serum and subsequently in the same medium without embryo extract. The cells survived for about months.

On starch-gel electrophoresis one of the principal components of the extract was a protein band with an electrophoretic mobility similar to that of α_2 -macroglobulin (Fig. 12). The extract also contained traces of another protein with an electrophoretic mobility approximately the same as that of albumin. At least three antigenically distinct components were present on immuno-electrophoretograms using a rabbit antiserum to whole human serum (Fig. 13). The protein extracts were also examined by gel diffusion, using a specific rabbit antiserum to human serum α_2 -macroglobulin which had been absorbed with the calf serum in which these cells had been cultured. The absorption step eliminated cross-reactions which might have occurred with calf serum proteins adsorbed on the cells (James, 1965b), but revealed serum proteins manufactured by the cells themselves (Fig. 14, overleaf). The antiserum to human IgM was not absorbed with calf serum as it did not cross-react with calf serum globulins (see Fig. 14). The analysis revealed that the synovial cell extract contained at least three immunologically distinct components, one of which appeared to be identical with human serum α_2 -macroglobulin while another reacted with an antibody in the antiserum to human IgM (see Fig. 14). The failure to detect more than one major component on starch-gel analysis is probably due to the failure of large molecular weight

Fig. 12.—Starch-gel electrophoresis of synovial cell extract. Normal human serum (A); human serum α_2 -macroglobulin (B); synovial cell extract (C). Electrophoresis conducted at 20 volts/cm. for 6 hrs.

Fig. 13.—Immuno-electrophoretic analysis of synovial cell extract.

A = human serum α_2 -macroglobulin
B = synovial cell extract
C = normal human serum
Antiserum = rabbit anti-human whole serum.

Fig. 14.—Gel diffusion precipitin analysis of synovial cell extract.

Antigens: A = normal human serum
B = human serum α_2 -macroglobulin
C = calf serum
D = synovial cell extract

Rabbit antisera to: (i) human α_2 -macroglobulin
(ii) human α_2 -macroglobulin absorbed with calf serum
(iii) human IgM (19S γ) globulin
(iv) whole human serum

proteins (e.g. IgM globulin) to penetrate the gel. The component moving with the same mobility as albumin did not apparently react with the polyvalent antiserum used. The control extract of Hela cells did not show these proteins when subjected to an identical procedure.

Labelling of Synovial Proteins *in vitro* with ^{14}C Lysine

Because of the difficulty in establishing synovial cell lines and the cell selection involved, it was decided to examine the proteins synthesized by freshly-isolated synovial cells. It was not possible to do this by simple extraction of the cells, partly

because of the small quantity of cells obtained from each joint and also because of the possibility that human serum proteins might be present on or in the cells. To avoid this source of error, the radioactive amino acid incorporation technique was used. On radio-immuno-electrophoresis of the extracts, a labelled α -globulin line was obtained. It corresponded to a faint precipitin line on the immunoelectrophoretogram which did not appear to be α_2 -macroglobulin (Fig. 15, opposite). Moreover, it appeared with only one of the antisera employed. The line did not appear with the polyvalent antiserum. It appeared with only one of the two preparations of joint cells tested.

Fig. 15.—Radio-immuno-electrophoresis of synovial cells grown in ^{14}C lysine.

A = immuno-electrophoresis of two synovial extracts (1 and 2) against human serum acting as a carrier

Antisera = (a) rabbit anti-human whole serum
(b) rabbit anti-human gamma globulin

B = autoradiographic picture of immuno-electrophoresis shown in A.

Note marked line in α region of synovial extract 1 when run against anti-human gamma globulin serum.

Discussion

Morphology

Fraser and Catt (1961) and Fraser and McCall (1965) described a method of trypsinization of knee joints at autopsy as a means of obtaining synovial cells for tissue culture. They concerned themselves mainly with the appearance of cells after culture and gave little account of the morphology of the cells immediately after isolation. That these cells were in fact synovial cells was implied by their cultural characteristics. Further evidence was offered by

histological examination of the synovial membrane after the cells had been harvested (Fraser and McCall, 1965). This method gives an excellent means of observing single cells and an opportunity for closer morphological study of synovial cells.

Several studies of normal human and other mammalian synovia using the electron microscope have been made during recent years (*e.g.* Lever and Ford, 1958; Barland and others, 1962, 1964). The work of Barland and others (1962) was of considerable interest in that they distinguished for the first

time two cell types (Types A and B) in normal human synovium. These have since been described in other mammalian synovia (Chapman, Muirden, Ball, and Hyde, 1962; Wyllie, More, and Haust, 1964).

In our study of preparations obtained from knee joints at autopsy it was possible to distinguish two cell types (Types 1 and 2). If it is presumed that these cells have originated from the synovial membrane, then it is probable that the predominant Type 1 cell corresponds to the predominant Type A cell of Barland, and similarly the Type 2 cell corresponds to the Type B cell.

The two cell types were morphologically distinct and it was not possible to distinguish an intermediate cell type as suggested by Chapman and others (1962) and Muirden (1964). However, variants of the Type 1 cell were frequently observed and it is probable that these represent the intermediate types, but in our preparation these cells bore little relationship to the Type 2 cells. Smears made from cultures of synovial cells demonstrated numerous variants and it was not possible to distinguish any specific cell types. Castor and Muirden (1964) described the ultrastructure of synovial cells in culture and they were unable to distinguish different cell types, for these cultured cells had features common to both Type A and B cells.

The staining characteristics of the Type 1 and 2 cells seen in our preparations were consistent with the ultrastructure of Type A and B cells as described by Barland and others (1962). Cytoplasmic basophilia with Jenner-Giemsma stain and deep pyroninophilia with methyl green-pyronin stain in the Type 2 cell suggest the presence of abundant ergastoplasm, whereas the less marked staining in the Type 1 cell is consistent with the sparse ergastoplasm described in Type A cells.

Comparable staining was obtained with sections of synovial membrane. This finding is contrary to that of Shaw and Martin (1962). Much confusion exists in the literature regarding the staining reactions of normal synovial membrane, so the use of smears may enable a clearer concept of the reaction of the two types of cell. The earlier work on the production of mucin and staining reactions of synovial membrane has been reviewed by Bauer, Ropes, and Waine (1940), Davies (1943), Ropes and Bauer (1953), and more recently by Hamerman and Schubert (1962) and Davies (1964).

In our preparations staining with toluidine blue gave faintly positive metachromasia in a small proportion of the Type 1 cells but we feel this to have little significance as it probably represents phagocytosed material.

Staining with periodic acid-Schiff (PAS) reagent gave faint but definite granular staining in the cyto-

plasm of almost all the Type 1 cells but negative staining in the Type 2 cells. It seems possible that the positive PAS staining which we observed was due to the action of this stain on the lipoprotein membrane of the lysosomes (DeDuve, 1963).

Having established the morphological and staining characteristics of synovial cells in our preparations, an attempt was made to recognize these cells in smears from normal and pathological synovial fluids. Two cell types were distinguishable. The cells simply designated as "synovial cells" by earlier workers (Coggeshall and others, 1940; Davies, 1943; Ropes and Bauer, 1953) apparently correspond to the Type 2 cells of our system. However the Type 1 cell was the predominant type of cell in normal synovial fluid and we presume that such cells correspond to the clasmatocyte and perhaps to some of the monocytes described by these earlier workers.

Staining for lactic dehydrogenase emphasised morphological detail of the cytoplasmic processes characteristic of synovial cells. However, there are still many mononuclear cells without processes.

Function

Since Vaubel (1933) gave the original description of the culture of explants of synovial cells many authors (*e.g.* Bartfeld, 1965; Stanfield and Stephens, 1963) have cultured synovia by a similar technique. No substantial difference in the behaviour of normal and rheumatoid synovia in culture has appeared. Bartfeld observed an increase in giant cell forms in rheumatoid synovia but Stanfield and Stephens found giant cells in about equal proportions in both normal and rheumatoid synovia. We have frequently seen multinucleate cells in smears from joints of normal people.

Fraser and Catt (1961) reported that the addition of serum from rheumatoid patients had a cytotoxic effect in culture on the cells obtained from knee joints at autopsy. We have not been able to confirm this. Myhre (1963) found that certain DEAE cellulose fractions of rheumatoid sera were toxic to human lines and synovial cells as well as being cytolytic to human leucocytes. In spite of our failure to find any specific cytotoxic effects of rheumatoid sera in our cultures, immunospecific phenomena may occur in this disease.

A vast quantity of literature has accumulated describing synovial cell structure and function, and variations encountered in disease states, and there has been much speculation on the origin of synovial fluid and the production of the mucin-like substance hyaluronic acid. The secretion of this mucosubstance in synovial cell cultures has been recorded by several authors (*e.g.* Vaubel, 1933a, b; Kling, Levine, and Weiss, 1955; Castor and Fries, 1961).

So far it has not been definitely established which of the synovial cell types is responsible for hyaluronate production. One view (that of Barland and others, 1962) is that the Type A cell has a dual function being both secretory and phagocytic.

The Type B cell is structurally adapted for a synthetic role. Coulter (1962) noted the frequent alignment of cisternae or elongated endoplasmic reticulum parallel to the plasma membrane in these cells, and in the opinion of Kemp (1957) this arrangement is characteristic of a protein-producing cell. The nature of the protein secreted is a matter of some interest for it has been presumed for some time that the proteins in synovial fluid all originate from the plasma and that synovial cells play no role in their formation (Decker, McKenzie, McGuckin, and Slocumb, 1959; Hamerman and Schubert, 1962). However, further study (Sandson and Hamerman, 1962) demonstrated the presence in normal synovial fluid of a protein bound to a small percentage of the hyaluronate, and it was thought probable that this protein might participate in the synthesis of hyaluronate in the synovial membrane cells, or that the protein might be added to the hyaluronate at the cell wall and play a role in the passage of hyaluronate into synovial fluid. Hamerman and Sandson (1963) described the formation of a much higher proportion of unusual hyaluronate protein complex in pathological fluids and they considered it possible that cells in the pathological synovial membrane synthesized this unusual compound of hyaluronate and protein. Sandson and Hamerman (1964) demonstrated that an alpha globulin was bound to this hyaluronate protein of pathological synovial fluids. Tests with specific antisera showed that this protein was not ceruloplasmin, haptoglobin, or α_2 -macroglobulin. An antiserum to hyaluronate protein has been employed by Blau, Janis, Hamerman, and Sandson (1965) to demonstrate by the immunofluorescence technique the presence of this protein in normal synovial lining cells, and it was presumed that this protein was synthesized by these cells.

Recently Kitlowski, Mooney, Rodnan, and Mankin (1965), using radioactive amino acid incorporation techniques with articular cartilage and synovium of rabbits *in vitro*, found a rapid incorporation of ^3H -tryptophan, suggesting a considerable synthesis of some protein or proteins other than collagen.

Our results suggest that a protein of α -globulin-like mobility which is antigenically related to serum α_2 -macroglobulin is synthesized by cultured synovial cells and that other proteins antigenically related to serum proteins are also synthesized by these cells. It should be borne in mind, however, that polyvalent antisera often contain antibodies to cellular (e.g.

erythrocyte and leucocyte) proteins as well as to serum proteins, and some cross-reaction of this sort cannot be completely excluded. However, similar findings were not observed with cultures of Hela cells used as controls.

The function of the synthesized α -glycoprotein still remains obscure, but it is of interest that Marr, Owen, and Wilson (1962) demonstrated the presence of an α -macroglobulin in foetal calf serum, and that this protein was one of two glycoproteins having a growth promoting effect in tissue culture. These workers felt that a possible function of these proteins was as carriers of smaller biologically active molecules. Recently James, Johnson, and Fudenberg (in preparation) demonstrated that α -globulin, especially α_2 -macroglobulin, appeared to interact with a large number of enzymes and hormones and this may be of extreme importance in cellular development. In synovial fluid this may assist in the nutrition of articular cartilage. Any changes in the level of synthesis of this α -macroglobulin may have a profound effect on the viability of the cartilage and further investigation of this subject is required in pathological conditions, especially in degenerative joint disease.

Summary

Two types of synovial cells (Type 1 and 2) were distinguished in smears of cells obtained from knee joints at autopsy after trypsinization of the joint.

The morphology and staining characteristics are described and these are compared with normal synovial membrane.

Comparisons are also made with the ultrastructure of synovial membrane as reported by other workers.

Similar types of cells were observed in both normal and pathological synovial fluid smears.

Synovial cells in culture were examined.

Evidence is presented for the production by synovial cells of a protein which is antigenically related to serum α_2 -macroglobulin. A possible role of this glycoprotein as a carrier protein to assist nutrition of articular cartilage is considered.

We are indebted to Dr. K. W. Walton, Dr. D. R. Stanworth, and Dr. D. S. Rowe for help and advice, and to Dr. C. F. Hawkins for the supply of blood and synovial fluids. This work was aided by a grant from the Nuffield Foundation.

REFERENCES

- Barland, P., Novikoff, A. B., and Hamerman, D. (1962). *J. Cell. Biol.*, **14**, 207.
- , —, — (1964). *Amer. J. Path.*, **44**, 853.
- Bartfeld, H. (1965). *Ann. rheum. Dis.*, **24**, 31.
- Bauer, W., Ropes, M. W., and Waite, H. (1940). *Physiol. Rev.*, **20**, 272.
- Blau, S., Janis, R., Hamerman, D., and Sandson, J. (1965). *Arthr. and Rheum.*, **8**, 432.

- Castor, C. W., and Fries, F. F. (1961). *J. Lab. clin. Med.*, **57**, 394.
- and Muirden, K. D. (1964). *Lab. Invest.*, **13**, 560.
- Chapman, J. A., Muirden, K. D., Ball, J., and Hyde, P. A. (1962). In "Electron Microscopy: Fifth International Congress for Electron Microscopy, Philadelphia, 1962", ed. S. S. Breeze, vol. 2, ss-12. Academic Press, New York.
- Coggeshall, H. L., Warren, C. F., and Bauer, W. (1940). *Anat. Rec.*, **77**, 129.
- Coulter, W. H. (1962). *Arthr. and Rheum.*, **5**, 70.
- Davies, D. V. (1943). *J. Anat. (Lond.)*, **77**, 160.
- (1964). In "Textbook of the Rheumatic Diseases", ed. W. S. C. Copeman, 3rd ed., pp. 35-80. Livingstone, Edinburgh and London.
- Decker, B., McKenzie, B. F., McGuckin, W. F., and Slocumb, C. H. (1959). *Arthr. and Rheum.*, **2**, 162.
- DeDuve, C. (1963). In "Ciba Foundation Symposium: Lysosomes", ed. A. V. S. de Reuck and M. P. Cameron, p. 17. Churchill, London.
- Fraser, J. R. E., and Catt, K. J. (1961). *Lancet*, **2**, 1437.
- and McCall, J. F. (1965). *Ann. rheum. Dis.*, **24**, 351.
- Grabar, P., and Williams, C. A. (1953). *Biochim. biophys. Acta*, **10**, 193.
- Grant, G. H., Rowe, D. S., and Stanworth, D. R. (1958). *Biochem. J.*, **69**, 13P.
- Hamerman, D., and Blum, M. (1959). *Arthr. and Rheum.*, **2**, 553.
- and Sandson, J. (1963). *J. clin. Invest.*, **42**, 1882.
- and Schubert, M. (1962). *Amer. J. Med.*, **33**, 555.
- , Stephens, M., and Barland, P. (1961). In "Inflammation and Diseases of Connective Tissue: A Hahnemann Symposium", ed. L. C. Mills and J. H. Moyer, pp. 158-168. Saunders, Philadelphia.
- James, K. (1965a). *Biochim. biophys. Acta*, **100**, 509.
- (1965b). *Immunology*, **8**, 55.
- , Johnson, G., and Fudenberg, H. H. In preparation.
- Kemp, N. E. (1957). *Univ. Michigan med. Bull.*, **23**, 197.
- Kitlowski, N. P., Mooney, V., Rodnan, P., and Mankin, H. J. (1965). *Arthr. and Rheum.*, **8**, 452.
- Kling, D. H., Levine, M. G., and Weiss, J. (1955). *Proc. Soc. exp. Biol. (N.Y.)*, **89**, 261.
- Lever, J. D., and Ford, E. H. R. (1958). *Anat. Rec.*, **132**, 525.
- Marr, A. G. M., Owen, J. A., and Wilson, G. S. (1962). *Biochim. biophys. Acta*, **63**, 276.
- Muirden, K. D. (1964). Reference to unpublished work in Castor and Muirden (1964).
- Myhre, B. A. (1963). *Fed. Proc.*, **22**, 499, Abstr. 2024.
- Nachlas, M. M., Walker, D. G., and Seligman, A. M. (1958). *J. biophys. biochem. Cytol.*, **4**, 29.
- Poulik, M. D. (1957). *Nature (Lond.)*, **180**, 1477.
- Ropes, M. W., and Bauer, W. (1953). "Synovial Fluid Changes in Joint Disease". Harvard University Press, Cambridge, Mass.
- Rowe, D. S. (1962). *Immunology*, **5**, 549.
- Sainte-Marie, G. (1962). *J. histochem. Cytochem.*, **10**, 250.
- Sandson, J., and Hamerman, D. (1962). *J. clin. Invest.*, **41**, 1817.
- (1964). *Science*, **146**, 70.
- Shaw, N. E., and Martin, B. F. (1962). *J. Anat. (Lond.)*, **96**, 359.
- Stanfield, A. B., and Stephens, C. A. L., Jr. (1963). *Texas Rep. Biol. Med.*, **21**, 400.
- Vaubel, E. (1933). *J. exp. Med.*, **58**, 63, 85.
- Wildy, P. (1964). Personal communication.
- Williamson, N., and Holt, L. P. J. (1966). *Lancet*, **1**, 799.
- Wyllie, J. C., More, R. H., and Haust, M. D. (1964). *Lab. Invest.*, **13**, 1254.

Les cellules synoviales

Étude de la morphologie et de la synthèse protidique

RÉSUMÉ

On distingue deux types de cellules synoviales (Type 1 et type 2) dans des frottis obtenus à partir de genoux lors d'autopsies après trypsination de l'articulation.

On décrit leur morphologie et leurs caractéristiques de coloration et on les compare avec ce qu'on observe dans une membrane synoviale normale.

On fait également des comparaisons avec l'ultrastructure de la membrane synoviale observée par d'autres auteurs.

Des types identiques de cellules ont été notés sur des frottis provenant de liquides synoviaux normaux aussi bien que pathologiques.

On examina des cultures de cellules synoviales.

On fournit des preuves de la production par les cellules synoviales d'une protéine antigéniquement apparentée à la macroglobuline- α_2 . On prend en considération le rôle possible de cette glycoprotéine comme support protéique facilitant la nutrition du cartilage articulaire.

Las células sinoviales

Un estudio de la morfología y un examen de la síntesis protéica de las células sinoviales

SUMARIO

Se distinguieron dos tipos de células sinoviales (tipos 1 y 2) en tomas de rodillas obtenidas durante autopsia después de triptinización de la articulación.

Se describe la morfología y características tincionales y se comparan con lo que se observa en membrana sinovial normal.

Comparaciones se hacen también con la ultraestructura de la membrana sinovial según descrita por otros autores.

Tipos semejantes de células se observaron en tomas de líquidos sinoviales tanto normales como patológicos.

Se examinaron cultivos de células sinoviales.

Se ofrecen datos de que las células sinoviales producen una proteína antigénicamente relacionada con la macroglobulina- α_2 . Se considera el posible papel de esta glycoproteína como un portador protéico para asistir nutrición del cartilago articular.