CULTURE OF SYNOVIAL CELLS *IN VITRO* * NOTES ON ISOLATION AND PROPAGATION

BY

J. R. E. FRASER AND J. F. MCCALL

From the University of Melbourne, Department of Medicine, Royal Melbourne Hospital, Victoria, Australia

The culture of synovial cells *in vitro* provides a useful means for the study of connective tissue function. Vaubel (1933) first used cultures of synovium to show that mucin was secreted in the joint itself, a conclusion since confirmed by others (Kling, Levine, and Wise, 1955; Castor, 1957; Hedberg and Moritz, 1958; Fischer and Torgerson, 1960). In recent years, Castor has studied synovial culture extensively in a variety of aspects (Castor, 1959, 1960, 1962; Castor and Fries, 1961; Castor and Prince, 1963). In all these studies synovial culture has relied on the growth of cells from pieces of synovium, or "explants". In a preliminary report (Fraser and Catt, 1961), we described an alternative method for the harvest of synovial intima in a monolayer suspension suitable for culture. This paper records our further experience with the initial isolation of synovial cells, and their serial propagation beyond the stage of primary culture. Evidence is also presented to show that the new method is more likely to yield cells derived solely from the synovial intima. Certain aspects concerned with specific experimental applications will be reported in detail later.

Initial Isolation of Synovial Cells

Method

In all studies, synovial tissue has been obtained from amputated limbs, or from limbs of subjects within 6 hours of death. The knee joint was used most often. The first method tried was the agitation of finely-chopped synovium in trypsin solution, but too few cells were obtained to establish satisfactory cultures. It was then found that cells would slowly grow from synovial fragments left undisturbed in a suitable medium, as in the studies quoted. However, dissatisfaction with the slow

and unpredictable growth in this system led us to attempt again to disperse cells from synovium before culture, by injecting appropriate solutions into intact joints. The following is the procedure now used.

An 18 S.W.G. needle with stylette is inserted beneath the patella. All solutions are heated to 37° C. The knee joint is rinsed twice with 50 ml. calcium- and magnesium-free Hanks's balanced salt solution or physiological saline, which is then withdrawn and replaced with 30 to 60 ml. trypsin (0.25 per cent. in phosphate-saline buffer). This is left in place for 15 min., then drawn back into the attached syringe to avoid increased tension in the joint during subsequent gentle massage of the synovial pouches. The trypsin solution is again injected into the joint cavity and withdrawn into the syringe where it is inspected in a strong light for opalescence. With experience, this visual assessment of the cell harvest is adequate. In case of doubt, the cell density may be gauged by examining the syringe through an inverted microscope, or by enumeration of an aliquot in a haemocytometer. If the yield is low, the trypsin is replaced in the joint and the same manoeuvres are repeated 5 minutes later. The cell suspension is aspirated, spun down at 1,000 r.p.m. for 10 min. and re-suspended in culture medium. If the suspension contains clumps of cells or flakes of cartilage (which suggests too vigorous handling of the joint) it may be filtered through a sterile stainless steel gauze before centrifugation.

Results and Comments

In eighteen studies, the yield from each knee ranged from 3×10^6 to 33×10^6 cells (average 11.0×10^6). Apart from other factors, the tissue temperature was an unknown variable and synovial surface areas differed widely as judged by the fluid capacity of the joints. However, it has been consistently possible to obtain sufficient numbers of cells to start cultures.

To ensure that the cells are in a suitable state for culture, two precautions are carefully observed:

(1) Donor joints are deliberately selected. We

* This study was made possible by grants from the Arthritis and Rheumatism Council for Research in Great Britain and the Commonwealth, the National Health and Medical Research Council of Australia, and the Australian Rheumatism Council.

prefer amputated joints where viability of the knee joint can be ascertained beforehand, and the specimen obtained quickly. Although it may be possible to obtain viable cells after longer intervals, we have avoided material more than 6 hrs old, and prefer it earlier, believing that the most specialized cells might be first to succumb. Rigor mortis also makes the technique difficult and the joints likely to rupture with distension. If the initial saline wash is turbid, the procedure is not continued.

(2) The time of exposure to trypsin and massage of the joint is kept to a minimum. Much heavier cell yields can be obtained by a further trypsin treatment or massage, but the suspension then contains flakes of cells embedded in fibrous matrix (Fig. 1), and fat globules appear on top after centrifugation, indicating breach of the synovial intima.

Fig. 1.—Cells embedded in fibrous matrix. See text. $\times 280$.

The foregoing precautions are intended to minimize cell damage before culture, and to extract only the superficial cells of the synovium. A series of twelve sections* taken from the massaged areas of four joints after the procedure showed in each instance that the intima was virtually intact, with no disruption of the subsynovial layer (Fig. 2). Although these sections sampled relatively small areas, they indicated that the majority of the detached cells

Fig. 2.—Section of synovium after trypsinization, with intima intact. $\times 100$.

were derived solely from the intima. If a cell suspension were intended for other uses, such as preparation of cell "antigen", a more vigorous approach might be used.

Effect of Hyaluronidase.—A solution of hyaluronidase was used before trypsin, in view of the concentration of mucopolysaccharide between cells of the synovial intima (Hamerman and Ruskin, 1959). In a pilot study, this yielded a dense suspension of cells. A comparison was therefore made in four subjects *post mortem*, in which one knee was previously treated with testicular hyaluronidase, 100 T.R.U. per ml., and the other with a control solution before trypsin. Hyaluronidase alone was ineffective, and did not enhance the harvest subsequently obtained by trypsin (Table I).

TABLE I
YIELD FROM TRYPSIN WITH AND WITHOUT
PRE-TREATMENT BY HYALURONIDASE

Strain No.	No. of Cells	
	After Hyaluronidase	After Control Solution
61-5	0.8×10^6	6.6×10^6
61-6	19.0×10^6	17.0×10^6
61-7	30.8×10^6	26.8×10^6
61-8	8.4×10^6	9.4×10^6

* We are indebted to Dr. J. D. Hicks, Pathologist to the Royal Melbourne Hospital, for his assessment of these sections.

Serial Propagation

Methods

Medium.—Cultures were established and maintained in Medium 199 (Morgan, Morton, and Parker, 1950) enriched with a minimum of 20 per cent. serum. All but two primary cultures were prepared in medium containing human serum with or without foetal calf serum, and subcultures in human or heterologous serum. All serum had been heated at 56° C. for 30 min. before use. Heterologous serum was specially prepared for tissue culture by the Commonwealth Serum Laboratories. Human serum was taken from fasting subjects, and was not used if lipaemia or haemolysis were visible.

Culture Vessels.—160-ml. "Pyrex" rectangular bottles or 1 litre "Pyrex" Roux flasks were used for stock cultures. Polystyrene Cooper dishes (Cooper, 1961) and culture flasks (Falcon Plastics) and acrylic slides with glass cover-slips were used for special studies. Other types of glass were tried with variable results, and were not used routinely.

Primary Cultures.—The cell sediment was gently suspended in culture medium and aliquots distributed in culture flasks. Additions and changes of growth medium were made when required by the fall in pH, but the bottles were left otherwise undisturbed until ready for subculture.

Subcultures.—The medium was decanted, the culture gently rinsed with calcium- and magnesium-free Hanks's balanced salt solution and exposed to 0.25 per cent. trypsin until microscopic examination showed beginning detachment of cells. This was completed by gentle agitation. The cells were then spun down at 1,000 r.p.m. for 10 min., re-suspended in fresh culture medium, and inoculated into fresh vessels, 10 to 15 ml. in the 160-ml. bottles, and 90 ml. in the litre flasks. Subculture was done before the vessel was completely covered with cells, and the fresh inoculum made with a density of $4 \cdot 10 \times 10^4$ cells per ml. With experience, this could be judged with sufficient accuracy without counting. In order to minimize damage, complete dispersal of cells was not sought unless accurate replicate cultures were required for specific experiments. A weekly routine of subculture was adopted wherever possible.

Assessment of Cytoplasmic Spreading and Cell Growth.—Methods were devised to assess the rate of cytoplasmic spreading of the cells after settling and attachment in new culture vessels, and to measure the rate of cell multiplication under various influences. These will be described in detail separately. Counts of suspended cells were made with a Coulter electronic cell-counter.

Culture of Cells in Suspension.—This was studied in a 50-ml. Bellco spinner flask with magnetic stirrer. The rate of agitation and nature of the medium were varied as stated below.

Deep-freeze Storage.—Cells were harvested in a phase of rapid growth, and stored in 2-ml. lots of $1 \cdot 5$ to $2 \cdot 0 \times 10^6$ cells in growth medium with 10 per cent. glycerol at -65°C . When required, the glass ampoules were rapidly warmed to 37°C ., and the contents added to 10 ml. of fresh growth medium.

Results and Comments

Primary Culture.—Complete failure of primary cell growth occurred in three of 37 cell dispersions. In one, failure was thought to be due to ischaemia of the donor specimen; in the other two instances, it was later found that well-established cultures grew poorly in the particular batch of glassware used. Another primary culture in the same glassware declined after early outgrowth. Two other cultures degenerated after 15 and 25 days respectively for no clear reason.

After attachment to the surface of the culture vessel, the first change in primary cultures was extension of cytoplasmic processes from the rounded state of cells after trypsin dispersal (Fig. 3).

Fig. 3.—Culture 18 hrs after isolation. Note cytoplasmic spreading. $\times 60$.

In some instances, these changes were delayed, and such cultures were best left with the least disturbance. Thereafter, multiplication was obvious, although the initial rate of growth varied widely. Addition or replacement of fresh medium became necessary with

the fall in pH, dead cells and debris being thus removed. In the first week, the apparent size of the cells increased as manifest by surface area of the cytoplasm. Subculture was usually required between 6 and 29 days (Table II). It seemed that rapid primary growth and early subculture were favoured by establishing the culture with a high cell density. Although this effect might have been illusory, it has been observed with other cell types (Ferguson and Wansbrough, 1962). With primary cultures, the optimal cell density appeared to be over 1×10^5 cells per ml., about twice as high as required for subcultures.

TABLE II
INTERVAL BETWEEN PRIMARY ISOLATION AND
FIRST SUBCULTURE

Interval (days)	8	8-14	15-28	29
Number of Cultures	5	14	7	2

Three primary cultures were lost by infection or overheating.

Long-term Propagation.—The remaining cultures grew well, and in the absence of infection or other accidents, could be sustained by serial subculture up to 6 months. Maintenance of a strain depended on sufficient multiplication between each passage to replace cells lost in transfer and used for experiments.

Each subculture cycle clearly consists of several distinct phases, which are not subject to the same conditions. Thus the first phases of cell attachment and cytoplasmic spreading are likely to be influenced more directly by the process of cell dispersal and by the previous subculture cycle than the next phase of cell multiplication. These phases were therefore studied separately to determine the optimal conditions for sustained culture.

CYTOPLASMIC SPREADING.—Cytoplasmic spreading was never observed in viable cells in free suspension, either after trypsin dispersal or after maintenance in suspension culture. As in other cell cultures, it appeared to be a consequence of cell attachment to a surface. On the other hand, when cytoplasmic spreading failed to follow attachment, the cells would usually detach, die, and disintegrate within 24 to 48 hrs. Excessive enzymic and physical damage readily inhibited subsequent cytoplasmic spreading, and cells which appeared morphologically damaged before subculture, for whatever reason, were more susceptible to otherwise tolerable exposure to trypsin.

Cultures which had formed dense multilayers

often re-established poorly after subculture, although apparently healthy on microscopic examination. Cultures in which cells were sparse and slow-growing behaved similarly, even if the new subcultures were established with an optimal cell density. In these sparse cultures, the cells often showed an unusually large amount of cytoplasm, and detached slowly with trypsin.

Apart from the recent history of the culture and its trypsinization, the culture medium played an important role in the phase of attachment and cytoplasmic spreading. This is still being studied; however, the following conclusions are relevant to routine culture:

(1) All fresh human sera were relatively toxic at high concentrations, and fresh canine serum was extremely toxic even at low concentration.

(2) After heating at 56° C. for 30 minutes, human sera were generally highly favourable for cell attachment and spreading at a concentration of 50 per cent., but lost this capacity to a marked degree at levels below 20 per cent.

(3) In contrast, heat-inactivated heterologous sera (foetal calf, bovine, and canine), although similar in activity to heat-inactivated human serum at higher concentrations, did not lose their potency at levels as low as 5 and 10 per cent. (Table III, opposite.)

CELL MULTIPLICATION.—In these studies, complete dispersal of cells was sought to allow accurate replication of test cultures, and the loss of cells in the first 24 hrs was undoubtedly higher than in routine culture. Cell proliferation was usually obvious after 24 to 48 hrs. Preliminary studies of the effects of serum thereon are shown in Table IV (opposite).

Increasing the concentration of heat-inactivated human serum increased the rate of cell division. Rapid cell division occurred from cell densities as low as 15,000 per ml. of medium, and mean generation times were recorded as low as 26 hrs. Rate of division was much slower when the initial cell density was very low, but this may have been due to greater injury by trypsinization, as indicated by the fall in cell numbers in the first 24 hrs. Addition of foetal calf serum to human serum did not accelerate cell multiplication. Human, bovine, and foetal calf serum were identical in their effect. Monkey serum was less effective, but the serum may have been from a toxic batch, as gross morphological damage was apparent. Chick embryo extract, heated to destroy any viable chick cells, appeared to be an effective growth stimulant, but was not often used as some batches were toxic.

CULTURE OF SYNOVIAL CELLS

355

TABLE III
CYTOPLASMIC SPREADING IN HEAT-ACTIVATED SERUM

Experimental Group*	Serum Concentration (per cent.)	Percentage of Cells with Cytoplasmic Spreading		
		At 24 hrs	At 48 hrs	Remarks
A. Human Serum	50 10	59 29	76 24	Means of 12 experiments
B. Human Serum	50 30 10	82 68 42	77 51 20	Means of 2 experiments
C. Human Serum	20 5	50 36	62 16	Means of 2 experiments
Bovine Serum	20 5	90 82	92 90	
D. Human Serum	20 5	52 25	44 17	Means of 3 experiments
Foetal Calf Serum	20 5	71 73	70 69	
E. Canine Serum	50 10	70 68	76 77	Single experiment

* Each experiment comprised a simultaneous comparison of the stated serum preparations.

TABLE IV
CELL MULTIPLICATION IN HEAT-INACTIVATED SERUM

Test Medium (concentration per cent.)		Initial Cell Count*	†Cell Count* after Experiment	Interval (days)
Human Serum	10	37,659	42,020	3
	50		74,631	
Human Serum	10	13,691	40,118	3
	20		57,002	
	30		62,300	
	50		92,266	
Human Serum Foetal Calf Serum Bovine Serum Monkey Serum	20	16,159	79,156	3
	20		79,312	
	20		77,230	
	20		41,096	
Human Serum Human Serum plus Chick Embryo Extract	20	14,410	79,918	3
	20 2·5		103,925	
Human Serum	10	3,097	5,901	7
	50		13,082	

* Each cell count represents the mean of counts from three replicate cultures.

† All replicate cultures established in standard medium and washed after 24 hrs before addition of test media.

Other Observations.—Extremes of pH impaired the survival and proliferation of cells, particularly at the time of subculture. Low pH was prevented by changes of medium every 2 or 3 days and avoiding very high cell densities. Excessive alkalinity was a less obvious but greater danger. Although the cells might appear healthy, division was slow and adhesion often poor, so that the culture gradually lost numbers. As the medium contained bicarbonate buffer, this situation could arise from:

- (1) Too large a gaseous phase in the culture flask;
- (2) Too few cells to start the culture;
- (3) Toxic agents in the system;

- (4) Leaks in stoppers;
- (5) Residual alkali on glassware;
- (6) Loss of CO₂ from prepared medium exposed too long to air.

With attention to the foregoing details, the chief risk to successful maintenance was infection, attributable in part to use of an open section of a busy general laboratory.

Deep-freeze Storage of Viable Cells.—Successful cultures were established from cells deep-frozen as early as the first subculture, and after storage at -65°C , for as long as 4 months (Table V, overleaf).

TABLE V
DEEP-FREEZE STORAGE OF CELLS

Strain No.	Age when Frozen (days)	Duration of Storage (days)	Growth after Thawing
62-2	28	(a) 112 (b) 121	Yes Yes
62-9	12	6	Yes
62-10	26	(a) 16 (b) 86 (c) 133 (d) 278	Yes No Yes No
62-11	24	(a) 112 (b) 113	No No
61-12	17	(a) 7 (b) 69	Yes Yes
63-3	58	131	Yes
	90	89	No
63-7	24	(a) 28 (b) 29 (c) 46	Yes Yes Yes
	58	21	Yes
63-11	22	56	No

Failures were usually attributable to one of the following reasons:

- (1) Freezing of cells not in phase of rapid growth;
- (2) Storage over 3 months;
- (3) Gas contamination in sealing ampoules;
- (4) Cracks in ampoules, shown by low pH in the sample after storage in solid CO₂.

After recovery from deep-freeze storage, the cultures behaved in all respects as other cultures, and proved a useful way of regaining stocks after our frequent infective accidents.

Cell Culture in Suspension.—It has not been yet possible to achieve cell proliferation in suspension culture. In the usual culture medium, the cultures died within 24 hrs. By using a minimum of agitation, and adding embryo extract to calcium-free modified Eagle's medium (Davis, Glover, and McLimans, 1958), the best result was the maintenance of a high proportion of viable cells (over 85 per cent.) as shown by trypan blue exclusion, with a slow decline in numbers. The cells, though viable, remained quite rounded, mostly single, with a few in groups of two and three. After 14 days in suspension culture, the cells attached, spread their cytoplasm, and proliferated quickly when placed in the usual culture bottles, with ordinary medium, or even in the modified Eagle's medium despite its low calcium content. On the other hand, HeLa cells were grown easily under the same conditions in the spinner flask and special medium. It would appear that the synovial cell strains are incapable of mitosis

unless spread on a solid surface, or require in suspension some particular factor far in excess of that needed in ordinary culture conditions. The former seems more likely.

Other Characters of the Cells.—Synovial cells grown in this manner are "fibroblastic" in appearance. The cytoplasm is relatively profuse in relation to nuclear size and spreads irregularly with numerous thin polar processes (Fig. 4, opposite).

Numerous motile organelles can frequently be seen in the inner aspects of the cytoplasm, but the peripheral cytoplasm is often so thin and featureless that it can only be recognized by phase contrast and high magnification if the edge is slightly crenated.

Cultured synovial cells tend to arrange themselves in a reticular pattern, which seems rather distinct from other "fibroblastic" cell strains. The network gradually fills in to form a dense multilayer if not subcultured. This layer rolls off the flask in a cohesive film in contrast to heteroploid cell lines such as HeLa. Detailed records of this event in fourteen instances showed that it occurred at cell densities of 163,300 to 442,200 cells per ml. medium, the mean density being 286,700. The range was wide, but cell densities varied between different parts of the slightly uneven stock culture vessels, so that peeling occurred in some parts while others were less densely covered. However, the maximum cell density achieved before detachment was usually far short of that possible with established heteroploid cell lines.

Fig. 4.—Synovial cells in established culture, with profuse cytoplasm and long processes. $\times 400$.

Chromosome studies of three strains of cultured synovial cells are shown in Table VI. The karyotypes appeared normal.

TABLE VI
CHROMOSOME ANALYSIS*

Strain No.	Age of Culture (days)	Mitoses Examined	Diploid (per cent)
63-6	106	20	100
63-7	71	20	100
63-8	58	20	100

Karyotypes normal.

* We are indebted to Miss Jean Ferguson, M.Sc., for these analyses.

In the few instances in which a culture was maintained without accident, certain changes gradually occurred after 3 to 5 months. The rate of proliferation slowed, the cytoplasm tended to spread more widely, and a fine granular debris appeared, which seemed to float over the groups of cells as though held in a gelatinous film. Such appearances might represent "senescence", or the limited life-span available to diploid cells in culture. On the other hand, we have observed the same changes under alkaline conditions early in the history of a cell strain, so that they may equally represent some unknown marginal deficiency in cultural requirements.

Hyaluronic Acid Synthesis.—An accurate micro-assay for hyaluronic acid in culture medium was not available to us during most of these studies, and hyaluronic acid secretion was therefore assessed by the mucin clot test using molar acetic acid, which allows only a relative assessment of concentration although it is a good index of polymerization. The specificity of this test was confirmed by the inhibition of mucin clot formation after prior treatment with highly purified bacterial hyaluronidase*. By this method, hyaluronic acid secretion was apparent in the cultures throughout their serial propagation, although it fluctuated widely. It appeared less in very dense or very rapidly growing cultures, and in damaged cultures, yet was often detectable despite low cell numbers or alkaline culture conditions.

Absence of sulphated mucopolysaccharides in media from three strains was confirmed by a study of the ionic conditions in which a polysaccharide complex was formed with cetyl trimethylammonium bromide (Scott, 1960).

Culture medium was also analyzed in a Kern micro-electrophoresis instrument in tris buffer, pH 7.0, $I = 0.05$, by the method of Curtain (1960).

The sole mucopolysaccharide demonstrated was hyaluronic acid, with mobility -7.6×10^{-5} cm.²/v. sec., and a characteristic narrow boundary (Fig. 5).

Fig. 5.—Electrophoretic pattern of culture medium. The slower peaks represent serum proteins included in the medium.

Discussion

The principle described for the isolation of synovial cells is simple, aseptic, and rapid. It can be applied to other hollow structures, although judging from our small experience with arteries different dispersing agents might be preferable for other tissues.

* Kindly donated by N. V. Organon Laboratories, Holland.

The chief advantages of the method are two-fold:

(1) The cells of the extremely thin synovial intima can be selectively separated from the much greater bulk of subsynovial tissue, with its variety of other cell-types.

(2) It is much quicker than dissection of a comparable area of tissue for explants.

It may be that the growth from dissected explants comes largely from the intima but, in the absence of any evidence on this point, the method described here has an important advantage in isolating the intimal cells.

With the use of a primary cell dispersion, some growth *in vitro* is usually apparent within the first few days after isolation, and cytoplasmic spreading within 24 hrs. This is generally more rapid than we have noted in explant cultures, and it is thus possible by the use of replicate preparations to perform experiments at a stage when most of the objections concerning loss of cellular differentiation can hardly apply. However, the treatment of cells in the primary isolation is unavoidably more vigorous than in subculture, and any specific experimental effects must be assessed against a greater background of clumping and other changes than in cells derived from subculture. This consideration, together with the increased supply of cells from subculture, warrants a close appraisal of the nature of synovial cells after serial propagation.

There is a strong belief that cells grown *in vitro* lose so much of their original character as to be virtually useless for many purposes. This is perhaps valid criticism in the case of the established cell lines which gain their infinite capacity for multiplication at the cost of profound changes in morphology, cultural characteristics, and chromosome constitution (Ross, Treadwell, and Syverton, 1962). These changes usually entail a loss of specialized functions such as mucopolysaccharide secretion (Castor, Prince, and Dorstewitz, 1961), but even well-established cell lines may retain at least some of their original antigenic identity (Goldstein, 1957). Nevertheless, "transformation" to an established cell line, with its attendant change from diploid to heteroploid chromosome patterns, presents the greatest hazard to the identity of cells in culture. In recent years, it has been recognized that cell cultures can be maintained for many months in the diploid state despite frequent subculture (Tjio and Puck, 1958; Hayflick and Moorhead, 1961; Ferguson and Wansbrough, 1962). Furthermore, most diploid cell strains degenerate within a year without "transformation" (Hayflick and Moorhead, 1961). The limited

studies of our cultures have confirmed their diploid chromosome complement and normal karyotypes within the period when we prefer to study them. None has exhibited any profound changes in morphology and behaviour. The striking cohesive detachment of multilayers at a relatively low cell density, and resistance to growth in suspension are further points of contrast with the behaviour of some of the heteroploid lines. The latter observation has been recorded before (Hayflick and Moorhead, 1961) and it seems likely that diploid cells from solid tissues require attachment to a surface to allow mitosis.

Maintenance of a normal chromosome pattern does not preclude profound changes in the character of a cell population in the course of long-term culture. The sustained rapid multiplication would rarely be matched *in vivo*, and must impose certain changes, such as increased protein synthesis. However, growth rate can be curtailed if necessary. Morphological appearances offer little help in the recognition of cell populations *in vitro*. Growth on a flat surface imposes a nearly two-dimensional structure, and it is difficult to typify cells in culture as other than "epitheloid" or "fibroblastic". This apparent limitation in structural character is undoubtedly one reason for suspected loss of identity. Nevertheless, there are points of distinction, though not easily measured. We have noted that a reticular pattern of growth is more prominent in synovial cells than in connective tissue cells from human skin grown in the same conditions, and the initial cytoplasmic spreading is mostly polar in the former, and commonly annular in the latter. Electron microscopy may in the future prove more useful in recognizing changes in culture. For example, it has been found that cultures from synovial explants exhibit in the one cell features of the two distinct types of cell found in the synovial intima *in vivo* (Castor and Muirden, 1964).

In the connective tissues of the body, hyaluronic acid occurs as the sole mucopolysaccharide only in the vitreous humour and synovial fluid (Meyer, Davidson, Linker, and Hoffman, 1956). However, the absence of other mucopolysaccharides in the secretion of the cells *in vitro* cannot be taken as certain evidence of their synovial origin, as the sulphated mucopolysaccharides seem to be less readily formed in culture (Grossfeld, Meyer, Godman, and Linker, 1957). In chemical analyses of cells grown from explants of different connective tissues, the content of certain enzymes—lactic acid dehydrogenase, acid phosphatase, and non-specific-esterase—and the soluble cellular proteins have been found to be similar (Castor and Prince, 1963).

However, this may have no more significance than does the morphology imposed by the environment.

The greatest hazard to cellular character is neither genetic nor environmental, but the tremendous opportunity for selection in the course of serial propagation. It is common experience that epithelial cells rarely survive through more than a few passages, whereas the "fibroblastic" cells proliferate freely. In a study of liver, thymus, spleen, and kidney, it has been found that even the primary isolation for culture selects cells showing an antigenic cross-reaction with non-cellular connective tissue elements (Zaroff, Sato, and Mills, 1961). Whether there is further selection within the range of connective tissue cells is not certain, but the possibility must be recognized. In the absence of any toxic influences in medium or glassware, cells are chiefly lost during the phase of attachment and cytoplasmic spreading after subculture. The higher the proportion of cells which is lost in each cycle, the greater is the risk that the cells surviving after a few cycles will represent but a small and hardy fraction of the original population. Apart from the usual care required for cell culture, the liberal use of serum in the culture of synovial cells, particularly in the primary isolation, will reduce this possibility.

Summary

A simple method is presented for the isolation of living cells from synovial intima.

Experience in culture of these cells *in vitro* is described, with an analysis of the optimal conditions for long-term propagation.

We are grateful to Miss Jean Ferguson, M.Sc., for chromosome analysis, and to Dr. C. C. Curtin for electrophoretic analyses; to Miss Beatrix Nadudvary for technical assistance; to Messrs. K. S. Inglis and J. Scrimgeour for photography; and to Prof. R. R. H. Lovell for advice and encouragement.

REFERENCES

- Castor, C. W. (1957). *Proc. Soc. exp. Biol. (N.Y.)*, **94**, 51.
 — (1959). *Arthr. and Rheum.*, **2**, 259.
 — (1960). *Ibid.*, **3**, 436.
 — (1962). *J. Lab. clin. Med.*, **60**, 788.
 — and Muir, K. D. (1964). *Lab. Invest.*, **13**, 560.
 — and Fries, F. F. (1961). *J. Lab. clin. Med.*, **57**, 394.
 — and Prince, R. K. (1963). *Lab. Invest.*, **12**, 38.
 —, —, and Dorstewitz, E. L. (1961). *Proc. Soc. exp. Biol. (N.Y.)*, **108**, 574.

- Cooper, W. G. (1961). *Ibid.*, **106**, 801.
 Curtin, C. C. (1960). *J. sci. Instrum.*, **37**, 190.
 Davis, E. V., Glover, F., and McLimans, W. F. (1958). *Proc. Soc. exp. Biol. (N.Y.)*, **97**, 454.
 Ferguson, J., and Wansbrough, A. (1962). *Cancer Res.*, **22**, 556.
 Fischer, W. B., and Torgerson, J. (1960). *Quart. Bull. Northw. Univ. med. Sch.*, **34**, 124.
 Fraser, J. R. E., and Catt, K. J. (1961). *Lancet*, **2**, 1437.
 Goldstein, M. N. (1957). *J. Immunol.*, **79**, 113.
 Grossfeld, H., Meyer, K., Godman, G., and Linker, A. (1957). *J. biophys. biochem. Cytol.*, **3**, 391.
 Hamerman, D., and Ruskin, J. (1959). *Arthr. and Rheum.*, **2**, 546.
 Hayflick, L., and Moorhead, P. S. (1961). *Exp. Cell Res.*, **25**, 585.
 Hedberg, H., and Moritz, U. (1958). *Proc. Soc. exp. Biol. (N.Y.)*, **98**, 80.
 Kling, D. H., Levine, M. G., and Wise, S. (1955). *Ibid.*, **89**, 261.
 Meyer, K., Davidson, E., Linker, A., and Hoffman, P. (1956). *Biochim. biophys. Acta (Amst.)*, **21**, 506.
 Morgan, J. F., Morton, H. J., and Parker, R. C. (1950). *Proc. Soc. exp. Biol. (N.Y.)*, **73**, 1.
 Ross, J. D., Treadwell, P. E., and Syvertson, J. T. (1962). *Ann. Rev. Microbiol.*, **16**, 141.
 Scott, J. E. (1960). *Meth. biochem. Anal.*, **8**, 145.
 Tjio, J. H., and Puck, T. T. (1958). *J. exp. Med.*, **108**, 259.
 Vaubel, E. (1933). *Ibid.*, **58**, 63.
 Zaroff, L., Sato, G., and Mills, S. E. (1961). *Exp. Cell Res.*, **23**, 565.

Culture des cellules synoviales *in vitro*.—Notes sur leur isolement et propagation

RÉSUMÉ

On présente une simple méthode pour isoler des cellules vivantes de l'intime synoviale.

On décrit des expériences avec des cultures de ces cellules *in vitro*, ainsi qu'une analyse des conditions optimum pour leur propagation prolongée.

Cultura de células sinoviales *in vitro*.—Notas sobre su aislamiento y propagación

SUMARIO

Se presenta un método simple para aislar células vivas de la intima sinovial.

Se describen experimentos con culturas de estas células *in vitro*, así como un análisis de las condiciones óptimas para su propagación a termino largo.