TISSUE CULTURE IN MEDICAL RESEARCH (II)

Editors:
R. J. Richards
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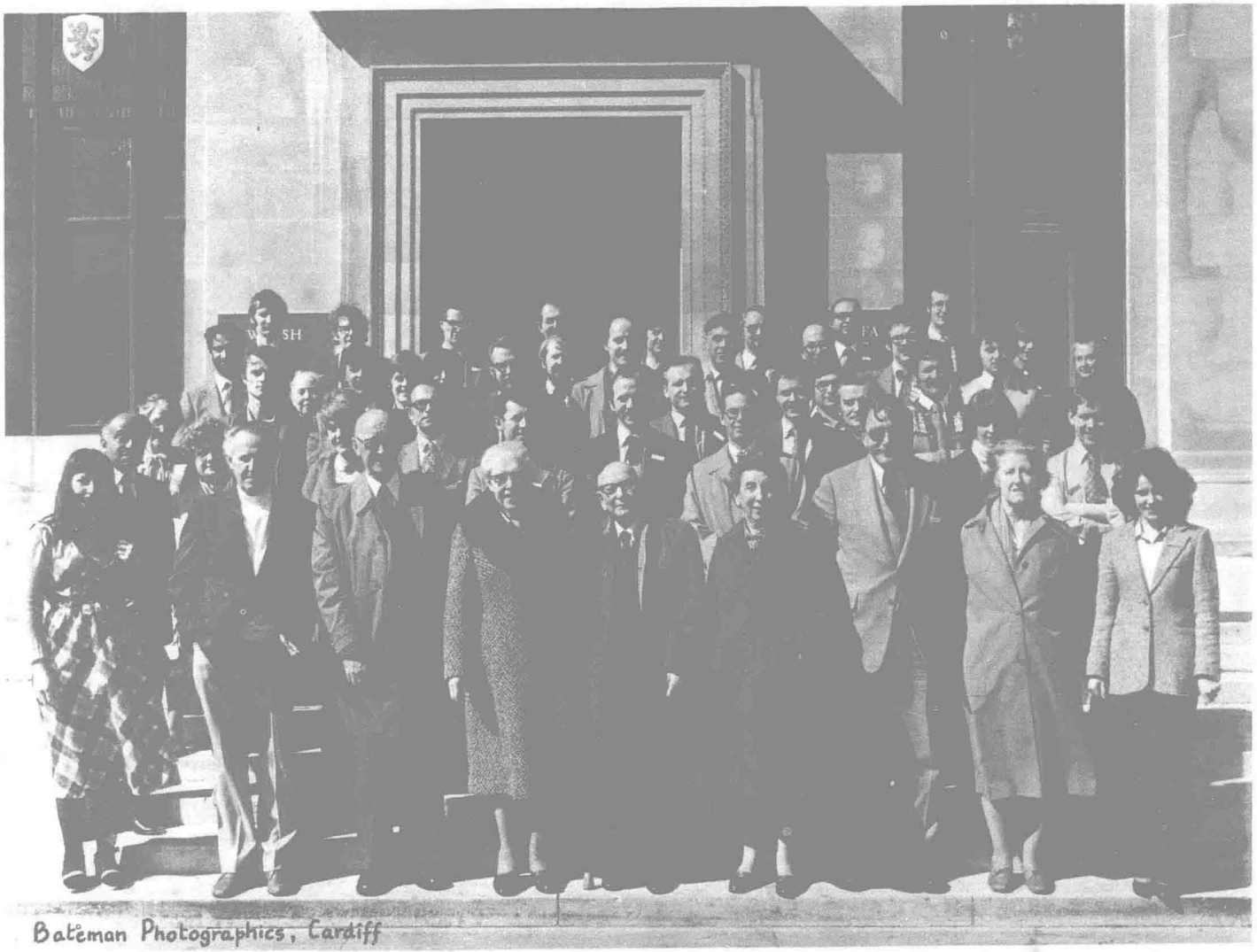
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INTRODUCTION

In 1971 the Tissue Culture Club of Cardiff was established and Dame Honor Fell agreed to be the Life President. In 1973 the first Symposium on Tissue Culture in Medical Research was held in Cardiff when experts from various disciplines met and exchanged ideas. The width of interest in this apparently specialised field prompted us to organise the Second Symposium in April 1980. It seemed appropriate to dedicate this Symposium to our Life President, Dame Honor Fell, F.R.S., who is 80 this year and has continued significantly to contribute to research on bone and cartilage using tissue culture methods. There must be very few Societies who have as distinguished a President as ours, whose work has been acclaimed world wide as honours have come from the Royal Society, French Academy of Science, Royal Netherland Academy, Harvard University and numerous other august bodies.

The intimate scale of the Symposium has been maintained by restricting the number of speakers and this invariably limited the number of subjects that could be covered. The subjects which appeared topical and in which advances have been made recently were specifically chosen. In spite of this it was possible to cover a large ground where Tissue Culture has made a contribution to medicine and these include Skeletal Tissue, Isolated Cells, Oncology, Genetics and Endocrinology. The free sessions and poster demonstration gave the opportunity to cover a much wider field.

The success of the Symposium can be gauged by the contribution not only from Great Britain but also from Netherlands, Germany, Yugoslavia, France, Sweden and the United States of America.

By restricting the numbers it was possible to create an informal atmosphere and to stimulate discussion. Although considerable time was allocated for discussion periods during the Symposium, because of the content of the papers, the interest created, the knowledge and wide ranging interest and experience of those present, these periods were always oversubscribed. The interest shown demands that another such Symposium should be considered before the 90th birthday of our President.

Such symposia, however small, involve considerable costs and we are most grateful to Gill Langley and David Paterson of the Dr Hadwen Trust for Humane Research, London, for securing generous financial support, and to all the trade exhibitors. We should like to thank Mr John Lavender, Managing Editor, Life Sciences, Pergamon Press Ltd., for his active interest in bringing the book to fruition, the contributors for their rapid submission of manuscripts and Carolyn Thomas for her patience in typing all the final corrections.

Finally, we are grateful to many individuals who have helped us with this symposium but special thanks are directed to Professor Dodgson for provision of facilities, the symposium chairmen and to Terry, Jen, Dick, Gerwyn and Marc for their excellent organisation and attention given to all of the participants.

K.T. RAJAN
R.J. RICHARDS

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ABSTRACT

Synovium from normal pigs when minced and explanted in organ culture acquires some of the features of inflammatory tissue in vivo. When associated with articular cartilage it causes breakdown of the cartilage matrix in two ways: (a) by a direct presumably enzymatic action on the matrix of either living or dead cartilage with which it is in contact, and (b) by an indirect action mediated through the chondrocytes for which contact is unnecessary and which affects living cartilage only. Similar changes are produced in isolated living cartilage cultivated in used synovial medium. The synovium also inhibits the incorporation of $^{35}SO_4$ by cartilage whether the two tissues are in contact or separated; this effect is reversible.

The behaviour of synovium and cartilage can be modified by environmental factors. For example serum in the medium increases both the uptake of $^{35}SO_4$ by isolated cartilage and the destructive action of the synovium; hyperoxia (50% O_2; Jubb, 1979) stimulates the destructuve ability of the aerobic synovium but inhibits that of the largely anaerobic chondrocytes.

Hydrocortisone (1 or 0.1 µg/ml) diminishes the destructive ability of the synovium but has little effect on the chondrocytes except to reduce their capacity for re-generating new matrix after depletion by trypsin (E. Lawrence, unpublished).

KEYWORDS

Arthritis, organ culture, synovium, cartilage, serum, hydrocortisone.

INTRODUCTION

As John Dingle (1973) has pointed out, in normal articular cartilage a delicate balance exists between the synthesis of matrix and its degradation. Disturbance of this equilibrium leads to pathological changes in place of the normal turnover, and is a crucial factor in the pathogenesis of various forms of arthritis. The work to be described concerns this balance in cartilage in organ culture and a number of ways in which it can be disturbed with profound effects on the tissue.

ORGAN CULTURES

The organ culture method was essentially the same as that described by Fell and Barratt (1973). Except where otherwise stated, the medium consisted of 15% normal heat-inactivated rabbit serum (NRS) + 85% Dulbecco's modification of Eagle's medium (DMEM). At 2-day intervals the used medium was withdrawn, stored for biochemical examination and replaced by fresh.

The tissues were obtained from the metacarpo-phalangeal joints of young pigs. The cartilage varies widely in different trotters, both in thickness and in the degree to which it responds to an experimental treatment. For this reason it was essential to use a series of paired explants for each experiment, one of each pair for the test and the other as its control. Such pairs were obtained as follows. A strip of cartilage was sliced from each condylar ridge above the level of the invading marrow; each strip was then cut in half, thus yielding a pair of comparable explants.

The synovium presented a more difficult problem, because it is technically impossible to cut equivalent explants of synovium. We got round this difficulty in the following way. Using curved iridectomy scissors we snipped off the synovial tissue from sheets of joint capsule, minced it, pooled the tissue from a number of trotters, then washed and centrifuged it. After decanting the fluid we dispensed measured amounts of the mince (usually 0.025 ml) to the culture vessels.

THE EFFECT OF SYNOVIAL TISSUE ON CARTILAGE

Two series of experiments were made in collaboration with Ronald Jubb, on the influence of the synovium on cartilage grown for 14 days under our standard culture conditions.

The Breakdown of Cartilage Matrix in Response to Synovial Tissue (Fell and Jubb, 1977).

1. Isolated cartilage. Cartilage cultured in isolation underwent little change. Sometimes there was a slight depletion of the matrix just above the cut surface; a variable number of chondrocytes crawled out of the explant, often forming nodules of new cartilage beneath the original fragment.

Isolated synovium. The fragments of synovial mince fused into a continuous mass of fibrous tissue. During cultivation it diminished in volume and acquired a lumpy appearance. In section few necrotic cells were seen, but there were areas where the cells were numerous and the collagen degraded.

In view of the results that follow, it should be emphasised that although the synovium had been obtained from a normal animal it was by no means normal synovium. Its morphology had been grossly disorganised and it was attempting repair in a somewhat chaotic manner. It had some of the characteristics of inflammatory tissue, with breakdown of intercellular material and migration and proliferation of cells.

2. Cartilage in contact with synovium. Contact between cartilage and synovium was achieved in two ways. In one set of experiments the cartilage was placed, articular surface downwards, on the top of a mass of synovial mince; in the other set a pannus was simulated by explanting the cartilage, articular surface upwards on a piece of Millipore filter and then depositing the synovial mince so that it overlapped one end of the cartilage explant. After 14 days' cultivation both arrangements gave essentially the same result, namely severe depletion of sulphated proteoglycan and a variable degree of collagen degradation.

Cartilage overlapped by synovium presented a very interesting histological picture.

In sections stained with toluidine blue, metachromasia was absent from the matrix beneath the synovium and from a tract immediately above the cut surface of the explant. Sections stained with Van Gieson's stain showed considerable breakdown of collagen. The most active part of the synovium was the leading edge of the overlap which scooped a large 'bite' out of the underlying cartilage, the chondrocytes of the degraded cartilage survived and especially in the hypertrophic zone, actively proliferated and gradually assumed a fibroblastic form.

Dead (frozen-thawed) cartilage was treated in a similar way. Proteoglycan was severly depleted, and there was some degradation of collagen, but in general breakdown was less drastic than in paired explants of living cartilage.

3. Cartilage and synovium separated. The fact that living cartilage in contact with synovium was more seriously affected than dead cartilage made us wonder whether the chondrocytes contributed to the degradation of the matrix. To investigate this point, we cultured the cartilage and synovium on the same supporting grid, but not in contact. Again there was breakdown of the matrix in living cartilage.

This result might have been due to hydrolytic enzymes released into the medium by the synovial tissue. This seemed unlikely, however, because although such enzymes are indeed shed into the medium they are in a latent form. However, to test this possibility the experiments were repeated with dead (frozen-thawed) cartilage. If the matrix of the dead cartilage also broke down, this would exclude any participation of the chondrocytes in the process and point to the action of synovial enzymes secreted into the medium. But the dead cartilage remained unaffected. So this result supported the hypothesis that the synovium was producing something which activated the chondrocytes to degrade their own matrix.

4. Isolated cartilage in used synovial medium. The next obvious step was to see whether used synovial medium would have any destructive action on isolated living cartilage. As we expected, the result was similar to that observed when cartilage and synovium were grown in the same culture vessel but not in contact.

What had the synovium done to the culture medium? At this point we handed the problem over to Dr. Dingle's group. As will be reported in Dingle's communication, Dr. Saklatvala succeeded in isolating a substance, which has been termed catabolin, from used synovial medium which when added to normal medium caused degradation of cartilage matrix (Dingle et al. 1979).

Conclusions. (1) Synovial tissue has a direct, presumably enzymatic action on the matrix of both living and dead cartilage with which it is in contact. (2) It also has an indirect action on living cartilage only, which is mediated through the chondrocytes and for which contact is not necessary.

Production of Matrix (Jubb and Fell, in press).

We next proceeded to study the effect of the synovium on the production of cartilage matrix. Steinberg, Sledge, Noble and Stirrat (1979) using bovine nasal cartilage and human rheumatoid synovia found that proteoglycan synthesis was significantly decreased in the presence of synovium. We have investigated the incorporation of radio-active sulphate ($^{35}SO_4$) into the articular cartilage in 3 of our experimental systems.

Explants were exposed either for 24-48 hrs. to medium containing 1.0 µCi $^{35}SO_4$/ml or for 2 hrs. to medium with 10 µCi/ml; the two treatments gave similar results. In some cultures the radioactivity of the explants was assayed biochemically in others the cartilage was fixed for autoradiography.

1. Isolated cartilage. As in our previous experiments, owing to the wide variation between trotters, the work was done entirely on paired explants. The validity of this procedure was tested by comparing the uptake of $^{35}SO_4$ by paired explants of isolated cartilage cultivated for 1-8 days. Biochemical assay showed no significant difference between the means of the pairs.

In autoradiographs, uptake was weakest in the fibrous articular layer, greater in the intermediate region and became very intense over the border between the intermediate and hypertrophic zones; it declined over the deeper hypertrophic cells.

2. The effect of synovium. When synovial mince was present in the culture either in contact with or separated from the cartilage, uptake of $^{35}SO_4$ was sharply reduced. This was clearly demonstrated both biochemically and in autoradiographs.

3. The formation of new cartilage in the presence of synovium. Although synovial tissue inhibited the incorporation of ^{35}S in the original cartilage it did not prevent the development of new cartilage on the cut surface, though it reduced its incidence. Even when the matrix of the original cartilage had lost most of its proteoglycan, chondrocytes crawled out of the explant and formed a thin layer of chondrogenic tissue which showed an intense uptake of $^{35}SO_4$.

In older (14-day) cultures this layer often differentiated into well developed cartilage.

4. Recovery from inhibitory action of synovium. Experiments were made in which pairs of cartilage fragments were explanted at a distance from synovial tissue but in the same dish, and cultivated for 8 days; one of each pair was then exposed to 10 µCi ^{35}SO /ml for 2 hrs. and either fixed for autoradiography or used for biochemical assay. The other was grown for a further 4 days without synovium before receiving 2 hrs. treatment with 10 µCi $^{35}SO_4$/ml and being fixed or harvested for assay. The results showed that when freed from the influence of the synovium the cartilage regained its capacity for incorporating ^{35}S and at the same time released less proteoglycan into the medium.

Conclusion

In our in vitro model, synovial tissue affects both sides of the dynamic balance in articular cartilage. On the one hand it causes destruction of existing matrix, and on the other it interferes with the production of new matrix.

ENVIRONMENTAL FACTORS

The behaviour of cartilage and synovium in our culture system can be modified by altering the environmental conditions. I am going to mention 2 ways in which this can be done: (a) by changing the composition of the culture medium and (b) as Ronald Jubb has shown (1979), by increasing the concentration of O_2 in the atmosphere.

Culture Medium

The object of the first set of experiments (unpublished), made in collaboration with R. W. Jubb, was to investigate the role of serum in the medium. All the experiments mentioned so far, were made with medium containing 15% normal heat-inactivated rabbit serum. We wondered what would happen if we reduced or omitted the serum. Our experiments are not yet complete, but so far we have studied the effect of different concentrations of serum both on the incorporation of radioactive sulphate by isolated cartilage, and on the breakdown of matrix in response to synovial tissue.