SYNOVIAL REGENERATION AND ARTICULAR CARTILAGE
CHANGES AFTER SYNOVECTOMY IN NORMAL AND
STEROID-TREATED RABBITS

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Little is known of the effects of synovectomy on articular cartilage. In order to investigate this matter, anterior synovectomy of the knee was performed in thirty-five normal adult rabbits and in thirty-five which were given 25 milligrams of hydrocortisone intramuscularly each week afterwards. The animals were killed at intervals from four to 110 days after synovectomy. Histological examination of the regenerating synovium in both groups showed complete structural and functional regeneration by eighty days in the first group and a delay in regeneration in the steroid group. 35Sulphur autoradiographs of the articular cartilage of femoral and tibial condyles revealed surface fibrillation and chondrocyte death in 23 per cent of normal knees after eighty days but only 1·8 per cent of knees of animals receiving hydrocortisone. Thus synovectomy in a healthy joint may have an unfavourable effect on the physiology of cartilage by alteration of synovial composition and hyaluronate content in normal joints. Systemically-administered hydrocortisone may reduce this harmful effect in normal cartilage.

In life, articular cartilage is bathed in synovial fluid, which is an ultradialysate of blood plasma essential for lubrication and nutrition of the cartilage. The nutrients are the oxygen, glucose, amino-acids and electrolytes of the fluid, but lubrication requires the presence of hyaluronate—that is, hyaluronic acid linked to a protein. If hyaluronate is removed or reduced in quantity by injection of hyaluronidase the articular cartilage will wear and erode (Barnett 1956).

Normal synovial membrane is composed of a surface layer one to three cells thick which lies on loose fibrous tissue containing a rich plexus of capillaries with associated plexuses of nerves and lymphatics. The fibrous tissue lies on joint capsule and its deep layer is continuous structurally with the capsule. Two types of surface cell have been described: Type A, which is thought to be derived from macrophages, is active in phagocytosis and contains a prominent Golgi apparatus, numerous vacuoles, many filopodia, mitochondria, intracellular fibrils and micropinocytotic vesicles; Type B contains large amounts of ergastoplasm with fewer mitochondria, vacuoles and vesicles, and is probably important in producing some proteins of the synovial fluid.

There is ample evidence from histochemical (Hamer- man and Ruskin 1959), electron microscopic (Roy and Ghadially 1967), and tissue culture experiments (Barland, Smith and Hamerman 1969) to show that hyaluronic acid is produced in the synovial lining cells, probably in the Golgi apparatus of the A cells.

Many clinical studies have indicated the beneficial effect of synovectomy in rheumatoid arthritis when performed at an early stage, before articular destruction is advanced, particularly in the knee and finger joints (Marmor 1966; Mason 1969; Preston 1969; Paradies 1969; Wilde 1973). These studies suggested that articular cartilage damage was delayed or even prevented by synovectomy. However, since synovectomy to be effective in rheumatoid arthritis involves removal of a large part of the membrane, it also involves removal of many cells which produce and control the substances required for nutrition and lubrication, and might therefore be harmful to the articular cartilage.

It has been shown that articular cartilage can obtain nutrition from the subchondral bone (Ekholm 1951; Cruess and Mitchell 1967), but in mature animals and man this almost certainly does not apply (Hodge and McKibbin 1969). Alternatively, it is possible that articular cartilage could be sustained from the tissue fluid of the surrounding fibrous tissue after synovectomy. The replacement of hyaluronate, probably reduced and certainly altered after synovectomy, could be explained either by secretion of more from the residual synovial cells or by secretion from the surrounding fibrous tissue cells.

If the removal of the synovium does alter the supply of hyaluronate or nutrients, albeit temporarily, it would be useful to know when the newly-formed synovium begins to produce normal fluid again, and particularly hyaluronate. This might be important in the management of joints after synovectomy, since the articular cartilage may be vulnerable and require protection until the synovial fluid is returned to normal.

There has been little experimental work in this field. Key (1925) and Wolcott (1927) showed regeneration of synovial membrane within sixty days after synovectomy.

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in rabbits, and Mitchell and Blackwell (1968) confirmed this by an electron microscopic study. Cruess and Mitchell (1967) showed that in rabbits the articular cartilage was almost unaffected by synovectomy; the only abnormality was a slight loss of metachromasia in the superficial and upper-mid zones of the cartilage from four to forty-five days.

An experiment was planned to assess the time of functional as well as morphological synovial regeneration by detecting the reappearance of hyaluronic acid in the regenerating synovial membrane cells after synovectomy, and also to assess the influence of systemic steroids on the regeneration process. For this purpose alcoholic toluidine blue was used to detect the faint metachromasia produced by hyaluronic acid in the cell cytoplasm. Hamerman and Ruskin (1959) had employed this method for indicating the presence of hyaluronic acid in synovial cells. The changes in articular cartilage and subchondral bone were traced simultaneously to detect any effect of synovectomy on the joint surfaces in the two groups of animals.

EXPERIMENTAL METHOD

Seventy mature male New Zealand white rabbits weighing 2.5 to 3.5 kilograms were used. The animals were housed under normal conditions and fed on normal diet. Under general anaesthesia with Nembutal a lateral parapatellar incision was made in the right knee joint and the synovium was removed from the medial and lateral compartments and from the suprapatellar pouch and the infrapatellar fat pad. The menisci were left intact because their removal might have had an effect on the behaviour of the articular cartilage. The posterior capsule was undisturbed. The opposite knee, not operated upon, served as a control. The capsule and quadriceps expansion were closed with plain catgut and the skin was closed with black silk. The animals were left free in their cages after operation and were killed in groups of six at intervals of from four to 110 days. Half of the animals were injected with 25 milligrams of hydrocortisone acetate (Merck, Sharpe and Dohme) intramuscularly at weekly intervals during the experiment, beginning three days after operation.

At death, the knee joints were opened and specimens of synovium were taken from the same area medial to the patella in every case. Plugs of articular cartilage were taken from the opposing surfaces of both femoral and tibial condyles and from the femoral groove (Fig. 1). Finally the whole joint was excised. The synovial specimens were fixed in 10 per cent neutral formalin and serial sections 6 µ thick were cut from paraffin blocks and stained with haematoxylin and eosin and alcoholic toluidine blue. The latter was employed to detect intracytoplasmic metachromasia in the synovial lining cells.

The articular cartilage plugs were prepared by incubating them in roller tubes containing Eagle Basal medium with 100 μcuries/millilitre of radioactive sulphate (35SO4) and penicillin 100 units/millilitre with streptomycin 100 μgrams/millilitre, for three hours at 37 degrees Celsius. The specimens were washed, fixed in 10 per cent formalin and decalcified in ethylene-diamine-tetracetic acid. Serial sections 6 µ thick were cut and stained with haematoxylin and eosin and also with toluidine blue and safranin O for glycosaminoglycans. Autoradiographs were prepared by the dipping technique of Jofes (1963) using Kodak NTB3 emulsion. The slides were developed after ten days in the dark at 4 degrees Celsius and stained with Ehrlich’s haematoxylin and eosin.

RESULTS

The results are summarised in Tables I to III. The thirty-five animals that received hydrocortisone lost on average 30 grams per week, and were obviously wasted. Three died during the course of the experiment and five developed infections of the experimental knee, compared with no deaths and two infections in the group not receiving steroids.

Macroscopically, the normal synovium was a thin, filmy, glistening membrane with a visible delicate vascular pattern. Histological examination revealed a surface intimal layer one to three cells thick with numerous
### Table I

**Synovial Regeneration after Synovectomy in Normal and Steroid-treated Rabbits**

<table>
<thead>
<tr>
<th>Time from synovectomy (days)</th>
<th>Number of animals</th>
<th>Surface layer</th>
<th>Cytoplasmic metachromasia</th>
<th>Vascularity</th>
<th>Subsynovial tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Steroid-treated</td>
<td>Normal</td>
<td>Steroid-treated</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>5</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>25</td>
<td>6</td>
<td>6</td>
<td>Incomplete</td>
<td>Incomplete</td>
<td>---</td>
</tr>
<tr>
<td>50</td>
<td>7</td>
<td>5</td>
<td>Complete 1 cell thick</td>
<td>Incomplete</td>
<td>---</td>
</tr>
<tr>
<td>85</td>
<td>6</td>
<td>5</td>
<td>Normal 1 to 3 cells thick</td>
<td>Single cell lining layer</td>
<td>Present</td>
</tr>
<tr>
<td>110</td>
<td>7</td>
<td>6</td>
<td>Normal 1 to 3 cells thick</td>
<td>Single cell lining layer</td>
<td>Present</td>
</tr>
</tbody>
</table>

### Table II

**Joint Changes after Synovectomy in Normal Rabbits**

<table>
<thead>
<tr>
<th>Time from synovectomy (days)</th>
<th>Number of animals</th>
<th>Articular cartilage</th>
<th>Femoral groove</th>
<th>Subchondral bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Medial femur</td>
<td>Lateral femur</td>
<td>Medial tibia</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>Normal</td>
<td>Normal</td>
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</tr>
<tr>
<td>50</td>
<td>7</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>85</td>
<td>6</td>
<td>3 fibrillation</td>
<td>1 superficial matrix depletion</td>
<td>2 fibrillation</td>
</tr>
<tr>
<td>110</td>
<td>7</td>
<td>3 fibrillation</td>
<td>2 fibrillation</td>
<td>1 superficial matrix loss</td>
</tr>
</tbody>
</table>

### Table III

**Joint Changes after Synovectomy in Steroid-treated Rabbits**

<table>
<thead>
<tr>
<th>Time from synovectomy (days)</th>
<th>Number of animals</th>
<th>Articular cartilage</th>
<th>Femoral groove</th>
<th>Subchondral bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Medial femur</td>
<td>Lateral femur</td>
<td>Medial tibia</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
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<td>Normal</td>
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<td>Normal</td>
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<tr>
<td>50</td>
<td>5</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>85</td>
<td>5</td>
<td>1—thinned disorganised cells in clusters</td>
<td>1 superficial matrix loss</td>
<td>Normal</td>
</tr>
<tr>
<td>110</td>
<td>6</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>
immediately subjacent capillaries and no basement membrane. Between the latter and skeletal muscle or fat there was well vascularised loose fibrous tissue. Whereas the nuclei of both the surface cells and the subintimal cells stained pale blue, the cytoplasm of the surface cells showed violet metachromasia with toluidine blue staining due to the presence of hyaluronic acid (Figs. 2 and 3).

Changes present four days after synovectomy

Synovial membrane—Four days after synovectomy the knee joints contained a moderate amount of blood-stained fluid. Histologically, the site of removal of the synovial membrane was covered by erythrocytes and the underlying fibroblasts were orientated roughly in line with the surface (Fig. 4).

In the steroid-treated group, that is, after one injection of 25 milligrams of hydrocortisone acetate, the subintimal tissue was oedematous and the fibroblasts were fewer, and less well-orientated to the surface than in the normal group. A few erythrocytes covered the denuded surface (Fig. 5).

Articular cartilage—The autoradiographs showed uniform uptake of $^{35}$ sulphur in the articular cartilage (Fig. 6). In the steroid-treated group there was uptake of $^{35}$ sulphur mainly in the deep Zone I and upper-mid Zone II (Fig. 7).
Regenerating synovium twenty-five days after synovectomy. The subsynovial fibroblasts are lying parallel to the surface and beginning to form a surface layer. (Haematoxylin and eosin, ×25.)

Twenty-five days after synovectomy. In the steroid-treated animals the subintimal fibroblasts are few, small and poorly organised. (Haematoxylin and eosin, ×25.)

Autoradiograph twenty-five days after synovectomy shows uniform, heavy uptake of radioactive sulphate (35SO₄) throughout the articular cartilage. (×12.)

Twenty-five days after synovectomy, in the steroid-treated animals, the autoradiograph shows light uptake of radioactive sulphate (35SO₄) throughout the articular cartilage. (×12.)

Autoradiograph of medial femur four days after synovectomy showing uniform light uptake of radioactive sulphate (35SO₄) throughout the articular cartilage. (×12.)

Autoradiograph of medial femur four days after synovectomy in a steroid-treated animal. There is light labelling in the superficial zone compared with the middle and deep zones. (×12.)

Autoradiograph of medial femur four days after synovectomy showing uniform light uptake of radioactive sulphate (35SO₄) throughout the articular cartilage. (×12.)

 Autoradiograph twenty-five days after synovectomy shows uniform, heavy uptake of radioactive sulphate (35SO₄) throughout the articular cartilage. (×12.)
Changes present twenty-five days after synovectomy
Synovial membrane—Twenty-five days after synovectomy the synovial membrane appeared hyperaemic at the margins of the femoral condyles and femoral groove. Histologically the orientation of the densely-staining fibroblasts with their long protoplasmic processes was seen (Fig. 8). In the steroid-treated group, in some areas a lining layer of cells appeared to be forming but beneath this was disorganised, oedematous fibrous tissue. Several large blood vessels with thin walls were present beneath the fibrous layer (Fig. 9).

Articular cartilage—The articular cartilage at this stage showed heavy uptake of $^{35}$S sulphur label in all layers (Fig. 10). In the steroid-treated group the uptake was less intense but was normally distributed throughout the cartilage (Fig. 11).

Changes present fifty days after synovectomy
Synovial membrane—Fifty days after synovectomy there was encroachment of synovium into the edge of the femoral groove and the membrane still bore a white scar at the site of the original operation. Otherwise the lining appeared shiny and normally-coloured in the first group. The lining appeared less shiny and pale grey in the steroid-treated group. The subchondral bone plate was obviously soft when the cartilage and bone samples were taken from the steroid-treated knees.

Histologically there was abundant healthy-looking fibrous tissue orientated to the surface with a well-defined single layer of surface cells (Fig. 12). There were numerous vessels deep to the fibrous tissue. With toluidine blue staining the nuclei of the lining cells stained blue but without metachromasia of the cytoplasm. In the steroid group there was obvious disorganised oedematous fibrous tissue with scattered cells which did not constitute a continuous lining layer (Fig. 13).

Articular cartilage—The autoradiograph showed uniform uptake of $^{35}$S sulphur throughout the articular cartilage at
this stage in both normal and steroid-treated animals (Figs. 14 and 15).

Changes present eighty-five days after synovectomy

Synovial membrane—Eighty-five days after synovectomy the membrane looked normal and glistening apart from the scarred area. The synovium in the steroid-treated group looked slightly pale and the scar was more widely spread and prominent. The subchondral bone was obviously softer in the steroid group.

The microscopic appearance confirmed that the synovial membrane had regenerated. There was a slightly hyperplastic and folded membrane with numerous vessels including some immediately beneath the surface layer. Faint metachromasia was seen in the cytoplasm of the intimal cells suggesting that hyaluronate production was occurring (Fig. 16). This contrasted with the steroid group where there was considerable fibrosis but only a tenuous lining layer one cell thick (Fig. 17).

Articular cartilage—Macroscopically all the articular surfaces appeared normal except for the femoral grooves which were overrun with synovium on their upper halves and borders. However, although most surfaces were normal, seven weight-bearing surfaces in six joints out of a total of thirty (23 per cent) showed focal microscopic surface fibrillation with loss of chondrocytes and the autoradiographs showed only labelling of basal cells and surrounding matrix in the cartilage beneath, confirming death of cells in these areas (Fig. 18). Five surfaces showed superficial depletion of the matrix of the cartilage extending into Zone II (17 per cent). In the steroid-treated group only one medial femoral condyle showed thinning of the articular cartilage with disorganised cells in clusters and one showed superficial matrix depletion (7 per cent). One cystic space was seen in Zone II but this is a normal appearance in adult rabbit cartilage. The subchondral bone plate was constantly thinner in the steroid group (Fig. 19).
Changes present 110 days after synovectomy

Synovial membrane—One hundred and ten days after synovectomy the synovial membrane looked normal. Microscopically, slightly hyperplastic, folded synovial membrane with many vessels was seen, which was indistinguishable from normal. The dense subsynovial fibrous tissue had been replaced by normal-looking loose fibrillar tissue. With toluidine blue there was blue staining of the lining cell nuclei with slight cytoplasmic metachromasia (Fig. 20). The synovium from the steroid-treated group showed a single layer of surface cells with little cytoplasm and toluidine blue staining revealed no metachromasia (Fig. 21). The subintimal tissue was heavily collagenised and poorly vascularised.

Articular cartilage—The articular cartilage of the animals not treated with hydrocortisone showed surface irregularity and superficial chondrocyte clusters in eight of the thirty-five weight-bearing surfaces of seven joints examined at this stage (23 per cent). Labelling with 35sulphur was absent indicating death of many chondrocytes in the affected areas (Fig. 22). In the steroid group the autoradiographs of the cartilage were normal in all of the thirty surfaces examined (Fig. 23).

DISCUSSION

The regenerated synovial membrane was not considered normal until eighty-five to 110 days from synovectomy because it was only at this stage that the following features were present: reformation of surface layer one to three cells thick; abundant capillaries immediately subjacent to the surface layer; loose fibrous tissue present between the surface layer and the underlying muscle or capsule; faint metachromasia of the surface cell cytoplasm. Although a surface layer one cell thick was seen in the steroid-treated group the subintimal tissue had progressed only from a poorly organised regenerative state to a highly collagenised and poorly vascularised state by the
end of 110 days, and there was no metachromasia of the surface cell cytoplasm. This was a constant point of differentiation between regenerating synovium in the steroid and non-steroid treated animals.

Study of numerous histological sections which showed junctions between pre-existing synovium and excised membrane served to show that the new membrane formed from the subsynovial tissue at the site of the excision.

Although the synovial membrane did not regenerate until between eighty-five and 110 days, the articular cartilage did not deteriorate rapidly in either group of animals. Grossfield, Meyer and Godman (1955) showed that fibroblasts in tissue culture produce hyaluronate, although Schubert and Hamerman (1968) suggested that these cells were not so metabolically active as the synovial lining cells. It is likely, therefore, that after synovectomy the fibroblasts of the surrounding capsular tissues usually produce enough hyaluronate-containing fluid to lubricate and sustain the joint surfaces. However, the articular cartilage in the non-steroid treated group of animals was fibrillated in sixteen of sixty-five surfaces (23 per cent) examined at or after eighty-five days from synovectomy, suggesting that the procedure may have a deleterious effect on articular cartilage in some instances over the long term. That the cartilage changes occurred at the time of regeneration of the synovium may indicate a lack of nutrients or hyaluronate or both in the period before synovial regeneration is complete. The relative lack of damage to articular cartilage in the steroid-treated animals even with poorly regenerated synovium may imply some protective effect of hydrocortisone on intact articular cartilage by the stabilising of lysosomes within the chondrocytes, a mechanism proposed by Thomas (1964).

The thinning effect of steroids on the subchondral bone was obvious at death from fifty days onwards. The bone felt soft on removal of the plugs of cartilage and bone and this impression was confirmed by the obvious thinning of the subchondral bone plate seen on the slides. Despite this the overlying articular cartilage was unaffected.

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REFERENCES