PRODUCTION OF ARTHRITIS IN THE RABBIT BY THE LOCAL INJECTION OF THE MUCOPOLYSACCHARIDE CARAGHEENIN

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In the laboratory study of rheumatoid arthritis the need has long been felt for a convenient animal replica of this disease. Very many techniques have been used during the past century for this purpose; they have recently been reviewed (Gardner, 1960). Forms of experimental arthritis have been studied in the monkey, horse, sheep, goat, pig, dog, rabbit, guinea-pig, rat, mouse, and chick-embryo, but none has provided a universally acceptable model. In the hope that an easily reproducible form of laboratory arthritis might lead to an advance in our understanding of the pathogenesis of rheumatoid arthritis, experiments have been made with the sulphated mucopolysaccharide caragheenin. This substance, obtained from the Irish moss *Chondrus crispus*, or from Scottish seaweed, has previously been used in the study of collagen fibre formation because of its remarkable capacity to stimulate local inflammation dominated by intense macrophage aggregation and by fibroblastic proliferation (Jackson, 1956a, b; Williams, 1957).

In the experimental study of arthritis, caragheenin appears to offer certain advantages as a stimulating material. Owing to its chemical structure, its position in the injected tissues is readily determined by the periodic acid-Schiff reaction. It is easily dissolved in isotonic saline, forming a gel at room temperature but becoming less viscous on warming. Sterilization can be effected by boiling without impairing the capacity of the material to cause inflammation, while the presence of sulphate groups on the molecule suggests that future studies with labelled caragheenin may be possible.

Material

A supply of a crude extract of caragheenin was obtained through the courtesy of the director of the Seaweed

Research Station at Musselburgh. Partial purification of the crude extract by dialysis did not alter the inflammatory reaction produced on injection. For use, the caragheenin powder was made up in isotonic normal saline and sterilized by boiling.

English rabbits of both sexes were used; the animals were mature and of good nutritional status, and were maintained on a diet containing all known essential factors.

Guinea-pigs were of a laboratory-bred strain. They were large, mature, and well-fed. Their diet contained all known essential nutritional factors.

For comparison with animal material, joints from twenty cases of rheumatoid arthritis were selected. These cases formed part of a series in which a *post mortem* examination had been conducted personally.

Methods

The injection of caragheenin was undertaken in three ways:

- (1) Intradermally, as a dilute solution, without anaesthesia, into the skin of guinea-pigs.
- (2) Under ether anaesthesia, into the joints of guinea-pigs.
- (3) Under pentobarbitone anaesthesia, into the joints of rabbits.

Material for biopsy was collected under ether anaesthesia. Animals were killed by the injection of pentobarbitone.

Tissue samples were placed in neutral formol saline immediately after biopsy or death. Bone was decalcified in a formic acid—sodium citrate mixture. Paraffin-embedded sections were cut at 4–8 μ , and were stained with haematoxylin and eosin, by the periodic acid-Schiff reaction, and by Masson's trichrome method.

Procedure

The experiment was conducted in three parts:

- (1) The optimal strength of a solution of caragheenin required to give a readily detectable inflammatory re-

sponse was determined by a series of injections into guinea-pig skin.

0.1 ml. of each of the following concentrations of the caragheenin solution were injected into the shaved dorsal skin of a guinea-pig: 0.25, 0.5, 1, and 2 per cent. A biopsy of each lesion was performed 36 hours later.

(2) A general assessment of the type of reaction to be expected following intra-articular injection, and the size and frequency of injections required to give a brisk articular response, were determined by the injection of amounts of the solution of optimal concentration into the joints of eight guinea-pigs.

Single injections of 0.1 ml. of a 1 per cent. solution of caragheenin were made into the large limb joints of guinea-pigs at the following intervals before biopsy: 1 month, 2 weeks, 1 week, 3 days, 1 day, and 6 hours.

Repeated injections of 0.1 ml. of the 1 per cent. solution were made at the following intervals:

- (i) Eleven injections at 3-day intervals
- (ii) Six injections at 7-day intervals
- (iii) Four injections at 7-day intervals
- (iv) Four injections at 14-day intervals
- (v) Three injections at 14-day intervals
- (vi) Three injections at 21-day intervals

(3) A more detailed study was made of the articular response to the injection at optimal intervals of moderate to large amounts of the solution of optimal strength, using for this purpose eleven rabbits.

The amounts of caragheenin injected into the principal limb joints at stated intervals are shown in the Table.

After these series of injections were completed, the animals were killed and the tissues examined histologically.

Results

(1) **Response of Guinea-pig Skin.**—An optimal response was given by the injection of 0.1 ml. of the 1 per cent. solution. An intense histiocytic reaction, with smaller numbers of polymorphonuclear leucocytes, was concentrated within the deeper layers of the dermis and the subcutaneous tissues.

(2) **Response of Guinea-pig Joints.**—After 6 hours a single injection of 0.1 ml. of the 1 per cent. solution produced an intense polymorphonuclear leucocytic inflammatory cell reaction. After 24 hours and after 3 days the cellular characteristics of the inflammatory infiltrate changed to include a progressively larger proportion of histiocytes and later fibroblasts. After 1 month no detectable abnormality remained.

Intra-articular injection of 0.1 per cent. and of 0.5 per cent. solutions produced slight responses only. A greatly exaggerated reaction was detected after the 1 per cent. solution and this strength was accepted as optimal. The viscosity of the 2 per cent. solution, which produced only a little more

TABLE
ARTICULAR RESPONSE IN TEN RABBITS

Series	Rabbit No.*	Dose (ml.)	Frequency (per week)	Duration (wks)	No. of Injections
Experimental	1	0.5 ml. of 1 per cent. solution rising to 1 ml. of 1 per cent. solution	Once	6	7
	2			10	11
	3	1 ml. of 1 per cent. solution	Twice	3	6
	4			3	6
	5			3	6
	8	Amounts of 1 per cent. solution increasing from 1 to 5 ml. per joint	Twice	4.5	9
	9			4	8
	10			3	6
Control	6	Increasing amounts intra-venously to a total of 19 ml.	Once	11	11
	7	0.5-1 ml. normal saline intra-articularly	Once	10	10

* Rabbit No. 11 died during the course of the experiment

inflammatory response, became so great that much difficulty was experienced in handling the material before injection, even with prior warming of the mixture and of the syringe.

(3) Response of Rabbit Joints.—A certain variability was observed, possibly related to the varying difficulty experienced in injecting the caragheen solution. Generally, to produce a prolonged arthritic response, it was necessary to inject caragheen at intervals of not more than 3 to 7 days; for this purpose the 1 per cent. solution was most convenient. With this procedure examination of the joints after intervals of 6 to 10 weeks revealed inflammatory proliferation of the synovial villi (Figs 1 and 2), with increased cellularity, and a marginal attachment of synovia to the superficial parts of the articular cartilages (Figs 3 and 4). Progressive replacement fibrosis of articular cartilage (Figs 5 and 6) by cellular connective tissue in continuity with that of the hypercellular synovial fringes commonly resulted in the formation of pannus, a change apparently analogous with the marginal "invasion" of articular cartilage seen in rheumatoid arthritis. The cellular reaction differed from that seen in rheumatoid arthritis, however, in that the predominant cell type present was the

lymphocyte and not the plasma cell (Figs 7 and 8). Nevertheless, the infiltrate was sometimes perivascular in distribution (Figs 9 and 10) and was accompanied by periarticular fibrosis (Figs 11 and 12). In the superficial margins of occasional hyperplastic synovial villi were accumulations of material staining positively for fibrin (Figs 13 and 14), but fibrinoid change was not an invariable characteristic of the reaction. Osteoclastic bone reabsorption was an occasional finding (Figs 15 and 16).

When a comparison was made between the animal joints and those from patients dying with rheumatoid arthritis, it proved possible, by the exercise of considerable selection, to find areas in both animal and human tissue which were morphologically very similar. Since the histological characteristics of the joints in rheumatoid arthritis are not individually specific, there was no justification for deducing that the rabbit arthritis produced by caragheen offered more than a crude replica of the human disease. Nevertheless, it was felt that the model offered certain practical advantages which might make it acceptable as a basis for future use, and in particular for the laboratory study of iron metabolism in animals with a chronic arthritis (Richmond, Gardner, Roy, and Duthie, 1956; Richmond, Roy, Gardner, Alexander, and Duthie, 1958).

Fig. 1.—Rabbit 1, seven injections at weekly intervals. Widespread lymphocytic reaction in hyperplastic synovial villi. Haematoxylin and eosin $\times 60$.

Fig. 2.—For comparison with Fig. 1. Synovial villi from an interphalangeal joint in a case of long-standing rheumatoid arthritis. Haematoxylin and eosin $\times 140$.

Fig. 3.—Rabbit 2, seven injections at weekly intervals. Mononuclear cells, fibroblasts, and cell debris form an irregular pannus in continuity with the superficial layer of articular cartilage.
Haematoxylin and eosin $\times 350$.

Fig. 4.—For comparison with Fig. 3. Marginal replacement fibrosis of articular cartilage from an interphalangeal joint in a case of long-standing rheumatoid arthritis.
Haematoxylin and eosin $\times 350$.

Fig. 5.—Rabbit 5, injections twice weekly for 3 weeks. Margin of articular cartilage undergoing replacement by lymphocytes and histiocytes.
Haematoxylin and eosin $\times 200$.

Fig. 6.—For comparison with Fig. 5. Replacement of border of articular cartilage of finger joint in a case of old active rheumatoid arthritis.
Haematoxylin and eosin $\times 200$.

Fig. 7.—The cellular response in arthritis induced by caragheen in is predominantly histiocytic. Haematoxylin and eosin $\times 640$.

Fig. 8.—In contrast with Fig. 7. The predominance of plasma cells is pathognomonic of active rheumatoid arthritis. Haematoxylin and eosin $\times 650$.

Fig. 9.—Rabbit 5, six twice-weekly injections. Both in caragheen in arthritis and in rheumatoid arthritis there is a tendency for the aggregation of inflammatory cells to become perivascular. Haematoxylin and eosin $\times 95$.

Fig. 10.—For comparison with Fig. 9. Perivascular fibrosis and inflammatory cells at margin of interphalangeal joint in active rheumatoid arthritis. Haematoxylin and eosin $\times 100$.

Fig. 11.—Rabbit 5. Lymphocytic foci within the para-articular soft tissues.
Haematoxylin and eosin $\times 200$.

Fig. 12.—Comparable lymphocytic foci are frequently seen in the soft tissues bounding involved metacarpophalangeal joints in rheumatoid arthritis.
Haematoxylin and eosin $\times 200$.

Fig. 13.—Rabbit 7, left fore-limb. Fibrin deposited superficially on hyperplastic cellular synovial tissue.
Haematoxylin and eosin $\times 95$.

Fig. 14.—For comparison with Fig. 13. Fibrinoid in margin synovial villus in metacarpophalangeal joint in a case of active longstanding rheumatoid arthritis.
Haematoxylin and eosin $\times 200$.

Fig. 15.—Rabbit 6. Osteoclastic bone reabsorption is frequent at the margins of involved joints in arthritis caused by caragheenine. Haematoxylin and eosin $\times 200$.

Fig. 16.—For comparison with Fig. 15. Similar appearances are found in active chronic rheumatoid arthritis. Haematoxylin and eosin $\times 200$.

Discussion

The experiment described in the present paper has shown that the large limb joints of guinea-pigs and of rabbits respond to the injection of a sterile solution of the mucopolysaccharide caragheenine in isotonic saline by the production of an inflammatory response, which can be perpetuated by repetitive injection, and which results after 6 to 10 weeks in the appearance of histological changes which individually may resemble closely those found in rheumatoid arthritis in the human. Very many alternative methods for the production of arthritis in laboratory animals have previously been described, but the present method is both simple, reproducible, and free from some of the uncontrolled incidental factors which have plagued so many of the methods described in the literature. Caragheenine is more readily obtained, more easily handled, and less disturbed by environmental changes than the mucopolysaccharides used by Jones and Carter (1954), Jones, Carter, and Rankin (1954), Jones and Carter (1957a, b), and Jones and Mayne (1958). Its reactivity appears to depend entirely upon a local stimulus to inflammation, and it is not known to be antigenic. Repeated injections maintain but do not exaggerate local responses, and repeated systemic

administration has caused no detectable change in the lungs, liver, and other viscera.

Whether future experiments will show that it is possible to reproduce in animals an arthritis comparable in every way to rheumatoid arthritis in the human is uncertain. It appears from the large amount of work performed on small rodents that these species are not likely to yield the answer to this question. The present author is among those who believe that it may prove necessary to turn to the primates before a complete animal replica of the human disease is found; this approach to the problem will be both arduous and costly.

Summary

(1) The highly sulphated mucopolysaccharide caragheenine extracted from Irish moss or from Scottish seaweed has been used to induce an articular inflammatory reaction in guinea-pigs and in rabbits. Experiment has shown that an optimal response is obtained when a 1 per cent. solution in sterile isotonic saline is injected at intervals of not more than 7 days for at least 6 weeks.

(2) Under these circumstances an arthritis may be produced the individual histological characteristics of which may closely resemble those of selected

cases of rheumatoid arthritis in the human. No claim can legitimately be made to have reproduced any particular form of human arthritis, but the procedure offers certain advantages for the future study of experimental arthritis.

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Production d'arthrite chez le lapin par injection locale de mucopolysaccharide *caragheen*

RÉSUMÉ

(1) Le mucopolysaccharide très sulfaté *caragheen*, extrait de l'algue marine irlandaise ou écossaise (*Irish*

moss ou *Scottish seaweed*) fut employé pour induire une réaction inflammatoire articulaire chez des cobayes et des lapins. On détermina expérimentalement qu'une réponse optimum s'obtient lorsqu'on injecte à des intervalles de 3 à 7 heures, pendant au moins 6 semaines, une solution à 1 pour cent en l'eau physiologique stérile.

(2) En ces circonstances on peut produire une arthrite dont les traits histologiques individuels ressemblent de près ceux des cas sélectionnés d'arthrite rhumatismale humaine. On ne peut pas prétendre légitimement qu'on ait reproduit une forme particulière d'arthrite humaine, mais le procédé offre certains avantages pour l'étude future de l'arthrite expérimentale.

Producción de artritis en el conejo por inyección local de mucopolisacárido *caragheen*

SUMARIO

(1) El altamente sulfatado mucopolisacárido *caragheen* extraído del alga marina irlandesa o escocesa (*Irish moss* o *Scottish seaweed*) fue usado para inducir una reacción inflamatoria articular en cobayos y conejos. Experimentalmente se determinó que la respuesta óptima se presenta cuando se inyecta con intervalos no superiores a 3-7 días por no menos de 6 semanas una solución al 1 por ciento en suero salino isotónico estéril.

(2) En estas circunstancias puede ser producida una artritis de características histológicas individuales con una semejanza cercana a casos seleccionados de artritis reumatoide en el hombre. No es posible afirmar que se haya reproducido una forma particular de artritis humana, pero el procedimiento ofrece ciertas ventajas para el futuro estudio de la artritis experimental.