

## Effect of intra-articular corticosteroid injections on primate cartilage

T. GIBSON, H. C. BURRY, D. POSWILLO\*, AND J. GLASS

*From Guy's Arthritis Research Unit, Guy's Hospital Medical School, London, and the Royal College of Surgeons Research Establishment, Downe, Kent\**

**SUMMARY** An attempt was made to ascertain whether intra-articular corticosteroids exert a harmful effect on primate cartilage. The knee joints of 10 *Macaca irus* monkeys were subjected to either one, two, or six injections of 20 mg methyl prednisolone or an equal number of control injections over a 12-week period. Minor degenerative changes of many femoral condyles were shown by India ink staining and by a system of histochemical grading. Changes in the joints injected with corticosteroid were not significantly different from those seen in control joints. The findings were in striking contrast to the severe degeneration reported by others in rabbit joints injected with corticosteroid. The experiment did not support the contention that intra-articular corticosteroids invariably have a deleterious effect on primate cartilage.

The use of intra-articular corticosteroid injections in the treatment of joint disorders remains controversial. Although widely used in the management of joint inflammation, evidence that they may accelerate cartilage destruction in man (Chandler and Wright, 1958) and rabbits (Salter *et al.*, 1967) has deterred universal acceptance.

Many reports describing harmful effects in man have been of isolated cases subjected to frequent injections (Chandler *et al.*, 1959; Steinberg *et al.*, 1962) and the induction of degenerative changes in rabbit joints is dependent on the frequency of injections (Salter *et al.*, 1967). Furthermore, the response of different species to corticosteroids is varied (Rehder and Enquist, 1967), and to what extent the observed effect on rabbit articular cartilage can be applied to man is uncertain.

This study was designed to assess the effect of intra-articular methyl prednisolone on the articular cartilage of a primate model (*Macaca irus* monkey) in the expectation that the results would have more relevance to man than animal experiments hitherto.

### Method

Ten mature monkeys (*M. irus*) of approximately equal age and size (mean weight 5.7 kg) were selected

for the experiment. Throughout the study they were fed a pathogen-free balanced diet suitable for humans. Using strict aseptic precautions and with the animals sedated with phencyclidine or ketamine hydrochloride, 0.5 ml (20 mg) methyl prednisolone was injected into one knee joint of each animal. An equivalent volume of vehicle used in the same commercial preparation of methyl prednisolone was injected into the opposite knee. The vehicle consisted of polyethylene glycol 29 mg, sodium chloride 8.7 mg, myristylgamma picolinium chloride 0.19 mg, and water to 1 ml. The accurate placing of the injections was confirmed by adding 0.2 ml of a radio-opaque dye (Conray) to the injection fluid and performing x-rays immediately after injection.

Tibial biopsies were obtained from both knees of 2 animals 4 weeks later and injections were repeated at the same time in the other 8 animals. 4 of these underwent a further series of four injections into each knee at 2-week intervals (total of 6 injections). X-rays of the joints were taken immediately before the experiment and again 14 weeks later at which time all 10 animals were sacrificed.

The surfaces of the femoral condyles, menisci, and tibiae were examined with the naked eye for surface defects. The fresh femoral specimens were coated with India ink diluted with physiological saline, examined for evidence of fibrillation in the manner described by Bullough and Goodfellow (1968) and then photographed.

The biopsies and the whole joints were fixed in

Accepted for publication May 2, 1976

Correspondence to Dr. T. Gibson, Guy's Arthritis Research Unit, Guy's Hospital Medical School, London Bridge, London SE1 9RT

Table 1 Details of the histological-histochemical grading method used to establish a score for each joint

Score	Structure	Cells	Staining intensity (Alcian blue)
0	Normal	Normal	Normal
1	Surface irregularities	Diffuse increase	Slight decrease
2	Clefts to midzone	Cloning	Moderate decrease
3	Clefts to calcified zone	Hypocellularity	Marked decrease
4	Complete disorganization		

formalin and decalcified. With the guidance of earlier photographs, sections were cut through areas of macroscopical abnormality and adjacent areas on the femoral condyles. Where no naked eye abnormality was apparent, sections were obtained through similar anatomic sites on the medial condyles.

Thin sections were obtained after embedding in paraffin wax. These were stained with haematoxylin and eosin and with Alcian blue 0.1% in 0.05 mol/l acetate buffer pH 5.8, containing  $MgCl_2$  at concentrations ranging from 0.3 mol/l to 0.9 mol/l. The latter critical electrolyte concentration technique stains both chondroitin sulphate and keratan

sulphate at low concentrations of magnesium chloride but a concentration of 0.9 mol/l detects only keratan sulphate (Scott and Dorling, 1965).

A system of histochemical grading was modified from that of Mankin *et al.*, (1971) (Table 1). The histological specimens were reviewed separately by two of us (T. G., H. B.) without knowledge of the source of the material.

### Results

X-rays taken before and on completion of the study showed no radiological abnormality of any of the joints. Tibial biopsies obtained 4 weeks after the initial injections had a normal macroscopical appearance and sections stained with haematoxylin and eosin showed no abnormality of either the two

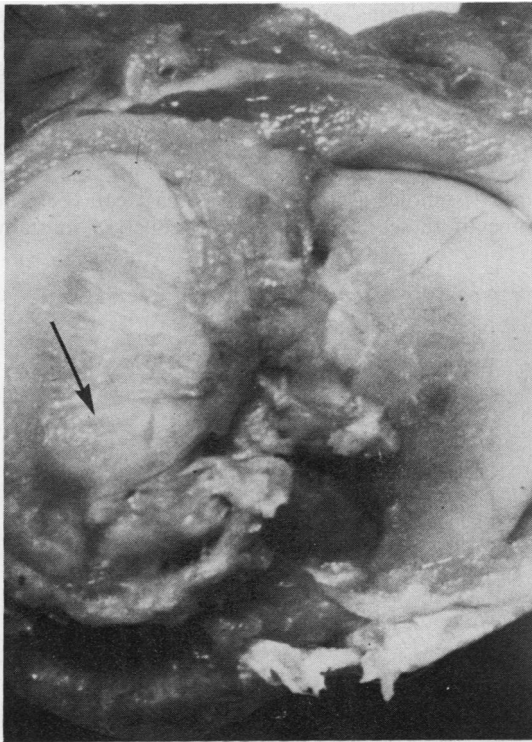


Fig. 1 Repair of tibial biopsy site by fibrocartilage (arrow) 10 weeks after biopsy.

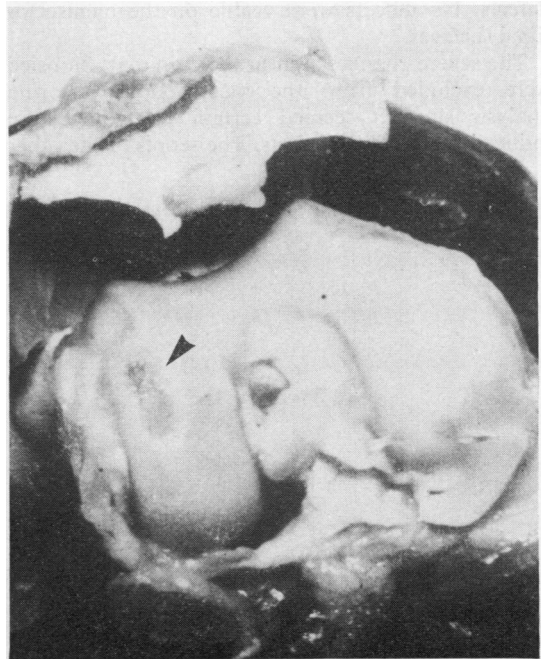


Fig. 2 Ulceration of femoral condyle (arrow) in monkey subjected to tibial biopsy 10 weeks previously.

Table 2 *Macroscopical scores in 8 animals receiving two or six injections*

Monkey	Macroscopical score	
	Steroid	Control
<i>2 injections</i>		
C	0	1
D	1	1
E	1	3
F	0	1
Total	2	6
<i>6 injections</i>		
G	1	6
H	2	0
I	2	4
J	5	0
Total	10	10

joints receiving one injection of steroid or the two control joints. At 14 weeks the biopsy defects were largely repaired by fibrocartilage (Fig. 1). However, examination of the femoral condyles of these 2 animals showed marked surface irregularities with cartilage roughening and ulceration (Fig. 2). The extent of these changes was identical in all four joints.

Macroscopical changes of this severity were not seen on the femoral condyles of the remaining 8 animals. These were either absolutely smooth or showed small areas of slight roughening or linear defects. No defects were visible on the menisci or tibial surfaces.

The severe changes seen in the 2 animals biopsied were excluded from the macroscopical scoring analysis since it seemed certain that they were induced by the tibial defects. The scores of the other

8 animals are given in Table 2., A slightly higher total score of 16 was obtained in the control joints compared with 12 for the steroid injected joints. The average score of the joints receiving six injections was the same in both steroid injected and control joints (2.5) but was higher than the average score of the joints receiving two injections (1.0).

A variety of histological changes was observed ranging from a normal appearance to surface defects, cartilage clefts, cloning of chondrocytes, and hypocellularity. The joints subjected to biopsy showed clefts in all four sections but the cellular configuration and staining densities were similar to those of several other animals and so their histochemical grading was incorporated in the total scores.

Cartilage clefts with and without cloning of chondrocytes were observed in five other joints. Such changes were not predominant in either the steroid injection or control joints. The knee joints of one animal not included in the experiment and which had received no injections showed clefts similar to those seen in the experimental animals (Fig. 3) when examined.

Alcian blue stain at 0.3 mol/l concentration was considerably denser than at 0.9 mol/l, and in some instances staining at the higher concentration was very faint. In general, differences between the joints were subtle and grading of stain intensity was difficult. There was no consistent relationship between the density of staining at 0.3 mol/l and 0.9 mol/l. Decreased staining density at one or both concentrations was noted in eight of the nine condyles showing clefts and was least equivocal in some of these. However, some reduction in stain

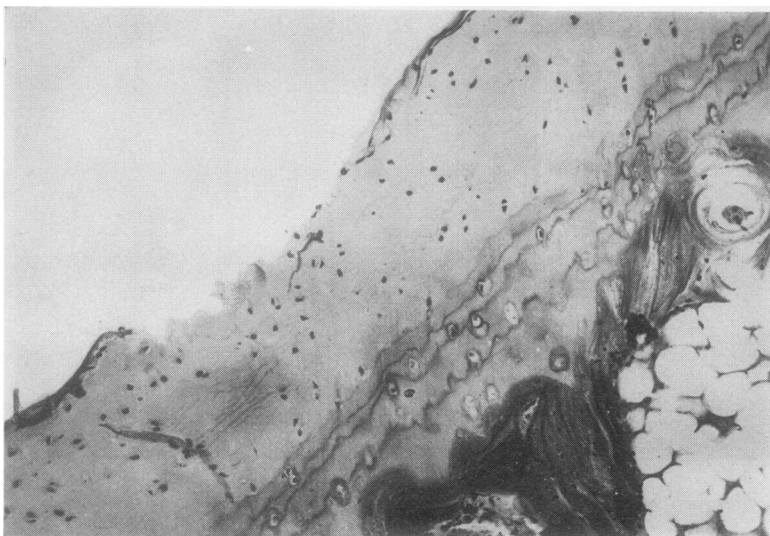


Fig. 3 *Cartilage cleft seen in femoral condyle of monkey not included in the experiment and identical to that observed in some of the experimental animals. Haematoxylin and eosin  $\times 76$ .*

Table 3 Detailed results of the histological-histochemical grading of 20 knee joints injected with methyl prednisolone or vehicle

Monkey	Structure		Cells		Staining intensity (Alcian blue)			
	Steroid	Control	Steroid	Control	0.3 mol/l MgCl <sub>2</sub>		0.9 mol/l MgCl <sub>2</sub>	
					Steroid	Control	Steroid	Control
<i>1 injection</i>								
A	2	2	3	2	1	1	3	1
B	2	2	2	1	3	3	2	1
Total	4	4	5	3	4	4	5	2
<i>2 injections</i>								
C	1	1	0	1	2	3	1	0
D	0	0	2	0	1	0	0	1
E	0	2	0	3	0	0	1	2
F	2	0	2	2	2	2	2	3
Total	3	3	4	6	5	5	4	6
<i>6 injections</i>								
G	1	1	0	0	0	0	1	0
H	2	0	2	0	0	1	3	2
I	0	2	1	0	2	1	2	3
J	2	0	2	1	2	2	2	1
Total	5	3	5	1	4	4	8	6

intensity was also seen in three joints with no or minimal structural changes.

The results of histochemical grading obtained by the two observers were in agreement and are summarized in Table 3. The average total score for all the histochemical parameters in the 2 animals injected once only was 9.0 for the steroid injected joints and 6.5 for the control joints. In the 4 animals injected twice the average total score was 4.0 for the steroid joints and 3.5 for the controls. Thus the highest average total scores were seen in the joints injected once and subjected to tibial biopsies. Differences between the steroid injected and control joints were small in each of these groups and the mean ( $\pm$ SD) total scores for the 10 animals were  $5.6 \pm 3.03$  for the joints injected with steroid and  $4.7 \pm 2.36$  for the control joints. The difference was not significant ( $P=0.17$ ; binomial test).

## Discussion

With few exceptions the weight of evidence incriminating intra-articular corticosteroids in the acceleration of joint destruction in man is based on anecdotal reports. Chandler *et al.*, (1959) recorded a single case of accelerated osteoarthritis in a hip injected repeatedly with hydrocortisone. A similar case was reported by Sweetnam *et al.*, (1960) although only three injections were given in the latter case compared with 18 in the former. Zachariae (1965) also reported rapid deterioration in knees or hips of 4 patients receiving intra-articular injections with as many as 50 injections over one year in one case. A Charcot-like arthropathy was observed by Steinberg *et al.* (1962) in a rheumatoid knee injected

with hydrocortisone on 22 occasions over a 2-year period and identical changes were reported in both knees of a patient who had received a total of 52 intra-articular injections of corticosteroid over a period of 14 months (Bentley and Goodfellow, 1969). Alarcon-Segovia and Ward (1966) cited the awesome example of a patient who received 3000 injections into the interphalangeal joints over a period of years and in whom joint disorganization ensued.

More convincing is the study by Chandler and Wright (1958) in which radiological deterioration occurred in more than half of 25 rheumatoid knee joints given four injections of hydrocortisone over a 48-week period despite symptomatic improvement. On the other hand, the same authors (Wright *et al.*, 1960) reported no radiological deterioration in 38 osteoarthrotic knees given an identical course of hydrocortisone injections. Furthermore, in a review of 123 patients over a 4-year period Keagy and Keim (1967) could find no evidence of accelerated joint destruction even among patients given up to four injections at the same site, and Hollander (1970) observed a deleterious effect in less than 1% of a large number of subjects.

It has been well established that systemic corticosteroids may modify metabolism of cartilage and other tissues in animals. Denko and Bergenstal (1961) showed an inhibition by hydrocortisone of <sup>35</sup>S incorporated in costal and articular cartilage of rats, and similar findings were later made by Anastasiades and Dziewiatkowski (1970). Decrease in the size of chondrocytes and cell death were observed by Silberberg *et al.* (1966) in the articular cartilage of mice given systemic cortisone. Layton (1951)

reported that cortisone inhibited the synthesis of chondroitin sulphate by chick embryo and wound tissues *in vitro*, and Mankin *et al.* (1972) observed marked reduction of *in vitro* glycosaminoglycan synthesis by cartilage removed from cortisone-treated rabbits.

Reconstitution of cartilage matrix in rabbit pretreated with papain was delayed by intra-articular injection of prednisolone and other corticosteroids (McCluskey and Thomas, 1959) and intra-articular hydrocortisone was shown to decrease glycine <sup>3</sup>H incorporation in rabbit cartilage (Mankin and Conger, 1966). These studies suggest a local inhibition by steroids of both glycosaminoglycan and protein synthesis. Additional experiments of intra-articular injection of corticosteroids in rabbits have shown exaggerated degenerative joint changes comprising loss of cartilage, subchondral cysts, and fissuring, the extent of which was dependent on the number of injections (Salter *et al.*, 1967; Moskowitz, *et al.*, 1970).

Using single doses of methyl prednisolone equivalent to 200 mg in a 60 kg man and far in excess of the usual amounts injected in human disease, we failed to reproduce the severe joint disorganization seen in rabbit joints. Minor degenerative changes were undoubtedly present in a large number of the knees but were also seen in an animal which had not undergone joint injection. Similar changes may also occur in rabbits not given intra-articular injections (Moskowitz *et al.*, 1970).

The India ink preparations of the femoral condyles showed on some surfaces a pattern that was identical to the 'minimal fibrillation' seen in human joints when examined at random by the same technique (Meachim and Fergie, 1975).

The pattern of staining obtained by the critical electrolyte concentration technique was remarkably similar to that of mature human joints (Stockwell, 1970). In man, at least, the results of this histochemical procedure correlate with the distribution of keratan sulphate and chondroitin sulphate as measured biochemically (Stockwell and Scott, 1967). Evidence suggests that degeneration of cartilage is associated with an increase of chondroitin sulphate, a decrease of keratan sulphate, and little change in total glycosaminoglycan content (Mankin and Lippiello, 1971). However, this conflicts with earlier observations in which reduction of chondroitin sulphate was reported (Matthews, 1953). For this reason we thought that the properties of the Alcian blue method would allow detection of any change in either the total or relative proportions of glycosaminoglycans even in the absence of structural abnormalities. There was a tendency for staining with Alcian blue at one or both concentrations of

magnesium chloride to be less intense in association with structural defects but there was no consistent pattern in the staining densities of the two concentrations relative to each other. It was not possible to make any firm conclusion about proportional changes of glycosaminoglycans. There was no overall difference between the steroid-injected and control joints with regard to staining density.

The observations of Poswillo (1970) suggested that steroid induced changes in primate joints might be transitory. We therefore attempted to examine this possibility by performing biopsies at an early stage of the experiment but could detect no abnormality.

After intra-articular injections of methyl prednisolone in man, absorption from the joint cavity may allow the preparation to exert a systemic effect (Holden and Kendall, 1962). Assuming this occurred in the monkeys used in our experiment it could be argued that the control joints were also exposed to corticosteroid. It is unlikely that such an effect influenced our findings because the histological and histochemical changes did not show any relationship to the frequency of injections, although a slightly higher macroscopical score did obtain for the joints injected six times compared with those injected twice.

Our observations do not imply that intra-articular corticosteroids in man are entirely devoid of the hazards suggested by previous experiments. In particular, the study does not examine the possibility that a harmful effect may occur in cartilage already compromised by inflammatory disease, as suggested by Chandler and Wright (1958). Nevertheless our results do show a marked difference of response to steroid injections in monkeys compared with the effects observed in rabbits in earlier experiments. These results emphasize that an effect in one species cannot be readily extrapolated to another. To this extent the experiment does not support the view that intra-articular corticosteroid injections are invariably harmful. The evidence in man suggests that very frequent injections may have a deleterious effect on articular cartilage. Whether infrequent injections may also accelerate cartilage destruction is far from certain and it may be that the benefits of occasional injections in reducing synovial inflammation far outweigh any effect of steroid on the articular surface.

We are grateful for the expert assistance of Mr. Carl Hobbs and Mr. Bruce Bidewell, and to Action Research for the Crippled Child for generous financial support.

#### References

- Alarcon-Segovia, D., and Ward, L. E. (1966). Marked destructive changes occurring in osteoarthritic finger

- joints after intra-articular injection of corticosteroids. *Arthritis and Rheumatism*, **9**, 443-463.
- Anastassiades, T., and Dziewiatkowski, D. (1970). The effect of cortisone on the metabolism of connective tissue in the rat. *Journal of Laboratory and Clinical Medicine*, **75**, 826-839.
- Bentley, G., and Goodfellow, J. W. (1969). Disorganisation of the knees following intra-articular hydrocortisone injections. *Journal of Bone and Joint Surgery*, **51B**, 498-502.
- Bullough, P., and Goodfellow, J. (1968). The significance of the fine structure of articular cartilage. *Journal of Bone and Joint Surgery*, **50B**, 852-857.
- Chandler, G. N., and Wright, V. (1958). Deleterious effect of intra-articular hydrocortisone. *Lancet*, **2**, 661-633.
- Chandler, G. N., Jones, D. T., Wright, V., and Hartfall, S. J. (1959). Charcot's arthropathy following intra-articular hydrocortisone. *British Medical Journal*, **1**, 952-953.
- Denko, C. W., and Bergenstal, D. M. (1961). Effects of growth hormone and corticosteroids on S<sup>35</sup> fixation in cartilage. *Endocrinology*, **69**, 769-777.
- Holden, G., and Kendall, P. H. (1962). The newer corticosteroids for local injection. *Annals of Physical Medicine*, **6**, 178-182.
- Hollander, J. L. (1970). Intrasynovial corticosteroid therapy in arthritis. *Maryland State Medical Journal*, **19**, 62-66.
- Keagy, R. D., and Keim, H. A. (1967). Intra-articular steroid therapy: repeated use in patients with chronic arthritis. *American Journal of the Medical Sciences*, **253**, 45-51.
- Layton, L. L. (1951). Effect of cortisone upon chondroitin sulfate synthesis by animal tissues. *Proceedings of the Society for Experimental Biology and Medicine*, **76**, 596-598.
- McCluskey, R. T., and Thomas, L. (1959). The removal of cartilage matrix in vivo by papain. Prevention of recovery with cortisone, hydrocortisone and prednisolone by a direct action on cartilage. *American Journal of Pathology*, **35**, 819-828.
- Mankin, H. J., and Conger, K. A. (1966). The acute effects of intra-articular hydrocortisone on articular cartilage in rabbits. *Journal of Bone and Joint Surgery*, **48A**, 1383-1388.
- Mankin, H. J., and Lippiello, L. (1971). The glycosaminoglycans of normal and arthritic cartilage. *Journal of Clinical Investigation*, **50**, 1712-1719.
- Mankin, H. J., Dorfman, H., Lippiello, L., and Zarins, A. (1971). Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *Journal of Bone and Joint Surgery*, **53A**, 523-537.
- Mankin, H. J., Zarins, A., and Jaffe, W. L. (1972). The effect of systemic corticosteroids on rabbit articular cartilage. *Arthritis and Rheumatism*, **15**, 593-599.
- Matthews, B. F. (1953). Composition of articular cartilage in osteoarthritis. Changes in collagen:chondroitin sulphate ratio. *British Medical Journal*, **2**, 660-661.
- Meachim, G., and Fergie, I. A. (1975). Morphological patterns of articular cartilage fibrillation. *Journal of Pathology*, **115**, 231-240.
- Moskowitz, R. W., Davis, W., Sammarco, J., Mast, W., and Chase, S. W. (1970). Experimentally induced corticosteroid arthropathy. *Arthritis and Rheumatism*, **13**, 236-243.
- Poswillo, D. (1970). Experimental investigation of the effects of intra-articular hydrocortisone and high condylectomy on the mandibular condyle. *Oral Surgery, Oral Medicine and Oral Pathology*, **30**, 161-173.
- Rehder, E., and Enquist, I. F. (1967). Species differences in the response to cortisone in wounded animals. *Archives of Surgery*, **94**, 74-78.
- Salter, R. B., Gross, A., and Hall, J. H. (1967). Hydrocortisone arthropathy. An experimental investigation. *Canadian Medical Association Journal*, **97**, 374-377.
- Scott, J. E., and Dorling, J. (1965). Differential staining of acid glycosaminoglycans (mucopolysaccharides) by Alcian Blue in salt solutions. *Histochemistry*, **5**, 221-233.
- Silberberg, M., Silberberg, R., and Hasler, M. (1966). Fine structure of articular cartilage in mice receiving cortisone acetate. *Archives of Pathology*, **82**, 569-582.
- Steinberg, C. L. R., Duthie, R. B., and Piva, A. E. (1962). Charcot-like arthropathy following intra-articular hydrocortisone. *Journal of the American Medical Association*, **181**, 851-854.
- Stockwell, R. A. (1970). Changes in the acid glycosaminoglycan content of the matrix of ageing human articular cartilage. *Annals of the Rheumatic Diseases*, **29**, 509-515.
- Stockwell, R. A., and Scott, J. E. (1967). Distribution of acid glycosaminoglycans in human articular cartilage. *Nature*, **215**, 1376-1378.
- Sweetnam, D. R., Mason, R. M., and Murray, R. O. (1960). Steroid arthropathy of the hip. *British Medical Journal*, **1**, 1392-1394.
- Wright, V., Chandler, G. N., Morison, R. A. H., and Hartfall, S. J. (1960). Intra-articular therapy in osteoarthritis. Comparison of hydrocortisone acetate and hydrocortisone tertiary butylacetate. *Annals of the Rheumatic Diseases*, **19**, 257-261.
- Zachariae, L. (1965). Deleterious effects of corticosteroids administered topically, in particular intra-articularly. *Acta Orthopaedica Scandinavica*, **36**, 127-136.