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OBSERVATIONS ON THE ACTION OF INTRAARTICULARLY ADMINISTERED PREDNISOLON TERTIARY BUTYL ACETATE (CODELCORTONE TBA) AND METHYLPREDNISOLON ACETATE (DEPOMEDRONE) IN THE NORMAL RABBIT KNEE JOINT

A vital microscopic and histologic study

By

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INTRODUCTION

Osteoarthritis has long proved a therapeutic problem. It was therefore with some relief that steroid treatment was accepted, not only for this ailment, but also for allied conditions in joints and periarticular tissues. Lessening of pain, increase in mobility and the withdrawal of exsudates and swollen capsules were features which proved steroids to be an efficient adjuvant in the general plan of treating various joint conditions. This was especially true when the steroid was applied locally.

Some authors have questioned whether the therapeutic effect of locally administered steroids in joints has been satisfactory. *E.g. Ansell et al.* (1956) stated that the one or three year results on treating rheumatoid arthritis with steroids and aspirin show no preference for either group. *Leveaux* (1957) compared the effect of intraarticularly deposited cortisone and procaine in cases of hip osteoarthritis without being able to show any advantage for either substance as regards mobility, gait and pain. Comparisons have also been made between steroids and local anaesthetics in cases of rheumatoid arthritis and *Fearnley* (1958) could

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not prove any improvement to the advantage of either preparation. *Chandler et al.* (1958) followed a series of patients with rheumatoid arthritis who received local steroid therapy. On X-ray he found that these patients as compared to a control group during the course of treatment developed an alarming amount of destructive changes in the articular cartilage which was thought to be due to the introduction of the steroid.

Nettelbladt & Sundblad (1963) have studied the effect of steroids on the composition of articular fluid in rheumatoid arthritis. They found that pain was relieved after only a few hours and a decrease of exsudate was seen within the same time. They believe that steroids stabilise the hyaluronic acid which is incompletely polymerized in states of inflammation. Hyaluronic acid plays an important part in capillary permeability and on its depolymerisation an increase in *e.g.* protein-leakage occurs. On the administration of steroids this capillary permeability decreases due to the stabilising effect of the steroids on hyaluronic acid. *Asboe-Hansen* (1963) states that steroids inhibit the mast cell activity and so impair capillary permeability.

McCarthy (1964), on the other hand, found that local steroid treatment caused an increase in articular exsudate and that the number of leukocytes was considerably higher.

In a recent study *Brånemark & Goldie* (1966) found that local administration of some steroid compounds in soft tissues causes a complete stop of blood flow in venules. About 90 per cent of the perivascular granular cells were disrupted, indicating tissue injury.

After two days the circulation had not been restored, but after two weeks the circulation was back to normal in the same vessels as observed before the steroid application.

No reports have been encountered on the effect of steroids on the circulation in synovial tissue. In a previous study (*Brånemark & Goldie* 1966) it was shown that the suspension medium of a locally applied steroid was the causative agent in provoking tissue injury with disturbances in the circulatory conditions. It was therefore thought to be of interest to investigate whether the same observations could be made on articular tissues. If such were the case, this might help to explain the adverse effects noted in steroid therapy of joint disease.

MATERIAL AND METHODS

The substances used were 1. prednisolon tertiary butly acetate and its suspension medium and also 2. methylprednisolon acetate with suspension medium. The compositions of these are

1. Prednisolon tert. butylacetate 20 mg
   Sorbimacropol. oleas, natr. citr. tribus
   Sorbitol, phenylcarbinol et aq. steril q.s. ad 1 ml.
   (Commercial name: Codelcortone TBA, MSD)

2. Methylprednisolon acetate 40 mg
   Macrogol 4000
   4-methyl-1-tetradecylpyridin chlorid
   et natr. chlorid. q.s.
   Aq steril. ad 1 ml
   (Commercial name: Depomedrone, Upjohn)

These preparations as well as the separate suspension media were either

1. applied directly onto the synovial tissue over the bone-cartilage border in the exposed knee joint of rabbits or

2. injected into the joint.

The amount used ranged from 0.5 to 1.5 ml. The experiments were carried out on 13 rabbit knee joints. Physiologic saline was injected in 5 controls.

After Urethane anaestesia with intubation the knee joints were carefully opened following microsurgical principles. Before incising the fibrous capsule and the synovial membrane at its attachment to the patella all vessels in the area of incision were cauterized with microdiathermy (for methodological details see *Lindström* 1963, 1966). Thus a haemorrhage free joint was obtained which left a good view of the intact vessels. The knee joints were all opened from the lateral aspect as the injections were made from the medial side thus leaving the bone-cartilage zone and synovial membrane free from possible interference by the injection-needles. As the joint was opened some drops amounting to about 0.5 to 1 ml of the media to be examined were applied directly onto the synovial membrane. Vitalmicroscopic observations were made immediately, as well as 15, 30 and 120 minutes after the application.

In the injection studies readings were made after 2 hours, 6 hours, 3 days and 2 weeks. The animals were permitted to run freely around as soon as they had wakened out of the anaesthesia.

The microsurgical procedure was carried out under a Zeiss operation microscope (x 6–40). The observations were made in incident light with a Leitz' Intravital Microscope equipped with an Ultropak Illuminator. A special apparatus for determinations of corpuscular blood flow velocity in different kinds of vessels was connected to the microscope (*Brånemark & Jonsson* 1961). By means of a closed-circuit TV-system with tape-recorder the analyses could easily be repeated. (*Brånemark* 1966).

RESULTS

1. *Action of Direct Local Deposition on Exposed Knee Joint*

a) *Steroid with Suspension Medium (Codelcortone TBA)*

Some difficulties arose in observing the individual vessels as the view was partly obstructed by the milky substance. Registrations could, however, be made and became easier after a short while when the preparation was dispersed. Immediately after deposition the blood flow was considerably slowed down and the evenness in corpuscular distribution was interrupted by marked plasma skimming. After 30 seconds the blood flow within the area of deposition had stopped in venules as well as in arterioles. The caliber of the vessels seemed to remain the same. No increase in white cells was observed, nor in thrombocytes. No thrombi were seen. The capillary endothelial cells remained normal and no oedema was present. The extravascular structures were normal. On following observations after 15 and 30 minutes the picture remained the same. After 120 minutes a slow corpuscular circulation had commenced in the periphery of the zone of deposition whereas in its centre there was no flow.

After 5 minutes' standstill the area was immersed with physiologic saline. The circulation then started, first with a comparatively slow flow in the most proximal short circuit vessels. Within the next 2 minutes corpuscular flow could be observed even in the most distal part of the capillary loops, which are typical for this kind of tissue. When the area was again inundated with the steroid preparation, the blood flow came to a stop within 45 seconds. After 10 minutes' application and later it was impossible to regain the circulation with physiologic saline. (Figures 1, 2 and 3).

b) *Suspension Medium Only (Codelcortone TBA)*

In these experiments the vessels were more easily visualized as the substance is more transparent without the steroid component. The observations became a repetition of what had been registered under a). Thus the blood flow stopped within 30 seconds after deposition of the suspension medium and remained so at the reading 2 hours later. The same phenomena with immersion of physiologic saline were repeated.

c) *Depomedrone*

The same experiments as above were carried out with Depomedrone and its suspension medium. In no instance did this preparation or its

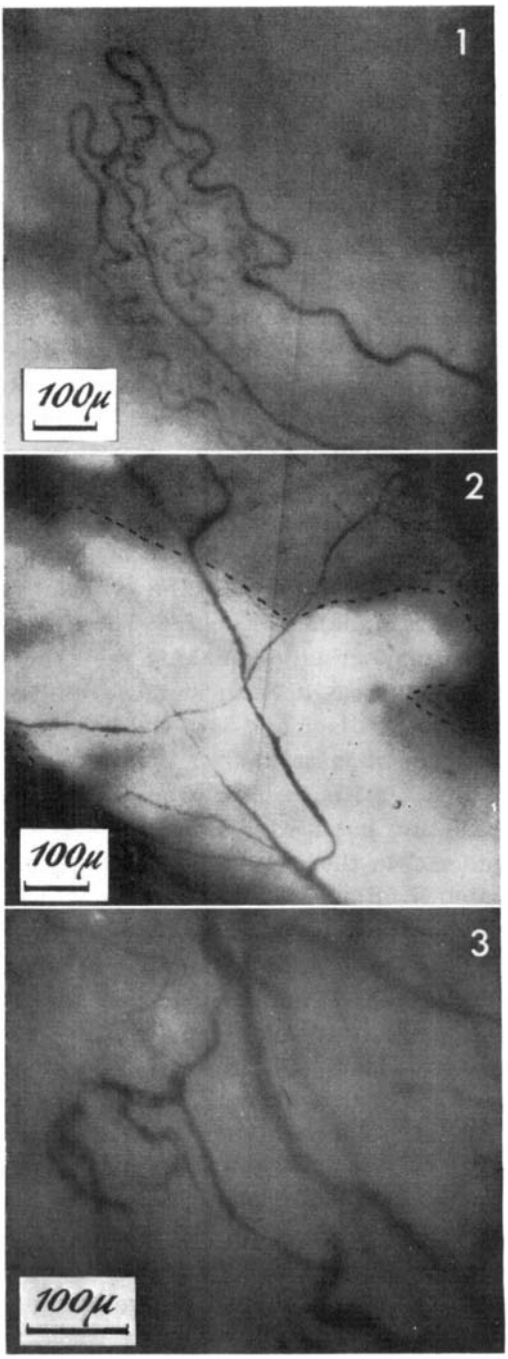


Figure 1. Normal capillary loop structure of synovial tissue in rabbit knee joint seen from lateral aspect after opening of the joint. Note evenness in structure and shunting capillaries between main branches of loop.

Figure 2. Same area as in Figure 1 after deposition of corticosteroid (Codelcortone). White areas are steroid deposits. Note irregularity of vascular structure, thinness of vessels, interrupted corpuscular continuity and also empty vessels.

Figure 3. Same tissue area as Figure 2 but under higher magnification. Note same changes as described in Figure 2.

suspension medium influence the microcirculation in a deleterious way. Microcirculatory structure and function remained completely intact when observed at the different intervals.

2. *Intraarticular Injections*

a) *Steroid with Suspension Medium (Codelcortone TBA)*

Macroscopic and vital microscopic: 1. On opening the joints *2 hours* after injection they were filled with a milky floccular mass which was suspended in the synovial fluid. The fibrous capsule was normal but the synovial tissue was moderately oedematous. The circulation in the vessels of the bone-cartilage zone had stopped, whereas in the free part of the synovium it was intact. No increase in white cells or thrombocytes was noted. The cartilage appeared normal with an even glossy surface. The joint was not bulging which was accepted as partial evidence that no greater increase in synovial fluid had occurred.

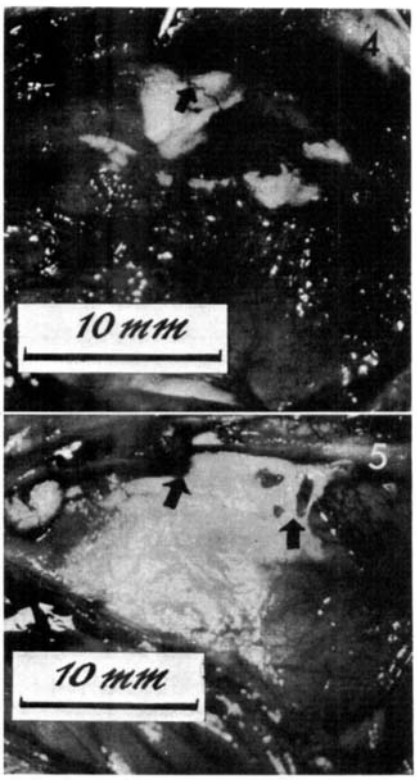
2. After *6 hours* a change of appearance was obvious. The fibrous capsule was normal and the synovial membrane was still oedematous but to a much lesser degree. The floccular masses were now dispersed into small granules, making the synovial fluid milky-like. It did not appear increased. Some small "granules" of the drug seemed to adhere to both cartilage and synovial tissue. Normal circulation was noted in all types of vessels.

3. After *3 days* the fibrous capsule and the synovium were normal excepting patches of white masses with a cheesy consistency which adhered to the membrane. No local reaction was noted around these patches (Figure 4). Vessels in which normal circulation was seen traversed the patches and also surrounded them. No signs of increase or "budding" as an inflammatory response were however noted. The fluid appeared normal in colour and consistency. Normal circulation was maintained in vessels of all types in the bone-cartilage zone and the synovial tissue. The various cell elements were all normal and no increase in thrombocytes or white cells was observed.

4. After *2 weeks* the capsule seemed uninterfered with except for the patches of white "cheese" material, which adhered to the synovium and now were somewhat more expansive. The fibrous capsule was easily detached from the synovial tissue layer including the areas where the deposits were found. These were now more obviously adherent to the articular cartilage and the fat pads. In fact some of them seemed embedded in the fatty tissue. The circulation in all types of

Figure 4. Right rabbit knee joint from the lateral aspect. White floccular masses in the upper, subpatellar synovial tissue area 3 days after injection of Codelcortone TBA. Slight general oedema of synovial tissue. No exaggerated local tissue reaction around cortisone deposit. Note unimpaired vessel running across cortisone patch (at arrow).

Figure 5. Right rabbit knee joint from the lateral view 2 weeks after injection of Codelcortone TBA. Note lack of reaction around cortisone deposits. (at arrows).



vessels was normal and the extravascular tissues including granular cells showed no abnormalities (Figure 5).

Histology: The steroid appeared as an inert amorphous mass surrounded by synovial tissue. In no place was the steroid broken up into spread particles but retained its compactness. There was no cellular reaction of either destructive or reparative type adjacent to the steroid mass. (Figures 6 a, b, c). The cellular layers of the synovial lining did not reveal any abnormal characteristics. No oedema was observed.

b) *Suspension Medium Only (Codelcortone TBA)*

Macroscopic and vitalmicroscopic: All the observations made at the appropriate times after the medium had been injected showed exactly the same conditions as were apparent under a). As no differences, excepting the white deposits decribed above, were present, the readings

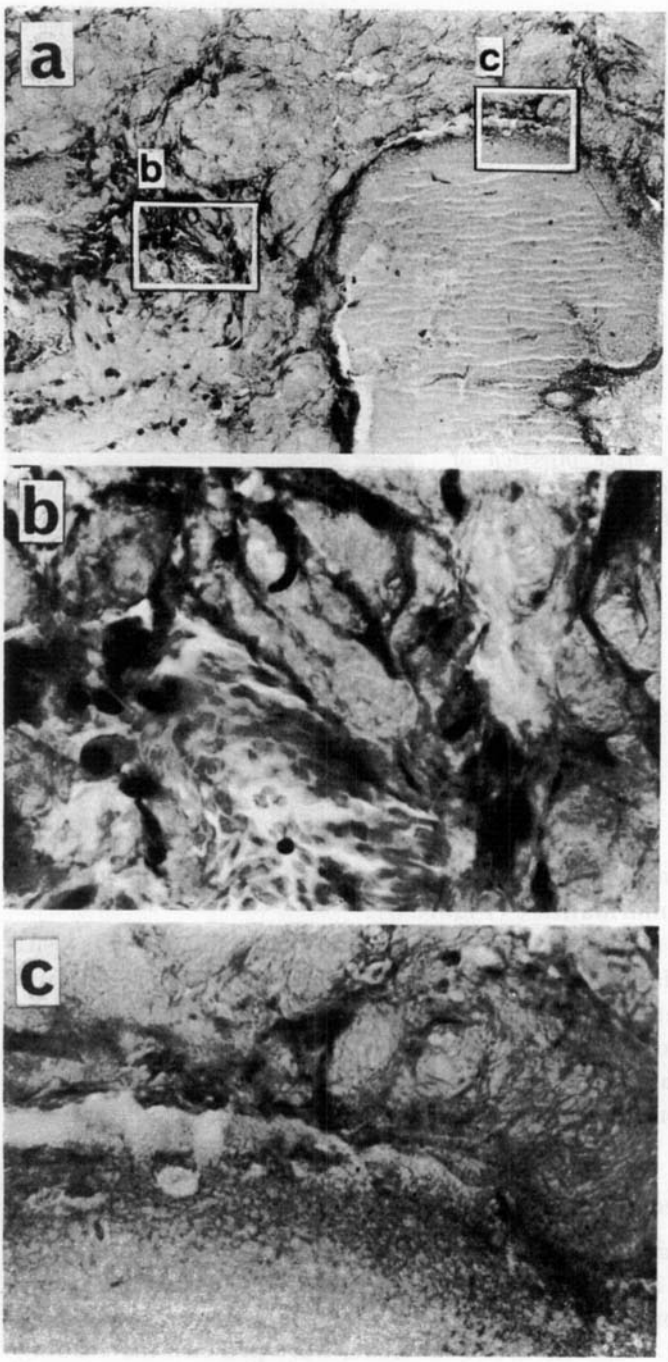


Figure 6. Histologic sections of local deposit of cortisone in synovial tissue. No apparent surrounding tissue reaction.

a) general view (× 75).

b) venule with a few surrounding round cells (× 400).

c) border zone between steroid and connective tissue (× 600).

will not be repeated. Further analysis showed that one component, sorbitol, caused the vascular reaction.

Histology: In the light microscope there were no signs of cellular or vascular reactions.

c) *Depomedrone* disclosed no changes as described under a and b.

d) *Physiologic Saline*

Macroscopic and vitalmicroscopic: The controls in which physiologic saline was injected disclosed no abnormalities irrespective of time-lag between injection and recording.

The histologic picture also remained normal.

COMMENTS AND CONCLUSION

It is generally accepted that the therapeutic value of steroid compounds in joint disorders is great but of late a certain resistance to their use has become prevalent. They are, however, still widely used despite the disadvantages their local application seem to cause. As previously mentioned *Chandler et al.* (1958) ascribed cartilage defects and destructions as they appeared on X-ray in cases of rheumatoid arthritis to the use of intraarticular steroids. This idea has been challenged by *Edström* (1961) who was of the opinion that the process of rheumatoid arthritis is such as to produce destructions of the kind described by *Chandler et al.* If, however, it is true that the use of cortisone may provoke cartilage destructions, it could partly be explained by the observations of *McCarthy* (1964) who found a richness in leukocytes in articular exsudate. These cells produce a proteolytic enzyme which influences the chondromucoproteins in a way as to cause destruction of cartilage. Thus steroids indirectly via the leukocytes would give rise to cartilage damage. On the other hand, the investigation by *Nettelbladt & Sundblad* (1963) indicates that there is no increase in the exsudate of a diseased joint treated with cortisone.

In view of this it was thought of interest to investigate the influence of inraarticularly administered cortisone in normal joints, and especially the vascular reaction. As it was known from previous investigations on soft tissues that steroid preparations cause microcirculatory disturbances (*Brånemark & Goldie* 1965) and that this in turn is due to the suspension medium, this investigation aimed at finding out if the joint vascular bed reacted in the same way.

The circulation in the synovial tissue and bone cartilage zone reacted with a stop in circulation exactly similar to that of soft tissues and the standstill in blood flow was due to the suspension medium used containing sorbitol (Codelcortone TBA). No such changes were, however, observed with Depomedrone the suspension medium of which contains macrogol with a lower molecular weight. As for cellular damage the perivascular cells were disrupted.

It is interesting to note that the deposition of cortisone in synovial tissue and the lack of its resorption do not cause any cellular or other changes as observed by light microscopy. In 1964 (*Goldie*) carried out a study on the pathogenesis of tennis elbow. In a number of cases a similar phenomenon as described above was observed with floccular masses of steroid deposited in the soft tissues surrounding the epicondyle. The time during which these depositions had lodged in the soft tissues varied from 1 to 3 years without any obvious interference with cellular structure but above all, which is just as important, without any cure of the disease. No analysis was made of the floccular substance but it would no doubt be interesting to find out if this contained any unabsorbed corisone or suspension medium. If this were the case how then does locally applied cortisone act—and for how long a time? Investigations have been instituted to further explore these problems.

The cause of cessation of blood flow is probably to be found in a direct damage to the exposed tissues including the vascular wall. The frequent finding of disrupted perivascular granular cell verifies this theory as such a mechanism is also an early sign of tissue injury.

It is important to point out that these observaions were made on normal tissues. What bearing they have in already damaged tissues with for example inflammatory changes remains to be investigated. It is, however, known from other studies that tissues, which have already been damaged to some extent and thus exhibit signs of tissue injury, have a lower threshold level for additional injury. Thus, it appears reasonable to assume that, if cortisone preparations of the kind tested here are applied to tissues affected by rheumaoid disease *e.g.*, the tissue would react with changes of at least the same magnitude as normal tissue, and most probably with even more pronounced disturbances in tissue structure and function.

SUMMARY

The use of corticosteroids in joint disease is controversial. Cartilage damage and soft tissue reactions have been ascribed to these agents.

A study was carried out in normal rabbit knee joints to clarify the action of locally administered steroids and a comparison was made between two usual commercial preparations. It was found that one of them (Codelcortone TBA) had a damaging influence on the synovial microcirculation in that it caused an arrest in the nutritive flow of blood. Perivascular granular cells were disrupted. On further analysis of this preparation it was found that a component, sorbitol, of the suspension medium, caused the microcirculatory disturbances.

The other preparation used (Depomedrone) did not cause any such damage.

RESUME

L'utilisation de corticostéroïdes dans les maladies des articulations est controversable. Des dommages cartilagineux et des réactions des tissus mous ont été attribués à ces agents.

Une étude a été pratiquée sur des articulations normales du genou de lapins, afin de clarifier l'action des stéroïdes administrés localement et une comparaison a été faite entre deux préparations courantes dans le commerce. Il a été trouvé que l'une d'elles (codelcortone TBA) avait une influence préjudiciable sur la microcirculation synoviale en causant un arrêt du courant nutritif de sang. Des cellules granulaires périvasculaires avaient éclaté. Une analyse plus approfondie de la préparation a dévoilé que c'est un composant, le sorbitol, dans le milieu de suspension, qui cause les troubles microcirculatoires.

L'autre préparation utilisée (Depomedrone) n'a a causé aucun dommage de ce genre.

ZUSAMMENFASSUNG

Die Verwendung von Cortico-steroiden bei Gelenkkrankheiten ist umstritten. Knorpelbeschädigung und Reaktionen der Weichteile sind diesen Agenden zugeschrieben worden. Um die Wirkung von lokal administrierten Steroiden zu untersuchen, ist ein Studium normaler Kniegelenke vom Kaninchen gemacht worden, nebst einem Vergleich zweier allgemein verwendeter kommersieller Präparate.

Es wurde gefunden dass das eine ihnen (Cordelcorton T.B.A.) eine schädliche Einwirkung auf die synoviale Mikrozirkulation hatte, indem eine Verlangsamung der nutritiven Durchblutung verursacht wurde. Perivasculäre granulierte Zellen wurden gesprengt. Durch fortgesetzte Analyse dieses Präparates wurde gefunden, dass Sorbitol—eine Kom-

ponente des Suspensionsmediums—die microzirkulatorischen Störungen verursachte.

Das andere der verwendeten Präparate (Depomedrone) verursachte keine derartigen Schäden.

REFERENCES

Asboe-Hansen, G. (1963) Connective Tissue. *Ann. Rev. Physiol.* **25**, 41–60.
Ansell, B. M., Bywaters, E. G. L, Isdale, I.C. (1956) *Brit. Med. J.* **1**, 1075–1077.
Brånemark, P.-I. & Jonsson, I. (1963) *Biorheology* **1**, 143–146.
Brånemark, P.-I. (1966) *Med. Biol. Ill. XVI:***2**, 100–108.
Brånemark, P.-I. & Goldie, I. (to be published).
Chandler, G. N., Wright, V. (1958) *Lancet* **II**, 661.
Edström, G. (1961) *Acta rheum.scand.* **7**, 151.
Fearnlay, G. R. (1958) *Practitioner* **180**, 58–64.
Goldie, I. (1964) *Acta chir. scand.*, Suppl. 339, diss.
Leveaux, V. M. (1956) *Ann. rheum. Dis.* **15**, 4, 330–337.
Lindström, J. (1963) *Acta rheum. scand.*, Suppl. 7, diss.
McCarthy, D. & Hogan, J. M. (1963) Arthritis and Rheumatism VII, 359–367.
Nettelbladt, E & Sundblad, L. (1963) Sv. Läkartidningen **60**, 1315–1320.