PERSISTENCE OF INTRA-ARTICULAR STEROID

EXPERIENCE WITH PREDNISOLONE
TRIMETHYLACETATE

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If intra-articular therapy is to prove satisfactory it would seem to be necessary to use a compound which not only has a marked local anti-inflammatory action but will persist in the joint for a long period.

Hydrocortisone, the drug most frequently used, has considerable anti-inflammatory activity but remains in effective concentration within the joint cavity for only a few hours (Wilson *et al.*, 1955; Oka, 1956).

In carrying out a clinical trial of a new intra-articular steroid, prednisolone trimethylacetate, we were impressed by the marked cytological and biochemical changes occurring in the synovial fluid (Murdoch and Will, 1959) as compared with those published for hydrocortisone. There was no clinical evidence of systemic absorption, and it was felt that the increased effectiveness of the drug might be due in part to its longer persistence in the joint cavity. The following experiments were designed to test this theory.

Procedure

At intervals of from 24 hours to 14 days after the intra-articular injection of prednisolone trimethylacetate the joints were aspirated as completely as possible.

Extraction.—The synovial fluid was extracted three times with chloroform in a separating funnel. This was done as soon after aspiration as possible, to minimize destruction of the steroid. To prevent emulsification, large volumes of chloroform were used. The chloroform extracts were evaporated to dryness under reduced pressure at a temperature not exceeding 40° C. The residue obtained was sometimes oily, containing phospholipids. These were eliminated by dissolving the residue in *n*-heptane and extracting with three separate changes of an equal volume of 70% v/v aqueous methanol. The aqueous methanol was then distilled off under reduced pressure, further small volumes of methanol being added during the process. The final residue obtained was redissolved in a small quantity of absolute alcohol and transferred to a micro-pipette.

Chromatography.—The solvent system of Bush (1952) was used. As prednisolone trimethylacetate was found to have a high *R_f* value the B1 system (water, methanol, petrol, and benzene) was considered most suitable. The residue, dissolved in absolute alcohol, was transferred to Whatman No. 1 paper from the micro-pipette, using a stream of dry nitrogen to assist evaporation and produce a spot of small diameter. At another origin a reference spot of prednisolone and prednisolone trimethylacetate was applied in similar fashion. A spot of Sudan IV red dye was used to mark the solvent front. The chromatogram was then placed

in a descending chromatography tank and was run through after satisfactory equilibration.

Detection of Steroid.—The dry chromatogram was examined by reflected ultra-violet light from a 253 mμ source, using a Hanovia chromatolite. The markers, and any steroid present in the synovial fluid extracts, appeared as dark spots on a fluorescent background. These were ringed in pencil. The papers were then dipped in a freshly prepared alkaline solution of blue tetrazolium (Burton *et al.*, 1951). They were blotted with filter paper and warmed gently to dry. With this technique, reducing steroids yield a blue coloration. Known quantities of prednisolone trimethylacetate were added to joint fluid *in vitro* and incubated to simulate natural conditions. After extraction similar spots appeared on the chromatograms in line with the markers. These did not occur in *in vitro* controls. It was thus possible to identify steroid present in the synovial fluid extracts by: (1) absorption of ultra-violet light at a wavelength of 253 mμ; (2) reduction of the tetrazolium reagent, and (3) position on the chromatogram in relation to the markers.

Results

Chromatograms were prepared from 50 specimens of synovial fluid (49 knees, 1 elbow) obtained at intervals of from 24 hours to 14 days after the injection of prednisolone trimethylacetate in a dosage ranging from 20 to 100 mg. Forty-five were obtained from rheumatoid patients and five from osteoarthritic joints. The investigation was planned in four stages.

Experiment 1.—A direct comparison was made between the persistence of hydrocortisone acetate and prednisolone trimethylacetate in the joint cavity. Sixteen joints were used. Eight were given hydrocortisone acetate in dosage ranging from 50 to 150 mg., eight received prednisolone trimethylacetate in dosage ranging from 20 to 100 mg. Twenty-four hours after injection the joints were aspirated completely. Whereas all eight joints injected with prednisolone trimethylacetate contained steroid, in no instance was hydrocortisone recovered from the eight joints into which it had been introduced. These results are summarized in Table I.

TABLE I.—*Detection of Steroid in Joint Fluid 24 Hours After Intra-articular Injection. A Comparison Between Prednisolone Trimethylacetate (P.T.M.A.) and Hydrocortisone Acetate*

Joint No.	P.T.M.A. (mg.)	Result	Joint No.	Hydrocortisone Acetate (mg.)	Result
1	100	P.T.M.A. and prednisolone present	9	50	No steroid detected
2	40	Prednisolone present	10	50	" "
3	30	" "	11	50	" "
4	30	" "	12	50	" "
5	30	" "	13	100	" "
6	20	" "	14	100	" "
7	20	" "	15	100	" "
8	20	" "	16	150	" "

Experiment 2.—As we had found it possible to recover steroid at 24 hours after the injection of small doses of prednisolone trimethylacetate we then progressively increased the interval before aspiration up to six days. This group comprises 12 joints, of which six were examined at 48 hours, having been given doses ranging from 20 to 50 mg. Of the six specimens, three contained steroid. The remaining six joints were given 50 mg. of prednisolone trimethylacetate and were aspirated at from three to six days after injection. Five

of the six contained steroid. These results are summarized in Table II.

Experiment 3.—It was now apparent that prednisolone trimethylacetate would persist for a considerable period in the knee-joint. In 16 patients the joints were aspirated after seven days, while the dosage varied from 30 to 100 mg. (Table III). It will be noted that steroid was not detected when the dose was less than 50 mg.

Experiment 4.—Table IV summarizes the results of aspiration at 10 to 14 days. Of three joints aspirated 10 days after injection, two contained steroid; and of three aspirated after 14 days, steroid was present in one. It is of interest that steroid was detected in the elbow-joint 14 days after the injection of only 25 mg. of prednisolone trimethylacetate.

TABLE II.—Detection of Steroid in Joint Fluid 48 Hours to Six Days After Intra-articular Injection of Prednisolone Trimethylacetate

Joint No.	P.T.M.A. (mg.)	Interval after Injection	Result
17	50	2 days	P.T.M.A. and prednisolone present
18	30	2 "	No steroid detected
19	30	2 "	" "
20	20	2 "	P.T.M.A. and prednisolone present
21	20	2 "	No steroid detected
22	20	2 "	Prednisolone present
23	50	3 "	" "
24	50	3 "	No steroid detected
25	50	4 "	Prednisolone present
26	50	5 "	" "
27	50	6 "	" "
28	50	6 "	" "

TABLE III.—Detection of Steroid in Joint Fluid Seven Days After Intra-articular Injection of Prednisolone Trimethylacetate

Joint No.	P.T.M.A.	Result
29	100 mg.	Prednisolone present
30	100 "	" "
31	60 "	No steroid detected
32	60 "	Prednisolone present
33	60 "	" "
34	60 "	" "
35	60 "	No steroid detected
36	50 "	Prednisolone present
37	50 "	No steroid detected
38	50 "	" "
39	50 "	" "
40	50 "	" "
41	50 "	Prednisolone present
42	50 "	No steroid detected
43	30 "	" "
44	30 "	" "

TABLE IV.—Detection of Steroid in Joint Fluid 10–14 Days After Intra-articular Injection of Prednisolone Trimethylacetate

Joint No.	P.T.M.A.	Interval	Result
45	100 mg.	10 days	Prednisolone present
46	60 "	10 "	" "
47	60 "	10 "	No steroid detected
48	100 "	14 "	" "
49	60 "	14 "	" "
50	25 "	14 "	Prednisolone present

Discussion

Our failure to recover hydrocortisone acetate from synovial fluid 24 hours after the injection of large doses is in accord with the findings of Wilson *et al.* (1955), who noted that 86% and 97% of an intra-articular dose of hydrocortisone had disappeared by one hour and three hours respectively after injection. Indeed, they recovered from the contralateral control knee significant amounts of hydrocortisone 30 minutes after the intra-articular injection. The results of Oka

(1956) are essentially similar. He blocked the endogenous output of hydrocortisone by the administration of 9 α -fluorohydrocortisone and estimated the plasma 17-hydroxycorticosteroid levels following intra-articular injection of hydrocortisone acetate. All cases, except one, showed a marked rise in plasma levels, with a peak at three hours after injection.

Gallagher *et al.* (1953), using intra-articular radioactive cortisone, showed that its clearance from the joint was as rapid as that of injected radiosodium.

It will be seen from the results of our experiments that steroid can be recovered from the synovial fluid at intervals of up to 14 days after the intra-articular injection of prednisolone trimethylacetate.

The effect of the drug appears to be local rather than systemic, owing to its persistence within the joint cavity. It is of interest that the steroid usually recovered was free alcohol prednisolone, whereas prednisolone trimethylacetate appeared in only three cases. This may be due to the low solubility of prednisolone trimethylacetate, which is presented in a microcrystalline form to act as a depot preparation. It seems likely that there is a gradual breakdown of the compound to the free alcohol prednisolone which exerts its anti-inflammatory action. We are at present engaged in *in vitro* studies to elucidate this point.

Summary

The persistence of prednisolone trimethylacetate was assessed in 50 arthritic joints following intra-articular injection of doses varying between 20 and 100 mg. and at intervals of between 24 hours and 14 days.

A control series of eight joints were injected with hydrocortisone acetate in doses ranging between 50 and 150 mg. No steroid could be detected after 24 hours.

The significance of these findings is discussed.

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Discussing the difficulty of finding staff for the domiciliary services, Dr. William Fielding, medical officer of health of Bournemouth, writes as follows in his Annual Report for 1958: "The choice of employment is now so wide that young people are tending to shun the social services as a career, where remuneration is often far behind the financial rewards in industry, and the development of the social services urged by an endless succession of Royal Commissions, Working Parties, and Ministry Circulars seems likely to be a very slow process. There is a tendency, also, to recommend the appointment of large numbers of social workers in an advisory capacity and to forget that many of the recipients of this advice are in no condition, either physical or mental, to act upon it. The great need, it would seem, is for far more help of a practical kind and fewer homilies."