Disposition of Intra-Articularly Injected Hydrocortisone Acetate, Hydrocortisone Free Alcohol and Cortisone Acetate in Arthritis[1]

Intra-articular injection of hydrocortisone acetate has been demonstrated by HOLLANDER, BROWN, JESSAR, and BROWN[2] to produce prompt alleviation of pains, and diminution of synovial swelling and effusion, with a fall to more normal intra-articular temperature in nearly 90% of patients with rheumatoid arthritis. In contrast to this, cortisone acetate in similar or even larger doses is far less effective, with only about 25% of the patients showing even partial improvement after intra-articular injection.

The reason for this difference in local anti-inflammatory action is obscure. Original assumptions that the difference was purely due to lower solubility of hydrocortisone acetate have proved inadequate.

The purpose of this report is to present the data on concentrations of hormone in the synovial fluid, its cells and the synovium at various intervals after intra-articular injection, i.e. the "disappearance rate", the relative distribution of the steroids in synovial cells and fluid, and the rate of hydrolysis of the acetate esters of cortisone and hydrocortisone.

The concentration of 17-hydrocorticoids in the joint fluid and cells was measured by a modification of the methods of PORTER and SILBER[3] and of NELSON and SAMUELS[4] for blood. The cells were separated from the fluid by centrifuging the joint fluid and washing the centrifugate with physiological saline. The cells were then exposed to ultra sound for 15 min to break them up. After extraction, the amounts of 17-hydrocorticoids were then determined for the cells, supernatant, and washings. The accuracy of the method was examined in 17 paired observations obtained within 75 min of injection. The average deviation of the observations from the mean of each pair was 4·3% with a maximum deviation of 14%. This degree of accuracy is believed to be due to the relatively high concentrations in the joint fluid during the first two hours after injection.

The relative proportion of the free form and the acetate present in the fluid and in the cells was determined by chromatographing appropriate aliquots of extract by a modification of the method of BURTON, ZAFFARONI, and KEUTMANN[5]. Approximations of quantities on the chromatogram were made by the method of TENNENT, WHITLA, and FLOREY[6].

We have now injected a total of 23 different patients; studying one patient with four paired experiments, and another with three paired experiments. Patients with bilateral arthritis of the knee joints were injected intra-articularly with approximately isomolecular amounts of cortisone acetate (*E*), hydrocortisone acetate (*F*), or free hydrocortisone. Patients with unilateral knee joint arthritis were given unilateral injections of either of these substances. The concentrations of 17-hydroxycorticoids were measured at various intervals after injection (less than 180 min). The initial specimens were obtained within approximately 3 min after injection.

Results

Change in concentration of 17-hydroxycorticoids in total joint fluid after intra-articular injection.–It has been noted that the rate of change in concentration was approximately the same over the period charted for the three compounds and that the rate of change in concentrations seemed to be more closely associated with physiological differences in the subject rather than with the chemical characteristics of the compounds.

Measurements were also made at 24, 48, and 168 h after intra-articular injection in some experiments. Low concentration of Porter-Silber reacting material was found 24 to 168 h after intra-articular injection. In general, *F* acetate injections resulted in higher concentrations than *E* acetate. Most of the Porter-Silber reacting material present after *F* acetate injection was in the cells, while most of the material found after *E* acetate injection was in the fluid phase. The accuracy, however, of these low values is questionable.

Distribution of 17-hydroxycorticoids in cells and fluid. It has been noted, considering the concentration of 17-hydroxycorticoids contained in the cells expressed as a percentage of the concentration in the uncentrifuged joint fluid, that a considerably greater proportion of 17-hydroxycorticoids was found in the cells after injection of *F* acetate than after injection of *E* acetate, and *F* free alcohol. It has also been noted that there was a sharp decrease with time of the proportion found in the cells after *E* acetate and *F* free alcohol.

Proportion of 17-hydroxycorticoids present as the acetate in cells and fluid.– The proportions of non-hydrolyzed *F* acetate and *E* acetate in the fluid decreased relatively rapidly over the first 75 min while the proportion of the material present in the cells as the non-hydrolyzed acetate remained high.

The following conclusions were suggested on the basis of our data: (1) the rate of decrease in concentration for *E* and *F* acetates and *F* free alcohol was approximately the same in any one individual; (2) the hydrolyzed form of both of the acetates was present in greater proportion in the fluid than in the cells; (3) the proportion of 17-hydroxycorticoids present in the cells after injection of *F* acetate was greater than after the injection of *E* acetate and *F* free alcohol.

Proportion of 17-hydroxycorticoids present in the lining cells of the synovial membrane – Preliminary results. We also injected 25 mg of cortisone acetate or hydrocortisone acetate into 5 arthritic knee joints at various intervals prior to synovectomy. At operation, the excised synovial tissue was immediately transferred to the endocrine laboratory, washed, and the inner layer dissected free. The lining layer of synovial tissue was then exposed to ultra sound and extracted for steroid analysis both by the Porter-Silber reaction and by paper chromatography. Our preliminary results showed that a large proportion of the intra-articularly injected *F* acetate was taken up and apparently stored by the lining cells of the synovial membrane at least for some days, whereas *E* acetate was absorbed in less than half the amount and disappeared much more quickly from the synovial lining.

We think these differences provide a clue to the reason for the greater local effectiveness of hydrocortisone in the arthritic joint. Further studies are in progress.

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Riassunto

In pazienti affetti da artrite reumatoide viene iniettato cortisone acetato, idrocortisone acetato, o idrocortisone, nel cavo sinoviale artritico.

La durata del riassorbimento di questi 17-idrossicorticoidi; la loro distribuzione nelle cellule e nel fluido articolare e nelle cellule del rivestimento sinoviale; le andamento del processo idrolitico dei composti acetati, studiati con mezzi chimici e cromatografici, si prestano a commenti utili ad interpretare la maggiore efficacia locale dell'idrocortisone.

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Flavines in Experimental Diabetes

It is known that in diabetes (alloxan diabetes in animals, and spontaneous diabetes mellitus in man), a decrease of cocarboxylase content of tissue[1] occurs, due to a disturbance of thiamine phosphorilation[2].

No study has so far been undertaken to provide information about the behaviour of some of the other coenzymes in diabetic conditions, probably owing to the difficulty of carrying out their routine determination in the tissue .

Using the very handy method recently developed by BESSEY *et al.*[1] for the determination of riboflavine and its coenzymes in tissues, we have investigated the behaviour of these cofactors in alloxan diabetes and comparatively in normal rats. In a number of experiments, FAD has also been determined enzymatically according to the method of WARBURG and CHRISTIAN.[2]

The results reported in the table show that in diabetic liver a significant decrease of FAD and a corresponding increase of the fraction "FMN + Rib" occurs. In animals with diabetic coma both total flavines and FAD values strongly decrease in respect to normal animals, while the fraction "FMN + Rib" increases.

The administration of riboflavine does not appreciably change the FAD content, either in normal or in diabetic animals; only "FMN + Rib" fraction is augmented. When riboflavine is injected together with ATP, FAD increases significantly mostly in diabetic livers. An analogous effect is noticeable after administration of FMN and of FMN + ATP.

The low content of FAD of diabetic animals is therefore restored to normal values when riboflavine + ATP or FMN are administered. The occurrence in biological materials of the two following reactions:

$$\text{ATP} + \text{Rib.} \longrightarrow \text{FMN} + \text{ADP}$$
$$\text{FMN} + \text{ATP} \longrightarrow \text{FAD} + \text{pyrophosphate}$$

discovered by KEARNEY *et al.*[3] and by KORNBERG *et al.*[4] respectively in yeast and in liver extracts, indicates that ATP is essential for the synthesis both of FMN and FAD.

In our experiments, the administration of riboflavine to the diabetic animals is as effective as FMN in restoring FAD to the normal values, provided that ATP is also injected. This is evidence that ATP, as indicated by these two reactions, is necessary for the sinthesis of FAD *in vivo*, and that most probably both these reactions take place in the living organism.

Since ATP has been found deficient in diabetic liver[5], the diminution of FAD observed in diabetic animals, may be attributed to this deficiency.

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Flavines μg/g of fresh liver tissue

Treatment or condition	Normal rats				Diabetic rats			
	N° of experiments	Total flavines	FAD	FMN + Rib.	N° of experiments	Total flavines	FAD	FMN + Rib.
	18	33·93 ± 4·11	29·33 ± 3·41 (27·75 ± 3·05)	4·60 ± 0·51	19	27·87 ± 3·01	21·40 ± 3·01 (19·80 ± 2·60)	6·47 ± 0·7
					(°) 8	19·83 ± 2·04	11·50 ± 1·51 (11·10 ± 1·30)	8·33 ± 1·10
Rib.(1)	11	39·22 ± 4·80	31·75 ± 3·60	4·47 ± 1·90	10	31·47 ± 3·50	20·92 ± 2·70	10·55 ± 1·40
Rib.+ ATP(2)	12	39·95 ± 4·20	32·45 ± 3·45	7·50 ± 1·65	9	34·45 ± 3·70	27·40 ± 3·10	7·05 ± 1·10
FMN(3)	15	38·02 ± 3·75	33·74 ± 3·65	4·28 ± 1·15	14	34·25 ± 3·20	28·55 ± 3·45	5·70 ± 0·85
FMN+ATP(4)	10	39·05 ± 4·20	34·80 ± 3·70	4·25 ± 1·20	10	33·65 ± 3·30	29·00 ± 3·55	4·65 ± 1·05

The values represent the means ± the standard error of the means. The substances were injected to the animals intraperitoneally in the following amounts: (1) Rib. 0·5 mg; (2) Rib. 0·5 mg + ATP.Na 1 mg; (3) FMN 0·6 mg; (4) FMN 0·6 mg + ATP.Na 1 mg. (°) In these experiments rats were in diabetic coma. ( ) FAD determined enzymatically according to WARBURG and CHRISTIAN (5).