Stimulatory action of steroidal anti-inflammatory drugs on plasma alpha$_1$-antitrypsin levels in the rat

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We have investigated a number of steroids and one non-steroidal drug—penicillamine—for their effect on alpha$_1$-antitrypsin synthesis *in vivo*. This plasma antiprotease is the most abundant in plasma and is capable of inhibiting many proteases believed to be involved in joint disease. (Harris, Di Bona & Krane, 1969; Ohlsson, 1971; Kaplan, Kuhn & Pierce, 1973). An antiarthritic role has been suggested for alpha$_1$-antitrypsin (Cox & Huber 1976).

Since liver function is involved in alpha$_1$-antitrypsin synthesis we also monitored plasma protein levels, (Lowry, Rosebrough, Farr & Randall, 1951), total and conjugated bilirubin (Sigma Technical Bulletin No. 605, 1977) and glutamic oxalacetic transaminase (GOT) (Sigma Technical Bulletin No. 505, 1977) plasma levels.

Initially oestradiol-17β, corticosterone, penicillamine, cortisone, hydrocortisone, betamethasome alcohol, prednisolone, methylprednisolone, triamcinolone acetonide and dexamethasone were administered as a suspension in 10% v/v dimethylsulphoxide in arachis oil at daily doses of 5 mg/kg as 0.2 ml portions s.c. into male Wistar rats (200 g) for 10 days. Plasma samples were taken the day preceding the first dose and on the day after the completion of dosing. Very highly significant increases were found in plasma alpha$_1$-antitrypsin levels (measured as trypsin inhibitory capacity—TIC) (Dietz, Rubinstein & Hodges 1974) in rats treated with hydrocortisone, betamethasone, prednisolone, methylprednisolone, triamcinolone and dexamethasone over initial and control levels and those of the other drugs.

In a dose response experiment hydrocortisone, prednisolone and dexamethasone were administered separately at daily doses of 0.25 mg/kg, 1.25 mg/kg and 2.5 mg/kg for 7 days. Plasma was obtained on the eighth day and the results are given in Table 1.

The results of the initial experiment showed that anti-inflammatory steroids elevated the plasma alpha$_1$-antitrypsin levels and that the synthetic steroids were the most active. The second experiment showed that the elevations of plasma alpha$_1$-antitrypsin followed the dose levels and were significant at doses regarded as being therapeutic. In this experiment there is evidence that steroids affected the other liver functions examined and that these too were dose responsive. However in the case of hydrocortisone and prednisolone the lowest dose, which is well within the therapeutic range, appeared to have no adverse effect on the liver.

Whether alpha$_1$-antitrypsin synthesis was due to the direct action of the steroid on the liver or indirectly due to liver damage (Lewis, Bird & Best 1979) cannot be concluded with certainty but the fact that hydrocortisone and prednisolone at the low dose elevated plasma alpha$_1$-antitrypsin without altering the other parameters suggests a direct action by the steroids on alpha$_1$-antitrypsin levels.

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Table 1 Effect of steroids on plasma alpha$_1$-antitrypsin, total protein, bilirubin and glutamic oxalacetic transaminase (GOT) levels in the rat

		Increase in values as % of control day 0 values			
Drug	*Dose/day × 7 in* (mg/kg)	*Alpha$_1$-antitrypsin*	*Total protein*	*Total bilirubin*	*GOT*
Hydrocortisone	0.25	107.2 ± 1.7**	96.4 ± 3.9	98.3 ± 4.0	99.3 ± 1.1**
Prednisolone	0.25	108.3 ± 1.5***	97.1 ± 3.7	144.9 ± 11.2**	109.7 ± 2.8
Dexamethasone	0.25	124.1 ± 1.1***	89.5 ± 5.5	173.7 ± 7.7***	122.7 ± 2.3***
Hydrocortisone	1.25	109.3 ± 2.3**	95.3 ± 5.1	122.9 ± 7.9*	102.4 ± 1.7***
Prednisolone	1.25	110.1 ± 1.3***	93.8 ± 5.0	176.3 ± 6.4***	113.0 ± 1.0
Dexamethasone	1.25	127.7 ± 3.1***	81.8 ± 4.0*	194.1 ± 12.5***	133.1 ± 1.8***
Hydrocortisone	2.50	111.7 ± 2.0***	91.3 ± 4.3	155.1 ± 6.2***	110.4 ± 2.5
Prednisolone	2.50	113.3 ± 1.4***	88.0 ± 2.3	205.1 ± 9.3***	119.0 ± 1.4
Dexamethasone	2.50	131.2 ± 1.2***	76.9 ± 6.5*	234.7 ± 13.2***	151.9 ± 2.7***
Control	vehicle only	101.2 ± 1.2	98.5 ± 6.9	103.4 ± 9.3	99.7 ± 1.1

Values are means of groups of 5 rats, expressed as % of control day 0 values ± s.e. mean.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by t test.

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Some possible mechanisms of action of an endogenous anti-inflammatory protein

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Inflammatory exudates obtained by implantation of polyester sponges in rats have been shown to be anti-inflammatory (Robinson & Robson, 1966) and the active component is known to be a protein (Billingham, Robinson & Robson, 1969). The mechanism of action of these proteins is not known but mechanisms suggested include lysosomal stabilization (Doherty & Robinson, 1976a), interaction with the complement system (Doherty & Robinson, 1976b) or by a counter irritant mechanism (Atkinson, Boura & Hicks, 1969).

The methods used to produce the inflammatory exudate was that employed by Robinson & Robson (1966) except that aseptic techniques were used throughout. Fractionation was carried out on a Sephadex G-150 column, (Billingham, Robinson & Robson, 1969), producing two pooled samples of material.

In this work we have examined the action of the crude exudate and its fractions on monocytes. The monocytes were isolated from guinea-pigs as previously described (Lewis, Best & Bird, 1977) and incubated with various concentrations of the crude exudate and its fractions (1 to 100 mg/ml). After incubation the degree of stabilisation was assessed by assaying for acid phosphatase (Symons, Lewis & Ancill, 1969). It was found that the crude exudate significantly stabilized the monocytes at all concentrations used. Both of the fractions stabilized the monocytes at lower concentrations, but exhibited lytic properties at the higher concentrations used.

It was found that the exudate possessed inherent proteolytic activity (Rinderknecht, Geokas, Silverman & Haverback, 1968) and is autolytic *in vitro* releasing dialysable peptides.

To determine if this autolytic property was of importance an *in vivo* model was developed. This model consisted of aseptically implanting small dialysis sacs, containing 100 mg of exudate in 0.5 ml saline, into rats and leaving them for 10 days to recover. A carrageenin oedema paw test was then carried out on the animals which were compared to groups of sham operated animals and animals implanted with dialysis sacs containing saline alone. The results showed that implantation of dialysis sacs alone was not anti-inflammatory, but that the exudate containing sacs were anti-inflammatory ($P < 0.05$). This could only be due to low molecular weight molecules produced by proteolysis inside the sacs entering into the rats circulation.

In conclusion, this particular inflammatory exudate exhibited a multivalent mode of action. That is, components within the exudate were capable of stabilizing inflammatory cells and also the exudate was capable of producing dialysable peptides by autolytic action which are either anti-inflammatory themselves or which acted as triggers for the production of an anti-inflammatory substance *in vivo*.

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