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STUDIES OF LYSOSOMES—VI.

THE EFFECT OF NEUTRAL STEROIDS AND BILE ACIDS ON LYSOSOMES *IN VITRO**

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Abstract—The effect of steroid hormones and bile acids on the release of hydrolases from lysosomes in granular fractions of rabbit liver and leukocytes has been studied in sucrose suspensions. At concentrations above $2{\cdot}5 \times 10^{-4}$ M, steroids such as etiocholanolone, pregnanedione, pregnanolone, lithocholic acid, and progesterone were found to release β-glucuronidase and acid phosphatase activity from granules of rabbit liver. Metabolic precursors of etiocholanolone, such as testosterone, dehydroepiandrosterone, or 11-desoxycortisol, were active at 37° but not at 20°. Whereas these 5-β-H and Δ4,5 or Δ5,6 steroids released enzymes from the granules into the suspending medium, no 5-α-H isomer, such as androsterone, had comparable activity. Oxygenation or hydroxylation of carbon-11 of pregnanolone or etiocholanolone decreased the activity on the granules of the steroids. Pretreatment of liver granules with cortisol acetate, cortisone acetate, or chloroquine diminished the release of acid hydrolases by etiocholanolone or progesterone. Both etiocholanolone and progesterone reduced the apparent absorbance of suspensions of isolated leukocyte granules, and released β-glucuronidase from the particles, without physically disrupting the majority of granules. The data are compatible with the hypothesis that lysosomes are disrupted by pyrogenic steroids; the relationship of this action *in vitro* to their fever-provoking capacity in man has yet to be determined.

PREVIOUS reports from this laboratory have suggested that the pharmacologic effects of adrenal glucocorticoids may be due, at least in part, to their stabilization of lysosomal membranes.[1–4] Both *in vitro* and *in vivo*, cortisol and cortisone antagonize the disruptive effects upon lysosomes of such agents and procedures as endotoxin,[2] hypervitaminosis A,[3] streptolysin O,[4] shock,[5] and u.v. irradiation;[1] de Duve and coworkers had previously shown that cortisone and cortisone acetate stabilized lysosome-rich fractions of rat liver against the effects of incubation at acid pH.[6]

Under perhaps different circumstances, cortisol antagonizes a pharmacologic effect of another steroid: etiocholanolone.‡ Fever in man, when induced by etiocholanolone,[7]

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‡ For the sake of convenience, generic names have been used for steroids. In the order listed in Table 1, these are: pregnanedione (5-β-pregnan-3,30-dione); progesterone (4-pregnen-3,20-dione); etiocholanolone (5-β-androstan-3-α-ol-17-one); pregnanolone (5-β-pregnan-3-α-ol-20-one); DHA acetate (5-androsten-3-β-ol-17-one acetate); epipregnanolone (5-β-pregnan-3-β-ol-20-one); lithocholic acid (5-β-cholanic acid-3-α-ol); 11-ketopregnanolone (5-β-pregnan-3-α-ol,11,20-dione); testosterone (4-androsten-17-β-ol-3-one); taurolithocholic acid [5-β-cholanic acid-3-α-ol N(2

may be reversed by the administration of cortisol; when both hormones are injected at the same site, the usual fever-producing action of etiocholanolone is diminished.[8] This antagonism suggested that etiocholanolone and structurally related steroids might act to disrupt lysosomes. One precedent for such a mechanism was the observation that bacterial endotoxins released acid hydrolases from lysosomes shortly before the induction of fever in rabbits; pretreatment of animals with cortisone retarded this effect of the pyrogen on lysosomes.[2]

The studies to be detailed below indicate that steroids of 5-β-H configuration (A:B ring junction *cis*), of which etiocholanolone is the prototype, but not of 5-α-H configuration (A:B ring junction *trans*), such as androsterone, release lysosomal hydrolases from granular fractions of rabbit liver and leukocytes. Steroids with an unsaturated bond between carbon atoms 4 and 5, but lacking an oxygen function at carbon-11, e.g. progesterone, also proved disruptive to lysosomes. Pretreatment of liver granules with cortisol acetate, cortisone acetate, or chloroquine diminished the subsequent release of enzymes by progesterone or etiocholanolone.

MATERIALS AND METHODS

Steroid compounds. In most cases these were obtained from Steraloids, Inc., New York; their purity was checked by melting point determination and/or chromatography in two solvent systems: A, benzene 100 methanol 50, H_2O 50; B, isooctane 1,000, tertiary butanol 900; H_2O 900. Estrone, 17-β estradiol, estriol, 11-ketopregnanolone, and the bile acids were generous gifts of Dr. Atallah Kappas of the University of Chicago. Acid compounds were neutralized with NaOH before use. Except where expressly stated, steroids were dissolved in anhydrous ethanol as in the studies of de Duve;[6] at the highest concentrations, neither cortisone acetate nor cortisol acetate was soluble in ethanol; they were added as fine suspensions. Dioxane successfully solubilized these, the pregnanediols, and allocholanic acid.

Preparation of a large granule fraction from rabbit liver and spleen. Details of this procedure have been published before.[3, 4] Briefly, homogenates of liver from young male rabbits were prepared in 0·25 M sucrose. A twice-washed granular fraction sedimenting between 800 *g* (10 min) and 20,000 *g* (20 min) was used in all studies. Aliquots of this fraction were incubated with the test steroids (temperatures and periods to be indicated below). After incubation, samples were again centrifuged at 20,000 *g* (20 min) and the β-glucuronidase and acid phosphatase content of the clear supernatant determined.

Preparation of the specific granules of leukocytes. Granules were obtained from the leukocytes of exudates induced in the peritoneum of rabbits by glycogen, by the sucrose disruption method of Cohn and Hirsch,[9] with modifications previously described.[10] Briefly, granules sedimenting between 400 *g* (10 min) and 8,200 *g* (15 min) were resuspended in 0·3 M sucrose containing 40 units of heparin (USP) per ml. Aliquots,

sulfoethyl)-amide]; androstenedione (4-androsten-3,17-dione); DHA (5-androsten-3-β-ol-17-one); 11-desoxycortisol (4-pregnen-17-α-21-diol-3,20-dione); androsterone (5-α-androstan-3-α-ol-17-one); 11-β-hydroxyetiocholanolone (5-β-androstan-3-α-11-β-diol-17-one); estradiol (1,3,5(10-estratrien-3,17-β-diol); estrone [1,3,5(10)-estratrien-3-ol-17-one]; estriol [1,3,5(10)-estratrien-3,16-α-17-β-triol]; pregnanediol (5-β-pregnan-3-α-20-α-diol); allopregnanedione (5-α-pregnane-3,20-dione); allocholanic acid (5-α-cholanic acid); allopregnanolone (5-α-pregnan-3-α-ol-20-one); corticosterone (4-pregnen-11 β-21-diol-3,20-dione); cortisol (4-pregnen-11-β-17-α-21-triol-3,20-dione); cortisone (4-pregnan-17-α-21-diol-3,11,20-trione); and desoxycorticosterone DOC (4-pregnen-21-ol-3,20-dione).

0·1 ml, were then dispensed into optically matched cuvets containing 3·0 ml of 0·3 M sucrose buffered at pH 7·4 with 0·02 M Tris. The turbidity of the suspensions was followed by measurements of the apparent absorbance of the samples at 520 mμ in a Beckman DB spectrophotometer. After exposure of the granules to test steroids, suspensions were centrifuged at 20,000 *g* (20 min) and the β-glucuronidase activity of the clear supernatants was determined.

Assay of enzymes and expression of results. β-Glucuronidase was determined by the method of Fishman *et al.*[11] and acid phosphatase by the method of Huggins and Talalay,[12] under conditions described previously.[2–4] The total activity of each enzyme present in the homogenate was determined by incubating the samples in the presence of 0·1% (v/v) Triton X-100 (Rohm and Haas, Philadelphia, Pa.) for 60 min. at 37°. After centrifugation of the homogenate for 20,000 *g* (20 min), the enzyme activities of the supernatant fluid were determined. When added to such supernatants at concentrations of 5×10^{-4} M, none of the steroids inhibited or augmented the activities of the solubilized enzymes. To permit comparison of homogenates from different tissues, and in conformity with previous studies,[2–4] all data have been expressed as per cent released from controls. The control samples consisted of tubes incubated for 60 min at the appropriate temperature with the solvents employed for the steroids: 5-ml granule suspension with 0·1 ml solvent, for liver; 3-ml granule suspension with 0·1 ml solvent, for leukocytes. Significance was calculated by "t" tests for paired samples.

RESULTS

Release of acid hydrolases by 5-β-H steroids

At concentrations above $2{\cdot}5 \times 10^{-4}$ M, three 5-β-H steroids (etiocholanolone, pregnanolone, and pregnanedione), released significant β-glucuronidase and acid phosphatase activity from liver granules into the suspending medium (Fig. 1). In direct contrast, the 5-α-H isomers of these steroids ("allo-") were inactive. Release of enzymes increased markedly at concentrations of the active steroids between $2{\cdot}5 \times 10^{-4}$ M and 5×10^{-4} M; the curves obtained for the activities of both β-glucuronidase and acid phosphatase were essentially similar.

Solubilization of the hydrolases by 5-β-H steroids, at 5×10^{-4} M, was approximately linear with the time (Fig. 2). Enzyme activities recorded at the ordinate (time = 0 min) presumably represent enzymes released from granules by the steroids during 20-min centrifugation at 4°. Although some activity was released under these conditions, this was considerably less at 4° than at 37°.

The relationship of steroid structure to release of enzymes from granules of liver

A relationship between the structure of steroids and their capacity to release enzymes from lysosomes of rabbit tissue may be seen in Table 1. Most steroids of 5-β-H configuration, such as etiocholanolone, pregnanedione, pregnanolone, and lithocholic acid, released β-glucuronidase and acid phosphatase from liver granules at 37° and 20°. Progesterone was also active in this system. Metabolic precursors of etiocholanolone, such as DHA, testosterone, androstenedione, or 11-desoxycortisol, released less enzyme activity from the granules at 37° than did etiocholanolone. Most

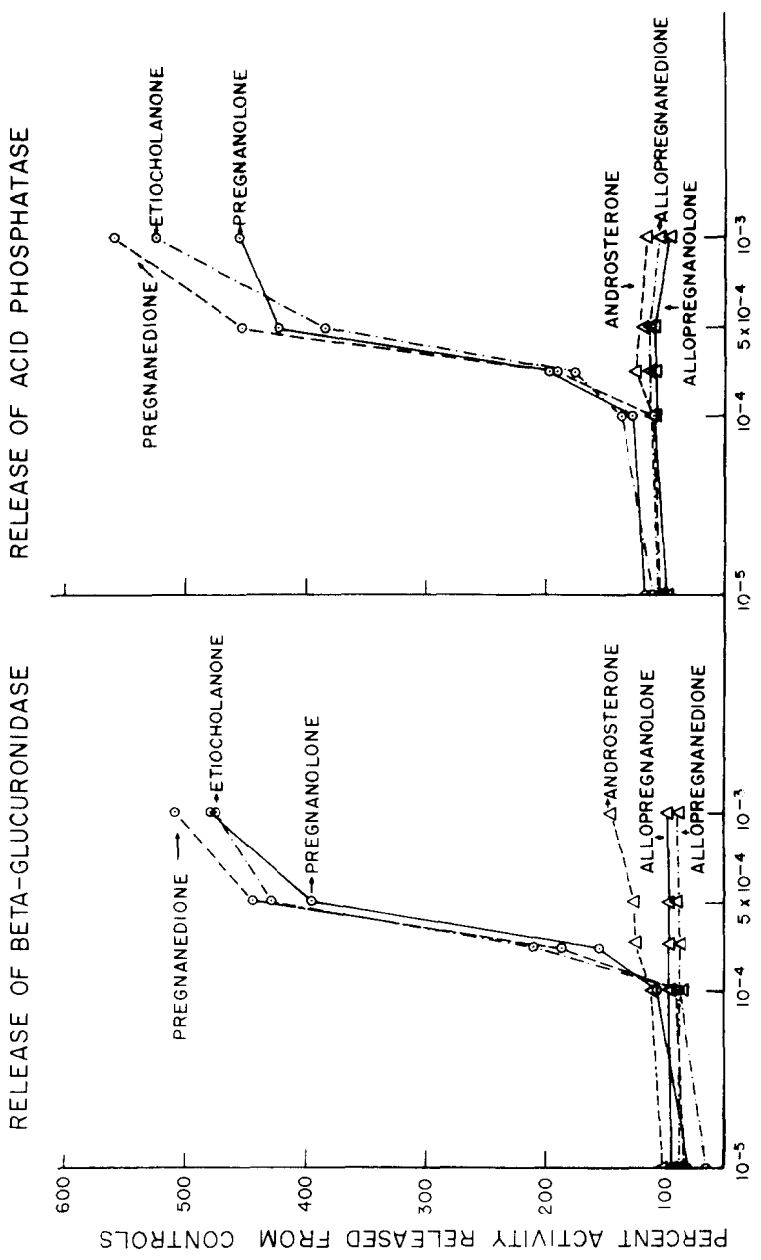

FIG. 1. Release of acid hydrolases from large granule fractions of rabbit liver in 0·25 M sucrose. Granules incubated for 60 min at 37° with concentrations of steroids indicated above, then centrifuged 20 min at 20,000 *g*. Determined activities of the supernatants are expressed as per cent released from control aliquots incubated with solvent alone (ethanol).

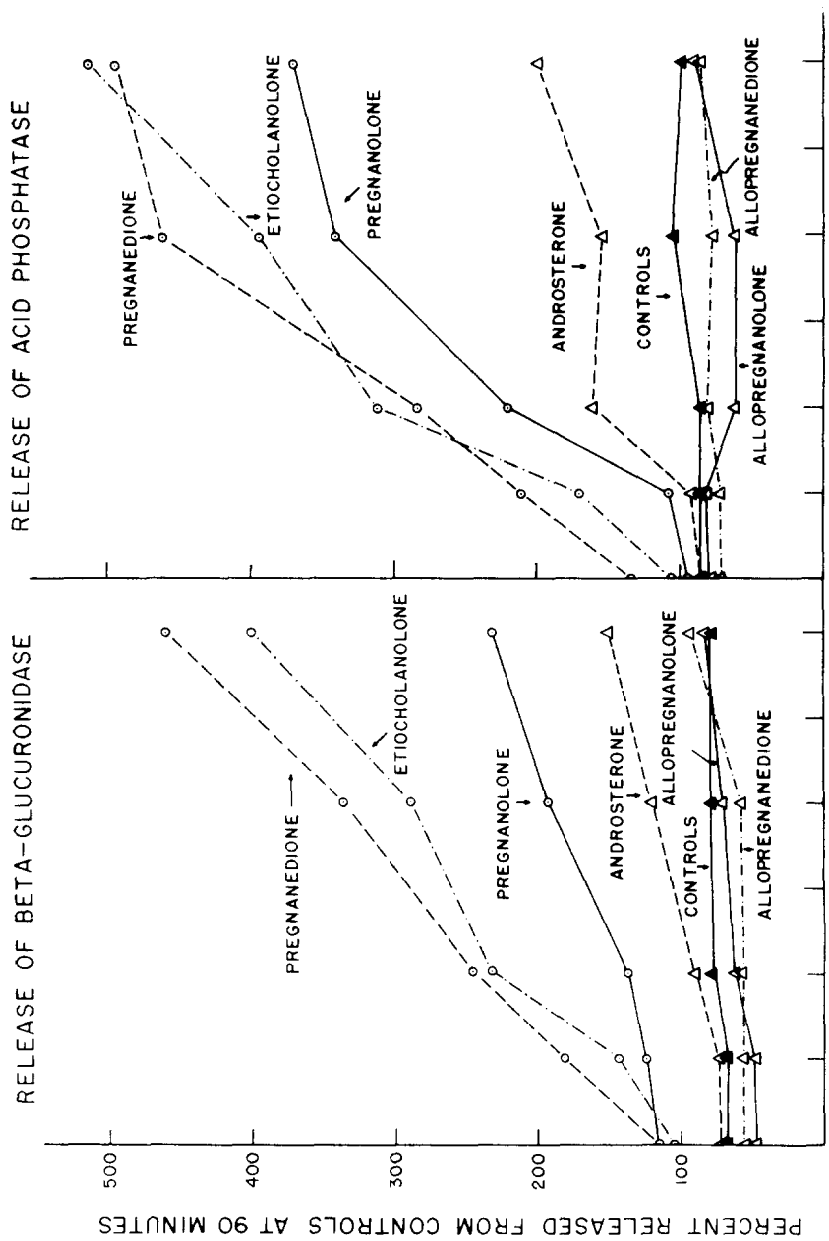

FIG. 2. Release of acid hydrolases from large granule fractions of rabbit liver in 0·25 M sucrose. Granules incubated for times indicated for Fig. 1 at 37° with all steroids at 5×10^{-4} M, then centrifuged for 20 min at 20,000 *g*. Determined activities of the supernatants are expressed as per cent released by control aliquots incubated with solvent alone (ethanol).

of their effects were lost at 20°, at which temperature formation of etiocholanolone from its precursors would be expected to proceed at slower rates.[13]

The effect of DHA acetate was remarkable: although at 37° its activity approached that of etiocholanolone, it was unable to release enzymes from the granules at 20°. At 37°, less enzyme was released from the granules by DHA acetate after 5–10 min incubation than by DHA (free alcohol). After 60-min incubation, however, enzyme activities released by DHA acetate approached those released by etiocholanolone,

TABLE 1. RELEASE OF ACID HYDROLASES FROM RABBIT LIVER GRANULES IN 0·25 M SUCROSE

Steroid	Melting point, uncorrected, °C	Liver, 37°			Liver, 20°		
		No. of expts.	β-Glucuronidase	Acid phosphatase	No. of expts.	β-Glucuronidase	Acid phosphatase
0. Controls		38	100[b]	100[c]	18	100[d]	100[e]
1. Pregnanedione[a]	119–121	9	562 ± 82[f]	611 ± 93[f]	4	283	248
2. Progesterone[a]	130–131	9	486 ± 87[f]	533 ± 98[f]	4	334	374
3. Etiocholanolone[a]	150–153	9	455 ± 52[f]	587 ± 63[f]	6	261 ± 48[g]	311 ± 53[g]
4. Pregnanolone[a]	143–144	9	445 ± 84[f]	579 ± 92[f]	4	306	302
5. DHA acetate	169–170	6	440 ± 49[f]	505 ± 51[f]	4	089	095
6. Epipregnanolone	142–143	4	364	400	4	258	284
7. Lithocholic acid[a]	184–187	4	360	480	4	206	286
8. 11-Ketopregnanolone[a]		4	266	320	2	150	161
9. Testosterone	152–153	6	260 ± 42[g]	224 ± 33[g]	4	170	124
10. Cholanic acid	165–166	3	242	316	2	200	226
11. Taurolithocholic acid		3	221	234	2	210	240
12. Androstenedione	172–173	3	198	191	2	108	088
13. DHA	149–150	6	164 ± 22	207 ± 26	2	109	118
14. 11-Desoxycortisol	209–210	4	155	157	4	129	111
15. DHA Sulfate	167–173	2	153	132	3	116	123
16. Androsterone	183	8	140 ± 23	123 ± 16	3	140	132
17. 11-β-Hydroxyetiocholanolone	237–239	4	140	132	4	120	109
18. Estradiol	175–176	3	130	208	3	110	117
19. Estrone	260–261	3	125	155	3	108	102
20. Estriol	281–283	3	111	153	3	106	101
21. Pregnanediol[a]	235–236	4	109	100	3	133	109
22. Allopregnanedione	201–203	6	104 ± 19	127 ± 20	3	100	084
23. Allocholanic acid		2	104	093	3	092	090
24. Allopregnanolone	194–195	3	101	102	3	108	104
25. Corticosterone	180–181	4	101	131	4	093	098
26. Cortisol	213–215	4	098	083	4	096	090
27. Cortisone	224	4	096	073	4	088	072
28. Cortisone acetate	238–240	4	084	075	4	075	080
29. Cortisol acetate	221–224	4	078	068	4	076	058

[a] Pyrogenic in man (Ref. 7 and Discussion).

[b] All enzyme activities are expressed as per cent, ± S.D., released from controls which released 14·2 ± 2·3% of "total" β-glucuronidase activity of liver granules at 37° (60 min).

[c] Controls released 12·0 ± 3·1% of total acid phosphatase activity of liver granules at 37° (60 min).

[d] Controls released 8·2 ± 1·6% of the total β-glucuronidase activity of liver granules at 20° (60 min).

[e] Controls released 8·9 ± 1·4% of the total acid phosphatase activity of liver granules at 20° (60 min).

[f] $P = < 0.01$ for paired samples versus controls.

[g] $P = < 0.05$.

whereas the hydrolytic activity solubilized by the free alcohol was considerably less. At 20°, the alcohol was more active than the acetate. Table 1 shows that these effects were not observed with DHA sulfate.

At both temperatures the presence of an –OH group at carbon-3 did not appreciably affect the release of hydrolases from granules, provided the 5-β-H configuration was maintained: epipregnanolone (3-β-OH) was almost as active as pregnanolone (3-α-OH). However, an oxygen function at carbon-11 decreased considerably the action of pregnanolone on the granules, and the presence of an 11-β-OH group resulted in the loss of activity upon the granules of etiocholanolone.

Release of hydrolases was not limited to C-19 or C-21 steroids. Three C-24 bile acids, derivates of cholesterol, liberated enzymes from liver and spleen fractions. Lithocholic acid was the most effective of these compounds. As with C-19 or C-21 steroids, a 5-α-H isomer, allocholanic acid, failed to liberate hydrolases from the granules. Nor were the unconjugated estrogens (as free alcohols) active on lysosomes. Pregnanediol proved a major exception to the general agreement between the 5-β-H configuration of a steroid and its effect upon lysosomal enzymes; this 5-β-H steroid was inactive, whether suspended in ethanol or dissolved in methanol or dioxane.

In the presence of the cortisol acetate and cortisone acetate, less acid hydrolase activity was released from granules than from controls.

Retardation of enzyme release by cortisol acetate, cortisone acetate, and chloroquine phosphate

Previous studies of the disruption of lysosomes have indicated that pretreatment of the granules with corticoids or with chloroquine diminished the subsequent disruptive effects of u.v. irradiation, vitamin A, streptolysin O, and lysolecithin.[1–4, 14] Therefore rabbit liver granules were preincubated with cortisone acetate, cortisol acetate, or

TABLE 2. EFFECT OF VARIOUS PRETREATMENTS ON SUBSEQUENT RELEASE OF ENZYMES FROM RABBIT LIVER GRANULES BY ETIOCHOLANOLONE OR PROGESTERONE

Pretreatment of granules (30 min, 37°)[a]	Subsequent exposure to: (60 min, 20°)[a]	Activity released[b] β-Glucuronidase	Acid phosphatase
Nil (control)	Nil	100 (16)	100 (16)
Chloroquine	Nil	081 ± 11 (6)	073 ± 14 (6)
Cortisol acetate	Nil	072 ± 13 (6)	081 ± 17 (6)
Cortisone acetate	Nil	074 ± 08 (8)	062 ± 13 (8)
Nil	Progesterone	372 ± 41 (8)	389 ± 38 (8)
Cortisol acetate	Progesterone	178 ± 22 (6)[c]	204 ± 30 (6)[c]
Cortisone acetate	Progesterone	192 ± 28 (6)[c]	223 ± 41 (6)[c]
Chloroquine	Progesterone	174 ± 32 (6)[c]	168 ± 43 (6)[c]
Nil	Etiocholanolone	284 ± 38 (8)	302 ± 42 (8)
Cortisol acetate	Etiocholanolone	198 ± 21 (6)[c]	207 ± 19 (6)[c]
Cortisone acetate	Etiocholanolone	184 ± 26 (6)[c]	214 ± 24 (6)[c]
Chloroquine	Etiocholanolone	182 ± 28 (6)[c]	160 ± 38 (6)[c]

[a] Large granule fractions of rabbit liver preincubated with steroids or chloroquine ($2{\cdot}5 \times 10^{-4}$ M), subsequently exposed to steroids (5×10^{-4} M).

[b] Enzyme activities released are expressed as per cent released from controls (10·3 ± 2·1 of total for β-glucuronidase, 11·3 ± 2·8 for acid phosphatase). Number of experiments in parentheses.

[c] $P < 0{\cdot}01$ for matched samples (Wilcoxon test) vs. non-preincubated samples with progesterone or etiocholanolone alone.

chloroquine phosphate (each at $2{\cdot}5 \times 10^{-4}$ M) for 30 min at 37°. Subsequently the granules were exposed to disruptive concentrations of etiocholanolone or progesterone for 60 min at 20°. Data listed in Table 2 demonstrate that this form of preincubation retarded the release of enzymes from the granules by etiocholanolone or progesterone. No retardation of enzyme release was observed when the granules were pretreated with a relatively inert steroid, allopregnanolone. When compared with control suspension, cortisol acetate-, cortisone acetate-, and chloroquine phosphate-treated suspensions released less enzyme activity into the suspending fluid.

The effects of steroids on the specific granules of leukocytes

Intact granules were isolated from rabbit leukocytes in suspensions relatively uncontaminated[9, 10] by other cytoplasmic organelles. Dilute suspensions of the granules were studied in 0·3 M sucrose; agents that disrupt the granules irreversibly decrease the turbidity of such suspensions and liberate β-glucuronidase into the surrounding medium.[10] When leukocyte granules were incubated with several 5-β-H steroids or their 5-α-H isomers at 20°, no change in turbidity was observed, nor was β-glucuronidase solubilized. However, at 37°, both progesterone and etiocholanolone reduced the turbidity of leukocyte granule suspensions (Fig. 3). Changes in apparent absorbance induced by etiocholanolone or progesterone were comparable to those induced by

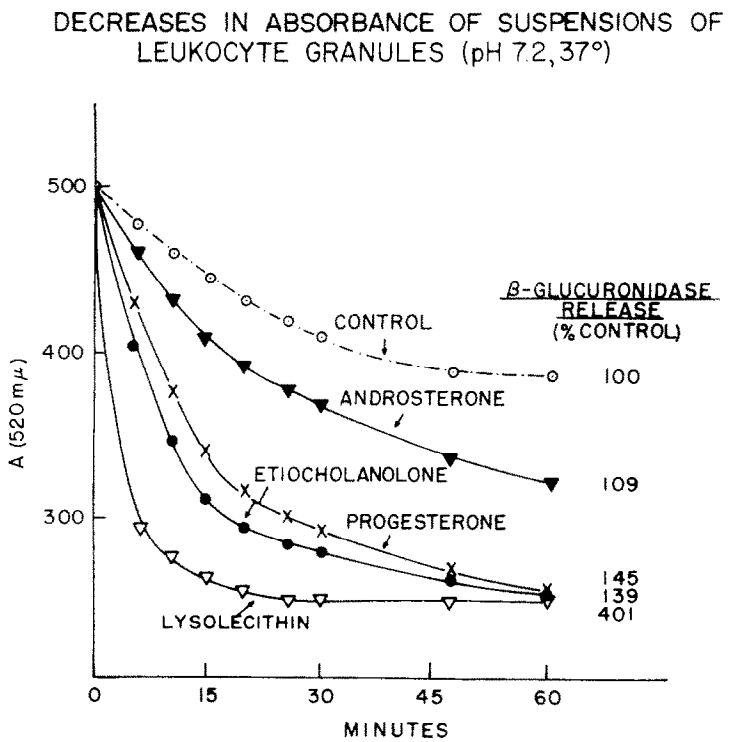

FIG. 3. Turbidity of leukocyte granule suspensions in 0·3 M sucrose, 0·02 M Tris buffer. Steroids, in ethanol, added at $2{\cdot}5 \times 10^{-4}$ M. Figures on the right represent β-glucuronidase liberated from the granules after centrifugation at 20,000 *g* for 20 min, and expressed as per cent released from control samples incubated with ethanol alone. All samples at 37°.

lysolecithin (10^{-4} M), an agent previously shown capable of disrupting leukocyte granules.[10] However, in contrast to granules exposed to lysolecithin, from which over 98 % of the total content of β-glucuronidase was released, granules treated with etiocholanolone released only 25–30 % more enzyme activity than did controls. Phase-contrast microscopy revealed that, whereas lysolecithin-treated granules were totally disrupted, those exposed to the active steroids remained largely intact. The 5-α-H steroid, androsterone, was as inactive on these granules as on those prepared from liver.

DISCUSSION

The data above demonstrate that several neutral steroids and bile acids release acid hydrolases from granular fractions of rabbit liver and leukocytes. Structural requirements for optimum activity are shared by C-19, C-21, and C-24 steroids of 5-β-H configuration, the 11-carbon of which is unoxygenated. An oxygen function at carbon 11 decreased considerably the disruptive effect of the steroid. An exception to these generalizations was pregnanediol, which was inactive either as the 5-α-H isomer or as the 5-β-H compound. It is quite unclear why DHA acetate should be more effective than DHA alcohol in releasing enzymes from lysosomes. It is possible that the acetate is metabolized to etiocholanolone more effectively than is the alcohol; this possibility is supported by the observation that the acetate lost its activity upon the granules at 20°. It is also possible that the solubility properties of the acetate among lipoprotein-bound particles in an ethanol:sucrose medium permit a more direct action of DHA acetate upon the lysosomal membrane. No evidence is available to support this possibility.

These studies, together with the observations that steroids affect the permeability of mitochondria[15] and of red cells,[16] indicate that at least one pharmacologic property shared by these steroids, of diverse biologic origin and fate, is a direct action upon membranes. This view supports the earlier studies of de Duve *et al.*,[6] who observed that progesterone and testosterone rendered rat liver lysosomes more permeable to β-glycerophosphate, a substrate for acid phosphatase. Scheib[17] confirmed these observations on sucrose homogenates of undifferentiated Müllerian ducts, finding that the tissue responded equally well to testosterone or progesterone. Leonard and Rustein[18] had shown that progesterone and DOC (a steroid also active on lysosomes[6]) affected the membranes of marine annelid eggs. Blecher and White[19] studied the swelling of mitochondria and activation of ATP-ase by progesterone, DOC, and testosterone derivatives; and Selye[20] had previously discovered the anesthetic effects not only of progesterone, DOC, and testosterone, but of many of the 5-β-H steroids active on lysosomes and on erythrocytes.

In short, considerable evidence is available to support the hypothesis of Willmer[21] and of White *et al.*[22] that steroids, at pharmacologic concentrations, affect the membranes of cells and their organelles. It is therefore probable that the action upon lysosomes of 5-β-H steroids and of progesterone is but a particular example of their general effect upon membranes.

Many of the steroids active on lysosomes share the property of inducing fever in man (Table 1) but not in lower animals such as the rabbit.[7] Only progesterone is a pyrogen in several species.[7] But, although lysosomes have been implicated in the fever provoked by exogenous pyrogens,[23] it is *not* possible, at present, to frame a consistent hypothesis relating the action of steroids upon lysosomes *in vitro* to their

pyrogenicity in man. It has not, for example, been demonstrated that any of the 5-β-H steroids releases enzymes from lysosomes in the living rabbit. Earlier studies[2, 23] have shown that two exogenous pyrogens, endotoxin and 2,4-dinitrophenol, are both active on lysosomes *in vivo* but not *in vitro*. However, although endotoxin fever is regularly accompanied by the appearance of endogenous pyrogen in the circulation,[24] 2,4-dinitrophenol does not appear to induce the formation of endogenous pyrogen,[25] nor has this substance been detected in the circulation of man after the injection of fever-producing steroids.[26] Thus, although it would be attractive to postulate that endogenous pyrogens are sequestered within, or result from the disruption of lysosomes, no direct evidence for this possibility exists as yet. Studies of Berlin and Wood[27] have shown that endogenous pyrogen is elaborated by intact leukocytes during phagocytosis. Since phagocytosis is accompanied by discharge of lysosomal contents into phagocytic vacuoles, it is possible that pyrogens are produced by this interaction. No data, however, are available on the subcellular distribution of endogenous pyrogen. We have observed above that etiocholanolone may induce marked physical changes in leukocyte lysosomes (decreases in absorbancy of granule suspensions) accompanied by only modest changes in the permeability of the granules to larger molecules such as β-glucuronidase. This may be a mechanism whereby granules are rendered more permeable to smaller molecules, such as endogenous pyrogen, without being completely disrupted. Recent studies of Atkins and Snell[28] have shown that endogenous pyrogens need not derive from leukocytes alone; suitable preparations of other tissues also provoked fever in animals. It remains possible that etiocholanolone might selectively release pyrogen in brain tissue or, alternatively, that the steroid might act on the lysosome-like organelles of the hypothalamus directly.[29]

The protection by cortisone acetate, cortisol acetate, and chloroquine of isolated lysosomes against rupture by etiocholanolone or progesterone is in keeping with earlier observations of the stabilization of lysosomes by the three anti-inflammatory compounds.[1–4, 14] Thus the antagonism between etiocholanolone and cortisol *in vivo*[8] has been demonstrated in the model *in vitro* system described above.

The concentration of steroids employed in these studies is above physiologic, and probably above pharmacologic, levels obtained *in vivo*. In view of the sparing solubility of the steroids in suspension, however, it is impossible to be certain of the concentration of steroid available at the surface of the organelles. Our recent studies employing dimethylsulfoxide as a solvent have indicated that the "protective" effects of cortisone and cortisol may be demonstrated at concentrations as low as 5×10^{-6} M. It remains to be seen whether procedures which serve to make steroid more available to the membranes of organelles will enable both lytic and protective effects to be demonstrated at more physiologic concentrations.

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