Correlation Between Chemical Constitution and Capillary Activity of Adrenalocortical Hormones.* (22452)

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It has been shown recently that a close relationship exists between the resistance of capillaries (as measured by negative pressure method) and certain steroids of the adrenal cortex. Cortisone increased capillary resistance of human beings(1) and in experimental animals(2) whereas findings with desoxycorticosterone were contradictory(2,3). In the present paper experiments are described in which a large number of steroid hormones, both naturally occurring and artificially produced, were systematically investigated for their potential action on capillary resistance and for possible correlation between chemical structure and capillary activity. Some importance is lent to such correlation by the fact that the anti-inflammatory (anti-rheumatic) effect of steroids was found to be dependent upon particular features in chemical structure(4) and by the possibility that anti-inflammatory effect and capillary activity may be closely related properties.

Method and procedure. Twenty-four mature female adrenalectomized albino rats of Sprague-Dawley strain weighing 220-300 g were used. Total adrenalectomy was performed at least 2 months prior to the experiments. The animals were fed Purina Checkers enriched with raw horse meat. One percent NaCl served for drinking water. Great care and much time was given to train these animals. They were not used until they became real pets and were perfectly accustomed to lying on their backs in the palm of the investigator with virtually no restraint, so that the testing procedure did not afford any emotional reaction. This treatment is indispensable because untrained animals are likely to develop changes in capillary resistance (emotional stress response), which makes testing and proper evaluation of results difficult if not impossible(5). Even though protracted changes in capillary resistance (late capillary stress response) do not occur after adrenalectomy, fluctuations of a few hours duration (immediate capillary stress response) may be frequently encountered(6). In addition, untrained adrenalectomized animals often show the phenomenon of emotional capillary spasm which may easily lead to erroneous readings (5). Capillary resistance was tested on the abdominal skin with the negative pressure method. The apparatus and details of procedure were described in previous papers(2, 7). The least negative pressure applied for 60 seconds and capable of eliciting one or more petechiae was considered the value for capillary resistance. In this manner a control base line of daily values was established, whereupon animals received, in the musculature of the thigh, daily injections of steroid to be tested for 3 days, after which period capillary resistance was again determined. The same animals were used for testing several substances, but were given at least 2 weeks of respite between test periods. Each substance was tested at least in 2 animals. The average daily dose was 1 mg/100 g body weight in a fine physiological saline suspension prepared by trituration and shaking. Volume of injected material varied from 0.1 - 0.3 ml. If no change in capillary resistance was noted, daily doses were increased by 50%, and in case of no response, by 100%. If test remained negative even with this final dose, the compound was considered ineffective. When a compound, in 1 mg/100 g dose, caused a marked change in capillary resistance, the tests were repeated with diminishing doses until the least effective dose was determined. Thirty-six different steroids were studied in this manner.

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TABLE I. Capillary-Active Steroids.

	Least effective dose, mg/100 g body wt
Cortisone acetate	.250
Hydrocortisone acetate	.070
Δ^1-cortisone	.020
Δ^1-hydrocortisone	.050
Fluorohydrocortisone acetate	.025

Results. Control values of capillary resistance of test rats were found to fluctuate between 4 and 12 cm Hg, mean 7.5 cm. Five of the 36 steroids increased capillary resistance by 50-60 cm Hg when injected in initial dose of 1 mg/100 g body weight for 3 consecutive days. These effective steroids are: cortisone acetate (11-dehydro-17-hydroxycorticosterone acetate), hydrocortisone acetate (17-hydroxycorticosterone), Δ^1-cortisone, Δ^1-hydrocortisone and fluorohydrocortisone acetate (9-α-fluoro-17-hydroxycorticosterone acetate). When tests were repeated with gradually decreasing doses the following results were obtained (Table I).

Comparing the least doses which proved effective we may state that hydrocortisone was more potent than cortisone, whereas Δ^1-cortisone, Δ^1-hydrocortisone and fluorohydrocortisone were more potent than hydrocortisone.

Thirty-one steroids (Table II), of the 36 investigated, were ineffective when injected in initial dose of 1 mg/100 g, as well as when

TABLE II. Capillary-Inactive Steroids.

Steroid compounds	Ketone group at C_3	Double bond between C_4 & C_5	Ketone group at C_{20}	Hydroxyl group at C_{21}	Stable orientation at C_{17}	Ketone or hydroxyl group at C_{11}	α-Hydroxyl group at C_{17}
Corticosterone (Compound B)							x
11-Dehydrocorticosterone							x
11-Desoxy-17-hydroxycorticosterone						x	
Dihydrocortisone acetate		x					
17-Hydroxyprogesterone						x	
21-Desoxycortisone				x			
4-Pregnentriolone diacetate						x	
Desoxycorticosterone acetate						x	x
Pregnane-3α,17α,21-triol-11,20-dione-21-acetate	x	x					
Pregnane-17α,21-diol-3,20-dione-21-acetate	x	x					
21-Acetoxyallopregnene-3,20-dione		x				x	x
Progesterone				x		x	x
17-Hydroxypregnenolone	x	x		x			
21-Acetoxypregnenolone acetate	x	x				x	x
Androstenedione			x	x		x	x
Allopregnane-3,20-dione		x		x		x	x
Δ^4-Transandrostene-3,17-diol	x		x	x		x	
16,17-Oxydopregnenolone acetate	x	x		x		x	x
Δ^5-Pregnene-3β,20β-diol-3,20-diacetate	x	x	x	x			x
Δ^5-Pregnene-3β,20α-diol-3,20-diacetate	x	x	x	x			x
$\Delta^{5,16}$-Pregnadiene-3β-ol-20-one	x	x		x		x	x
α-Estradiol	x	x	x	x			x
16,17-Epoxypregnenolone acetate	x	x		x		x	x
Equilenine	x	x	x	x			x
16-Dehydropregnenolone	x	x		x		x	x
Etiocholane-3α,11β-diol-17-one	x	x	x	x		x	x
Estriol	x	x	x	x		x	x
Estrone	x	x	x	x		x	x
Androsterone	x	x	x	x		x	x
Dehydroisoandrosterone acetate	x	x	x	x		x	x

x indicates absence of structural features essential for capillary activity.

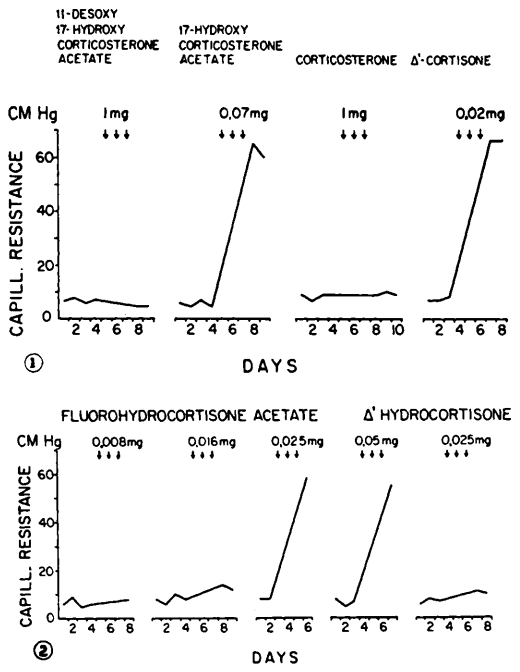


FIG. 1 and 2. Representative experiments showing tests for capillary activity of various corticoids. Individual tests are separated by at least 2 wk intervals. Arrows indicate intramuscular injections of the compounds, doses are given/100 g body wt.

dose was increased by 50% and later by 100%. Two figures serve for visualization of the testing procedure.

Comment. The chemical structure of the *5 steroids found to be "capillary active"* (ability to increase capillary resistance) has the following features in common: a ketone group at C_3; a double bond between C_4 and C_5; a ketone group at C_{20}; a hydroxyl group at C_{21}; a ketone or hydroxyl group at C_{11}; an α-hydroxyl group at C_{17}; stable orientation of the side chain at C_{17}.

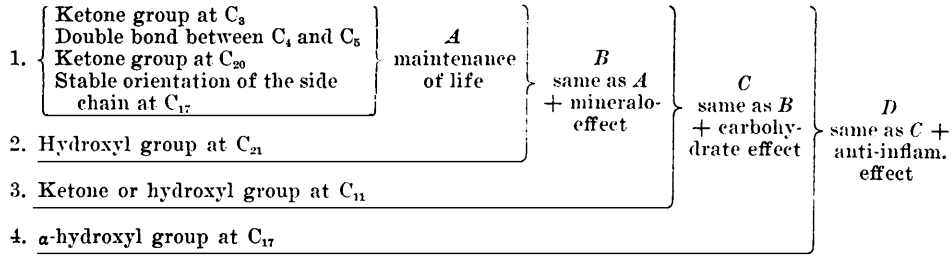
The *31 steroids found to be capillary inactive* are similar in one respect: at least one of the above 7 prerequisites is missing in their chemical structure (Table II).

To evaluate these findings, it is necessary to compare the already known relations between chemical constitution and biologic activity of corticosteroids. This relationship may be visualized in scheme shown at bottom of this page.

Items in 1 are necessary for maintenance of life. Items 1 and 2 are required for mineralo-effect beside maintenance of life. When items 1, 2 and 3 are present in the molecule there is an additional carbohydrate effect. For anti-inflammatory activity all items, 1, 2, 3 and 4 must be present in the molecule.

A comparison of chemical structural prerequisites, found in this study to be essential for capillary activity with those required for other types of cortical activity, indicates that they are identical with the prerequisites of the *anti-inflammatory effect* (*D* in the scheme). Our findings therefore suggest that the *capillary activity of corticoids, as measured by capillary resistance test, and their anti-inflammatory activity are closely related physiological properties.* The same relation is borne out by the comparative study of the 5 capillary-active steroids as to their least effective dose. We found that any alteration in the cortisone molecule which improves the anti-inflammatory activity also augments the capillary activity as measured by an increase in capillary resistance.

The present study suggests that capillary resistance test on the adrenalectomized rat may find a new practical application. From the clinical point of view the anti-inflammatory activity is one of the most important properties of a corticosteroid. Several meth-



ods have been devised to demonstrate and measure this activity. The described procedure is proposed as a new, simple, and convenient method for screening new compounds, either artificially prepared or later to be isolated from the amorphous fraction of the adrenocortical extract.

Summary. 1. To be able to increase capillary resistance a steroid must possess the same chemical structural prerequisites as required for anti-inflammatory properties. 2. Alterations in cortisone molecule which potentiate its anti-inflammatory activity also increase capillary activity. 3. It seems that capillary activity, as measured by the capillary resistance test, is intimately related with the mechanism involved in the anti-inflammatory effect. 4. Capillary resistance test on adrenalectomized rat is proposed as a new bio-assay method for the demonstration of the anti-inflammatory activity of corticoids.

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Effects of Lysergic Acid Diethylamide upon Performance of Trained Rats. (22453)

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Although lysergic acid diethylamide (LSD) is known to produce symptoms in man resembling those of certain psychotic disorders, a quantitative measure of the effects of this drug in animals has been lacking. Woolley(1) has described a syndrome observed in mice injected with LSD, and Shore *et al.*(2,3) have shown that LSD antagonizes the potentiating effects of reserpine and serotonin upon hypnotic agents. In the present paper, we demonstrate that the effects of LSD upon trained rats bear a linear relationship to the log dose of the drug, and that this phenomenon can be used in the study of LSD-antagonists.

Methods. Holtzman male rats of 100 to 225 g were used. Preliminary evidence indicates that animals outside this range may not be suitable, but this has not been established with certainty. Animals, fasted for 24 hours or longer, were trained to climb a vertical rope of about 172 cm as described previously(4). Incentive for climbing was food cup placed on platform at top of the rope; in addition, there was an electrified wire grid at the bottom. Also at bottom of rope was a small "tweeter" type of loud speaker which emitted brief bursts of noise at intervals of about a half-second. Untreated animals needed neither grid nor noise, but these facilitated climbing in rats affected by drugs, and decreased variability of performance. Observations were made of behavior of animals while in their cages as well as while negotiating the climb. Time required for the climb was obtained to the nearest 0.1 second. Three control times were obtained before administration of drug, and the average value was used as base line for drug effect, after it was established that in saline-injected animals climbing time remained constant for 5 hours. Evaluation of effects of drugs on climbing per-