17103. Some Chemical and Morphological Changes Elicited in the Adrenal by Stilbestrol and LAP.

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Preliminary observations suggested that a lyophilized anterior pituitary preparation (LAP), when administered simultaneously with stilbestrol, inhibits the discharge of adrenal sudanophilic material which is normally a characteristic effect of this synthetic estrogen. Hence the following experiment was planned to study in a more comprehensive manner the chemical and morphological changes produced in the adrenal by LAP, stilbestrol and combined treatment with both these substances.

Materials and methods. 48 female piebald rats averaging 140 g in weight were divided into 4 groups of 12 rats each and treated as follows: Group I served as normal controls. Group II received 20 mg of LAP in 0.2 cc of saline twice daily subcutaneously. Group III was given 1 mg of stilbestrol dissolved in 0.2 cc of corn oil once daily subcutaneously. In addition this group received a saline injection on the 9th day (to equalize the injection-trauma with Group IV). Group IV was given both LAP and stilbestrol in the same manner as Groups II and III respectively. At the end of 10 days the rats were killed by ether anesthesia. The adrenal glands from 6 rats of each group were immediately removed and weighed separately to 1 mg of accuracy. One gland of each rat was then placed into 4% trichloracetic acid for ascorbic acid determination (method of Roe and Kuether[1]) and the other into acetone-alcohol for cholesterol estimation (method of Sperry and Schoenheimer, modification of Sperry[2]).

The adrenals of the other 6 rats of each group were used for histological study as follows: one was fixed in 10% formalin for 48 hours after which frozen sections were made at 10 μ. These were stained for lipids with Sudan IV and for "Plasmalogens" with the Schiff reagent (method of Feulgen and Voigt as described by Lison[3]). The other adrenal and thymus were fixed in Heidenhain's Suza fixative for H and E paraffin sections cut at 6 μ.

Results. A summary of our results is given in Table I which includes a statistical comparison of the values obtained.

Weight changes. As thymus and adrenal weight changes are inversely related to each other, they can be considered together. In all experimental groups there was adrenal enlargement and thymus involution. These changes were most pronounced in Group IV and least marked in Group II.

Chemical changes. The cholesterol and ascorbic acid values are expressed in mg/100 mg of adrenal tissue (concentration) and in mg/100 g of body weight (total content).

After LAP treatment (Group II) there is a decrease in the concentration and total content of adrenal *cholesterol;* only the decrease in concentration being significant. Stilbestrol (Group III) causes a very marked and significant decrease in both concentration and content of adrenal cholesterol. After LAP and stilbestrol (Group IV) these values were still lower, but not significantly different from the former group (Group III).

In a progressive order, LAP (Group II), stilbestrol alone (Group III) and in conjunction with LAP (Group IV) significantly decreased the adrenal *ascorbic acid* concentration. It is of interest that while the changes in the total content and concentration of cholesterol run parallel, no such parallelism is evident as regards ascorbic acid.[4]

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1 Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1943, **147**, 399.

2 Sperry, W. M., *Am. J. Clin. Path.*, 1938, **2**, 91.

3 Lison, L., Histochimie Animale, Gauthier-Villars, Paris, 1936.

4 Ludewig, S., and Chanutin, A., *Endocrinology*, 1947, **41**, 135.

TABLE I.
Effect of LAP* and Stilbestrol on Thymus and Adrenal Weight and Adrenal Cholesterol and Ascorbic Acid.

Group	Treatment	Thymus weight: Mg/100 g body	Thymus weight: P†	Adrenal weight: Mg/100 g body	Adrenal weight: P
I	0	116 ± 22		32 ± 2.5	
II	LAP	47 ± 6.5	II-I <.01	36 ± 0.5	II-I <.01
III	Stilbestrol	56 ± 4.8	III-I <.02	79 ± 6.6	III-I <.01
IV	Stilbestrol + LAP	21.5 ± 1.5	IV-I <.01 IV-II <.01 IV-III <.01	85.9 ± 14.5	IV-I <.01 IV-II <.01 IV-III >.1

Group	Treatment	Adrenal cholesterol: Mg/100 g body	P	Mg/100 mg adrenal tissue	P
I	0	.623 ± .088		2.674 ± .168	
II	LAP	.375 ± .076	II-I >.1‡	1.047 ± .231	II-I <.01
III	Stilbestrol	.300 ± .058	III-I <.02	.385 ± .087	III-I <.01
IV	Stilbestrol + LAP	.186 ± .021	IV-I <.01 IV-II <.05 IV-III <.1‡	.227 ± 0.15	IV-I <.01 IV-II <.01 IV-III >.1

Group	Treatment	Adrenal ascorbic acid: Mg/100 g body	P	Mg/100 mg adrenal tissue	P
I	0	.105 ± .014		.442 ± .050	
II	LAP	.117 ± .008	II-I >.1‡	.322 ± .020	II-I <.05
III	Stilbestrol	.168 ± .027	III-I <.1‡	.222 ± .050	III-I <.02
IV	Stilbestrol + LAP	.097 ± .014	IV-I >.1‡ IV-II >.1‡ IV-III <.05	.122 ± .021	IV-I <.01 IV-II <.01 IV-III >.1‡

* Lyophilized Anterior Pituitary.
† Probability of a chance occurrence of the difference between the means, from Fisher's table of T values.
‡ Difference not statistically significant.

Histology. As the results of Sudan and Schiff stains were in the same direction, only the former will be discussed.

The sudanophilia of the *adrenal* cortex was greatly increased by LAP. In contrast stilbestrol caused a marked discharge of sudanophilic granules. Curiously, combined stilbestrol and LAP treatment actually increased the cortical lipid content above normal, with a maximal concentration in the outer fasciculata.

Examination of H. and E. sections revealed a predominant hyperplasia of the adrenals with slight hyperemia in the group treated with LAP. The stilbestrol and especially the LAP and stilbestrol treated rats evidenced a degree of hyperplasia which was completely overshadowed by a marked congestion and edematous imbibition resulting in severe disruption of the glandular structure.

In the *thymus* there was nuclear pyknosis which appeared to parallel the degree of atrophy.

Discussion. Hypertrophy,[5,6] loss of sudanophilic material[7–9] as well as depletion of cholesterol[10–12] and ascorbic acid[13–15] followed by

5 Selye, H., *Brit. J. Exp. Pathol.*, 1936, **17**, 234.
6 Tepperman, J., Engel, F. L., and Long, C. N. H., *Endocrinology*, 1943, **32**, 373.
7 Dalton, A. J., Dosne, C., and Selye, H., *Anat. Rec.*, 1940, *Suppl.* **76**, 85.
8 Zwemer, R. L., *Am. J. Pathol.*, 1936, **12**, 107.
9 Darrow, D. C., and Sarason, E. L., *J. Clin. Invest.*, 1944, **23**, 11.
10 Popjack, G., *J. Path. and Bact.*, 1944, **56**, 485.
11 Levin, L., *Endocrinology*, 1945, **37**, 34.
12 Long, C. N. H., *Rec. Progr. in Horm. Res. Proc. Laur. Horm. Conf.*, 1947, **1**, 99.

a return of these substances to or above normal levels, have been repeatedly described as characteristic of the adrenal response to stress. The alterations in cholesterol, ascorbic acid and stainable lipids have been considered by Sayers and Sayers[16] to closely parallel each other. Indeed the observation by these workers of a simultaneous decrease in cholesterol and sudanophilic material has prompted them to identify the latter with cholesterol esters. The present study shows that it is possible to dissociate the sudanophilia from the cholesterol content of the adrenal cortex. LAP, either alone or in conjunction with stilbestrol, decreases the cholesterol but increases the sudanophilia of the adrenal. This suggests that the sudanophilic substance of the cortex is probably not cholesterol. Earlier experiments[17] have demonstrated that, under certain conditions, purified ACTH can cause a similar dissociation. Finally it appears from the above data that LAP, given with stilbestrol, markedly inhibits that loss of sudanophilic granules from the cortex which is characteristic of stilbestrol treatment.

[13] Torrance, C. C., *J. Biol. Chem.*, 1940, **132**, 575.

[14] Harkins, H. N., and Long, C. N. H., *Am. J. Physiol.*, 1945, **144**, 661.

[15] Sayers, G., and Sayers, M. A., Tsan-Ying Liang, and Long, C. N. H., *Endocrinology*, 1945, **37**, 96.

[16] Sayers, G., and Sayers, M. A., *Rec. Progr. in Horm. Res., Proc. Laur. Horm. Conf.*, 1948, **2**, 81.

[17] Constantinides, P., Fortier, C., Skelton, F. R., Timiras, P., and Selye, H., in press.

Further experiments are under way to study the effects of purified corticotrophic preparations on the adrenal changes elicited by nonspecific stress.

Summary. The adrenal cholesterol and ascorbic acid levels were studied in relation to the stainable lipids of the gland in rats treated with a lyophilized anterior pituitary preparation (LAP), given alone or in conjunction with a synthetic folliculoid (stilbestrol):

1. In stilbestrol treated rats, an almost total depletion of stainable lipids was observed in conjunction with low cholesterol and ascorbic acid values.

2. LAP caused accumulation of sudanophilic lipids in the adrenal cortex but significantly lowered the cholesterol and ascorbic acid levels.

3. A similar dissociation of sudanophilic material from cholesterol and ascorbic acid, was even more evident when LAP was administered concurrently with stilbestrol. The effect of stilbestrol on sudanophilia was counteracted by LAP.

4. These observations show that the corticotrophic effect of folliculoids cannot be solely ascribed to the liberation of anterior pituitary corticotrophin such as is present in LAP.

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17104. Effect of Various Drugs on Epinephrine-Induced Pulmonary Edema in Rabbits.*

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Large doses of epinephrine are known to cause a fulminating pulmonary edema in a number of species of animals.[1-3] Various

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[1] Meltzer, S. J., *Am. Med.*, 1904, **8**, 191.

[2] Emerson, H., *Arch. Int. Med.*, 1909, **3**, 368.

[3] Bovet, D., and Bovet-Nitti, F., *Structure et activité pharmacodynamique des médicaments du système nerveux végétatif*, S. Karger, Bale, 1948, p. 23.