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THE ACTION OF GONADOTROPHIC HORMONE AND OF PITUITARY CORTICOTROPHIC HORMONE ON THE CHOLESTEROL CONTENT OF THE ADRENALS

By R. A. CARREYETT, Y. M. L. GOLLA and M. REISS
From the Endocrinological Department, Burden Neurological Institute, Bristol

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It was found, when standardizing gonadotrophic hormone from pregnant-mare serum, that injections of amounts of this hormone sufficient to produce oestrus in young hypophysectomized rats were attended by a considerable mortality. However, none of the experimental animals died if the injected extracts of gonadotrophic hormone had been previously inactivated by heating. The symptoms following lethal injections of the gonadotrophic hormone resembled in many respects those exhibited by rats dying after adrenalectomy. Principal among such symptoms were the anorexia, the stupor with intervening convulsive attacks and the respiratory failure. It was, therefore, decided to study the effect of the gonadotrophic hormone of pregnant-mare serum on the adrenal cortex and particularly on the concentration of cholesterol and of sudanophilic substances in the cortex. The effects of injections of corticotrophic hormone of the anterior pituitary on the cholesterol content of this organ was also investigated, since it is known that this hormone influences the sudanophilic reaction of the adrenal cortex (Reiss, Balint, Oestreicher & Aronson, 1936).

Methods

Male Wistar rats weighing between 30 and 50 g. were used. All injections were made intraperitoneally. Control animals received the same volume of physiological salt solution. The hypophysectomy was made by parapharyngeal approach (Reiss, Druckrey & Hochwald's (1933) modification of Smith's (1930) method) in rats weighing 100–130 g.

The cholesterol content of the adrenals was determined by Bloor's (1922, 1926) method. Since this method was unlikely to yield sufficiently exact figures when applied to the extraction of a single pair of adrenals, it was decided to use the glands of groups of animals treated in the same way. The pooled glands, after careful dissection, were weighed on a torsion balance, ground and extracted with Bloor's mixture. The groups of rats were formed by evenly distributing litters. It will be seen from Table 1 that the cholesterol content of pooled adrenals of groups formed in this manner remains comparable if each group of animals, having received control injections of saline, is killed on different days.

Histological methods. Frozen sections were stained with haematoxylin and Sudan III.

Hormone preparations used. (1) Gonadotrophic preparation: pregnant-mare serum was purified by fractional precipitation with acetone; the crude preparation was re-extracted with 50%

TABLE 1. Cholesterol content of pooled adrenals of control rats, determined on different days after the formation of groups

Days after formation of groups	No. of animals	Cholesterol content of pooled adrenals in mg./100 g. adrenal tissue
1	10	2280
2	10	2320
3	6	2210
4	8	2430
6	10	2370
8	10	2420

alcohol and reprecipitated by increasing the alcohol concentration to 80%. The different preparations used contained 250–300 i.u. gonadotrophic hormone.

(2) Corticotrophic preparation: the crude pituitary extract was prepared by picric acid precipitation of a 0·25% acetic acid extract. This extract was purified by salting out at half-saturation with ammonium sulphate, and finally by isoelectric precipitation at *p*H 4·7. 1 mg. of the preparation used contained between 5 to 20 sudanophobic units (see Reiss *et al.* 1936; Simpson, Evans & Li, 1943).

RESULTS

It will be seen from Table 2 that the cholesterol content of the adrenals of rats killed 24 hr. after administration of gonadotrophic hormone, showed a significant increase when compared with the cholesterol content of the adrenals of a group of control animals. However, in the adrenals of rats injected for

TABLE 2. Cholesterol content of adrenals of male rats treated with gonadotrophic hormone (of pregnant-mare serum)

No. of rats in group	Total amount of gonadotrophic hormone injected (i.u.)	Duration of treatment	No. of injections administered each day	Total cholesterol content in mg./100 g. of the pooled adrenal tissue	Percentage change in cholesterol content
10 untreated controls		—	—	2260	—
6	40	6 hr.	1	3175	+80
6	40	12 hr.	1	2960	+31
6	40	1 day	1	2750	+22
6	40	2 days	1	2320	+ 2
6	40	3 days	1	1980	- 3
10 untreated controls		—	—	2080	—
5	10	7 days	1	787	-62
5	30	7 days	1	464	-78
10 untreated controls		—	—	2630	—
10	40	4 days	1	480	-82
10	40	7 days	1	295	-89

4 days and longer, a marked depletion followed upon the initial increase of the cholesterol content. Table 3 shows that injections of pituitary corticotrophic hormone had the reverse effect on the cholesterol content of the adrenal cortex. The initial effect of the injections of corticotrophic hormone was a decrease in the cholesterol content, while a prolongation of the injections beyond 3 days produced an increase which ranged from +32 to +147% in the various groups of injected animals.

TABLE 3. Cholesterol content of adrenals of male rats treated with corticotrophic hormone

No. of rats in group	Total amount of corticotrophic hormone injected (sudanophobic units)	Duration of treatment	No. of injections administered each day	Total cholesterol content in mg./100 g. of the pooled adrenal tissue	Percentage change in cholesterol content
10 untreated controls		—	—	2940	—
6	8	6 hr.	1	1620	− 45
6	8	12 hr.	2	1850	− 37
6	8	1 day	3	2385	− 19
6	8	2 days	3	3230	+ 10
6	8	3 days	3	3895	+ 32
12 untreated controls		—	—	2600	—
3	14	7 days	3	3600	+ 38
3	28	7 days	3	3880	+ 49
4	21	7 days	3	3430	+ 32
2	42	7 days	3	4325	+ 66
9 untreated controls		—	—	2020	—
8	32	3 days	3	3670	+ 79
6 untreated controls		—	—	2820	—
4	20	3 days	3	4390	+ 56
4	10	3 days	3	4070	+ 44
5 untreated controls		—	—	2630	—
5	1	3 days	3	4920	+ 87
5	2	3 days	3	6230	+137
5	4	3 days	3	4750	+ 81
5	8	3 days	3	5020	+ 91
5	16	3 days	3	6225	+137
5	32	3 days	3	6500	+147

The effect of injections of the pituitary corticotrophic hormone was further investigated in hypophysectomized rats. Such animals show as a rule a diminution of the cholesterol content of the adrenals. The present series of experiments (Table 4) demonstrates that the decrease of the cholesterol content, if it occurs, can be influenced by subsequent injections of corticotrophic hormone. Small doses of this principle are liable to diminish the

TABLE 4. Total cholesterol content of adrenal of (*a*) normal untreated rats, (*b*) hypophysectomized rats, and (*c*) hypophysectomized rats treated with corticotrophic hormone

(Body weight 100–130 g.)

No. of rats in group	Days after hypophysectomy	Total no. of units of corticotrophin injected	Duration of treatment (days)	No. of injections each day	Total cholesterol content in mg./100 g. of the pooled adrenal tissue	Percentage change compared with cholesterol content in normal untreated animals	Percentage change compared with cholesterol content in untreated hypophysectomized animals
8 unoperated and untreated controls				—	2445	—	—
7	17–18	—	—	—	1010	−58	—
2	18	½	7	2	502	−79	−50
4	14–18	1	7	2	708	−71	−21
3	17	2	7	2	995	−59	− 1
2	17	4	7	2	1350	−45	+24
3	16	8	7	2	1932	−21	+91

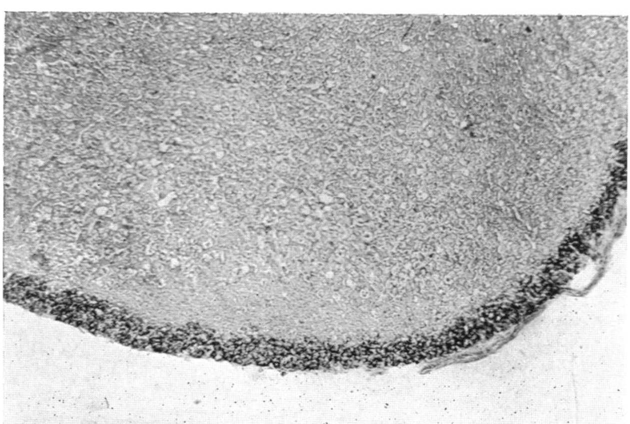

Fig. 1. After 7 days' treatment with 70 i.u. (total dose) of gonadotrophic hormone.

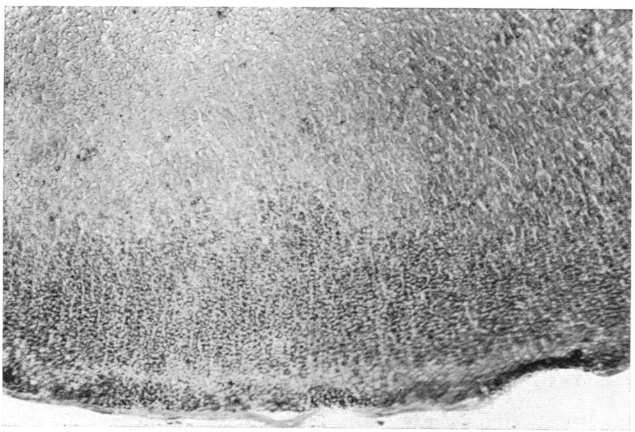

Fig. 2. Untreated control.

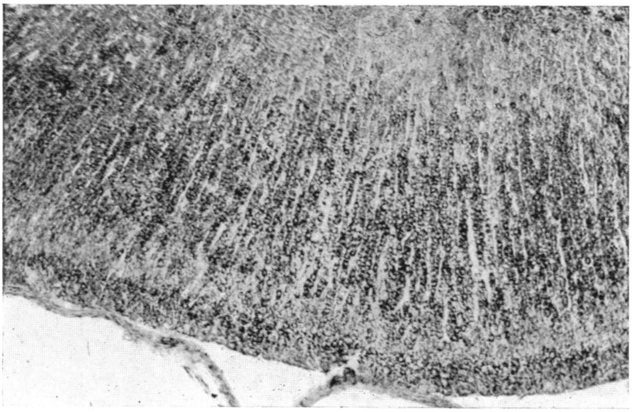

Fig. 3. After 7 days' treatment with 28 sudanophobic units (total dose) of corticotrophic hormone.

Adrenals of 50 g. male litter-mates. Frozen sections. Haematoxylin—Sudan III. ×80.

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cholesterol content even further. However, larger doses of the corticotrophic hormone restored the cholesterol content, with the result that, as shown in Table 4, the values for normal untreated animals were approached.

Histological changes. Changes in the lipid content of the adrenals were also investigated by histological methods (see Pl. 1). It was found that the adrenals of rats, which for 7 days had been injected with gonadotrophic hormone, showed an almost complete loss of sudanophilic granules in the fasciculate and the reticulate zones. The agreement between these findings and the changes in the cholesterol content will be noted. The adrenals of rats injected with corticotrophic hormone for 7 days, showed a marked increase of sudanophilic granules compared with adrenals of a control litter mate. A comparison between these findings and the results of the cholesterol determinations (Tables 2 and 3) shows good agreement between the histological and the biochemical data.

Discussion

The results presented show a diphasic effect of both the corticotrophic and the gonadotrophic hormones on the cholesterol content of the adrenal cortex. The results observed with injection of corticotrophic hormone are in full agreement with those of Sayers, Sayers, White & Long (1943).

The antagonistic action of the gonadotrophic and corticotrophic hormones has also been shown to apply to the blood (Reiss & Langendorf, 1929; Reiss, 1930) for the cholesterol content has been observed to increase after injection of gonadotrophic hormone and to decrease after injection of the corticotrophic hormone.

It seems likely that changes of the cholesterol content of the adrenal cortex reflect changes in the production of the cortical steroid hormones. For instance, it has been shown that changes in the cholesterol metabolism of the adrenal cortex are connected with changes in the production and excretion of 17-ketosteroid hormones. Hemphill, Macleod & Reiss (1942) found that a diminished ketosteroid excretion in certain mental patients could be considerably increased by treatment with corticotrophic hormone. More recent investigations showed, on the other hand, that administration of gonadotrophic hormone decreased the ketosteroid excretion in patients with primary excessive ketosteroid excretion.

Variations in the sudanophilic substances of the adrenal cortex have previously been studied by Reiss *et al.* (1936). It was found that in the hypophysectomized rat, the sudanophilic granules became larger and tended to form clumps; and it was assumed that, in view of the diminished hormone production, this phenomenon was not concerned with the hormone production. When hypophysectomized rats are treated with corticotrophic hormone the earliest change noted is the fragmentation and uniform dispersal of the coarse clumped granules, followed by the gradual elimination of the sudanophobic

zone by the deposition of sudanophilic granules in the zona fasciculata. It will be remembered in this connexion that the cholesterol content of the adrenal cortex of hypophysectomized rats was also shown to increase, provided that a sufficiently large dose of corticotrophic hormone had been administered.

Weaver & Nelson (1943), who recently investigated changes in the birefringent material of the adrenal cortex, obtained essentially similar results.

The histological changes seen in the adrenal cortex after gonadotrophic hormone had been injected for 7 days, are probably due to a mobilization of testosterone or androsterone. This assumption is supported by the findings of Hall & Korenchevsky (1938) who achieved a nearly analogous decrease of sudanophilic lipins after 4–7 weeks' daily injection of small doses (0·1–0·9 mg.) of androsterone or testosterone to castrated male rats.

Summary

1. Injections of corticotrophic hormone of the pituitary anterior lobe and of the gonadotrophic hormone of pregnant-mare serum have an antagonistic influence on the cholesterol metabolism of the adrenal cortex.

2. Gonadotrophic hormone decreases the cholesterol content after a preliminary increase, corticotrophic hormone increases the cholesterol content after a preliminary decrease.

3. The probable connexion of these changes with the production of adrenal cortical hormone and of the 17-ketosteroid excretion are discussed.

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