TREATMENT OF SENESCENT MALE RATS WITH CORTISONE ACETATE ALONE OR TOGETHER WITH SEX AND THYROID HORMONES

V. KORENCHEVSKY

(From the Gerontological Research Laboratory, Whittington Hospital, London)

In the present experiments the effects of comparatively small, non-toxic doses of cortisone alone, or in combination with testosterone and estradiol compounds and desiccated thyroid, were investigated.

An extensive literature refers to the effects of cortisone on various organs, tissues, and functions, and it was reviewed in several publications (2, 6, 7, 11, 16, 17, 18, 19, 21).

While changes in the weight and histologic structure of the adrenals and lymphoid organs were investigated in most cases, those in other organs were examined less frequently (1, 2, 8, 12, 13, 24). Moreover, since large doses of cortisone are used for therapeutic purposes, chiefly the effects of these doses were investigated. Large doses, however, possess toxic properties (2, 7, 21, 26, 27) which prevent the investigation of physiologic effects.

Thus, large doses of cortisone compounds produced atrophic changes in the adrenals, thymus, spleen, and lymph nodes (1, 11, 24). For example, in the experiments of Antopol (1) the respective weights of the adrenals, thymus, and spleen were 2.34, 48.1, and 164 mg. in control mice, while in the cortisone treated animals they were 1.56, 8.05, and 51 mg., respectively. In the adrenals a decreased size of cortical cells, depletion of lipoid granules, and occasional development of small adenomas were observed. The adrenal atrophy is explained at least partly by a compensatory suppression of the cortex, produced by administration of excessive amounts of the adrenal hormone.

In the spleen, besides atrophy and destruction of cells, Teilum and associates (23) recorded a hyalinosis of the splenic reticulum in perifollicular and perivascular zones and a decrease in number of plasma cells.

The weight and structure of the kidneys were usually not changed (24, 25), but in some cases a thrombosis of glomerular capillaries and granulomatous nests were found (2).

In the thyroid a flattening of the epithelium and increased amount of hard colloid were observed, while the foci of interstitial inflammation or necrosis were found in the pancreas (2). However, in other experiments (8, 24, 25) no changes were recorded in these organs.

Contradictory observations were also reported on the changes in cardiac and skeletal muscles. While in some experiments (2, 13) degenerative lesions and even nests of necrosis were observed, in other investigations (8, 12, 24, 25) a normal weight and structure of the heart were found.

The testes, seminal vesicles, and prostate in male mice and the ovaries in females appeared smaller in Antopol's experiments (1), but by other authors (23) the male sex organs were found to be normal.

In guinea pigs Harris and associates (8) did not notice any significant changes in the weight of the thymus, spleen, heart, pancreas, and lungs, but they found a greatly increased weight of the liver (from 50 to 123 per cent) and a less pronounced increase of the kidneys (from 8 to 20 per cent) and testes (up to 33 per cent). These authors observed the same effect on the liver of rabbits, but not in mice, rats, or chicks. Histologically hepatic cells of guinea pigs were enlarged. Chemical investigation showed an increased nitrogen, water, and glycogen content in the liver of guinea pigs. Glycogen accumulation in the liver cells after cortisone injections was also observed in mice, rats, and rabbits (2, 20, 23).

The above mentioned changes were also produced by ACTH administration. Therefore, according to these results the hypertrophy of the liver produced by cortisone or ACTH should be explained by protein and glycogen deposition and by water retention. In contrast to this, Winter and associates (24) found no changes in the fat, nitrogen, and phosphorus content of the liver and kidneys of cortisone injected rats.

Various lymphoid tumors, rhabdomyosarcomas, ependymyomas, and malignant leukemia were temporarily inhibited or regressed by cortisone compounds in experimental animals (3, 4, 9, 10, 11, 22, 27, 28), but Antopol and asso-

Submitted for publication September 15, 1955.

ciates (1, 2) observed a development of adenomas in the adrenal cortex of cortisone injected mice. Cade (5) reviewed the literature on the subject and reported his own results on the effects of double adrenalectomy on breast cancer in women. Their life after the operation was maintained by cortisone injections. In about 60 per cent of Cade's cases a subjective and objective improvement was obtained.

MATERIALS AND METHODS

Two experiments were performed on a total of 68 male normal rats.

The arrangement of the groups in both experiments was the same, the difference being only in the dosage of hormones administered. The changes in the weight or organs were similar in both experiments, being somewhat more pronounced in the Experiment II with larger doses. For economy of space the tables of relative weights of organs and those of measurements of cells and nuclei in the kidneys and adrenals were omitted. These data are, however, discussed in the text. The number of rats in each group of experiments is recorded in tables 1 and 2. Control animals were untreated littermates of rats in experimental groups.

Relative weights of organs were calculated per 200 Gm. of fat-free body weight, the total body fat being calculated by the formula used in previous experiments (14).

The histologic technique and the paper replica method used for the measurement of cells and nuclei were the same as in previous experiments (14, 15).

The statistical method and designations used were also the same as in previous investigations.*

Experiment I was performed on 34 rats, 6 of which served as controls. The duration of the experiment and of hormonal treatment was 67 days. The average age of animals at the end of the experiment was about 31.5 months. Cortisone acetate was injected subcutaneously in crystals suspended in 5 per cent gum acacia solution, in the dose of 0.3 mg. twice a week. Testosterone propionate was implanted subcutaneously in 20 mg. pellets containing 5 mg. of the hormone + 15 mg. cholesterol. The absorption from these pellets was very small, about 0.22 mg. per week. Desiccated thyroid was given orally by pipette 25 mg. twice a week during the first 53 days and once a week during the last two weeks. Two subcutaneous injections of 0.022 mg. of estradiol-3-benzoate-n-butyrate (estradiol B.B.) were given only during the first week of the experiment.

Experiment II was performed on 34 rats, 9 of which served as controls. The duration of the experiment was 45 days. The average age of rats was about 21 months. Cortisone acetate was injected subcutaneously in crystals suspended in 5 per cent gum acacia solution, 1.5 mg. once a week during first 30 days of the experiment, and 2 mg. once a week during the remaining period. Testosterone dipropionate was also injected in crystals in the gum acacia solution, 0.5 mg. every tenth day during the first half of the experiment and every fourteenth day during the remaining period. Desiccated thyroid was given by mouth, at first 20 mg. once a week and during the last 4 weeks of the experiment 8 mg. once a week. Two injections of 0.03 mg. of estradiol B.B. were given subcutaneously during the first week only.*

RESULTS

As compared with Experiment I, in Experiment II the doses of cortisone acetate and especially of testosterone compound were considerably larger; those of estradiol B.B. were slightly larger, while those of desiccated thyroid were smaller. Accordingly, the effects of cortisone acetate and especially of testosterone compound were more pronounced in Experiment II.

Testes.—As in a previous study (14) a 4 stage system was used for rating testicular degenerative changes. In present experiments the aging degenerative changes in control untreated rats were more pronounced than in the previous investigation (14, 15). Thus, Stage 1 changes were present in all control rats; Stage 2 or 3 changes were observed as well in about 25 per cent. Even the extreme Stage 4 of degeneration was observed in a few seminiferous tubules of some control rats in Experiment I on older rats.

Cortisone had no significant effect on the weight or histologic structure of the testes.

A larger dose of testosterone compound (Experiment II), administered alone or together with other hormones, had the usual atrophying effect on these organs. This effect was shown both by a decreased weight of the testes (table

* Statistical evaluation of the results obtained was performed by C. W. Shaddick, the statistician of the Health Department of the London County Council.

* The hormones used were supplied by Ciba Ltd., by courtesy of Dr. K. Miescher.

TABLE 1. EFFECT OF HORMONES ON ACTUAL WEIGHTS OF ORGANS IN EXPERIMENT I.

Organs	Controls	Testosterone Propionate	Cortisone Acetate		
			Alone	+ Testosterone Propionate	+ Testosterone Propionate + Estradiol B.B. + Desiccated Thyroid
Testes (Gm.)	0.90	0.98	1.15	0.77	0.92
Seminal vesicles (Gm.)	0.85	0.84	0.76	0.84	0.74
Prostate (Gm.)	1.30	1.56	1.52	1.37	1.58
Penis (mg.)	355	381	344	370	367
Preputial glands (mg.)	185	229	241	234	259
Adrenals (mg.)	57.8	65.2	51.4	57.2	61.6
Thyroid (mg.)	31.0	34.7	30.3	32.9	31.2
Hypophysis (mg.)	16.0	22.0	15.3	17.0	20.1
Liver (Gm.)	12.03	12.55	12.76	12.15	13.22
Kidneys (Gm.)	2.45	2.82	2.75	2.56	2.76
Spleen (mg.)	910	923	975	1006	1002
Heart (mg.)	1209	1360	1298	1263	1395
Brain (Gm.)	2.10	2.07	2.12	2.06	2.06
Sex fat + retroperitoneal fat (Gm.)	33.5	26.4	32.5	27.5	23.0
Final body weight (Gm.)	410	386	409	396	361
Number of rats in each group	6	7	7	8	6

TABLE 2. EFFECT OF HORMONES ON ACTUAL WEIGHTS OF ORGANS IN EXPERIMENT II.

Organs	Controls	Testosterone Dipropionate	Cortisone Acetate		
			Alone	+ Testosterone Dipropionate	+ Testosterone Dipropionate + Estradiol B.B. + Desiccated Thyroid
Testes (Gm.)	2.82	1.36	2.92	1.34	1.38
Seminal vesicles (Gm.)	0.69	1.27	0.87	1.07	1.11
Prostate (Gm.)	1.29	1.98	1.73	1.96	2.02
Penis (mg.)	359	392	370	412	376
Preputial glands (mg.)	105	163	143	169	137
Adrenals (mg.)	36.1	40.5	37.5	35.2	39.1
Thyroid (mg.)	21.7	23.2	24.2	25.9	24.6
Hypophysis (mg.)	11.7	12.0	13.9	12.5	12.5
Liver (Gm.)	9.57	10.75	10.94	10.92	10.03
Kidneys (Gm.)	2.19	2.52	2.52	2.47	2.59
Spleen (mg.)	1304	1384	1299	1297	1350
Heart (mg.)	1122	1281	1297	1230	1202
Brain (Gm.)	2.16	2.12	2.19	2.19	2.19
Sex fat + retroperitoneal fat (Gm.)	18.4	21.3	21.4	24.9	20.7
Final body weight (Gm.)	379	386	389	391	373
Number of rats in each group	9	6	6	6	7

2), and histologically by more pronounced degenerative changes.

Secondary sex organs.—While a larger dose of testosterone compound in Experiment II, alone or with other hormones, produced the usual hypertrophying effects on the seminal vesicles, prostate, preputial glands, and penis (table 2), these effects in Experiment I were slight and statistically not significant, except in the case of the prostate in the group of rats to which all hormones used were administered simultaneously ($P < 0.05$). These hypertrophic changes were confirmed by histologic examination.

Unexpectedly cortisone had a similar but weaker effect which was statistically significant only in the case of the prostate with a larger dose of cortisone (table 2, $P < 0.001$). However, in the groups to which testosterone and cortisone compounds were administered simultaneously, no definite cooperative effect of cortisone and testosterone compounds was noticed.

The slight androgenic effect of cortisone might be explained by a weak androgenic property of this hormone or by the presence in the compound used of impurities in the form of other definitely androgenic corticoadrenal hormones (e.g., adrenosterone).

Experiments on castrated rats with cortisone especially carefully purified from corticoadrenal androgens would clarify this point.

Adrenals.—Comparatively small and definitely non-toxic doses of cortisone acetate used in the present experiments did not produce any effect on the weight of the adrenals (tables 1 and 2) or size of cortical cells and their nuclei. For example, the average size of fasciculata cells of control rats in Experiments I and II was 10.49 and 10.74 mg., respectively, (as measured by their paper replicas). In "cortisone" groups the respective figures were 11.34 and 10.97 mg.

Since the doses used of testosterone compounds were also small and therefore did not cause atrophy of the corticoadrenal cells (10.58 and 10.08 mg.), the combined administration of both hormones also did not produce any statistically significant atrophying effect in both experiments (11.13 and 12.83 mg., respectively), showing even a slightly larger size of cells. Therefore, the atrophying effect of cortisone recorded by all workers using large doses apparently should be explained by a toxic action of excessive doses.

The only change in the adrenals observed in the present experiments was a partial or, in some cases, a complete depletion of lipoid granules in the zona glomerulosa. This change was found in about 50 per cent of rats treated with cortisone alone, while it occurred in nearly all rats treated with testosterone compounds alone or together with other hormones.

Liver.—In previous experiments (15) it was found that androgenic hormones produced definite hepatotrophic effects in castrated rats, but in normal animals the increase in the weight of the liver and hypertrophy of cells were small or absent, while nuclei could become hypertrophic.

In the present experiments, possibly due to more pronounced changes in the testes, in control intact rats the cytoplasm of hepatic cells was as small (8.22 and 8.77 mg., table 3) as observed previously in castrated rats of similar age (8.66 mg., Korenchevsky and associates (14, table 7)) while in normal intact rats it should measure about 11.79 mg. (14).

Possibly this explains why in the present experiments testosterone compounds, producing a small but statistically significent increase ($P < 0.05$ in Experiment I and $P < 0.02$ in Experiment II) in relative weights of the liver, greatly hypertrophied the cytoplasm of hepatic cells (table 3, $P < 0.01 - 0.001$). Nuclei, however, were significantly enlarged only in Experiment II ($P < 0.05$).

Similar changes were observed in the groups in which testosterone compounds had been administered together with other hormones.

Cortisone in both experiments produced only a slight increase, statistically not significant, in the weight of the liver (tables 1 and 2), but about the same hypertrophy of the cytoplasm as did androgens ($P < 0.01 - 0.001$). The size of the nuclei, however, did not change significantly.

Therefore, contrary to the results obtained by Harris and associates (8), not only in guinea pigs and rabbits but in rats as well the hepatotrophic effect of cortisone could be observed. Probably the discrepancy can be explained by different doses used, since Harris and associates recorded the loss in body and liver weights of their rats. This suggests that probably too large and therefore toxic doses of cortisone were employed (unfortunately the dosage was not stated in the paper).

Those pathologic changes which are usually observed in the liver of aging rats, namely small nests of round cell infiltrations and bile duct hyperplasias (14, 15), were present both

TABLE 3. EFFECT OF HORMONES ON LIVER CELLS.

Groups	Average Weight of Paper Replica (Mg.)					
	Experiment I			Experiment II		
	Cytoplasm	Nucleus	Ratio of Nucleus to Cytoplasm	Cytoplasm	Nucleus	Ratio of Nucleus to Cytoplasm
Controls	8.22	1.16	0.141	8.77	1.09	0.126
Cortisone acetate	10.48	1.15	0.110	10.47	1.09	0.104
Testosterone propionate (Exp. I) or dipropionate (Exp. II)	11.79	1.19	0.103	10.95	1.18	0.110
Cortisone acetate + Testosterone propionate (Exp. I) or dipropionate (Exp. II)	10.68	1.23	0.116	10.66	1.17	0.110
Cortisone acetate + testosterone propionate (Exp. I) or dipropionate (Exp. II) + estradiol B.B. + desiccated thyroid	11.22	1.39	0.105	10.55	1.12	0.106

in control and treated rats in about the same number and were of about the same size.

Kidneys.—Previously (15) it was found that the renotrophic effect of androgens was very pronounced in castrated rats but usually was weak or absent in normal rats. In the present experiments it was also weak, the renal enlargement being statistically significant only in relative weights ($P < 0.5$ in Experiment I and < 0.02 in the Experiment II). The cells of the convoluted tubules were enlarged only slightly and statistically not significantly (as measured by the paper replica method).

Cortisone in the doses used had an effect similar to that of testosterone compounds. The increase in weight of the kidneys valued about 15 per cent and it was statistically significant only in relative weights ($P < 0.05 - 0.02$). Although the enlargement of the cells in the renal convoluted tubules was statistically not significant, a definite enlargement of their nuclei was observed in Experiment II (1.78 mg. in control rats and 2.19 mg. in cortisone injected animals, $P < 0.05$).

Thus, the results obtained in the present experiments on rats were similar to those on guinea pigs by Harris and associates (8), who, however, did not find these changes in rats.

Combined administration of testosterone and cortisone compounds did not significantly increase the effects produced by each of these hormones separately. When, however, all 4 hormones used were given simultaneously, the renotrophic effects, especially with a larger dose of cortisone acetate, in Experiment II, were more pronounced. This effect was shown by the relative weight of the kidneys (23.6 per cent increase, $P < 0.001$) and the enlargement of renal cells and nuclei (7.67 and 1.78 mg., respectively, in control rats; 8.50 and 2.02 mg., respectively, in treated rats, $P < 0.02$).

Heart.—A cardiotrophic effect of androgens on senescent rats was established previously (15).

In the present experiments a stimulating effect of testosterone and cortisone compounds on the heart was similar to that on the liver and kidneys, being slight and more pronounced in Experiment II. It was statistically not significant in actual weights but was significant ($P < 0.05$) in relative weights when these hormones were administered alone or together or simultaneously with thyroid and estradiol hormones.

The number of enlarged cardiac fibers was not counted in present experiments, but previously (15) it was found that after testosterone administration this value increased in castrated, but not in normal male senescent rats.

Spleen.—As previously (14) observed in intact senescent rats, in control animals of the present experiments the number of mitoses and giant cells was also small as compared with

these values in young rats. No difference between control rats and treated animals was noted in actual or relative weights, histologic structure, number of mitoses, and giant cells in the spleen. Therefore, the atrophic changes in this organ after the injection of large doses of cortisone compounds, observed by authors using these doses, should be explained by their toxicity.

Other organs.—Winter and associates (24) recorded that, after cortisone injections, the hypophysis and thyroid of normal rats were normal both histologically and by weight.

In the present experiments no statistically significant difference was found in the weight of the hypophysis, thyroid, and brain, nor in the histologic structure of the thyroid and pancreas. The thyroid had a structure of an active gland in rats of all groups. The number of metaplasias, small cysts, and adenomas seemed to be slightly increased in the thyroids of treated animals. The number of rats in the various groups, however, was too small for definite conclusions on adenomagenic effects of hormones.

SUMMARY

1. In 53 intact male senescent rats the effect of comparatively small non-toxic doses of cortisone acetate administered alone, or together with testosterone compounds, estradiol B.B., and desiccated thyroid was investigated on the weights and histologic structure of various organs. Fifteen animals served as controls.

2. Some effects of cortisone were found to be similar to, but weaker than, those of androgens, being more pronounced with a larger dose of cortisone.

3. In the case of the sex organs, cortisone produced a hypertrophy of the prostate and a similar, but considerably lesser effect on the seminal vesicles, penis, and preputial glands. In contrast to the usual androgenic effect on the testes, the doses used of cortisone did not produce atrophy of these organs.

4. Like androgens, cortisone also produced a definite, but limited stimulating effect on the liver, and a less pronounced stimulation of the kidneys and heart.

5. Since the effects of androgenic hormones are always much more pronounced in castrates than in normal intact animals, it was suggested that "androgenic" properties of cortisone should be also studied on castrates.

REFERENCES

1. Antopol, W.: Anatomic Changes Produced in Mice Treated with Excessive Doses of Cortisone. *Proc. Soc. Exper. Biol. & Med., 73:* 262-265, 1950.
2. Antopol, W., Glaubach, S., and Quittner, H.: Experimental Observations with Massive Doses of Cortisone. *Rheumatism, 7:* 1-10, 1951.
3. Brzustowitz, R. J., Svien, H. J., Bennett, W. A., and Higgins, G. M.: The Effect of Cortisone on the Growth of Transplanted Ependymomas in Mice. *Proc. Mayo Clinic, 26:* 121-128, 1951.
4. Burchenal, J. H., Stock, C. C., and Rhoads, C. P.: The Effect of Cortisone and ACTH on Transplanted Mouse Leukemia. *Cancer Research 10:* 209, 1950.
5. Cade, Sir S.: Adrenalectomy for Breast Cancer. *Brit. Med. J., 1:* 1-5, 1955.
6. Cameron, G. R.: A Survey of Tissue Responses to ACTH and Cortisone. *Proc. V Symposium of Colston Res. Soc.,* 155-166, Butterworths Sc. Publications, London, 1952.
7. Davidson, L. S. P.: The Physiological and Clinical Effects of Cortisone and ACTH. *Edinburgh Med. J., 57:* 317-340, 1950.
8. Harris, L. J., Bland, M. N., Hughes, R. E., and Constable, B. J.: Effect of ACTH and Cortisone on Vitamin-C Metabolism and on the Weight and Composition of the Liver. *Lancet, 1:* 1021-1024, 1953.
9. Heilman, F. R., and Kendall, E. C.: The Influence of Compound E on the Growth of a Malignant Tumor in the Mouse. *Endocrinology, 34:* 416-420, 1944.
10. Higgins, G. M., Woods, K. A., and Bennett, W. A.: The Influence of Cortisone upon the Growth of a Transplanted Rhabdomyosarcoma in CZH Mice. *Cancer Research, 10:* 203, 1950.
11. Ingle, D. J.: The Biologic Properties of Cortisone. *J. Clin. Endocrinol., 10:* 1312-1354, 1950.
12. Kendall, E. C.: In: *Metabolic Aspects of Convalescence, Including Bone and Wound Healing. Tr. 10th Conference, Josiah Macy, Jr. Foundation,* New York, pp. 81-97, 1945.
13. Kochakian, C. D.: In: *Metabolic Aspects of Convalescence, Including Bone and Wound Healing. Tr. 6th Conference, Josiah Macy, Jr. Foundation,* New York, pp. 13-35, 1944.
14. Korenchevsky, V., Paris, S. K., and Benjamin, B.: Effects of Castration and the Processes of Aging in Male Rats and Man. *J. Gerontol., 8:* 6-32, 1953.
15. Korenchevsky, V., Paris, S. K., and Benjamin, B.: Treatment of Senescence in Male Rats with Sex and Thyroid Hormones and Desoxycorticosterone Acetate. *J. Gerontol., 8:* 415-434, 1953.
16. Ralli, E. P.: Editor, *Adrenal Cortex. Tr. 2nd Conference, Josiah Macy, Jr. Foundation,* New York, 1950.
17. Ralli, E. P.: Editor, *Adrenal Cortex. Tr. 3rd Conference, Josiah Macy, Jr. Foundation,* New York, 1951.
18. Ralli, E. P.: Editor, *Adrenal Cortex. Tr. 4th Conference, Josiah Macy, Jr. Foundation,* New York, 1952.

19. Ralli, E. P.: Editor, *Adrenal Cortex. Tr. 5th Conference, Josiah Macy, Jr. Foundation,* New York, 1953.

20. Reinecke, R. M., and Kendall, E. C.: A Comparison of the Influence of Some Crystalline Hormones of the Adrenal Cortex on the Deposition of Glycogen in the Liver. *Endocrinology, 32:* 505-508, 1943.

21. Sprague, R. G., Power, M. H., Mason, H. L., Mathieson, D. R., Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F.: Observations on the Physiological Effects of Cortisone and ACTH in Man. *Arch. Int. Med., 85:* 199-258, 1950.

22. Sugiura, K., Stock, C. C., Dobriner, K., and Rhoads, C. P.: The Effect of Cortisone and Other Steroids on Experimental Tumors. *Cancer Research, 10:* 244-245, 1950.

23. Teilum, G., Engbaek, H. C., and Simonsen, M.: Effects of Cortisone on Plasma Cells and Reticulo-Endothelial System in Hyperimmunized Rabbits. *Acta Endocrinol., 5:* 181-193, 1950.

24. Winter, C. A., Silber, R. H., and Stoerk, H. C.: Production of Reversible Hyperadrenocortinism in Rats by Prolonged Administration of Cortisone. *Endocrinology, 47:* 60-71, 1950.

25. Woodbury, D. M., Rosenberg, E. F., and Sayers, G.: Personal communication to Ingle, reference 11, p. 1334.

26. Woodruff, M. F., and Boswell, T.: The Effect of Cortisone and ACTH on Adrenal Transplants in the Rat. *J. Endocrinol., 10:* 86-92, 1953.

27. Woolley, G. W.: Some Effects of Cortisone and Related Steroids on Transplanted Tumors of the Mouse. *Tr. New York Acad. Sc. 13:* 64-66, 1950.

28. Woolley, G. W., and Peters, B. A.: Prolongation of Life in High-Leukemia AKR Mice by Cortisone. *Proc. Soc. Exper. Biol. & Med., 82:* 286-287, 1953.