4. The biochemical properties of the tyrosinase preparations from the horse and fish melanomas are similar to the properties of the preparation obtained from mouse melanoma.

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Effect of Adrenocorticotrophic Hormone, Cortisone and Desoxycorticosterone on Brain Excitability.* (18211)

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It has been demonstrated in this laboratory that desoxycorticosterone acetate (DCA) decreases brain excitability as measured by the electroshock seizure threshold (EST)(1,2), and that brain excitability of DCA-treated rats can be restored to normal by the administration of adrenocorticotrophic hormone (ACTH) or adrenocortical extract (ACE)(2). The following experiments are an extension and elaboration of these earlier studies.

Methods. Adult male rats from the Sprague-Dawley farm were employed. The technic for determining EST has been presented in detail(3). EST measurements were made at 2- to 3-day intervals for a period of about two weeks before the beginning of hormone administration. Thresholds tend to be more variable and slightly higher at the beginning than at the end of such a period of stabilization. Hence, the alterations in EST observed throughout the course of the experiments have been expressed as a percentage of the control value obtained at the *end* of the stabilization period. When thresholds had stabilized, some of the rats were unilaterally nephrectomized, implanted subcutaneously with DCA† (twelve 15-mg pellets per rat) and given a 0.9% solution of sodium chloride to drink. EST measurements were made every 2 to 3 days throughout the experiments. The dose of ACTH‡ is expressed in all instances as the mg equivalent of the ACTH standard, La-1-A. Preparation 74 Ax, employed in Experiment A, is 50% as potent as La-1-A; it has posterior pituitary pressor activity of less than 0.06 unit per mg equivalent of La-1-A. Preparation 83 ASI, employed in Experiment B, is 160% as potent as La-1-A; it has posterior pituitary pressor activity of less than 0.05 unit per mg equivalent of La-1-A. ACTH was dissolved in a 0.9% solution of sodium chloride at pH 1; each dose was injected subcutaneously in a volume of 0.2 ml. Cortisone acetate was suspended in 10% ethanol; each dose was injected subcutaneously in a volume of 0.2 ml. Posterior pituitary substance (Pitressin, Parke, Davis and Co., 20 pressor units per ml) was diluted with 0.9% sodium chloride solution to a concentration of 0.1 unit per 0.2 ml; this volume was injected subcutaneously. When combinations of hormones were employed, the preparations were injected throughout the entire course of the experiment, starting at the time of DCA implantation. This is in contrast to the previous study(2) in which ACTH and ACE were not injected until the rats had been under the influence of DCA for some time.

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1. Woodbury, D. M., and Davenport, V. D., *Am. J. Physiol.*, 1949, v157, 234.

2. Woodbury, D. M., Cheng, C. P., Sayers, G., and Goodman, L. S., *Am. J. Physiol.*, 1950, v160, 217.

† Percorten, generously supplied by Dr. Ernst Oppenheimer of the Ciba Pharmaceutical Co.

‡ ACTH was obtained through the kindness of Dr. E. E. Hays of the Armour Co.

TABLE I. Treatment of Experimental Groups.

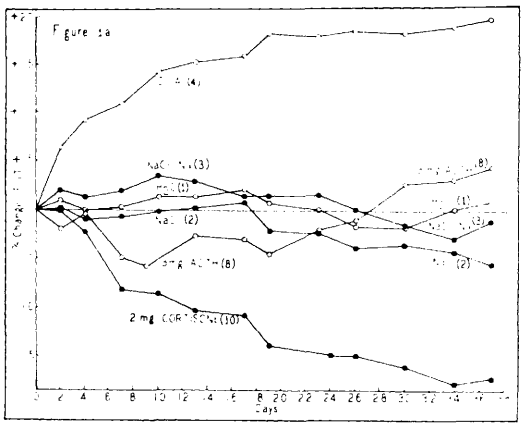
Exp.	Group	No. rats	Unilateral nephrectomy	Drinking fluid	DCA 15 mg pellets	ACTH* total dose, mg	Cortisone,* total dose, mg	Initial body wt, g and ± S.E.	Final body wt, g and ± S.E.
A	1	4	No	H_2O	0	0	0	350 ± 19	380 ± 25
	2	5	"	NaCl	0	0	0	350 ± 17	375 ± 16
	3	6	Yes	"	0	0	0	342 ± 18	368 ± 17
	4	5	"	"	12	0	0	337 ± 9	305 ± 18
	5	6	"	"	12	3(3)	0	345 ± 6	322 ± 9
	6	6	"	"	12	.5(1)	0	306 ± 5	278 ± 14
	7	5	"	"	12	.05(1)	0	344 ± 15	348 ± 12
	8	4	"	"	0	3(3)	0	325 ± 20	313 ± 20
	9	5	"	"	12	0	2(2)	336 ± 6	260 ± 8
	10	6	"	"	0	0	2(2)	333 ± 11	270 ± 12
B	11	7	"	"	0	0	0	263 ± 5	353 ± 6
	12	7	"	"	12	0	0	273 ± 4	319 ± 8
	13	7	"	"	12	.001(1)	0	266 ± 6	298 ± 11
	14	7	"	"	12	.01(1)	0	271 ± 6	312 ± 8
	15	6	"	"	12	10(5)	0	277 ± 9	256 ± 11
	16†	7	"	"	12	.5(1)	0	274 ± 7	323 ± 9
	17	7	"	"	12	0	.1(1)	263 ± 12	307 ± 13

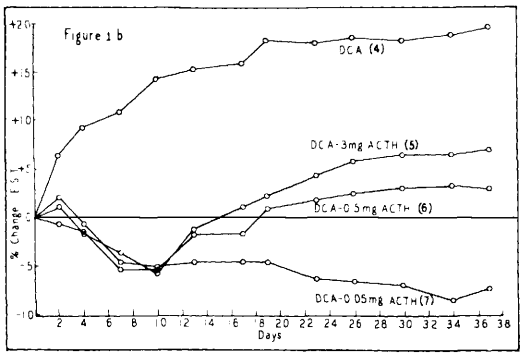
* Numbers in parentheses indicate number of divided doses per day.
† 0.1 unit posterior pituitary 5 times daily.

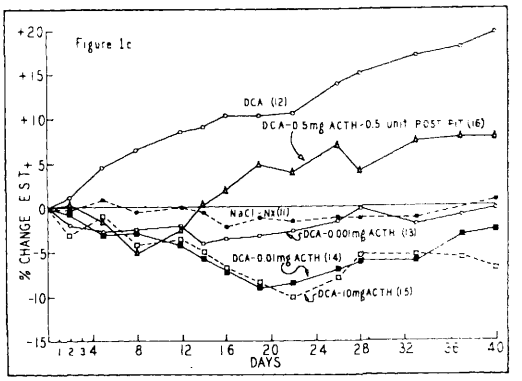
Results. Two experiments, A and B, were performed. Information regarding the nature of the drinking fluid, hormone treatment and body weight changes of each group of rats is presented in Table I.

Fig. 1 illustrates the data obtained. Fig. 1a and 1c indicate that control rats, *i.e.*, Groups 1, 2 and 3 of Experiment A and Group 11 of Experiment B, showed little or no change in EST throughout the course of the experiment. A slight elevation of EST occurred in Group 3 of Experiment A from the 8th to the 12th day and a slight decline of EST occurred in Group 2 of Experiment A beginning on the 20th day. It may be concluded that excess dietary sodium and unilateral nephrectomy do not appreciably influence brain excitability in rats as measured by EST. In both Experiments A and B, DCA induced an elevation in EST similar to that previously reported from this laboratory(1,2). In the earlier studies rats with intact kidneys were implanted with six 15-mg pellets of DCA and were given water to drink. It thus appears that an increase in the dose of DCA from 6 to twelve 15-mg pellets, combined with unilateral nephrectomy and 0.9% sodium chloride solution to drink had no additive effect on brain excitability. It is of interest in this connection that the concentration of sodium in the plasmas of rats in Experiment A (153.2 ± 3.9 mEq per kg of plasma) was not significantly greater than the concentration of this cation (151.2 ± 1.2) in the plasma of rats implanted with six 15-mg pellets and given water to drink. The rats given ACTH alone in a dose of 1 mg thrice daily showed a slight initial decrease in EST, followed by a return to normal and a subsequent slight increase (Fig. 1a).

All doses of ACTH effectively antagonized the action of DCA on EST (Fig. 1b and 1c). The EST actually decreased below control levels during the first 10 to 12 days in all groups of DCA-rats treated with ACTH. The EST remained at a level lower than normal during the rest of the experimental period in rats given 0.01 mg, 0.05 mg or 10 mg of ACTH daily (Fig. 1b and 1c). At the 0.001 mg dose level (Fig. 1c) the EST returned to normal during the subsequent course of the experiment. A progressive rise in EST, beginning on the 10th day, occurred in DCA-rats treated with 0.5 mg or 3 mg ACTH (Fig. 1b). With the 3 mg dose the EST was significantly increased above normal during the







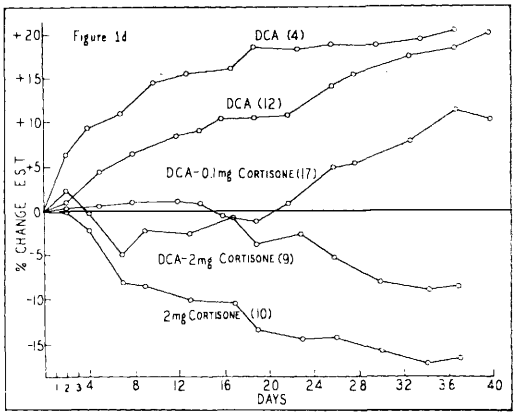


FIG. 1a and b. Average changes from control level (0) in electroshock seizure threshold (EST) with time in rats given various treatments. See text for explanation. Nx = unilateral nephrectomy. Numbers in parentheses are the group numbers (Table I).

FIG. 1c and d. Same legend as for previous figures.

last 14 days of the experiment, but the value did not approach that of animals given DCA alone. In DCA-rats treated with 0.5 mg ACTH plus 0.5 unit of posterior pituitary (Fig. 1c), the pattern of change in EST was similar to that of the DCA-animals treated with 3 mg ACTH (Fig. 1b).

Cortisone alone in a dose of 1 mg twice daily induced a marked reduction in the EST (Fig. 1d). Furthermore, this steroid effectively antagonized the EST-elevating properties of DCA (Fig. 1d). In a dose of 2 mg the action of the cortisone predominated and the ultimate EST of animals treated with the combination was significantly less than normal. Cortisone in a dose of 0.1 mg once daily maintained the EST of DCA-treated animals at a normal level until the 22nd day when there began a progressive increase in EST which, at the end of the experiment, was 10% above normal.

Discussion. A number of factors have been shown to affect the excitability of the brain as measured by the EST. The concentration of extracellular sodium has an important influence, the EST varying directly with the concentration of plasma sodium. Adrenalectomized rats have a hyponatremia in association with a decreased EST(3). DCA or sodium chloride restores to normal both the concentration of plasma sodium and the EST of adrenalectomized rats(3); DCA induces a hypernatremia and an elevation in EST in intact(1,2) and adrenalectomized(2) rats. Furthermore, both the hypernatremia and the decreased brain excitability induced by DCA are corrected by ACTH(2).

3. Davenport, V. D., *Am. J. Physiol.*, 1940, v156, 322.

Nutritional factors also have a pronounced effect on the excitability of the brain. It has been demonstrated in this laboratory(4) that restricted caloric intake, accompanied by failure to grow or loss in body weight, results in a marked reduction in EST. Although loss of weight in starved animals is accompanied by an increase in concentration of plasma sodium, there is a marked decrease in the EST(4,5). It is thus apparent that the influence of extracellular sodium on brain excitability can be antagonized, or even overshadowed by other factors.

The animals in Exp. A and B were given 0.9% sodium chloride solution to drink, whereas in a previous study(2) the animals were given water to drink.§ As pointed out above, ACTH prevented the hypernatremia and the elevation in EST in DCA-rats given water to drink. As yet incomplete electrolyte data indicate that there is no significant difference in extracellular sodium concentration among the various experimental groups, despite marked differences in EST; the available electrolyte data suggest that ACTH and cortisone can antagonize the action of DCA on brain excitability without producing an accompanying decrease in the concentration of plasma sodium.

In the previous experiments reported from this laboratory(2), ACTH and ACE were administered after EST had been elevated by DCA. It was demonstrated that ACTH and ACE could reverse the change in brain excitability induced by DCA. In the present experiments, it is demonstrated that the EST-elevating action of DCA can be prevented by ACTH or cortisone administered during the entire course of the experiment. ACTH, therefore, can both prevent and reverse the effect of DCA on the excitability of the central nervous system. It is likely that cortisone and ACE can also both prevent and reverse the action of DCA, although additional studies are required definitely to establish this point.

All doses of ACTH employed, 0.001 to 10 mg per day, completely antagonized the effect of DCA during the early phase of the experiments. Subsequently, the intermediate doses were not as effective as either the small or the very large doses of ACTH in lowering the EST of DCA-treated animals. It is remarkable that a dose of ACTH as small as 1.0 μg daily is effective. A dose of 10 μg of ACTH appears to have a slightly greater effect than 1.0 μg. The possibility that the ACTH-DCA antagonism may provide the basis of a new method of assay for ACTH is undergoing experimental test in this laboratory.

The secondary rise in the EST of animals treated with 0.5 and 3 mg doses of ACTH cannot be adequately explained at the present time. Several possible explanations have been considered, three of which are the following: (1) antihormone formation; (2) contamination of ACTH with a substance which influences brain excitability, *e.g.*, posterior pituitary; (3) changes in the qualitative nature of the secretion of the adrenal cortex. The data suggest that the first 2 possibilities are unlikely. The third is difficult to rule out in the present state of our knowledge regarding the nature of the secretion of the adrenal cortex.

That the 10 mg dose of ACTH acted similarly to the 0.001, 0.01 and 0.05 mg doses emphasizes the fact that numerous variables are operating to alter the EST in these experiments. It was pointed out above that restriction of food intake results in a marked reduction in the EST. The 10 mg dose of ACTH exerted a considerable catabolic effect as shown by the weight loss (−21 g, Group 15, Table I) in animals that normally grew at a rapid rate (+90 g, Group 11). The 0.5 and 3 mg doses of ACTH induced relatively less change in body weight (−28 g, Group 6 and −23 g, Group 5) in comparison to controls (+26 g, Group 3). The difference in effect on EST produced by the 10 mg dose and by the 0.5 and 3 mg doses of ACTH appears to be due in large measure to the weight loss

4. Davenport, V. D., and Davenport, H. W., *J. Nutrition,* 1948, v36, 139.

5. Davenport, V. D., *Fed. Proc.,* 1950, v9, 28.

§ Unilateral nephrectomy and sodium chloride were employed in the present investigation in connection with parallel pathological studies on the influence of ACTH and cortisone on the toxic effect of DCA on tissues.

in animals injected with the inordinately large dose of ACTH. However, no final conclusion can be reached until the effects of these various doses of ACTH are compared under experimental conditions in which body weight changes are rigidly controlled.

Cortisone alone in a dose of 2 mg daily produced a marked reduction in the EST (Group 10). However, a marked reduction in body weight occurred, and the final evaluation of the experimental data, which suggest an increased brain excitability after cortisone, must therefore await additional studies with smaller doses of the steroid and with adequate control of the factor of body weight change. Baehr and Soffer(6) discontinued cortisone treatment in a young woman with lupus erythematosis because of the occurrence of repeated epileptiform convulsive seizures.

The effect of DCA on EST was effectively antagonized throughout the entire experiment by the daily administration of 2 mg of cortisone. The weight loss in the DCA-cortisone group was actually greater than that in the group given cortisone alone. However, despite the tendency of weight loss to decrease EST, the threshold of the animals on the combined steroid treatment was significantly higher than that of the animals given cortisone alone. A dose of 0.1 mg of cortisone counteracted the action of DCA during the first 20 days of the experiment. However, an appreciable, steady rise subsequently occurred. It is possible, although unlikely, that cortisone at this dose level acts similarly to the 0.5 and 3 mg doses of ACTH in inducing a secondary rise in threshold. It is noteworthy that the increase in EST induced by the 0.5 and 3 mg doses of ACTH started earlier than that induced by 0.1 mg of cortisone. In order to clarify this aspect of the problem and to explore the possible use of the cortisone-DCA antagonism as a method of assay for cortisone-like substances, additional experiments are being conducted in which doses of cortisone both smaller than 0.1 mg and intermediate between 0.1 and 2 mg are employed.

6. Baehr, G., and Soffer, L. J., *Bull. New York Acad. Med.*, 1950, v26, 229.

The concept has been developed in this laboratory(2,7-9) that administration of DCA induces a state of steroid hormone imbalance characterized by an excess of exogenous DCA and a deficiency of endogenous 11,17-oxysteroids (cortisone-like steroids); the deficiency is considered to be caused by (a) inactivity of the adrenal cortex as a result of inhibition of adenohypophyseal adrenocorticotrophic activity by DCA, and (b) antagonism between DCA and the 11,17-oxysteroids at their loci of action in tissues. The results presented here give additional experimental support to this concept. In a previous study(2) it was demonstrated that ACTH and ACE antagonize the action of DCA in decreasing brain excitability. The present experiments confirm the previous findings in regard to ACTH and in addition demonstrate that cortisone also antagonizes the EST-elevating property of DCA.

Summary. Rats treated with ACTH alone, in a dose of 1 mg thrice daily, initially showed a slight decrease in EST followed by a return to normal and subsequently a slight increase in threshold. ACTH in doses ranging from 0.001 mg to 10 mg effectively antagonized the action of DCA on the EST. Beyond the 12th day of hormone treatment, 0.001, 0.01, 0.05 and 10 mg of ACTH maintained the EST of DCA-treated rats at a normal or slightly less than normal level. Intermediate doses (0.5 and 3 mg) produced a secondary rise beginning about the 12th day of the experiment. Nevertheless, the final EST values in animals treated with 0.5 and 3 mg of ACTH were significantly less than in rats given DCA alone. Cortisone alone, in a dose of 1 mg twice daily, markedly reduced the EST of rats. This dose of cortisone given in combination with DCA resulted in an EST which was lower than normal but higher than that for animals treated with cortisone alone. Cortisone in a dose of 0.1 mg daily was able to counteract the EST-

7. Sayers, G., and Sayers, M. A., Recent Progress in Hormone Research, 1948, v2, 81.

8. Woodbury, D. M., Cheng, C. P., and Sayers, G., *Fed. Proc.*, 1949, v8, 172.

9. Cheng, C. P., and Sayers, G., *Endocrinology*, 1949, v44, 400.

elevating property of DCA for the first 22 days of the experiment. Thereafter the EST increased to a final value intermediate between normal and that of rats treated with DCA alone.

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Alterations in Long Bones of Young Albino Rats Induced by Dihydrotachysterol. (Hytakerol).* (18212)

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In the stages of transformation of ergosterol by ultra-violet radiation into a substance with antirachitic potency, Holtz and Schreiber(2) had determined the presence of two different factors, the antirachitic factor and a separate intermediate factor which they called the "Kalcinosefactor." A year later Windaus(3) reported the isolation of a toxic substance, tachysterol which he thought was a contaminant in highly effective antirachitic agents. Animal experimentation indicated that the dihydro-derivative of tachysterol had marked hypercalcemic effects, which suggested its use as a therapeutic agent in parathyroprivia (4,5). This drug was first used clinically by Holtz and its nature exhaustively restudied by von Werder(6). Albright and Ellsworth (7) had considered the action of parathormone was to raise the level of urinary phosphate excretion, and Albright(8) later demonstrated that the actions of parathormone, vitamin D and dihydrotachysterol were similar but that the action of the latter was more nearly like that of parathormone because it tended to increase urinary phosphorus excretion in contrast to that of vitamin D which increased intestinal absorption of calcium.

The present investigation was undertaken to determine the nature of changes occurring in growing bone resulting from toxic dosage of dihydrotachysterol and to compare these with changes reported, using other factors regulating bone architecture. The only such reference in English that we have found is a statement by McLean(9) that the lesions resulting from administration of vitamin D and dihydrotachysterol were similar and that no fibrous infiltration was observed as seen in hyperparathyroidism.

Methods. A group of 9 rats (A) 1 month old, were given daily subcutaneous injections over a period of from 30 to 35 days, total dosage varying from 3.75 mg to 5.5 mg of the crystalline dehydrotachysterol. Two litter mates were given daily injections of sesame oil and 2 others served as untreated controls (Table I). In 2 rats (Group B), 2 months old, the drug was given daily by stomach tube over a period of 10 days, total dosage being 1.03 mg and 1.16 mg of crystalline product. Again 2 litter mates were given an equivalent amount of sesame oil over the same

* Some of these experiments were presented by title at the American Association of Anatomists, April 5, 1950(1).

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2. Windaus, A., *Proc. Roy. Soc. London.*, Series B., 1931, v108, 568.

3. Holtz, F., and Schreiber, F., *Z. Physiol. Chem.*, 1930, v191, 1.

4. v. Brandt, T., Holtz, F., and Putschar, W., *Arch. f. exp. Path. u. Pharmakol.*, 1932, v167, 113.

5. Holtz, F., Gursching, J., and Kraut, H., *Arch. f. exp. Path. u. Pharmakol.*, 1934, v174, 51.

6. v. Werder, F., *Z. f. Physiol. Chem.*, 1939, v260, 119.

7. Albright, F., and Ellsworth, R., *J. Clin. Invest.*, 1929, v7, 183.

8. Albright, F., *Trans. Am. Assn. Phys.*, 1938, v53, 221.

9. McLean, F. C., *J.A.M.A.*, 1941, v117, 609.