Effect of Amphenone "B"* on Adrenal and Thyroid Function of Adult Ovariectomized Rats.† (19553)

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Hertz. Allen, and Tullner(1,2) have shown that the administration of Amphenone "B" causes hypertrophy of the thyroid and adrenal glands of rats. This observation has been confirmed(3,4) but, thus far, the degree of function of these hypertrophied glands has been studied to only a limited extent(3,4). Hertz, Tullner, and Allen(2) have, however, expressed the suggestion that Amphenone "B" might be blocking the function of the adrenal gland much like thiouracil blocks the thyroid gland. With this in mind, experiments are here reported on the effect of the drug on thyroid function as measured by oxygen consumption. and on adrenal function as measured by electroshock seizure threshold (EST), thymus weight, and histological examination of the sectioned adrenal.

Methods and materials. Twenty-six adult rats ovariectomized 42 days prior to the time of drug administration, and 8 of which had been adrenalectomized 10 days previous to that time, were divided into 6 groups as shown in Table I. All rats received either tap water or 1% saline *ad libitum* in addition to pulverized stock diet (Purina Dog Chow) with or without the incorporation of Amphenone "B" (5) at a concentration of 0.6%. Inasmuch as saline is given advantageously to adrenalectomized rats, it was made available to 2 groups of animals (Table I) because of the possible decreased function of the adrenal(2). The Amphenone "B" as mixed in the food provided approximately 200 mg/kg/day to the average rat; the approximate average food consumption after the incorporation of the drug into the diet, was 33 g/kg body weight/day, as compared with a control intake of 50 g/kg body weight/day. For one week prior to the time of drug administration, control determinations of EST were made on all rats by the general technics previously reported by Woodbury, Sayers, Davenport, Goodman, and Cheng(6). The third control EST determination, made just before the first day of drug administration, was used as the control EST from which percentage changes were calculated for the following test period. During this period, the EST was determined 3 times per week. After 9 days of drug administration, the animals were fasted for 24 hours in preparation for oxygen consumption measurements. On the morning of these tests, 25 mg of Amphenone "B" in water solution (or a comparable volume of saline in the case of the controls) was injected subcutaneously into each test rat in lieu of the drug which the animal would normally have received in its food. The mean oxygen consumption of each rat was determined during 3 consecutive 15-minute periods(3). Immediately following the measurement of oxygen consumption, each rat was allowed stock diet (no drug) and water, or 1% saline, *ad libitum,* for a period of 30 minutes. Following this feeding period, the rats were put into individual metabolism cages, the post-prandial urine was collected for a 3-hour period, and a qualitative Fehling's test for urinary reducing substances was made thereon.‡

At the end of 17 days of drug administration, all rats were anesthetized with ether. After axillary blood had been collected from each for a blood glucose level determination (7), the rats were sacrificed and autopsied. The uteri as well as adrenal, thyroid, pituitary, and thymus glands were removed and weighed on a torsion balance. One adrenal gland from

*1,2-bis (p-Aminophenyl) - 2-methylpropanone-1 dihydrochloride.

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‡ Fehling's test using 12 drops of urine per 5 cc of total solution.

TABLE I. Effect of Amphenone "B" on Oxygen Consumption, Possible Glycosuria and Adrenal Sudanophilia of Ovariectomized Adult Rats.*

Group No.	No. of rats†	Description‡	Drinking fluid	Oxygen utilization§ (l/m²/hr)	"P"	Possible glycosuria‖	Histological adrenal sudanophilia
III	5	Ovarex control	Water	7 ± .6		1/4	1+
IV	5	" test	"	5.8 ± .6	.02	4/4	3+
VI	4	" control	Saline	6.5 ± .9		1/4	1+
V	4	" test	"	5.9 ± .7	.4	3/3	4+
II	4	Adrenex-ovarex control	"	5.8 ± .8		1/4	
I	4	" " test	"	5.3 ± .4	.3	2/2	

* All values expressed as mean ± S.D.

† During the experiment, 2 rats died in Group I, 1 in Group IV, and 1 was sacrificed in Group VI (Fig. 1-3).

‡ All test rats received Amphenone "B" concentration of .6% in pulverized stock diet for 17 days prior to autopsy.

§ Oxygen utilization of the rats was determined 10 days after onset of Amphenone "B" treatment. "P" was calculated by the "t" test(9).

‖ Qualitative post-prandial glycosuria expressed as No. of rats having detectable reducing substances in the urine (Fehling's test) over No. of rats tested.

each rat was placed in 10% neutral formalin. Following a >24-hour period of fixation, these adrenals were sectioned on a freezing microtome, and representative sections from each gland were stained en masse in a single staining rack. The stains employed were Sudan IV, Sudan IV counterstained with hematoxylin, and hematoxylin counterstained with phloxine(8).

Results. The results of the EST experiments indicate that the administration of Amphenone "B" caused an elevation of EST in adrenalectomized, ovariectomized rats (Fig. 1) and that the elevation was not as great in the Amphenone "B"-treated ovariectomized rats with adrenals (Fig. 2 and 3). In the rats that had adrenals, there was also a reversal of trend in EST concurrent with the 24-hour fast. This reversal in trend was continuous for the group receiving water (Fig. 2), but only temporary for the group receiving saline (Fig. 3).

The degree of trend reversal was in inverse proportion to the amount of sudanophilic ma-

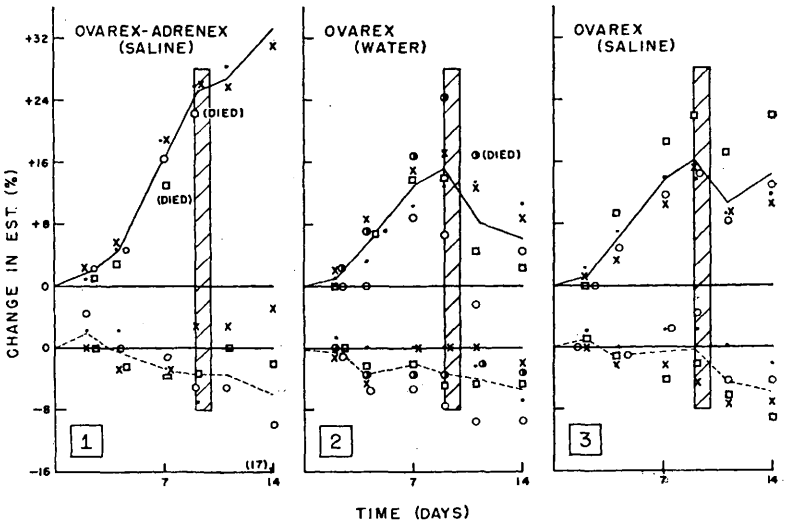

FIG. 1-3. Electroshock Seizure Threshold: Curves represent means; symbols represent EST of individual rats of each respective group; bar shows time of 24-hr fast. Upper curve, Amphenone "B"-treated with .6% in food. Lower curve, respective controls.

TABLE II. Effect of Amphenone "B" on Relative Weights of Endocrine Glands at Time of Autopsy of Ovariectomized Adult Rats.*

Group No.†	Final body wt (g)	Pituitary wt (mg/100 g)	Adrenal wt (mg/100 g)	Thymus wt (mg/100 g)	Thyroid wt (mg/100 g)	Uterine wt‡ (mg/100 g)
III	237 ± 24	4.4 ± 1	19 ± 7	223 ± 38	5.3 ± 1	11 ± 4
IV	185 ± 20	5.3 ± 1.4	33 ± 7	78 ± 19	14.2 ± 1.8	25 ± 6
VI	240 ± 13	5.3 ± .9	18 ± 1	209 ± 39	4.8 ± .7	9 ± 4
V	191 ± 9	5.3 ± .8	34 ± 9	128 ± 30	14.6 ± 2	19 ± 5
II	215 ± 39	4.5 ± .7	—	298 ± 25	8 ± 1	13 ± 2
I	190 ± 2.8	5.7 ± .3	—	176 ± 57	13.4 ± 3.5	23 ± 1

* All values expressed as mean ± S.D., with gland weights given as mg/100 g final body wt. See Table I for description of groups.

† Group numbers refer to same animal groups as those shown in Table I.

‡ Uterine portion weighed was only the body itself (often termed cervix) and not the cornua.

terial present in the adrenal (Table I), in that the group of rats having only a temporary reversal of the rising EST showed the greatest concentration of sudanophilic material.

As another measure of adrenal activity, it should be noted that the mean thymus weight (Table II) of the rats decreased according to the following order of animal groups: II, adrenalectomized, ovariectomized, control rats receiving saline; III, ovariectomized, control rats receiving water; VI, ovariectomized control rats receiving saline; I, adrenalectomized, ovariectomized test rats receiving saline; V, ovariectomized test rats receiving saline; and IV, ovariectomized test rats receiving water. The adrenal glands of all Amphenone "B"-treated rats were increased in weight (Table II).

The thyroid weight of all Amphenone "B"-treated rats was increased (Table II). However, only in the group of ovariectomized rats receiving water as the drinking fluid was there a statistically significant lowering of the oxygen consumption of the treated rats as compared with the control rats (Table I).

The uterine weight was increased consistently with Amphenone "B" treatment (Table II), whereas it is doubtful that the pituitary weight was affected.

Every animal receiving the drug showed a positive test for reducing substances in the urine, whereas only one-fourth of the control rats showed the same (Table I). Measurement of the blood glucose level showed that in no case was it above 150 mg% in either the control or Amphenone "B"-treated groups. It should be mentioned that Amphenone "B" failed to give a positive test when 10 mg were heated in 5 cc Fehling's solution; however, it is not known whether this positive reducing test is due to glucose, a metabolite of Amphenone "B", or some other undetermined substance.

By gross appearance, the adrenals of Amphenone "B"-treated rats were larger and more yellow in color than those of the normal animals. Histologically, there was a tremendous amount of sudanophilic material in all 3 layers of the adrenal cortex, but it was most evident in the *zona fasciculata*. In general, many of the cells of the cortex appeared to show a fatty metamorphosis-like change with small vacuoles observable in the cytoplasm but with little nuclear change. Adrenals from rats receiving Amphenone "B" with saline as the drinking fluid showed much greater changes of the type mentioned above than did those from rats receiving Amphenone "B" and water.

Discussion. The lower oxygen utilization of all Amphenone "B"-treated rats (statistically significant in one of 3 groups) would suggest a possible decrease in function of the thyroid gland. Related experiments have shown that Amphenone "B" is also a potent depressant of radioactive iodine uptake in the rat thyroid (4,10). On the other hand, the present evidence suggests an increase in function of the adrenal gland of Amphenone "B"-treated rats. Cortisone has been reported to depress the EST(6), and in this experiment, the presence of the adrenal in Amphenone "B"-treated rats

is associated with a smaller elevation of EST than that observed in Amphenone "B"-treated rats from which the adrenal had been removed (Fig. 1-3). This would suggest that increased endogenous cortisone production was counteracting the primary effect of the Amphenone "B". The super-imposed stress of the 24-hour fast may have caused an even greater activity of the adrenal as noted by reversal in trend of the rising EST of Amphenone "B"-treated rats (Fig. 1-3); this is a further suggestion of increased endogenous cortisone production.

If the degree of thymic involution is considered a measure of adrenal activity(11) then these data are in agreement with the EST data. For instance, of the Amphenone "B"-treated rats: the ovariectomized, adrenalectomized rats receiving saline showed the greatest elevation of EST as well as the greatest thymus weight; the ovariectomized rats receiving saline showed a lesser elevation of, and temporary reversal of EST, as well as lesser thymus weight; the ovariectomized rats receiving water showed the least elevation of, and continued reversal of EST, as well as smallest thymus weight.

The histological study of the adrenals showed the adrenals of Amphenone "B"-treated rats on saline to contain more sudanophilic material and to display a greater degree of fatty metamorphosis-like change than the glands of Amphenone "B"-treated rats receiving water. This suggests that either the adrenal desoxycorticosterone-like function may be implicated in the responses to Amphenone "B" administration, or that the increased sudanophilia is merely an indication of altered adrenal activity in response to the additional saline intake. Morphologically, it would appear as if the glands from the rats receiving water would be more functional than those of rats receiving saline and this is borne out by the EST test as well as the thymus weight measurements.

Glycosuria has been reported to follow the administration of cortisone to normal rats(12). Therefore, the possible glycosuria here observed might be the result of increased adrenal cortical activity which in turn would be in agreement with the EST and thymus weight studies. On the other hand, however, two adrenalectomized rats also showed an unexplained possible glycosuria.

The increase in uterine weight was similar to that previously reported by Hertz, Allen, and Tullner(1).

All data of this report point toward, or are consistent with, an increase in activity of the cortisone-like aspect of the adrenal in the Amphenone "B"-treated rats. However, it is unknown as to whether this is a direct action of the drug or whether it is mediated through the general alarm reaction.

Summary. When compared with appropriate controls, it appears as if rats treated with Amphenone "B" show a decrease in function of the hypertrophied thyroid gland as measured by oxygen consumption but a probable increase in cortisone-like function of the hypertrophied adrenal gland as measured by electroshock seizure threshold, thymus involution, reaction to 24-hour fast, and histological study of the adrenal.

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