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Effects of Amphenone "B"\* on Thyroid, Adrenals, and Genital Tract  
of the Female Rat. (18286)

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We have previously described the progesterone-like effect of Amphenone "B"\* on the rabbit(1). The present report will describe the marked adrenal and thyroid hypertrophy as well as the trophic effects on the genital tract exerted by this compound in the female rat (Fig. 1).

*Materials and methods.* Amphenone "B" was synthesized by the method of Allen and Corwin(2). The compound was stored in a vacuum desiccator and for administration dissolved in triple-distilled water not more

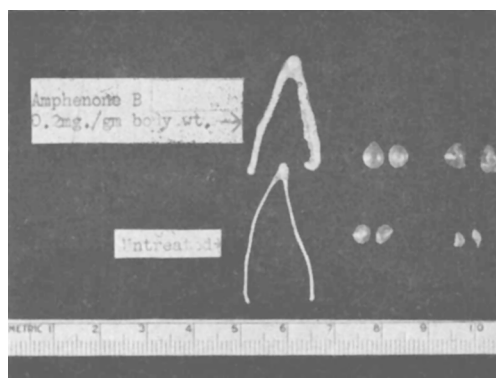


Fig. 1.

Uterus, adrenals, and thyroid of treated and untreated ovariectomized rats.

\* 1,2-bis-(p-Aminophenyl)-2-Methylpropanone - 1 Dihydrochloride.

1. Allen, Hertz, and Tullner, *Proc. Soc. Exp. Biol. and Med.*, 1950, v74, 632.

2. Allen and Corwin, *J.A.C.S.*, 1950, v72, 117.

than 3 days before use. Solutions were stored in the refrigerator when not in use. The

TABLE I. Effect of Amphenone "B" on Adrenal, Uterine, and Thymus Weight.

Series	Age at ovariectomy (days)	Age at autopsy (days)	No. of rats	No. of rats surviving	Dose* in mg	Body wt at autopsy (g)	Adrenal wt (mg)	Uterine wt (mg)	Thymus wt (mg)
A	49	112	10	10	50	208 ± 17	164 ± 42	101 ± 27	226 ± 49
"	"	"	"	9	45	226 ± 14	151 ± 18	102 ± 14	222 ± 21
"	"	"	"	10	40	231 ± 9	139 ± 23	92 ± 23	249 ± 29
"	"	"	"	9	30	244 ± 14	116 ± 17	78 ± 20	373 ± 157
"	"	"	"	10	25	252 ± 7	103 ± 10	75 ± 15	373 ± 62
"	"	"	"	10	0	260 ± 31	59 ± 3	55 ± 7	539 ± 96
B	25	105	10	3	75	184 ± 1	175 ± 10	84 ± 9	172 ± 36
"	"	"	"	10	50	211 ± 15	188 ± 27	80 ± 12	245 ± 53
"	"	"	"	10	25	230 ± 10	98 ± 8	63 ± 8	359 ± 101
"	"	"	"	10	12.5	263 ± 8	76 ± 10	45 ± 6	565 ± 73
"	"	"	"	10	0	268 ± 15	67 ± 7	34 ± 8	648 ± 50
C	28	84	5	5	50	225 ± 12	205 ± 16	66 ± 6	236 ± 40
"	"	"	"	5	25	231 ± 8	108 ± 14	43 ± 7	391 ± 29
"	"	"	"	5	12.5	239 ± 6	86 ± 5	35 ± 6	499 ± 115
"	"	"	"	5	0	279 ± 19	70 ± 5	27 ± 7	818 ± 24

\* Daily oral dose given in from 0.5 to 1.0 cc aqueous sol. for 5 consecutive days; autopsy 24 hr after last dose.

TABLE II. Effect of Amphenone "B" on Thyroid, Adrenal, and Uterine Weight.

Group	Treatment in addition to Amphenone "B"	Days treated with Amphenone "B"	Daily dose, § mg	No. of animals	Age at autopsy (days)	Body wt at autopsy, g	Adrenal wt, mg	Uterine wt, mg	Thyroid wt, mg	Thymus wt, mg
I	Adrenalectomy† and ovariectomy*	3	40	10	93	234 ± 19		73 ± 13		749 ± 94
		0	0	3		217 ± 9		29 ± 4		972 ± 90
II	Cortisone‡ and ovariectomy*	4	40	6	91	185 ± 7	61 ± 5	70 ± 12	12 ± 3	91 ± 12
II(a)	Ovariectomy*	4	40	7		226 ± 9	129 ± 11	59 ± 5	15 ± 4	388 ± 96
III	Ovariectomy*	22	12	5	50	111 ± 5	75 ± 7	69 ± 17	30 ± 9	232 ± 23
		0	0	5		125 ± 10	40 ± 2	22 ± 3	9 ± 1	522 ± 94
IV	Ovariectomy*	15	38	4	87	182 ± 9	103 ± 10	145 ± 10	42 ± 11	288 ± 71
		0	0	4		238 ± 12	66 ± 6	27 ± 3	14 ± 2	748 ± 47
V	Ovariectomy*	16	18	5	79	175 ± 13	141 ± 20	58 ± 7	40 ± 4	284 ± 51
		0	0	5		219 ± 12	66 ± 13	30 ± 3	16 ± 3	686 ± 61

\* Ovariectomized at 24 days of age.

† Adrenalectomized on day before starting Amphenone "B"; maintained on saline for drinking water.

‡ 5 mg cortisone acetate given daily subcutaneously in 0.2 cc aqueous suspension beginning 2 days before Amphenone "B".

§ Amphenone "B" given daily in 0.5 to 1 cc aqueous solution by stomach tube.

daily dose was administered by stomach tube in from 0.5 to 1.0 cc of water. Female rats of the Holtzman strain were employed. Ovariectomy was done when the animals were 3 to 7 weeks of age. The animals were sacrificed by exposure to ether or chloroform and their

organs were weighed to the nearest milligram on a Roller-Smith torsion balance. Tissues were fixed in 10% formalin and stained by routine hematoxylin and eosin.

*Results and discussion.* The genital tract of previously ovariectomized adult and juven-

ile rats exhibited an atypical trophic response to Amphenone "B" (Tables I, II; Fig. 1). The uteri were grossly enlarged and were distended with a mucoid secretion somewhat more viscous than that usually seen after estrogen administration. The vaginal membrane was broken down in most cases and a mucoid fluid could be readily expressed from the vagina. Microscopic examination of vaginal smears revealed a mixed smear consisting of nucleated and cornified epithelial cells in about equal numbers, leucocytes being only rarely seen. The uterine weights following any given dosage were quite variable and reached a maximum at about a 3- to 4-fold increment above the controls.

The adrenals of adult animals treated for 3 to 5 days showed a remarkable degree of hypertrophy (Tables I, II; Fig. 1). These enlarged adrenals have a normal yellow color and are readily differentiated from the red or brown adrenal seen in non-specific intoxications. Table I shows the quantitative relationship between the dose of drug and the degree of adrenal stimulation. This indicates that the effect is not a "trigger-response," but is quantitatively related to the amount of drug administered.

The daily ingestion of Amphenone "B" over a period of 15 to 22 days in the growing rat leads to a striking hypertrophy of the thyroid (Table II; Fig. 1). In some cases this represents a four-fold increment in thyroid weight. These enlarged glands are grossly hyperaemic and very friable.

The adrenal enlargement was absent in 7 hypophysectomized animals treated for 4 to 7 days with daily doses of 0.1 mg to 0.2 mg per gram of body weight of Amphenone "B". This indicates that the adrenotrophic effects are mediated through the pituitary gland. Moreover, the adrenal stimulation can be prevented by the simultaneous administration of cortisone which is known to suppress endogenous adrenotrophin formation(3),(Table II).

It is apparent that the hypertrophied adrenals are producing corticoids because the thymus involutes substantially in all animals

showing adrenal stimulation following Amphenone "B" and such thymic involution is lacking in previously adrenalectomized animals treated with the Compound (Tables I, II). That the uterine hypertrophy seen in ovariectomized Amphenone "B"-treated animals is a direct effect on the genital tract is shown by the persistence of this response after either hypophysectomy or adrenalectomy (Table II).

Preliminary histological study of the grossly affected organs indicates that the adrenals retain essentially normal zonal relationships except for the compression of the medulla by the enlarged cortex. The cortical cells are laden with lipid and the individual cells are substantially enlarged.

The hyperplastic thyroids are almost colloid-free and the secretory epithelium is of a tall, columnar type. The vascular and lymph channels are widely dilated.

The acute toxicity of Amphenone "B" has been found to be quite high. Thus a dose of .4 mg/g body weight given orally in 0.5 cc H<sub>2</sub>O will regularly kill adult female rats. Since its maximum effective dose in terms of uterine, thyroid, and adrenal stimulation is about 0.25 mg/g, the margin of safety of this compound must be considered fairly small. Moreover, the nature of the acute toxic effects is of some interest, since animals exposed to a sublethal dose show a profound degree of narcosis lasting for from 1 to 6 hours, after which the animal resumes normal behavior. At lethal doses this narcosis will persist for 10 to 12 hours preceding the animal's death.

It is apparent that Amphenone "B" not only exerts a marked trophic action on the genital tract of the ovariectomized rat, but that it also stimulates increased thyrotropic and adrenotropic activity in the anterior pituitary. These endocrine effects coupled with its depressant action on the central nervous system and its acute toxicity render Amphenone "B" and its analogues worthy of further biological and pharmacological investigation.

*Summary.* Amphenone "B" administered either orally or parenterally, exerts an atypical trophic action on the genital tract of the

ovariectomized female rat. It also effects a rapid hypertrophy of the adrenals and thyroid. These adrenal effects are lacking in the hypophysectomized rat but the uterine stimulation is unaffected either by hypophysectomy or adrenalectomy. The compound also pos-

sesses an acute toxicity which is characterized by marked narcosis leading to death. The maximum effective dose is approximately two-thirds of the lethal dose.

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### Failure of Cockerel Comb and Testis Development on Sesame Meal and its Prevention by Vitamin B<sub>12</sub>.<sup>\*†</sup> (18287)

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During the course of a nutrition experiment designed by one of us (A.R.P.), it was observed that when the ration contained 70% sesame meal there was marked failure of development of combs and testes. This condition was prevented by adding Vit. B<sub>12</sub> to the diet. The source of the Vit. B<sub>12</sub> was Merck APF Supplement No. 3.

**Methods and materials.** Single comb White Leghorn cockerels were fed a Vit. B<sub>12</sub> depletion diet based on soy bean (sodium proteinate) which contained 0.05% iodinated casein (Protamone). These birds were distributed evenly by weight into groups of 15 each, eliminating extremes in weight. The experimental rations consisting of sesame meal 70%, yellow corn meal 26%, dicalcium phosphate 2%, pulverized limestone 1%, salt mixture 0.5%, choline chloride 0.17%, Vit. A, D<sub>3</sub>, E, and K in oil 0.25%, B-complex Vitamins (except B<sub>12</sub>) in starch 0.10%, and sufficient iodized calcium carbonate to supply 1 mg iodine per lb were fed for 2 weeks. For purposes of comparison, a group of chicks depleted of Vit. B<sub>12</sub> was also fed a standard chick starter. Because the basal diet was deficient in lysine(1,2), certain groups were

fed additional lysine in the form of DL-lysine monohydrochloride monohydrate to provide 0.2% L-lysine. Since the requirement for Vit. B<sub>12</sub> on high protein diets is increased(3), sufficient APF supplement was added to provide for this deficiency (25 µg B<sub>12</sub> per lb of ration). This amount supplied approximately 10 times more Vit. B<sub>12</sub> than the standard chick starter mash used for comparison.

**Results.** Table I gives the gross effects of the various supplements at four weeks of age. During the experiment it was observed that the birds on the high sesame meal-Vit. B<sub>12</sub> deficient diet (Group 2) had underdeveloped, pale combs, whereas the combs of chicks receiving the APF supplement with or without lysine (Groups 3 and 5) showed normal red color and size comparable to those receiving the standard chick starter (Group 1).

Since comb growth and color are influenced by testicular function, the testes were examined to determine the state of their development. Testes produced on the basal diet were subnormal in weight, whereas the ones produced by an addition of both lysine and Vit. B<sub>12</sub> showed an increase in weight of 88% over those on the basal diet. The histological differences were even more remarkable. As expected, the testes from chicks on

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† Colorado Agricultural Experiment Station Scientific Journal Series No. 336.

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2. Grau, C. R., *J. Nutrition*, 1948, v36, 99.

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