TABLE I. Surgical Procedures Under Amphenone Anesthesia.

Animal	Body wt (kg)	Dose for induction (mg/kg)	Length of induction (min)	Total dose (mg/kg)	Duration of total injection (min)	Duration of anesthesia (min)	Surgical procedure*	Length of operation^T (min)	Recovery (min)
Dog 1	10.15	118	5	197	22	70	1	30	140
2	2.39	119	4	218	26	46	1, 2	24	49
3	3.5	230	4	320	27	34	1	17	82
Rabbit 1	1.98	150	3	200	4	16	1, 2	11	16
2	2.20	153	4	254	7	9	1	9	19
3	1.69	118	3	213	7	13	1	7	39
4	2.82	84	3	200	5	17	1	10	32
5	3	195	4	293	11	20	1	12	—

* 1 = bilateral ovariectomy; 2 = subtotal hysterectomy.
^{T} = time from incision to closure. — = not observed.

sufficient to permit complete anesthesia without major respiratory depression and with rapid recovery. The combination of endocrinological and anesthetic properties of Amphenone "B" parallels that previously observed for progesterone and other steroids.

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Effect of Amphenone "B" on Adrenal, Thyroid and Testes.* (19267)

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Recent work by Allen, Hertz, and Tullner (1,2) has shown that when Amphenone "B"‡ (3), a substituted desoxybenzoin, was injected subcutaneously into female rabbits or given intragastrically to female rats, it produced marked thyroid and adrenal enlargement as well as progestation. The adrenomegalic effects were prevented by the administration of cortisone without reducing the goitrogenic effect and, conversely, the administration of thyroxine inhibited thyroid enlargement without affecting the adrenal enlargement(4).

These observations, and the fact that Amphenone "B" did not cause enlargement of the adrenal and thyroid in hypophysectomized animals, suggested that "These responses were brought about through a pituitary mechanism similar to that by which thiouracil induces thyroid hypertrophy"(5). Whereas, the evidence indicates that Amphenone "B" increases the secretion of ACTH and TSH, the data available do not preclude the possibility of an increased production of thyroid and adrenal hormones rather than a decreased production, as would be expected in the case of a blocking agent such as thiouracil. We have extended the foregoing studies in an effort to explain more fully the alterations of thyroid and adrenal size and function and are reporting the results in this paper.

Experimental. Sprague-Dawley rats were

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‡ 1,2-bis-(p-aminophenyl)-2 methyl propanone-1 dihydrochloride, kindly supplied by Dr. E. J. Fellows of the Smith, Kline and French Co., Philadelphia, Pa.

used throughout. Two types of experiments were conducted, one relatively chronic and one acute. In the chronic study, 22-day-old male rats (34-44 g) were injected subcutaneously with 5 mg of Amphenone "B" in 0.5 ml of saline at 8:00 A.M. and 5 mg in 0.2 ml of sesame oil at 7:00 P.M. (25 mg per 100 g body weight daily) for a period of 11 days. Eight rats were given Amphenone "B" and 12 served as controls. Twenty-four hours before sacrifice all animals were given 50 μc of I^{131} subcutaneously. Approximately 15 hours after the last dose of Amphenone "B" in oil, the animals were anesthetized with nembutal and immediately before death the adrenal glands were removed, weighed and one gland placed in 30% KOH. Blood was drawn from the aorta for eosinophile counts(6). The thyroids were then removed, weighed and one lobe placed in *2N* NaOH and finally, thymi and testes were removed and weighed. The remaining lobe of the thyroid, the other adrenal gland and the testes were placed in formalin prior to sectioning and staining. Adrenal cholesterol determinations were carried out using a modification of the Schoenheimer-Sperry method(7). The thyroid tissue was homogenized to facilitate dissolution in the NaOH, aliquots pipetted onto planchets and the radioactivity determined by means of a Geiger-Müller counting system. In an effort to determine the effects of Amphenone "B" over a shorter period of time, and, especially to establish more precisely its effect on thyroid I^{131} concentration, an acute experiment was devised. This was divided into three sections. Female rats weighing between 130 and 220 g were used for each determination, the weights being relatively constant in each section; all injections were by the subcutaneous route. The first section of the acute experiment was planned in such a way that Amphenone "B" was given both before and after the injection of radioiodine in order that a maximum effect on thyroid I^{131} concentration might be produced. TSH§ was given to compare its action with that of Amphenone "B". The short-term effects of the compound on adrenal cholesterol concentration were also observed in this section. Seven groups of 4 rats each were used. Three groups were given Amphenone "B" (10 mg in saline at 0 hours, 5 mg in saline at 4 hours, 10 mg in sesame oil at 7 hours, and 10 mg in saline at 18 hours; a total of approximately 20 mg per 100 g of body weight), 50 μc of I^{131} at 6 hours, and killed at 24 hours. Two groups received I^{131} alone and served as controls. In all 5 groups the measurements consisted of thyroid weight and I^{131} concentration, adrenal weights and cholesterol concentration and thymus weight. A sixth group received I^{131} at 6 hours, 12.5 mg of TSH at 19 hours and were sacrificed at 24 hours. A seventh group was given Amphenone "B", I^{131}, and TSH at the times mentioned above. Thyroid weights and I^{131} concentrations alone were determined in the last 2 groups. In the second section of the experiment, radioiodine was given 16 to 17 hours before the administration of Amphenone "B" or TSH. It was hoped that this dosage schedule would demonstrate whether or not Amphenone "B" produced an increased release of thyroid I^{131}. Eight animals were used in each group. The dosage schedule was as follows: 50 μc of I^{131} was given at 0 hours, 10 mg of Amphenone "B" in saline at 16 hours, 12.5 mg TSH at 17 hours and the rats were killed at 22 hours. One group was given all the above, one group was given I^{131} and TSH, one group was given I^{131} and Amphenone "B", and a fourth, control group, was given I^{131} alone. Thyroid weight and I^{131} concentrations were determined as above. In a third experiment, 3 groups of 8 animals were used. Here, the dosage schedule was arranged so that the possible effects of Amphenone "B" on iodine uptake by the thyroid might be demonstrated. The animals were given Amphenone "B" (10 mg in saline at 0 hours, 10 mg in saline, and 10 mg in oil at 6 hours, and 10 mg in saline at 16 hours) 12.5 mg of TSH at 16 hours, 50 μc of I^{131} at 16 hours and were killed at 22 hours. One group received all the above, one group received TSH and I^{131}, and one group, the control, I^{131} alone. Thyroid size and radioiodine concentration were determined.

Results. In the chronic experiment (Fig.

§ Kindly supplied by Dr. Harley Cluxton, Armour and Co., Chicago, Ill.

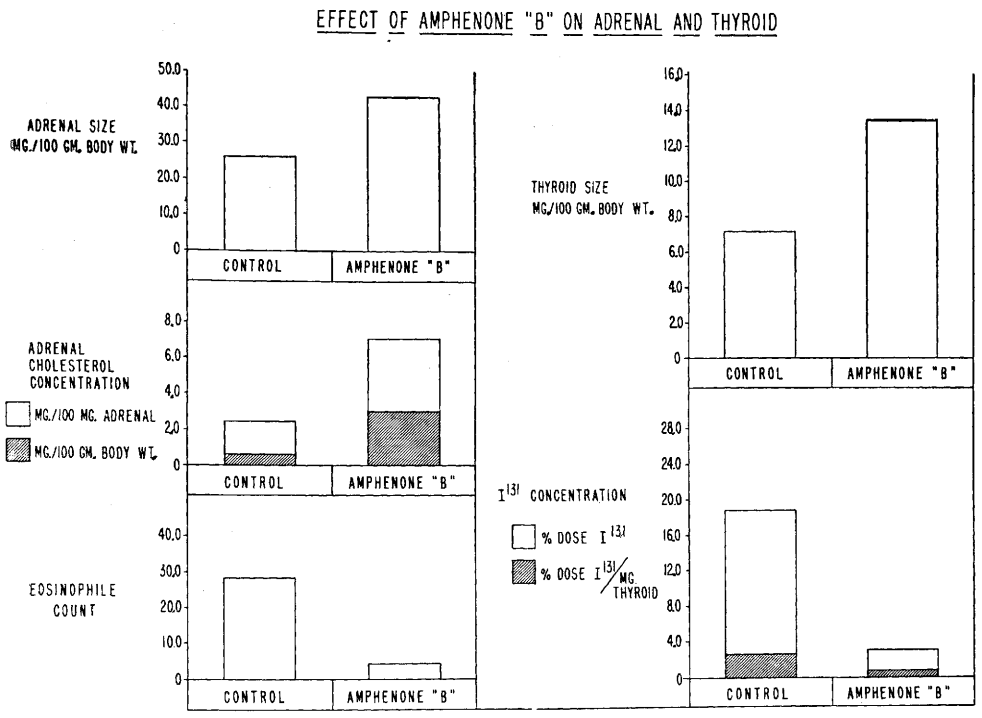

FIG. 1.

1) the adrenal size increased approximately 60% with the administration of Amphenone "B". Moreover, the adrenal cholesterol concentration increased about 200%. At the same time the number of circulating eosinophiles decreased to 12% of that of the controls. The thyroids increased in size from an average of 7.4 mg per 100 g body weight in the controls to 13.6 mg in the treated animals, while the I^{131} content in the Amphenone "B" rats fell to 16% of that of the controls. All animals were weighed before, during and at the end of the experiment and both the controls and the Amphenone "B" treated animals exhibited a constant weight gain.

Thymic weight decreased from 300 $\pm$ 11.8 mg per 100 g of body weight (mean $\pm$ standard error) in the controls to 252 $\pm$ 25.3 mg in the Amphenone "B" treated animals. The testes decreased in size from 773 $\pm$ 48.6 mg per 100 g body weight to 624 $\pm$ 41.2 mg. The figures for the testes are statistically significant, the P value being less than 0.05. Those for the thymi are significant only at the 10% level as P is greater than 0.05.

The acute effects of Amphenone "B" on the thyroid are shown in Fig. 2. It will be seen that in this experiment, with the administration of Amphenone "B" on four occasions within a 24-hour interval, the effect on I^{131} concentration is even more striking than in the chronic group. The concentration of I^{131} in the thyroid was less than in the controls whether Amphenone "B" was given before or after the administration of radioiodine. In addition, TSH when given alone at the time intervals indicated, produced a decrease in I^{131} concentration whether expressed as total content or as concentration per mg of thyroid. The combination of Amphenone "B" and TSH resulted in no significant difference in I^{131} concentration from that seen with Amphenone "B" alone. Where both substances were given before the administration of I^{131}, although the total radioiodine in the hypertrophied gland was equal to that of the control the concentration in the gland was low. In the acute experiment there was no significant change in adrenal size or adrenal cholesterol concentration. There was no change in thymic size of

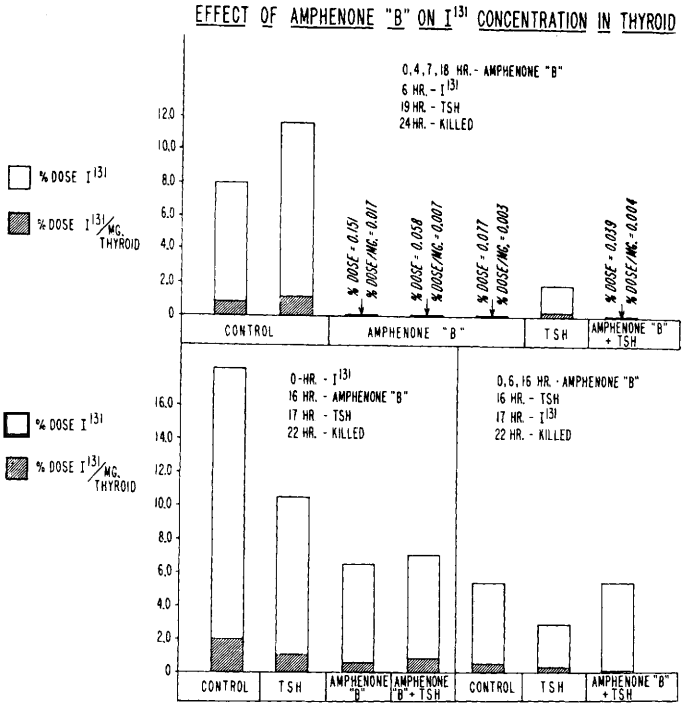

FIG. 2.

Amphenone "B" treated animals within this short period.

Discussion. Adrenal. The increase in adrenal size observed in our experiments confirms that reported originally by Hertz *et al.* (2). The fact that they did not find this effect in hypophysectomized animals and that the response was diminished or absent in animals given cortisone is good evidence that Amphenone "B" produces an increased secretion of adrenocorticotropin. Not only an absolute but a marked relative increase in adrenal cholesterol was noted in all Amphenone "B" treated animals. Sayers and coworkers(8) have shown that although single doses of ACTH result in depletion of adrenal cholesterol, more prolonged administration of this hormone may produce an elevation in adrenal cholesterol concentration, providing determinations are made 16 hours or more following the last injection of ACTH. This evidence suggests that Amphenone "B" causes adrenal enlargement by increasing the secretion of ACTH. A marked fall in circulating eosinophiles was noted in animals treated with Amphenone "B" over an 11-day period. This finding indicates that despite accumulation of large amounts of cholesterol, the hypertrophic adrenals are capable of secreting sufficient quantities of steroid to cause such a response. Hence, Amphenone "B" does not have an effect on the adrenals analogous to the effect of thiouracil on the thyroid since the adrenals are able to produce an increased amount of steroid. Were this a "thiouracil-like" effect of such a degree as to produce the marked hypertrophy and increase in cholesterol concentration seen in these experiments, one would expect to find almost complete blocking of adrenal hormone secretion.

Thyroid. Despite an almost 2-fold increase in thyroid size observed in Amphenone "B" treated animals, there was a marked reduction in the total amount of I^{131} and hence an even more marked decrease in I^{131} concentration. The latter effect was observed in both chronic and acute experiments and was most pronounced in the acute, where Amphenone "B" was given at shorter intervals over a relatively short period of time. It is known that TSH

promotes both an increased uptake of I^{131} by the thyroid and an increase in its secretion into the blood as thyroxine. With the dosage schedules of all sections of our acute experiment the latter phase apparently predominated, as in all instances I^{131} concentration was significantly lower following the administration of TSH than in the control. Amphenone "B" produced an effect on thyroid I^{131} concentration similar to that of TSH. The acute experiments were originally designed to show whether the drug exerts its effect primarily on the uptake of I^{131} or on its release. Since, contrary to expectations, thyroid I^{131} concentration was low whether Amphenone "B" was given before or after a tracer dose of I^{131} it may be that both factors are involved or, more likely, the action of the drugs administered was exerted over such a length of time as to obscure interpretation as to mode or site of action. Studies are in progress to clarify this point. These observations indicate therefore, that the action of Amphenone "B" on the thyroid may be through direct and immediate stimulation of production of TSH by the pituitary and/or due to a blocking effect such as that seen with thiouracil.

Testes. In the chronic experiment, Amphenone "B" produced a decrease in the size of the testes, although no consistent diminution in size or number of any one cell type was observed. Inasmuch as the compound is known to have a progestational action, it may be postulated that its presence caused a diminished secretion of FSH by the pituitary leading to testicular atrophy. Similarly, the presence of excessive amounts of adrenal corticoids following Amphenone "B" administration may also have caused a reduction in FSH secretion.

Summary. (1) Enlargement of rat adrenal and thyroid glands following the administration of Amphenone "B", observed by Hertz, Allen, and Tullner, was confirmed. (2) The adrenal enlargement was associated with an increase in adrenal cholesterol concentration. At the same time, the adrenals remained capable of secreting sufficient steroid to produce a marked fall in circulating eosinophiles. Amphenone "B" may exert its adrenomegalic effect by direct stimulation of the adenohypophysis to produce ACTH rather than indirectly as seen, for example, in thiouracil-induced goiter. (3) In addition to thyroid enlargement, a marked decrease in I^{131} concentration in the gland was observed. This could be due to a thiouracil-type of action, although the effects noted within the time relationships of our experiments were similar to those produced by TSH and hence possibly the result of increased secretion of radioiodine rather than a decreased uptake. (4) Decrease in size of the testes was observed in animals given Amphenone "B". It is postulated that this may be due to a diminished production of FSH brought about either by elevated blood levels of Amphenone "B" itself or by an increase in adrenal corticoids as a result of adrenal stimulation.

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