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EFFECT OF RESERPINE ON GROWTH AND SEXUAL DEVELOPMENT OF CHICKENS

BY

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One-month-old male chickens were given injections of 1 mg, 2 mg and 4 mg/kg of reserpine. The injections were repeated at weekly intervals for three months. The chickens receiving reserpine grew at half the rate of untreated control chickens and failed to grow combs. At 4 months of age the testes of reserpine-treated chickens were only about one-tenth the weight of the testes of the control chickens and histologically they showed extreme hypoplasia of the seminiferous tubules. Two large doses of reserpine one month apart could cause atrophy of the testes of adult roosters.

In studies in which reserpine was used to deplete chicken adrenals of their adenine nucleotides and catechol amines (Burack, Weiner & Hagen, 1960) and in which roosters treated with stilboestrol served as experimental animals because they were cheap and readily available, the observation was made that control birds, which had not received reserpine, grew combs when kept for several months. On the other hand, those birds which had received a single injection of reserpine two or three months previously continued to look like capons. Thus, reserpine apparently prevented the appearance of this secondary sex characteristic (comb growth) which appeared in the control animals as the effect of the stilboestrol wore off.

The experiments reported in this paper were carried out in order to determine whether reserpine has an inhibitory effect on sexual development or can cause regression of gonadal activity in adult birds. The results of these experiments indicate that reserpine, injected once weekly in doses as low as 1 mg/kg, can inhibit both growth and sexual development of young male chickens, and that two large injections of reserpine with one month's interval between can cause atrophy of the testes of full-grown roosters. A preliminary report of this work has already been given (Hagen & Wallace, 1960).

METHODS

Effect of reserpine on maturation and on the development of secondary sex characteristics

One-month-old male (Sussex–Barred Rock) chickens were obtained from a commercial hatchery. They were divided into three groups of nine each and placed in three cages. In the first group six chickens received an intramuscular injection of reserpine 1 mg/kg body

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weight each week and three untreated chickens were kept in the same cage. In the second group six chickens received reserpine 2 mg/kg weekly and three untreated chickens were kept in the same cage. In the third group five chickens received 4 mg/kg reserpine weekly and four untreated chickens were kept in the same cage. The injected animals were identified by a band around the leg. After five weeks the control animals were so much more active that they were removed to separate cages lest their general ebullience might upset the less active reserpine-treated birds.

The chickens were fed a commercial growing-mash daily and excess wheat was always available on the floor of the cage. They were weighed at intervals of two weeks.

After ten weeks one chicken from each injected group and two of the control birds were killed and their testes, combs and wattles, thyroid glands and adrenal glands were removed and weighed. Their testes, thyroids and adrenals were also examined histologically.

Three and a half weeks later (13.5 weeks from the beginning of the experiments) all the chickens (now 17.5 weeks old) were killed and autopsied, the weights of their testes, combs and wattles determined and the testes examined histologically.

Effect of reserpine on adult roosters

Twenty White Leghorn roosters each of between 1.8 and 2.2 kg weight were divided into two groups of ten. Each of the members of one group received 30 mg reserpine in a volume of 0.3 ml. by intramuscular injection; each of the control group received 0.3 ml. of reserpine solvent (Burack, Weiner & Hagen, 1960). One month later each of the experimental group received a second injection of 25 mg of reserpine. Four of the reserpine-treated roosters died during the experiment. One of the remaining roosters was killed one week after the second injection and a control rooster killed at the same time. The remaining five were killed at the end of the second month after the initial injection and five control birds killed at the same time. The weights of the testes were recorded and portions of the testes fixed in formalin for histological investigation. The diet of these animals consisted of growing-mash and wheat *ad libitum*. However, food was removed from the cages of the control animals for three to four days following the administration of reserpine to the experimental birds, who showed no inclination to eat for several days after each injection. The food was returned to the control birds when the injected animals began eating again.

RESULTS

Effect of reserpine on body growth

The growth curves of the groups treated with 1 mg/kg/week and 4 mg/kg/week reserpine and of the control groups are shown in Fig. 1. Those birds receiving the lowest dose of reserpine (1 mg/kg) did not grow at a significantly faster rate than those receiving 4 mg/kg.

Effect of reserpine on comb growth

Four weeks after the beginning of the experiment combs had begun to develop on the control chickens but had failed to appear on those receiving reserpine. As the experiment progressed slight comb growth was evident on the injected birds, but the difference in comb growth between the controls and the treated animals became more marked (Fig. 2). The mean weight of the comb and wattles of all control animals was 9.6 ± 3.6 g. The mean weight of comb and wattles of all animals receiving reserpine was 1.2 ± 1.4 g (Table 1).

Effect of reserpine on testis development

With three exceptions, in animals receiving only 1 mg/kg reserpine, the testes of all animals receiving reserpine weighed less than 1 g. The mean weight of the

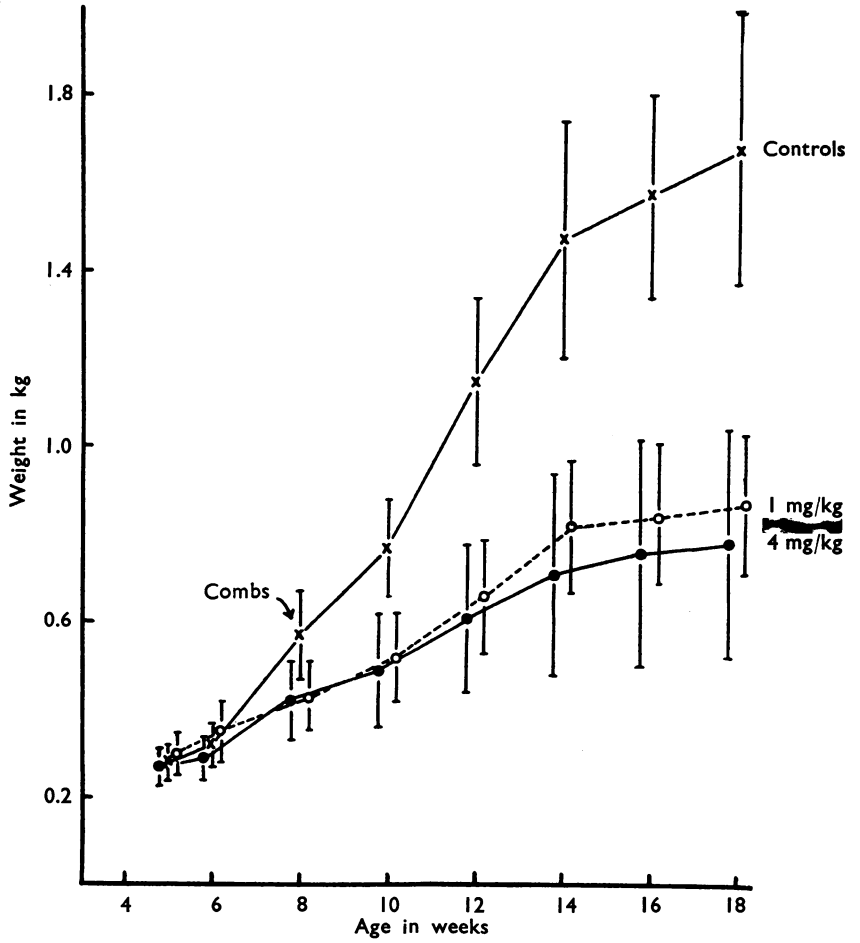

Fig. 1. Growth curves of male chickens showing the effect of weekly injections of reserpine begun at 5 weeks of age. The size of the weekly doses of reserpine is indicated on each graph. The time of appearance of definite combs is indicated on the graph. The vertical lines indicate standard deviations of the individual readings from the mean.

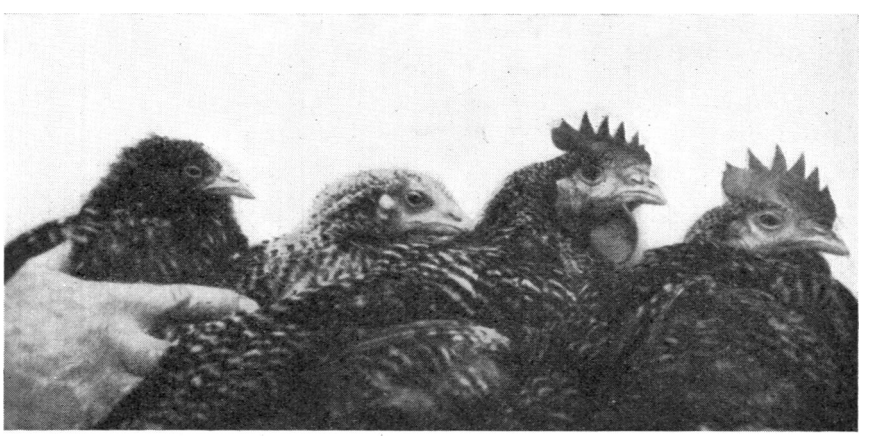

Fig. 2. Photograph of two of the chickens receiving reserpine 4 mg/kg weekly and of two control chickens all at 3 months of age.

TABLE 1

EFFECT OF RESERPINE ON WEIGHT OF CHICKENS AT 4 MONTHS OF AGE

Each chicken received weekly doses of reserpine since their fifth week of life

Dose of reserpine mg/kg	Number of animals	Mean weight (g)±s.d.		
		Whole animal	Testes	Comb and wattles
0	10	1,682±316	8·10±2·20	9·60±3·60
1	6	870±160	1·42±1·83	2·00±2·00
2	6	960±172	0·26±0·28	0·98±0·55
4	5	780±264	0·43±0·57	0·42±0·26

control testes was 8.1±2.2 g; of the testes of reserpine-treated birds 0.72±0.52 g (Table 1). Histologically, the testes of all animals receiving 4 mg/kg of reserpine weekly showed extreme hypoplasia. The tubules were small in diameter and were lined almost invariably by a single epithelial layer. Most of the cells in the lining were Sertoli cells, although occasional spermatogonia were present. The interstitial tissue was very small in amount. In some testes very few Leydig cells could be seen, while in others they were fairly abundant; in no case did there appear to be hyperplasia of these elements. No spermatozoa were seen in the testes of this group (Figs. 3 and 4). Testes of those animals receiving reserpine 2 mg/kg weekly showed more variable degrees of atrophy. In most there was some reduction in

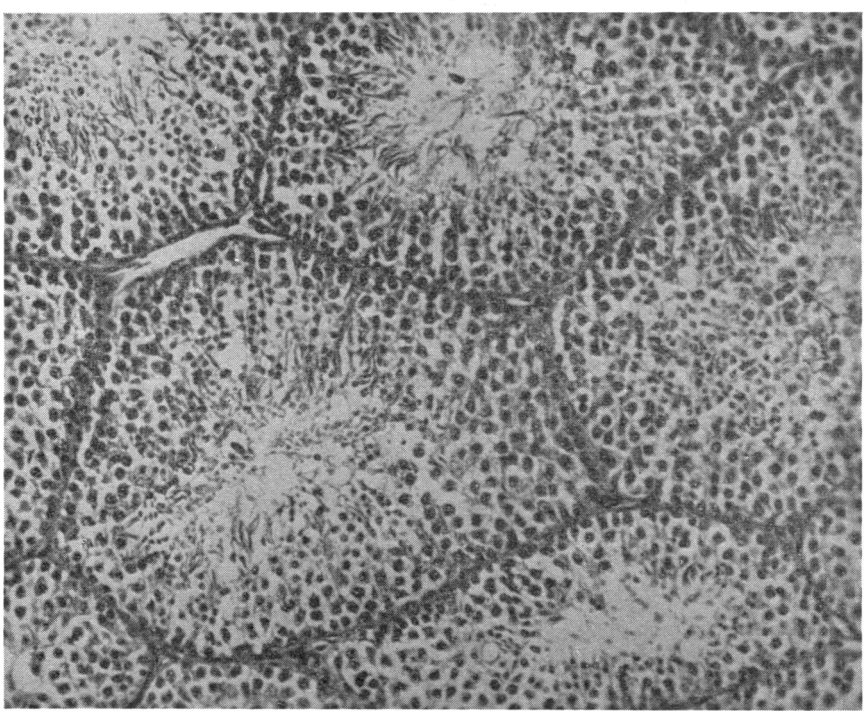

Fig. 3. Normal rooster testis showing all stages of spermatogenesis and tubule lumen filled with mature spermatozoa. The epithelium is many layers thick, and the interstitial tissue is relatively small in amount. (H. & E., ×250.)

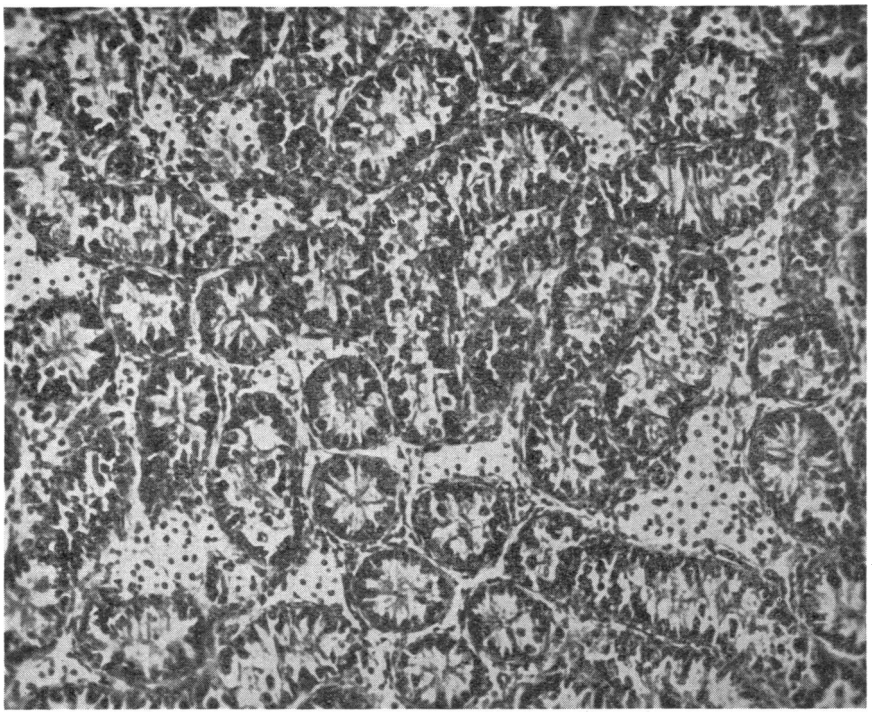

Fig. 4. Testis from animal receiving 4 mg/kg of reserpine weekly beginning at 1 month of age. There is an extreme degree of hypoplasia, the diameter of the tubules being about 15% of normal. The tubular epithelium consists for the most part of a single layer of Sertoli cells. No mature spermatozoa are present. Leydig cells are quite numerous in this section, but this was not the case with all the animals under treatment. (H. & E., ×250.)

tubular diameter, epithelial lining of about two cells in thickness, and a marked reduction in spermatogenesis. In the animals that received 1 mg/kg, the testes varied from completely normal to the picture of complete hypoplasia identical to that seen with 4 mg doses.

Effect of reserpine on thyroid and adrenal gland development

The weights of the thyroid and adrenal glands of the animals treated with reserpine did not differ significantly from those of the control roosters. They appeared normal on histological examination.

Effect of reserpine on testes of mature roosters

The weights of the testes of the six surviving adult roosters which received reserpine and of six control birds are shown in Table 2. The mean weight of the testes of the control birds was 9.3 ± 3.8 g and of the reserpine-treated birds 2.6 ± 1.7 g. Hypofunction of the testes was indicated in the course of the experiment by a marked reduction in size of the combs of some of the roosters receiving reserpine. However, some of the reserpine-treated animals did retain large combs throughout the experiment despite atrophy of the testes.

TABLE 2

BODY AND TESTICULAR WEIGHTS OF CONTROL ROOSTERS AND ROOSTERS TREATED WITH RESERPINE

* One control and one reserpine-treated rooster killed five weeks after the beginning of the experiment

Initial weight (kg)	Final weight (kg)	Testicular weight (g)
	Controls	
2·0	2·1	8·0
1·9*	1·8*	4·0*
2·2	2·3	12·0
2·3	2·2	12·0
2·1	2·1	14·0
2·2	2·1	6·0
	Reserpine treated	
2·0	1·9	0·7
1·9	1·9	4·0
2·0	1·9	3·0
2·2*	1·8*	1·0*
2·2	1·9	2·0
1·8	1·7	5·0

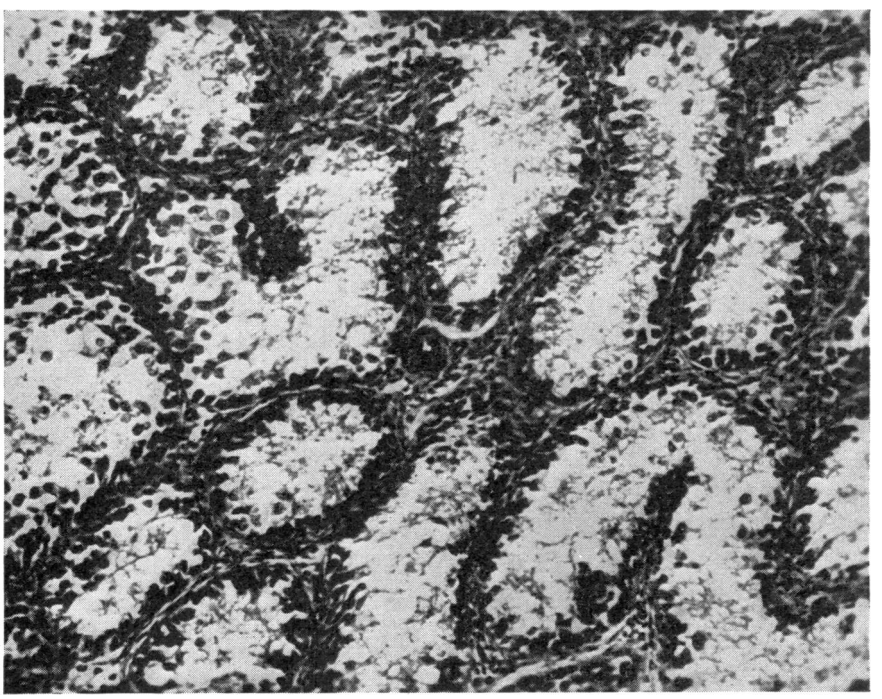

Fig. 5. Testis of a rooster receiving a total of 55 mg of reserpine in two doses. The tubular lumen is not as small as in the case of the testes of the immature animals treated, but the diameter is only 30% of normal. The epithelial lining is mainly a single layer of Sertoli cells, with occasional spermatogonia. No mature spermatozoa are present. In this section, the interstitial tissue is small in amount. (H. & E., ×250.)

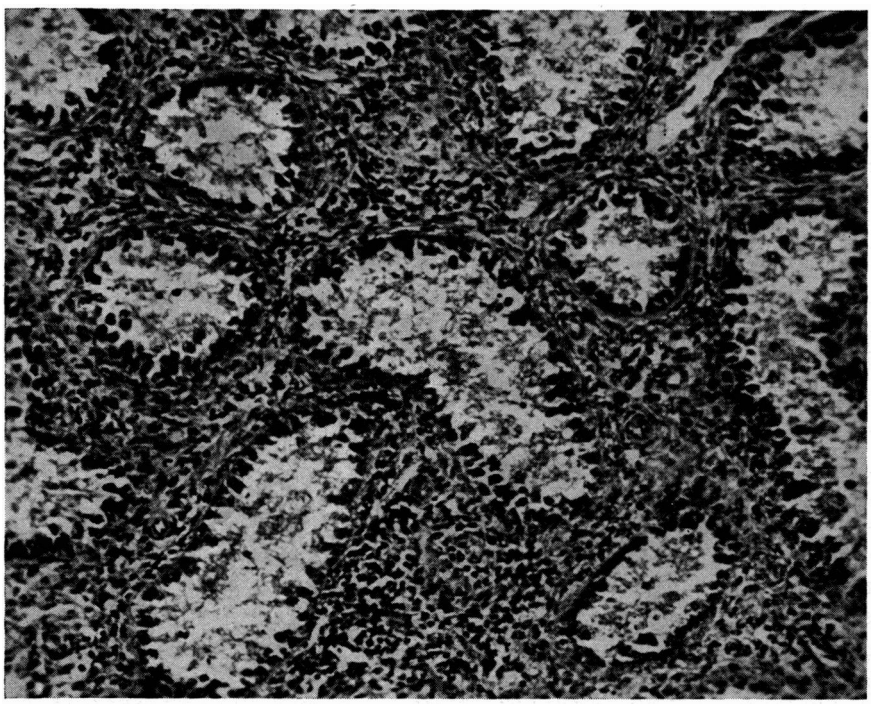

Fig. 6. Testis from another rooster receiving the same dose of reserpine as the animal whose testis is seen in Fig. 5. The appearance of the tubules is the same, but the interstitial tissue is much greater in amount. It appears to consist largely of fibrous tissue with chronic inflammation, and Leydig cells are not prominent. (H. & E., ×250.)

The histological appearance of the testes was essentially that of atrophy, similar to that described for the testes of roosters treated with female sex hormones (Emmens, 1939) or subjected to hypophysectomy (Nalbandov, Meyer & McShan, 1946). Animals given two doses of reserpine all showed profound testicular atrophy, although the reduction in tubule size was not as marked as that seen in the hypoplastic testes of the immature roosters. Spermatogenesis was totally absent; tubules were lined with epithelial cells only one to three layers in depth, contrasted with a depth of about eight cells in the tubules of the control animals. The profound effect on spermatogenesis is contrary to reports of other workers who examined the testes of rats treated with reserpine (Albeaux-Fernet, Bellot, Breant, Bugard, Chabot, Deribreux, Gelinet & Romani, 1959). Most of the cells present were Sertoli cells. The interstitial tissue varied somewhat. In some animals it formed a narrow, acellular framework (see Fig. 5), while in others it was of considerable thickness (see Fig. 6). In the latter case the interstitial tissue was quite fibrous and contained abundant lymphocytes and monocytes. Leydig cells were not a prominent feature in any of the sections, and, judging by their lack of vacuolation, were not active. The rooster killed one week after the second dose of reserpine showed only a moderate degree of testicular atrophy, and spermatogenesis persisted at a reduced rate in both testes. Control animals' testes were entirely normal except for occasional small focal zones of atrophy, which comprised only a minute portion of the total area.

DISCUSSION

The results of the experiments described leave little doubt that reserpine can cause impairment of growth of young male chickens and slight loss of weight in adult roosters. In addition, reserpine can cause retardation of development of the testes so that at 18 weeks of age the testicular weights of those chickens receiving 2 or 4 mg/kg weekly were only one-sixteenth the weights of the testes of the controls. A dose of 1 mg/kg, which caused approximately the same reduction in weight, was less effective in inhibiting testicular growth, so that the weight of the testes was only about one-sixth the weight of the control testes.

In considering the site at which reserpine may be acting to cause testicular atrophy or hypoplasia several possibilities come to mind. A direct effect on the testes might either interfere with the normal growth of the testicular cells or antagonize the action of gonadotrophic hormone on the testes. On the other hand, it is possible that reserpine might act on the adenohypophysis to inhibit either the synthesis or secretion of gonadotrophins. Failure of these animals to grow suggests a possible inhibition of growth hormone secretion. In fact, an action of reserpine on the adenohypophysis to inhibit the secretion of not only growth hormone and gonadotrophic hormones but possibly also other hormones might be postulated. A more general action of reserpine on the hypophysis is suggested when one remembers that effects of reserpine on the secretion of other pituitary hormones are already known. Thus reserpine has been shown to reduce egg production and the fertility and hatchability of eggs of female chickens (Hewitt & Reynolds, 1957); it can cause release of corticotrophin and it can also prevent the secretion of corticotrophin in response to stress (Wells, Briggs & Munson, 1956). It can reduce thyroid activity and secretion of oxytocin in rats (Moon & Turner, 1959a and b). Although the evidence is insufficient to substantiate such a claim it may be justifiable to speculate that, in addition to its action to deplete nervous and other tissues of catechol amines (Holzbauer & Vogt, 1956) and 5-hydroxytryptamine (Paasonen and Vogt, 1956; Pletscher, Shore & Brodie, 1956), reserpine might well be able to deplete also the pituitary gland of the stores of several of its hormones.

In view of the other actions of reserpine on the endocrine glands discussed above, it would seem unlikely that the effects of reserpine on chicken testes are merely secondary to an inanition resulting from depression of appetite by this drug. Further evidence against such a mechanism is the much more marked effect of reserpine on the testes when given in a dose of 4 mg weekly than the dose of 1 mg weekly, although both doses of reserpine caused approximately the same retardation of growth.

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REFERENCES

ALBEAUX-FERNET, M., BELLOT, L., BREANT, P., BUGARD, P., CHABOT, J., DERIBREUX, J., GELINET, M. & ROMANI, J.-D. (1959). *L'Année Endocrinologique*, 11th ed. Paris: Masson & Co.

BURACK, W. R., WEINER, N. & HAGEN, P. B. (1960). The effect of reserpine on the catecholamine and adenine nucleotide contents of adrenal gland. *J. Pharmacol. exp. Ther.*, **130**, 245–250.

EMMENS, C. W. (1939). The effect of prolonged dosage with oestrogen on the adult brown leghorn cock. *J. Physiol. (Lond.)*, **95**, 379–382.

HAGEN, P. & WALLACE, A. C. (1960). An effect of reserpine on growth and sexual maturation. *Fed. Proc.*, **19**, 168.

HEWITT, O. H. & REYNOLDS, R. E. (1957). Tranquilizers in rearing game birds. *Cornell Nutrition Conference for Feed Manufactures.*

HOLZBAUER, M. & VOGT, M. (1956). Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. *J. Neurochem.*, **1**, 8–11.

MOON, R. C. & TURNER, C. W. (1959a). Effect of reserpine on thyroid activity in rats. *Proc. Soc. exp. Biol. & Med.*, **100**, 679.

MOON, R. C. & TURNER, C. W. (1959b). Effect of reserpine on oxytocin and lactogen discharge in lactating rats. *Proc. Soc. exp. Biol. & Med.*, **101**, 332.

NALBANDOV, A. V., MEYER, R. K. & MCSHAN, W. H. (1946). Effect of purified gonadotropes on the androgen-secreting ability of testes of hypophysectomized cocks. *Endocrinology*, **39**, 91–104.

PAASONEN, M. K. & VOGT, M. (1956). The effect of drugs on the amounts of substance P and 5-hydroxytryptamine in mammalian brain. *J. Physiol. (Lond.)*, **131**, 617–626.

PLETSCHER, A., SHORE, P. A. & BRODIE, B. B. (1956). Release of brain serotonin by reserpine. *J. Pharmacol. exp. Ther.*, **116**, 46.

WELLS, H., BRIGGS, F. N. & MUNSON, P. L. (1956). The inhibitory effect of reserpine on ACTH secretion in response to stressful stimuli. *Endocrinology*, **59**, 571–579.