The pituitary preparations employed in these experiments were previously found to possess both follicular stimulating and luteinizing properties when injected subcutaneously into sexually immature rats. These findings show the extraordinarily high threshold of anterior pituitary material required for the luteinization of the primate ovary, and indicate the widely different responses to be obtained from subcutaneous and intravenous injections of the same preparation.

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Gonadotropic Action of Phyone on Juvenile Female Rabbit.*

R. HERTZ, A. HELLBAUM AND F. L. HISAW.

Department of Zoology, University of Wisconsin.

Van Dyke and Wallen-Lawrence[1] have prepared an extract of fresh beef pituitary tissue (phyone) possessing marked growth promoting properties on the hypophysectomized as well as on the normal immature rat. This preparation was without effect on the gonads of such test animals. Phyone was consequently thought to contain only the growth promoting hormone of the pituitary gland, and to be free of the sex-stimulating factors known to exist in this gland.

We have assayed 3 different batches of phyone† upon juvenile chinchilla female rabbits from 12 to 13 weeks of age. Four litters of 4 rabbits each were employed. In each litter one rabbit was retained as an uninjected control. Two of the members of each litter were given 0.5 cc. of phyone either intravenously or subcutaneously for from 3 to 5 days. Since Leonard[2] has shown that a female rabbit, if fully adult, will ovulate upon the intravenous injection of phyone, the remaining rabbit in each litter was given a single intravenous injection of 1 cc. of phyone from the batch used on its own litter-mates. This single injection was without effect upon the ovaries of the juvenile rabbits, but 1 cc. samples from any

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[1] Van Dyke, H. B., and Wallen-Lawrence, Z., *J. Pharm. and Exp. Ther.*, 1930, **40**, 413.

† This material was obtained from The Wilson Laboratories through the courtesy of Dr. David Klein.

batch invariably produced ovulation upon intravenous injection into an adult rabbit in oestrus. From this difference in response it is apparent that the young rabbits employed here were not sexually mature.

When given repeatedly, as described above, phyone produced marked follicular enlargement and extensive luteinization in the ovaries of the juvenile rabbits. No ovulation occurred, but numerous blood-points were produced. The degree of the response was proportional to the amount of phyone administered, and the route of injection caused no difference in the reaction obtained.

The uteri of all these rabbits reflected the conditions found in their ovaries. Where follicular enlargement predominated, the uterus showed a marked oestrous picture. In those cases where the ovary was more extensively luteinized, the uterus showed a definite pseudopregnant condition. The control uteri in all cases showed only the very slight prooestrous development of the mucosa characteristic of the prepubertal uterus.

It is to be emphasized that each batch of phyone used here was found to be without effect upon the ovaries of sexually immature rats, even when given in the same absolute doses administered to the juvenile rabbits.

On the basis of the negative response of the rat's ovary to phyone, a number of observers have considered this preparation to be free of any of the recognized gonadotropic hormones of the pituitary. Thus, Leonard[2] has presented the possibility of the presence in phyone of a distinct substance causing ovulation in the rabbit. Our findings indicate that such an effect may be due to the gonadotropic content of the phyone employed.

The present work further demonstrates the wide variations in response which may be obtained from different species of test animals with a given tissue extract. It is essential that such differences be checked before attributing a definite hormone content to the preparation being tested.‡

[2] Leonard, S. L., *Am. J. Physiol.*, 1931, **98**, 406.

‡ Dr. S. L. Leonard, Columbia College of Physicians and Surgeons, has recently favored us with a personal communication confirming these findings on juvenile rabbits.