

These rats gained in weight in most instances and were apparently in better physical condition than untreated rats.

The effectiveness of the parenteral administration of any given amount of cortical hormone probably varies to some degree with the total cortical hormone which may be still available in the involuted suprarenal cortex.‡

These observations further support the hypothesis that the depression in natural resistance following pituitarectomy is due to the impairment of the function of the suprarenal cortex resulting from the withdrawal of the adrenotropic hormone of the anterior lobe.

7866 C

The Age Factor in Responsiveness to Gonadotropic Hormones.

H. SELYE, J. B. COLLIP, AND D. L. THOMSON.

From the Department of Biochemistry, McGill University, Montreal.

We reported^{1, 2} that in very young rats (6-12 days old) A.P.L. (anterior pituitary-like hormone of pregnancy urine) leads only to the luteinization of theca cells but not to the formation of corpora lutea from granulosa cells. This theca-reaction is accompanied by continuous vaginal oestrus. This type of reaction to A.P.L. is very similar to that observed in hypophysectomized rats³ and therefore the possibility of its being due to insufficient pituitary function at this early age had to be considered.

Corey⁴ and Swezy and Evans⁵ stated that pituitary implants have no effect on the ovaries of the rat during embryonic development, and during the first days of life. In order to see whether the effect of A.P.L. is different from that of the pituitary gonadotropic hormone during the first days of life, we repeated our earlier A.P.L.

‡ It is of interest that in a pituitarectomized rat in which treatment was begun 95 days after operation, the rat gained in weight, but did not survive 800 mg. of histamine per kg. of body weight. Its suprarenals were found to be reduced to the size of a pinhead.

¹ Selye, H., Collip, J. B., and Thomson, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 647.

² Selye, H., Collip, J. B., and Thomson, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 780.

³ Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.

⁴ Corey, E. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1928, **25**, 498.

⁵ Swezy, O., and Evans, H. M., *Anat. Rec.*, 1931, **50**, 189.

experiments with a potent sheep pituitary extract. This extract was prepared as follows:

Sheep glands which had been partly defatted and dehydrated with acetone were extracted twice with 4 volumes of 70% acetone containing 1% ammonia. The residue was extracted with 1% aqueous ammonia, $\text{Ca}_3(\text{PO}_4)_2$ being used as an aid to filtration. The filtrate was saturated with $(\text{NH}_4)_2\text{SO}_4$ and the resultant precipitate was dissolved in water. Two volumes of acetone were added to this solution and the precipitate which formed was collected and dissolved in water, so that 1 cc. of the solution represented $\frac{1}{4}$ gm. of the original glands. Immature rats injected subcutaneously twice daily with $\frac{1}{2}$ to 1 cc. of this extract manifested vaginal oestrus in 72 to 96 hours and showed enlarged luteinized ovaries (40 to 60 mg.) on the fifth day. A standard powder was also prepared from the above mentioned acetone precipitate.

Eleven female rats were injected with $\frac{1}{8}$ to 1 cc. of this extract twice daily over a period of 8 to 44 days. They were killed 24 hours after the last injection. The treatment did not induce oestrus and the ovaries of 2 animals killed on the 8th day, and of 2 killed on the 11th day, showed no detectable changes, except that in the 2 latter cases a few hemorrhages into the theca were seen. Thecal luteinization did not occur. The first vaginal oestrus was observed on the 14th day of treatment—that is, at a time when the animals were 26 days of age. Thenceforward the ovaries reacted with formation of corpora lutea of granulosa origin, and irregular cycles appeared. The maximum ovarian weight increase was observed around the 25th day of treatment (120 mg.); thereafter the ovaries involuted in spite of the continued treatment, the animals having lost their sensitivity to this preparation. It appears from these experiments that in the very young rat the gonadotropic preparations from pregnancy urine do not act in the same way as those obtained from the pituitary. While the former lead to theca-luteinization and continuous vaginal oestrus, the latter have no effect on the ovary and do not cause oestrus.

In another experiment the ovaries of six 12-day-old rats were implanted into the spleens of 6 females which were ovariectomized 2 weeks before the transplantation was performed. Even though the transplant “took” in all these cases, no follicle maturation, corpus luteum formation or theca luteinization was observed, and the hosts showed no vaginal oestrus. This experiment corroborates the view that the rat ovary is insensitive to the pituitary sex hormone during the first days of life.

The development of the Graafian follicle and its capacity to undergo transformation into a corpus luteum may be regarded as divisible into 3 different periods. The first stages of development are found in the ovary of rats between the first and the eighteenth day of life. At this period the granulosa cells do not respond to any known gonadotropic hormone, and the only effect that one can see after the administration of A.P.L. is the transformation of theca cells into theca-lutein cells,^{1, 2} while anterior pituitary sex hormone does not seem to have any effect at all. The irresponsiveness of such immature ovaries to gonadotropic hormones is in the ovary itself and not in its hormonal environment, because these ovaries will not form mature follicles and corpora lutea even if they are transplanted into adult female castrates.

The second stages of development are found in the ovaries of rats after the 18th day of life, and in the ovaries of hypophysectomized animals. Up to this stage of maturation no pituitary hormones are needed. The experiments of Swezy⁶ have shown that ovogenesis is not impaired after hypophysectomy, and since we have frequently found mitotic figures in the granulosa of hypophysectomized rats we feel that the maturation of the granulosa must also be independent of the pituitary hormones up to a certain stage in the development of the follicle. In this second stage, the follicle is able to respond to the pituitary follicle-stimulating hormone, but not to A.P.L. If A.P.L. is administered to hypophysectomized animals, only thecal luteinization will take place. If it is administered to the immature animal (around the 18th day of age) the follicles of which are also in this stage of development, the same preparation will produce follicle maturation with the subsequent luteinization of the granulosa only because the experimental animal's own pituitary is able to participate in the stimulation of the ovary,^{7, 8}

The third stages of development are found in the ovaries of animals in full oestrus or of hypophysectomized animals after administration of follicle-stimulating hormone as obtained from castrate urine or pituitary tissue. This is the stage of full follicular development. At this time, and only at this time, A.P.L. has a direct effect on the granulosa cells, which it is able to transform into corpus luteum cells.

This explains why A.P.L. may lead to the formation of a true

⁶ Swezy, O., *Ovogenesis and Its Relation to the Hypophysis*, Science Press, Lancaster, Pa., 1933.

⁷ Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinol.*, 1933, **17**, 494.

⁸ Selye, H., *Proc. Soc. Exp. Biol. and Med.*, 1933, **31**, 262.

corpus luteum if injected into a rabbit immediately after hypophysectomy, since in this species fully mature follicles are almost continuously present in the ovary. It further explains why the injection of A.P.L. into guinea pigs does not lead to the formation of corpora lutea except in the presence of a large follicle. Since this species responds to A.P.L. in a manner similar to the hypophysectomized rat or rabbit, one may assume that the hypophysis of the guinea pig does not participate with the injected A.P.L. in follicular stimulation. This interpretation would also make Engle's⁹ experiments on monkeys more comprehensible. The monkey seems to react very much like the guinea pig, in that here again A.P.L. produces luteinization only after the follicle has responded to hypophyseal stimulation.

It seems necessary at the present time to postulate 2 hypophyseal gonadotropic hormones, one follicle-stimulating and one that luteinizes the theca and the mature granulosa while it has no effect on the immature granulosa cells. The so-called "Prolan A" of menopausal urine appears to consist chiefly of the former, or at least to resemble it closely, whereas the placental hormone "A.P.L." of pregnancy urine ("Prolan A plus Prolan B" of Zondek's original terminology) is more comparable, in its biological relations, to the luteinizing fraction.

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Effect of Iodine and Desiccated Thyroid on Anterior Pituitary of Goitrous and Thyroidectomized Rabbits.*

DAVID MARINE, S. H. ROSEN, AND CHARLES SPARK.

From the Laboratory Division, Montefiore Hospital, New York City.

It has been known for nearly a century¹ that individuals and animals with endemic goiter or cretinism have hypertrophy of the anterior pituitary. In man pituitary weights up to 3 gm. have been recorded, while in rabbits of approximately 2 kg. body weight with large parenchymatous goiters we have observed pituitary weights of 0.070 gm., whereas our normal average weight of the pituitary of rabbits of this size is 0.020-0.022 gm. Rogowitsch² first demon-

⁹ Engle, E. T., *Endocrinol.*, 1934, **18**, 513.

*Aided by a grant from the Ella Sachs Plotz Foundation.

¹ Nièpee, B., *Traité du goître et du crétinisme*, Paris, 1851.

² Rogowitsch, N., *Ziegler's Beitrag.*, 1889, **4**, 453.