

agar-agar, 2; McCollum's salts No. 185, 4; filtered butter fat, 10; and dextrin, 64. On this ration, deficient only in the vitamin B complex, we secure depletion in 2 and generally in not more than 3 weeks.

For our uncomplicated vitamin B studies we have introduced autoclaved round steak as a source of vitamin G, because muscle meats are known to be deficient in the antineuritic and abundant in the antipellagric factor. The autoclaving is carried out as follows: Thirty pounds of round steak are cut in small cubes and heated for 6 hours at 20 pounds pressure, dried at 100°C., and ground. The composition of the ration deficient only in vitamin B (No. 2345) is as follows: Casein (purified with acidulated water and 25% alcohol), 10; salts No. 185, 4; autoclaved dried round steak, 15; butter fat, 10; dextrin, 61. On this ration depletion of vitamin B reserves generally take place 2 to 3 weeks after all storage of the vitamin B complex has been depleted on Ration No. 1751. The most satisfying feature about ration 2345 is that all negative controls lose weight on this diet rapidly and invariably die.

We have determined that this ration is abundant in vitamin G for optimum growth with a large number of animals, receiving supplementary additions of small amounts of highly concentrated vitamin B preparations.

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### Further Studies on Production of Thecal Luteinization by Means of A. P. L.

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We have reported that if A.P.L (anterior-pituitary-like hormone) is given to very young rats (6-8 days old) no follicle maturation or corpus luteum formation occurs, but the thecal cells increase in size and store large amounts of fat, so that their appearance becomes very similar to that of real corpus luteum cells. Normal reactions to A.P.L—that is, follicle maturation and corpus luteum formation—is regularly found in prepubertal rats if the injections are started around the 21st day of life. We found, however, that rats treated daily with this hormone, beginning at the age of 6 days, react with

thecal luteinization only, even when they reach the age of 21 days or more.<sup>1</sup>

In the present experimental series, we wished to see (1) if the formation of thecal lutein cells precedes the appearance of the squamous vaginal reaction and, (2) how long these rats continue to react to A.P.L. with exclusively thecal luteinization and continuous oestrus. For this purpose, 43 female rats were injected with increasing amounts of A.P.L. (6-25 units daily), beginning at the age of 6 days. We examined the vaginal smears every day and killed an animal every few days in order to study the histological changes produced in the ovary. These experiments showed that at the time when the smears first became oestrous—on the seventh to the thirteenth day of treatment—the formation of thecal corpora lutea was already very conspicuous. All the animals killed during the next 4 to 5 weeks showed a similar reaction, inasmuch as in all of them only thecal cells turned into lutein cells. As the treatment progressed, severe follicular atresia set in, so that most of the primary follicles disappeared and histological preparations in these stages showed almost nothing but small islands of luteinized thecal cells. In some cases inconspicuous remnants of the granulosa or the ovum could be detected on serial sections in the middle of these islands, but later even these traces disappeared. The reason for this severe follicular atresia is yet unknown, but considering that the granulosa has no blood vessels of its own, and is therefore entirely dependent upon the theca for its nutrition (as is the ovum itself) the disappearance of these parts of the follicle may simply be the result of the changes produced in the theca.

Between the thirtieth and fortieth day of treatment the oestrus became discontinuous and irregular cycles appeared. The ovaries examined at this stage of the experiment showed several mature follicles and real corpora lutea in various stages of development. These corpora lutea were undoubtedly formed from maturing follicles, the granulosa cells of which became luteinized. At the same time the thecal corpora lutea disappeared. At this stage of our experiment some new factor must have appeared which enabled A.P.L. to produce its normal effect on the ovaries.

According to our conception of the mechanism of A.P.L. action<sup>2, 3</sup> the factor responsible for the change in the reaction of the

<sup>1</sup>Selye, H., and Collip, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 647.

<sup>2</sup>Collip, J. B., Selye, H., and Thomson, D. L., *Virchow's Arch.*, 1933, in press.

<sup>3</sup>Collip, J. B., Selye, H., Thomson, D. L., and Williamson, J. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 590.

ovary to this hormone at this stage must be a complementary substance furnished by the pituitary. In hypophysectomized animals one finds thecal luteinization only<sup>4</sup> unless suitable pituitary extracts are given together with A.P.L.<sup>2, 3</sup> It is most likely that the immature pituitary is unable to furnish the complementary substance necessary for the normal action of A.P.L. on the ovary, but it becomes able to do so before normal puberty is reached, and at this time A.P.L. will produce precocious puberty.

Many animals do not react as well to A.P.L. as the rat does. Jares<sup>5</sup> stated that intravenous administration of pregnancy urine fails to induce ovulation in adult virgin guinea pigs, nor could he detect any histological changes in the ovaries of these animals. As it is impossible to induce a normal ovarian reaction in the guinea pig, the effect of A.P.L. has not been studied adequately in this species. Thinking that the reason for this lack of reaction might be the inability of the guinea pig's pituitary to furnish the complementary substance necessary for A.P.L. action, we studied the histological effect of this hormone on the ovary more closely in order to deter-

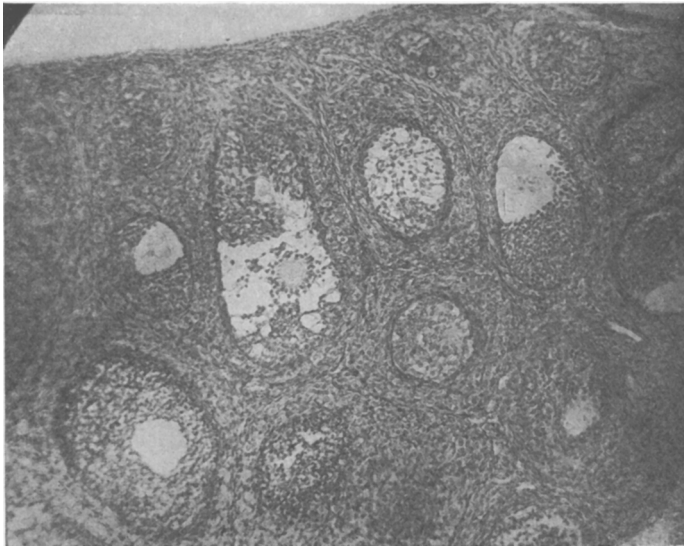


FIG. 1.

Ovary of guinea pig weighing 126 gm. treated daily for 6 days with 300 units of A.P.L. Picture shows proliferation of thecal cells which assume the appearance of lutein cells.

<sup>4</sup> Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.

<sup>5</sup> Jares, J. J., *Anat. Rec.*, 1931, **49**, 185.

mine whether changes would appear similar to those seen in the hypophysectomized rat—that is, thecal luteinization.

Six virgin guinea pigs, weighing 136-321 gm., received various amounts of A.P.L. (200-700 units per day for 6-14 days); the ovaries of these animals were enlarged, but showed no signs of follicle maturation or corpus luteum formation. The thecal cells, however, had changed into luteal cells.

These experiments seem to support the conception that species differences in the reaction to A.P.L. may be due to differences in the ability of the pituitary to furnish the complementary substance necessary for the normal reaction of A.P.L. on the ovary.

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### A Lactation Hormone of the Adrenal Cortex.\*

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One of us<sup>1</sup> showed that adrenalectomized rats could bear young if treated with cortin. No attempt was made to raise the young. Carr<sup>2</sup> found that cortical extract made according to the Swingle and Piffner method did not support lactation. Swingle and Piffner<sup>3</sup> have recently shown that their extract will support lactation in the dog.

Extract made according to our method which removes fatty substances by chilling to  $-12^{\circ}\text{C}$ . does not enable adrenalectomized rats to supply milk (B, Table I) even when used in large amounts (C), yet it is potent as far as the cortin content is concerned. On the other hand, if the chilling is not carried below  $3^{\circ}\text{C}$ ., our extract supports lactation (D). The material chilled out between 3 and  $-12^{\circ}\text{C}$ . contains a substance necessary for milk production, since if an extract of this material is added to cortin and injector into adrenalectomized mother rats, they raise a much larger proportion of their young (E).

\* Aided by a grant from the Carnegie Corporation of New York to the Carnegie Institution of Washington.

<sup>1</sup> Hartman, F. A., *Endocrin.*, 1930, **14**, 229.

<sup>2</sup> Carr, J. L., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 131.

<sup>3</sup> Swingle, W. W., and Piffner, J. J., *Med.*, 1932, **11**, 371.