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# Specificity of Reactions Produced by Injection of Urine from Pregnant Cows into Immature Female Guinea Pigs.*

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Recent experiments have shown that the anterior hypophysis, in addition to its primary growth inducing function, has a secondary, apparently specific effect upon the sex organs. Under the influence of transplants of anterior hypophysis taken from various mammals, immature mice and rats have been brought to precocious sexual maturity (Smith and Engle,[1] Zondek and Aschheim[2]). One of the most striking effects produced by the anterior hypophyseal transplants has been the luteinization of follicles in the ovaries of the female host animal. Within 2 days after the first transplantation the ovaries began to show hyperemia accompanied by progressive growth of follicles. About 2 to 3 days later a distinct luteinization process was initiated in a number of follicles, which changed into atretic corpora lutea. Similar phenomena had been previously recorded by Evans and Long[3] after intraperitoneal injections of fresh anterior hypophyseal substance. These authors believe the whole reaction to represent "a powerful, specific stimulus to lutein cell transformation". This interpretation of specificity has been expanded by later investigators who attribute all the changes, caused by the transplants upon sex glands, to a separate "extra-gonadal" or "sex maturing" anterior pituitary hormone. Through the discovery of this particular hormone, in large quantities, in the urine of pregnant women, Aschheim and Zondek[4] developed an important method for diagnosing pregnancy. Injections of urine from pregnant women given to immature female mice resulted as with anterior hypophyseal transplants in the development of atretic corpora lutea and of hemorrhagic spots in the ovaries. The application of this test gave very satisfactory results with human urine. On the

* This work has been aided by the Committee for Research on Sex Problems of the National Research Council and by the Mount Hope Farm of Williamstown, Mass.

1 Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, **40**, 159.

2 Zondek, B., and Aschheim, S., *Arch. f. Gynak.*, 1927, **130**, 1.

3 Evans, H. M., and Long, T. A., *Proc. Acad. Sciences*, 1920, **8**, 38.

4 Aschheim, S., and Zondek, B., *Klin. Wochenschr.*, 1928, **7**, 1404.

other hand, tests made with the urine of pregnant cows, and of other mammals have been reported as negative.[5, 6]

In the course of experiments with cows, carried out with the cooperation of Mount Hope Farm,† we obtained material for the study of the effect of urine injections upon immature female guinea pigs.

Each animal received 6 injections of 1-2 cc. of cow's urine on 2 consecutive days. Vaginal smears were taken the third and fourth days and then the animals were killed and autopsied.

The ovaries of test animals, which received injections of pregnant urine did not show formation of corpora lutea or the large, typical hemorrhagic spots. On the other hand there was a definite increase in the vascularization of follicles and in their atretic degeneration. A number of follicles evidently began to grow, but, before reaching maturity, their theca interna became highly vascularized and caused their early disintegration. This excessive vascularization of medium sized follicles was one of the most outstanding features of the ovarian changes.

Outside of the follicular theca this reaction was less pronounced. Blood extravasations were observed but not as extensive nor typical as those described in mice.

The vascular phenomena, which appear after the injection of pregnancy-positive urine are not limited to the ovary or the genital tract. Other glands, like the adrenals, the thyroids, and the hypophysis display certain vascular changes.

These changes are particularly evident in the adrenals. In the most typical cases, the large vessels of the medulla and those of the reticular zone of the cortex become conspicuous and show a characteristic state of turgescence and a regularity in their general conformation. At the same time certain morphological changes are apparent in the various zones of adrenal cortex and especially in the reticularis. This last zone is greatly stimulated and shows a pronounced hypertrophy. Its cells are well defined with normal, rounded nuclei and numerous aggregations of secretory granules.‡

[5] Aschheim, S., *Am. J. Obst. and Gyn.*, 1930, **19**, 335.

[6] Allan, H., and Dickens, F., *Lancet*, 1930, **238**, 39.

† Experimental work related to various problems of reproduction has been conducted for the last 2 years at the Mount Hope Farm at the suggestion of Mr. E. Parmalee Prentice.

‡ These changes appeared in a large number of guinea pigs injected with pregnancy-positive urine. They were absent in control animals, injected with normal (non-pregnant) urine. A disturbing factor has been found in the occasional infection of some of the injected animals. This caused a congestion of the adrenal cortex and an apparent stimulation of the two external zones, the

These effects upon the adrenals and the vascular system may offer an explanation for the changes occurring in the ovaries after injections of urine from pregnant women. When an immature female rodent is injected with such urine its ovarian follicles and especially their theca interna become highly vascularized. Under normal conditions the process of the vascularization of the follicles ocurs at the completion of their ripening and prior to their rupture and their transformation into corpora lutea. In the injected animals the vascularization occurs prematurely and its effects are different according to the size of the affected follicles. A large, almost mature follicle, may turn into an atretic corpus luteum or may rupture and form a typical corpus luteum. A smaller immature follicle will become atretic and disintegrate or may become hemorrhagic through the rupture of the abnormally congested vessels of its theca. The transformation of granulosa or theca cells into luteal elements is an inherent property of these cells and may be completed under the influence of certain external stimuli.

In view of these considerations, one may explain why various species of rodents react differently to the injection of pregnancy-positive human urine. In animals with a very short sex cycle, such as mice or rats, in which the ripening of the follicles occurs rapidly, the vascular stimulation of the follicles, running its course within 4 to 5 days, leads to the development of large follicles and atretic corpora lutea. In animals having a longer cycle, such as the guinea pigs, in which the follicular growth is completed at a much slower rate, the vascularization of the theca does not induce the full development of ripe follicles and their transformation into corpora lutea. The stimulation here results in a relative growth of follicles leading to their final atretic degeneration. In rabbits, in which the absence of a spontaneous ovulation permits the use of larger animals for pregnancy tests, the formation of corpora lutea or hemorrhagic follicles can be induced within 24 hours after the injections of pregnancy-positive urine.[7] This is made possible by the presence of large follicles within the ovaries.

The existence of a specific hormone, the so-called "sex-maturing hormone", which is supposed to affect the sex glands primarily and to cause a luteinization of the ovarian follicles as well as a precocious sex-maturity becomes highly improbable in the light of these investigations. This active substance, whatever it is, seems to have

glomerulosa and fasciculata, with an increase in mitotic figures. This reaction could be recognized as being of pathological nature and should not be confused with the above described normal reaction.

[7] Friedman, M. A., *Am. J. Physiol.*, 1929, **90**, 617.

a general effect upon the vascular system, possibly through the adrenal glands, causing secondary effects in various organs, including the ovaries. The need for a stimulation of the glandular system and the growth of vascular tissues like the placenta during pregnancy might account for the existence of a general reaction of this type.

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## Is Levulose Converted to Dextrose in the Process of Absorption from the Small Intestine?

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A number of experiments were carried out by injecting levulose into closed loops of small intestine in normal unanesthetized dogs. After one hour the remaining loop fluid was aspirated and its total reducible sugar determined. A polariscope reading was made on a cleared specimen and a levulose determination made by the colorimetric method of Corley.[1] No dextrose was indicated in the aspirated fluid by these methods. However, this did not exclude the possibility of conversion in the mucosa with none demonstrable in the loop fluid. It became necessary for us to know whether or not levulose was present in the mesenteric vein from the loop during absorption. Forty-five minutes after giving levulose by loop the animal was anesthetized, abdomen aseptically opened, mesenteric vein from the loop exposed, and 10 cc. of blood withdrawn. A similar amount of blood was taken from the heart immediately following. Blood sugar was determined in each specimen and both were tested for levulose. In 5 experiments levulose was found in the mesenteric blood with the systemic blood negative. The mesenteric blood sugar was greater than that of the systemic blood by the amount of levulose found in mesenteric blood. Since the amount of levulose given (1.5 to 2 gm.) was shown not to cause a rise in systemic blood sugar in separate experiments, these findings indicate that no levulose is converted to dextrose in the process of absorption from the small intestine.

[1] Corley, R. C., *J. Biol. Chem.*, 1929, **81**, 81.