as the result of hypophysectomy. Two other hypophysectomized dogs failed to respond, although several times the amount of the hormone found necessary to induce lactation in normal dogs was injected in these animals. The failure, however, to obtain lactation with the hormone in these 2 dogs may probably be ascribed to lack of mammary development, inasmuch as one of these animals was sexually immature with infantile mammae, while the other was a senile bitch with atrophied mammae and ovaries.

All dogs in which lactation was induced continued secreting milk for about 2 weeks after the last injection. Even a non-parous bitch was stripped of approximately 100 cc. of milk on 3 consecutive days. A mother that had been removed from her litter and had been dry for a week was brought back to full lactation to continue suckling.

The response of completely depancreatized dogs to the lactation hormone has been reported elsewhere.[1] It is interesting to note in this connection that no secretion of milk was observed in 5 depancreatized dogs following injections of the hormone, whereas in a single dog lactation was obtained.

7103 C

Availability of the Rabbit for Assay of the Hypophyseal Lactogenic Hormone.*

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Two years ago, while investigating various preparations of the anterior pituitary for their lactogenic effect we employed castrated virgin rabbits, as had Corner[1] before us. While satisfied with the results in the rabbit, we prefer at present to use the virgin guinea pig ovariectomized in oestrus and injected immediately thereafter (Nelson and Pfiffner[2]) or the 4 to 5 weeks old squab (Riddle[3]) as

[1] Chaikoff, I. L., and Lyons, W. R., *Am. J. Physiol.*, in press.

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[1] Corner, G. W., *Am. J. Phys.*, 1930, **95**, 43.

[2] Nelson, W. O., and Pfiffner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 1.

[3] Riddle, O., Bates, R. W., and Dykeshorn, S. W., *Am. J. Phys.*, 1933, **105**, 191.

more convenient forms. It would seem, however, that the same degree of satisfaction with the castrate virgin rabbit has not been experienced by some other investigators. This report restates our position on the action of the lactogenic hormone in rabbits, and submits further information concerning our experiences with this animal.

We have reported[4] that a mature mammary gland in a virgin rabbit normally matured, then castrated, will respond by growth and lactation to the lactogenic hormone, but will merely atrophy following administration of potent growth hormone, or of potent gonadotropic hormone from pregnant mares' serum, or pregnant women's urine. That no continuing ovarian factor plays a rôle in the resultant growth of the ducts and alveoli of such positive reactions seems plausible since we always remove the ovaries just before beginning injections. Should ovarian secretions remain in the body after castration, their influence on the mammary apparatus could only be a synergistic one with the lactogenic hormone, since without administration of the latter the castrate rabbit's mammary glands gradually atrophy.

The rabbits used by us have been 5½ to 6 months old virgin New Zealand Whites and Reds weighing around 3 kg. If the nipples of these just mature females show any appreciable growth over and above the immature or male-sized nipples, then lactation may be expected following treatment with lactogenic hormone. Precaution must be exercised to ascertain that the animal is not already secreting milk. Stripping of the glands throughout their extent and observation of a control biopsy spread assures one on this point. We have often observed "spontaneous" lactation in virgin rabbits isolated from males and other females, but we have never found anything but atrophy of the mammary glands of untreated castrate rabbits not lactating at the time of operation. Spontaneous lactation has been observed in young virgin rabbits, the ovaries of which upon serial sectioning showed no evidence that a corpus luteum had ever been formed (although a considerable amount of interstitial tissue around atretic follicles was present), and such animals have been observed to lactate continuously for a month following removal of the ovaries, apparently in response to lactogenic hormone from the animals' own hypophyses.

For uniform results with the lactogenic hormone in rabbits one should use virgins, the ovaries of which show no evidence of corpus luteum formation. The ovaries of a just mature rabbit previously

[4] Catchpole, H. R., and Lyons, W. R., *Anat. Rec.*, 1933, **55**, 49.

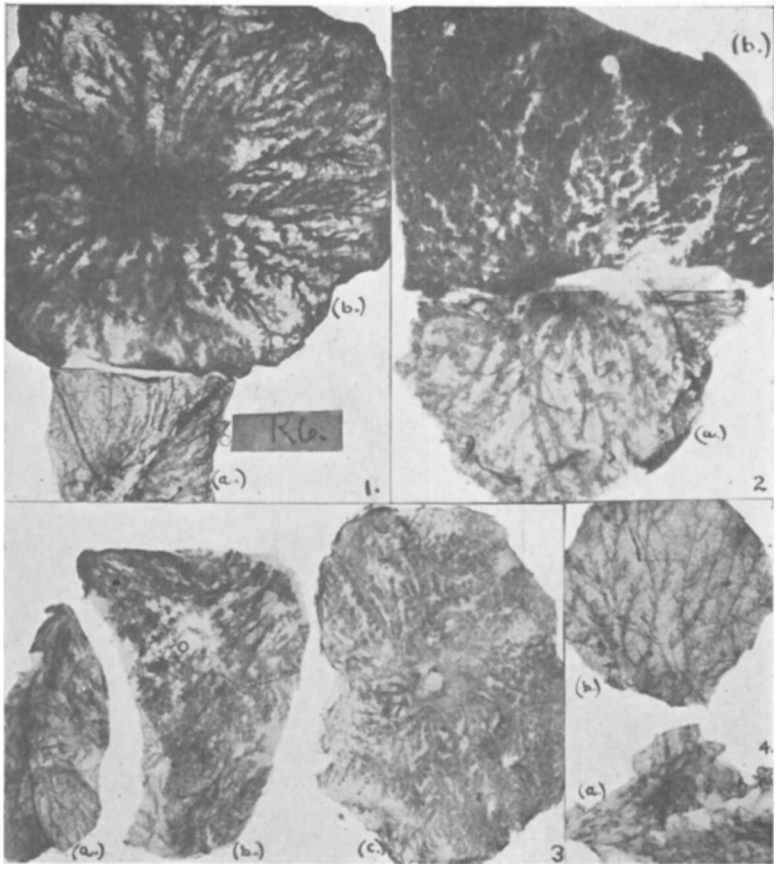

FIG. 1. Shows the control mammary spread (a) and the reaction (b) to the lactogenic hormone. This rabbit lactated after 7 daily injections (each 5.0 cc. of a 1% solution) of an acetone-ammonia preparation (sheep anterior lobes) free of growth but contaminated with the gonadotropic hormone.

FIG. 2. Shows the control spread (a) and the reaction (b) to two daily injections (each 5.0 cc. of a 1% solution) of a preparation similar to above.

FIG. 3. Shows the control spread (a) and the reaction (b) (lactating) after 5 daily injections (each 2.0 cc. of a 1% solution) and the reaction (c) to 5 more injections (each 5.0 cc. of a 1% solution) of an acid-acetone preparation (bovine anterior lobes) free of gonadotropic but slightly contaminated with growth hormone.

FIG. 4. Shows the control spread (a) and the negative reaction (b) to 12 daily injections (each 5.0 cc. of a 1% solution) of a preparation potent for growth but free of gonadotropic and lactogenic hormones.

isolated at weaning should show a crop of fairly large follicles but no corpora. Typical mammary spreads of such rabbits may be seen in the (a) parts of Figs. 1-4. Fig. 2a shows a mammary gland more developed than the others, but still not lactating. We feel that such glands (esp. 1, 3 and 4) are from rabbits just over the border-

line of mammary (and sexual) maturity. Since in our hands such slightly developed glands respond to the lactogenic hormone alone by the growth shown in the (b) parts of Figs. 1-3, we should be inclined to stress a physiological rather than a morphological definition of mammary maturity by defining a mature mammary apparatus in the rabbit as one that is capable of responding to the lactogenic hormone by duct and alveolar growth, and by secretion of milk.

Such a statement is at variance with that made by Riddle *et al,*[3] who state that they have become "fully convinced that prolactin† has not in the least favored the growth and development of mammary tissue in the particular individuals with which we have worked," (guinea pigs and rabbits); and that of Gardner and Turner,[5] who state that "growth of the mammary gland in the rabbit has never been stimulated, at least to any marked degree, by injection of pituitary extracts," also that "Galactin† was ineffective in stimulating the growth of mammary glands of young, ovariectomized rabbits." When our photographs are compared with those of Gardner and Turner (loc. cit., Plate III, p. 26) it becomes apparent that we have produced growth as well as lactation in glands considered by them as non-reactive or infantile. With the establishment of a proper lactogenic hormone unit such differences in results will probably be cleared up.

We would suggest that if the rabbit is to be used as an assay animal rather than the squab or the castrated virgin guinea pig, it would consume less time and allow for less chance of error if mature, non-ovulated, non-lactating virgin rabbits castrated just prior to testing were used. It may be objected that ovariectomy need not be done, but a warning seems timely that solutions containing no lactogenic factor but rich in gonad-stimulating hormone may set up a condition in the ovary simulating that in pseudopregnancy, and lactation frequently follows this condition in the rabbit apparently through secretion of lactogenic hormone by the animal's own hypophysis. Consequently although Gardner and Turner‡ use the pseudopregnant rabbit as a basis for their assay, we consider it difficult to differentiate between the effects caused by injected sub-

†Names employed for the hypophyseal lactogenic hormone.

[5] Gardner, W. W., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 196, (1933).

‡ These workers use 12-16 day pseudopregnant rabbits made pseudopregnant by injection of gonadotropic substances, or by sterile coitus. They treat with the lactogenic hormone solution for 7 days. According to their method a +++ or a ++++ but not a ++ reaction identifies the unit.

stances and those induced by the pseudopregnant state itself on the mammary glands of the rabbit.

7104 P

Effect of Castration on Fasting Ketonuria in Rats Following Diacetic Acid Administration.*

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Deuel and Gulick[1] have shown that women are much more susceptible to fasting ketosis than are men. Although no sexual difference was observed by Butts and Deuel[2] in the level of the slight ketonuria which develops in rats during fasting, it was noted that the administration of diacetic acid by stomach tube to fasting rats in proportion to their surface areas was followed by a greater excretion of acetone bodies in the urine of the females than in that of the males. Similar results were obtained with guinea pigs.

In the present investigation a comparison has been made in the acetone body excretion in normal male and female rats with castrated ones of both sexes, some of which received theelin after the administration of diacetic acid. The experimental technic was similar to that employed in the earlier work.[2] Rats which were 4 to 5 months old were used. The number of rats used in each group is as follows: normal males, 24; castrate males, 12; castrate males with theelin, 9; normal females, 23; castrate females, 12; and castrate females with theelin, 9.

The acetone body excretion was uniformly about twice as high in the female as in the males. Castration in the male resulted in a small decrease in the level of ketonuria. However, the acetone body excretion in the oöphorectomized female rats was reduced to a value about one-fourth that of the normal females and about one-half that of normal males. This level showed no tendency to rise during the 5 days during which the experiment was carried on. The administration of 3 units of theelin per 140 gm. rat to castrate male

* This work was aided by Grant 289 of the Committee on Scientific Research, American Medical Association.

1 Deuel, H. J., Jr., and Gulick, M., *J. Biol. Chem.*, 1932, **96**, 25.

2 Butts, J. S., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1933, **100** 45.