intestinal nature, in others upper respiratory and possibly in others one, or both, of these clinical pictures with the predominating effects soon after onset manifest in the central nervous system, the pathology being that of an encephalitis. The clinical history of this disease, the lack of discovery of the true etiological agent over these many years, permits at least the raising of the question again as to its possible specific relation to epidemic influenza, most probably caused by an ultramicroscopic virus.

7100

Assay with the Guinea Pig of the Lactogenic Hypophyseal Hormone.*

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Corner's[1] results on mammary stimulation in virgin ovariectomized rabbits by injections of hypophyseal extracts were confirmed by Nelson and Pfiffner[2] in guinea pigs. They obtained lactation in 3 ovariectomized, virgin guinea pigs following injections of hypophyseal extracts alone.

In our own investigations of the lactogenic hypophyseal hormone,† rats, rabbits, guinea pigs, cows, dogs, a monkey and an opossum have been used. Lactation was readily induced in all forms except the rat, which was poorly responsive. We have also used the squab test‡ of Riddle *et al*[3] for lactogenic hormone on some hun-

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

[2] Nelson, W. O., and Pfiffner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **25**, 1.

† Lactogenic hormone has been prepared by making acid-acetone extracts (approx. 7.0 cc. conc. HCl were added to 1 liter of 66% acetone) of acetone-dried anterior pituitary powders from cattle, pigs and lambs. The active principle is precipitated out of the cleared extract at a concentration of acetone between 83-90% at a pH of about 2.0. This white precipitate represents a crude lactogenic fraction and is free of gonadotropic hormone, but may sometimes be slightly contaminated with growth hormone.

By isoelectric precipitation, powders readily soluble in water-clear salt solutions at pH 6.0 and 7.0, but insoluble at 6.4 have been obtained, which were active for

dred birds. While satisfied with it, more particularly because the test animals may be used for simultaneous assay of other anterior pituitary hormones, we have nevertheless been interested in using the guinea pig as a favorable mammalian form in which to study mammary response. We have observed lactation in more than 100 virgin guinea pigs (600-700 gm.) ovariectomized in oestrus and injected immediately thereafter with lactogenic hormone. Such animals, contrary to Riddle's[3] opinion, are very reactive without previous injections of ovarian hormones (the influence of preceding ovarian cycles probably suffices to "prepare" the gland). Virgins in oestrus are chosen because at this time of the cycle a few drops of clear, watery fluid containing colostrum-corpuscles may be expressed from the gland, indicating that the gland is at the height of its cyclical growth.[4] To date we have never observed milk secretion in normal or ovariectomized virgin guinea pigs uninjected with the lactogenic hormone. As early as 8 hours following the injection of the lactogenic principle, the serous secretion may be replaced by a turbid one; and it has been possible following potent hormonal injections to express milk in streams within 24 hours.

It is preferable to remove the ovaries of the test animal, especially if there is a suspicion that the extracts being tested contain the gonadotropic factor. It is very difficult even at this time to ascertain which of the mammary reactions in non-castrates reported in the earlier literature were due to injected lactogenic hormone and which to the test animal's own hypophyseal lactogenic hormone released because of induced ovarian conditions. That the gonad-stimulating hormone of the anterior lobe is not in itself responsible for direct mammary stimulation has been definitely shown.[3, 5, 6]

It is possible in the guinea pig to grade the mammary response by judging the spreads as Gardner and Turner[5] do with the rabbit, but this has little advantage over the expression of milk by manipulation.

lactogenic hormone when tested on squabs and guinea pigs in doses of less than 1 mg. and which were negative for the growth and gonadotropic hormones.

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

‡ In this test we believe that one need not depend upon a weight increase in the crop-gland over and above the control, to determine a positive reaction, since beginning growth changes may be seen in crops that weigh less than the average normal, as early as 48 hours after the injection of potent hormone. Stained spreads may be made of these crops just as is done with mammary gland, although a qualitative reaction may be recognized by merely holding them to the light.

[4] Loeb, L., and Hesselberg, C., *J. Exp. Med.*, 1917, **25**, 285.

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

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

Both are qualitative tests, but the minimal effective dose may be arrived at quite as easily by qualitative methods as quantitative. With further purification of the lactogenic hormone it may be possible to use guinea pigs repeatedly for tests, as is done in assays of oestrin. We have on several occasions induced a second lactation 2 weeks after an earlier positive reaction had subsided in ovariectomized virgins. On the other hand, with crude lactogenic extracts of beef hypophysis we have observed anaphylactic death following the second injection.

7101

Induction of Lactation in Heifers with the Hypophyseal Lactogenic Hormone.*

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(Introduced by Herbert M. Evans.)

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We present here results of preliminary experiments on the possibility of producing lactation in the virgin heifer by the injection of the hypophyseal lactogenic hormone. A 1% solution of crude lactogenic hormone† prepared from bovine anterior lobes was used. We employed 2 Holstein heifers 16 months old and 2 Ayrshire heifers 12 months old.

Holstein Experiments. The udders of these animals H-1 and H-2 were manipulated twice daily for 1 week. At the end of this time H-1 showed a very slight serous secretion. 10.0 cc. of the hormone were then injected subcutaneously daily for 11 days into this animal. The secretion became milky on the fourth day of injection and increased rapidly in amount up to 500 cc. daily by the eleventh day when milking was stopped and the heifer allowed to go dry. Through the courtesy of Dr. G. A. Richardson of the Division of Dairy Industry, University of California at Davis, samples of the tenth and eleventh days secretions were analyzed. The fat content, pH, and co-

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† This preparation was free of gonad-stimulating hormone (immature rat test) but gave rise to reducing substances in the urine of injected dogs. For method of preparation see accompanying note by Lyons and Catchpole.