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.

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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.

agulation times of the milks were within the usual limits of variation. Without knowledge of the source, the flavor was criticized as "not definitely pleasing". The most prominent taste was salty, due to low lactose and high chloride content. The consistency of the milk was somewhat thick, probably due to the high protein content. Peroxidase was present. It was agreed that in the course of 10 days the hormone had induced a secretion at least sensibly approaching normal constitution, and we would expect the discrepancies to vanish should the milk secreting mechanism get fully under way with continued treatment.

The control animal H-2 was manipulated daily for 22 days. A few drops of a salty, serous fluid could be expressed on the 15th day and after. Beginning on the twenty-second day, a total of 100 cc. hormone was injected over a period of 13 days. By the tenth day the secretion was milky in appearance. On each of the fourteenth and fifteenth days about 250 cc. of milk were obtained. Milking was continued until the eighteenth day, when the secretion had begun to fall off in amount, since hormone was no longer being given.

Ayrshire Experiments. The results obtained with these animals, A-1 and A-2 gave important leads for further experimentation. A-2 was used as a control animal for the first 21 days. Manipulation during this time gave nothing beyond a few drops of serous, salty fluid. A total of 115 cc. hormone was then given over a period of 14 days. The secretion became milky on the fifth day and increased in amount to a 300 cc. yield on the sixteenth day. By the nineteenth day with continued milking (injections having stopped on the fourteenth day) the yield fell off as in the case of H-2 above.

The heifer, H-1 showed an udder development rather less than any of the other experimental animals, and injection of a total of 150 cc. of hormone over 22 days provoked no response. Even the slight serous secretion was absent. This cow was in heat on the twentieth day of the experiment and on the twenty-third day a scanty serous secretion appeared. A further 100 cc. hormone injected over the next 12 days led to the production of 80 cc. milk by the fourteenth day. This fell off on ceasing the injections.

The results with the heifer A-2 may be viewed in the light of our finding in the rabbit that the mammary apparatus of the sexually immature animal is apparently insensitive to the lactogenic hormone.[1] Preparation of the mammary gland with an ovarian factor seems essential for stimulation by the lactogenic hormone. Whether in the heifer the degree of mammary sensitiveness to lactogenic hormone

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

action is normally determined by the number of cycles that the animal has passed through will be determined by further experiments with heifers at these borderline stages.

A comparison of our data with those of E. I. Evans[2] makes it appear that our heifers were just at, or just over the borderline of mammary reactivity. This author does not, however, state the ages of the virgin heifers that were used in his experiment.

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Experiments with Hypophyseal Lactogenic Hormone on Normal Ovariectomized and Hypophysectomized Dogs.

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The present report deals with the effects of the lactogenic hormone in normal dogs and in dogs following the removal of ovaries and hypophysis. The hormone used in this investigation was obtained in powder form from an acid-acetone extract of the anterior pituitary.

One mg. of the dry powder was equivalent to 40 mg. of fresh anterior lobe. Gonad-stimulating hormone could not be demonstrated in 100-mg. doses of such powders tested on immature pigeons and rats. No attempt was made in these experiments to free these powders of some slight contamination with growth hormone, although potent isoelectric precipitations at pH 6.4 have yielded growth-free lactogenic hormone. Full details of the method of preparation are submitted separately.

The results are shown in tabular form. It was found that 2 subcutaneous injections representing a total of 20 mg. of crude lactogenic hormone sufficed to cause the secretion of milk in normal parous and non-parous mature bitches.

Lactation was obtained in 3 bitches ovariectomized one day previous to administration of the hormone.

The hormone induced lactation in a single bitch hypophysectomized one week prior to treatment. The mammary glands of this animal were well developed and did not yet show regressive changes

[2] Evans, E. I., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 1372.