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Preparation and Assay of Mammotropic Hormone.

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It has been possible to prepare mammotropic hormone with an average minimal effective dose of 0.1 mg. when tested in squabs by means of the 4-day (4 injections) "systemic" test introduced by Riddle, *et al.*,[1] and 0.1γ when tested by means of the 2-day (1 injection) "local" test.[2] This hormone (unheated) does not stimulate the thyroids or gonads of immature squabs, rats or guinea pigs in doses of 100 mg. (4 daily doses of 25 mg.) whereas purified thyrotropin and gonadotropin are effective in doses of less than one mg. Furthermore, hypophysectomized rats show no increase in body weight, nor repair of adrenals, thyroid, gonads, when injected with purified, unheated mammotropin (10) mg. per day for 20 days.

One method of preparing mammotropin is presented here because it yields equivalent amounts of mammotropic and adrenocorticotropic hormones, sufficiently separated to prove them distinct substances.

Grind thoroughly 1 kg. undissected sheep pituitaries (equivalent to approximately 500 gm. anterior lobe). Add 4 litres acetone (to which has been added 100 cc. 35% HCl) one litre at a time, mixing thoroughly by putting the mash through the grinder several times after each addition.

Centrifuge,† and to the supernatant add 5 litres acetone. Agitate rcibly and if possible allow to settle overnight at a low tempera-e. Dissolve the gummy precipitate in a mixture of 100 cc. H_2O 100 cc. acetone, and reprecipitate by adding 800 cc. acetone. olve precipitate in 200 cc. H_2O, add 100 cc. NH_4OH (28%) and allow to stand at room temperature for about 3 hours. ı add 2 volumes of acetone, centrifuge and discard precipitate reëxtract it by dissolving in 100 cc. H_2O and adding 50 cc. OH and 2 volumes of acetone as before). To supernatant(s) 1 volume acetone and 10 cc. 35% HCl. Agitate forcibly to pitate.

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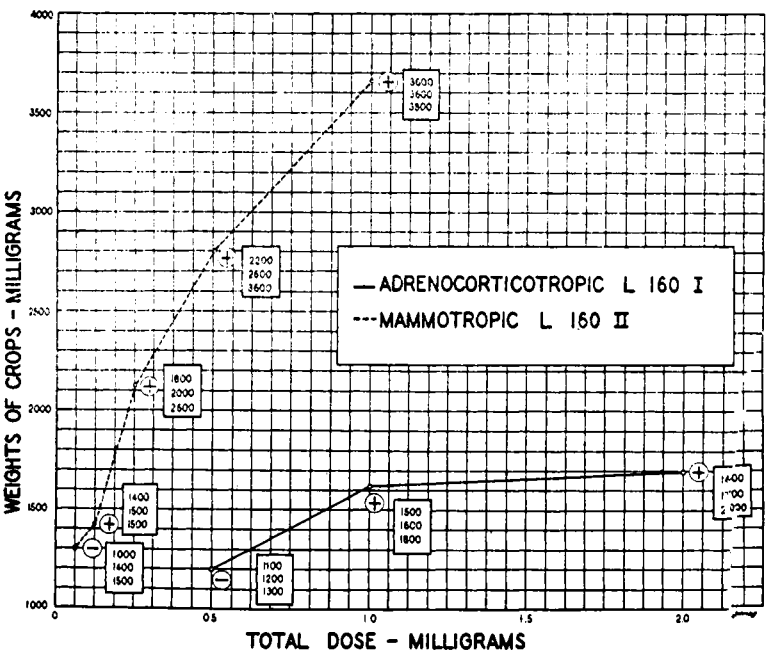
rease yield, the mash may be re-extracted with 2 litres 85% acetone ›H 1.5 and the supernatants pooled. In the first extraction with ›, consideration is given to the H_2O in the tissue.

Discard supernatant and evaporate acetone-NH_3 from precipitate. Dissolve in 200 cc. H_2O with sufficient N/1 NaOH to obtain a clear solution. Adjust to pH 6.5 with N/1 HCl and centrifuge out precipitate.‡ Adjust to pH 5.5 and freeze out precipitate. Several adjustments of the supernatant should be made with HCl and NaOH and freezing in order to increase the yield of isoelectric precipitate, which usually approximates 1.0 gm. Dissolve precipitate in as little H_2O and N/1 NaOH as is necessary to give a clear solution at pH 8.0. After ascertaining per cent of organic matter, adjust solution to 1% at pH 7.6, add Hexylresorcinol to 1:15,000 or Merthiolate 1:5000, and sterilize 20 minutes at 100°C. on 2 successive days.

The accompanying graph shows the average weights of the dissected, reactive areas of both crop sacs after injections of the mammotropic (pH 5.5 iso-electric) and adrenocorticotropic (pH 6.5 iso-electric) fractions respectively. Three birds were used at each level, and were sacrificed 24 hours after the fourth daily injec-



Graph showing the difference in crop sac reactions of 1-month-old squabs to 4 subcutaneous, daily injections of different amounts of incompletely separated fractions of mammotropin and adrenocorticotropin.

A + accompanies such positive reactions as fall within the weight range of normal crop sacs (1000-1500 mg.).

‡ This fraction has been investigated by Mr. Henry D. Moon and is the subject of an accompanying paper.

tion. It would appear that the pH 5.5 iso-electric contains about 10 times as much mammotropin as the pH 6.5 iso-electric or adrenocorticotropic fraction. This represents the typical finding in about 50 batches prepared in the above manner.

The "local" intradermal method of testing mammotropin (Fig. 1) while as yet lacking a gravimetric basis, requires less hormone and time than the "systemic" test, and is indispensable in studies of urinary mammotropin (especially that of normal males). The histology of the local reaction is the same as seen in the "systemic" test, but the maximal reactions (Fig. 5) show less growth because of the shorter period (48 hours). The minimal effects are usually limited to circumscribed areas (3-4 cm. in diameter) better seen after tearing the muscle layer off the stretched crop (the fingernail does this nicely). Histologically these areas may show as much

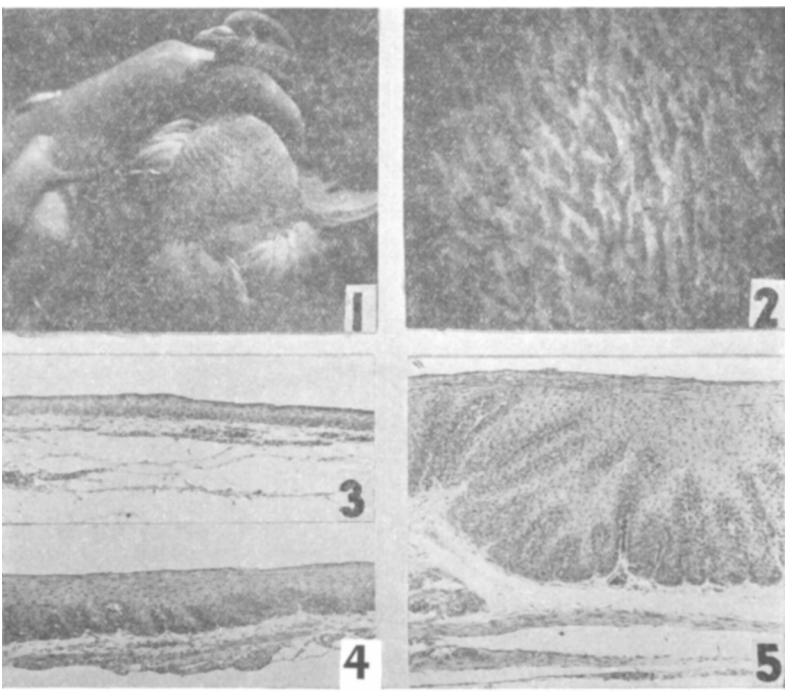


FIG. 1. The plucked crop sac area of a 1-month-old Silver King squab. 0.1 cc. of solution is injected into a feather ridge at the point indicated. A different dilution is tested on the opposite side.

FIG. 2. The appearance of a positive "local" reaction to mammotropin when the stretched crop sac is held to the light. ×2.

FIG. 3. Section of normal crop sac membrane of a 1-month-old squab. × 40.

FIG. 4. Section showing a low grade reaction obtained 48 hours after 1 "local" intradermal injection of 0.1 microgram of mammotropin. × 40.

FIG. 5. High grade "local" reaction to 6.4 micrograms of same. × 40.

growth as seen in Fig. 4 while the surrounding membrane is similar to the crop of control squabs (Fig. 3). The gross appearance of the "local" reaction when the crop sac is stretched in front of a light is shown in Fig. 2. It has been possible to show that round discs 3 cm. in diameter cut with a cork-borer from the reactive area of crop sacs stretched tightly over a cork and washed with water, when desiccated in the 110 C. oven overnight show weights proportional to the amount of hormone injected in the dose range from 0.1 to 10.0 micrograms. For those who prefer a gravimetric unit, this observation may have something to offer, but the qualitative minimal effective dose as is used in tests for the female sex hormone[3] seems sufficient at present. Larger numbers of squabs for each level would have given our results greater statistical significance, but we have not found it economically practical to use more than 5, and we customarily use 3. Our squabs coming from 2-egg nests are chosen one month from hatching for their uniformity, from a large local loft; they are all Silver Kings and may occasionally vary 100% in reactivity to the hormone. Of 5 birds tested on the right side with 0.05γ of L 160 II only one reacted, while 4 reacted to 0.1γ injected on the left side. Only in large doses is a local injection on one side carried over (probably systemically) to affect the opposite sac.

Conclusion. A method has been described for extracting mammotropic hormone from sheep pituitaries involving its solubility in HCl-acetone and NH_3-acetone, and its precipitation with isoelectric protein at pH 5.5. A fraction made by the same procedure but precipitated at pH 6.5 has been shown to contain relatively little mammotropin, and is identified in the following paper as the adrenocorticotropic hormone. The "local" intradermal squab test has several advantages over the "systemic" tests of Riddle, *et al.*, when only mammotropic hormone is to be tested for, but the latter test has the very great advantage of allowing one to assay for the gonadotropic, thyrotropic, adrenotropic and mammotropic hormones on the same bird.

[3] McShan, W. H., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 50.