Cell populations in control cultures averaged 3.17 million/ml after 5 days; had utilized an average of 0.99 mg of glucose and 4.0 mg of protein/ml of medium. 3) Fluoride ($10^{-3}$ M), azide ($10^{-4}$ M) and arsenite ($10^{-5}$ M) decreased cell numbers by 24, 63 and 81%, respectively. Although urethan ($10^{-1}$ M) killed the cells, a concentration of $10^{-2}$ M had little effect. 4) Fluoride ($10^{-2}$ M), arsenite ($10^{-4}$ M) and urethan ($10^{-3}$ M) inhibited glucose uptake by 28, 23 and 8%, respectively. Azide ($10^{-3}$ M) produced 10% acceleration of glucose uptake. 5) Azide ($10^{-3}$ M) and fluoride ($10^{-3}$ M) inhibited protein uptake by 92 and 39%, respectively. Arsenite and urethan accelerated protein uptake in all concentrations. 6) The mitotic index of untreated cells was 10.5. All inhibitors except azide produced a lowered index. 7) In control cultures, 4% of cells were multinucleate. Azide ($10^{-4}$ M) increased these forms to 17%.

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## Extraction and Concentration of Mammogenic Fractions from Anterior Pituitary Gland.* (25877)

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The concept of homogeneity of lactogenic and mammogenic (mammary gland growth promoting) hormones has been disputed for many years. Older studies(1) showing wide differences in relative amount of these hormones have been repeated using improved assay technics for both hormones(2). Since recent evidence(3) indicates that lactogenic hormone discharge from anterior pituitary gland (AP) does not occur until late pregnancy, or until after over 50% of mammary gland growth has taken place, it would appear incongruous with physiological facts to accept the idea that lactogenic hormone is pituitary agent primarily responsible for mammary gland growth during pregnancy. Critics of the mammogen concept have suggested need of extracting a preparation from the pituitary rich in mammogen and free of lactogenic hormone. The present report indicates our progress. It will be shown that mammogenic hormone is poorly extracted by solvents which effectively remove other established hormones (4). Evidence will be presented indicating that after other hormones are removed from AP, a mammogenic factor may be extracted and concentrated from the residue.

*Materials and methods.* To remove gona-

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dotropins, ACTH, TSH, growth and lactogenic hormone, acetone dried AP powder was thoroughly mixed with 16 volumes of 56% ethanol and 4% butanol. The suspension was brought to pH 12.5 by addition of 1 N NaOH, then titrated back to pH 10.0 with 1 N HCl. After stirring for 1 hour, residue was removed by bottle centrifuge. The residue was extracted 3 additional times, and each time pH of solvent was "over-shot" to 12.5, then adjusted to pH 10.5, 11.0, and 11.5 on second, third and fourth extractions, respectively. The residue was dried with acetone and ether and stored in a desiccator. Two mammogenic fractions were extracted from the residue, the first obtained by use of a sodium phosphate buffer solution and the second by one of isotonic saline. Phosphate buffer solution was composed of 0.6 g monosodium phosphate monohydrate and 2.2 g disodium phosphate anhydrous per liter distilled water. Isotonic saline was composed of 9 g C.P. sodium chloride per liter distilled water. A ratio of 1 part residue (by weight) to 40 parts solvent was used. Suspension was stirred at room temperature for 45 minutes, centrifuged, and clear supernatant saved. Residue was extracted in a similar manner 3 additional times and supernatants were pooled. In case of phosphate buffer system, pooled supernatants were brought to concentration of 56% ethanol and 4% butanol. During addition of 2 N NaOH, a precipitate formed at approximately pH 12.0. The precipitate was collected and dried with acetone. Buffer salts were removed by dissolving precipitate in water and dialyzing against distilled water which was agitated slowly for 8 hours. Four changes of water were required to remove phosphorous from solution. Precipitate was recovered on a Whatman No. 50 filter paper, dried with acetone and weighed. Yield was approximately 0.12% of initial residue, or 0.08% of starting acetone dried AP powder. This fraction was an amorphous, grey powder (fraction A). Upon assay(5), this fraction contained less than 0.03% lactogen, $p = .99$. For the saline solution, all extracts were pooled and saturated with sodium chloride, then chilled in deep freeze. Precipitate was collected on Whatman No. 50 filter paper, dried with acetone, and weighed. Yield was approximately 0.10% of the initial residue, or about 0.07% of the starting acetone dried AP powder. This fraction was more crystalline and white (fraction B). This fraction contained no trace of lactogen. To produce a suitable standard mammary gland growth curve for comparison of mammogenic potencies of a highly purified lactogenic preparation(6) and mammogenic fraction A, 0.75 μg estradiol benzoate (EB) plus varying amounts of progesterone were injected/day for 10 days to male mice previously primed with diethylstilbestrol. Graded amounts of lactogenic hormone plus 0.75 μg EB/day and graded amounts of fraction A plus 0.75 μg EB/day were administered to groups of estrogen primed male mice for 10 days. Mammary gland growth determined by amount of desoxyribonucleic acid was compared, and relative potencies of the 2 protein fractions were calculated (Table I). In second experiment, female mice were ovariectomized, and groups injected with estrogen and estrogen plus progesterone for 19 days, to approximate gestation period in mouse. To determine relative

TABLE I. Lobule-Alveolar Growth Stimulated by Progesterone, Mammogen and Lactogen in the Male Mouse.

| Substance | Total 10-day dose (mg) | No. of animals | Total DNA mean (mg) |
|---|---|---|---|
| Progesterone* | 10.0 | 9 | 3.25 |
| | 5.0 | 7 | 2.96 |
| | 1.25 | 8 | 2.68 |
| Lactogen (Cole & Li)* | 4.0 | 8 | 3.65 |
| | 2.0 | 9 | 2.80 |
| | 1.0 | 10 | 2.16 |
| | .5 | 5 | 2.10 |
| Conc. mammogen* (fraction A) | .1 | 10 | 3.44 |
| | .05 | 8 | 3.07 |
| | .025 | 8 | 3.05 |
| | .0125 | 9 | 2.82 |

* Plus .75 μg estradiol benzoate/day.

SUMMARY

| Summary | a | b | Syx | Relative potency |
|---|---|---|---|---|
| Progesterone | 2.60 | .612 | .078 | 1.0 |
| Lactogen (Cole & Li) | 1.71 | 1.76 | .286 | 1.59 .89–2.87 |
| Conc. mammogen | 2.75 | .631 | .140 | 223.3 96.6–515.5 |

TABLE II. Effects of Anterior Pituitary Fractions on Mammary Gland Growth in Ovariectomized Female Mice.

| Treatment, daily dose | No. of animals | DNA/mg DFFT, μg | DFFT, mg | Total DNA, mg | Body wt mean, g | Total DNA per 10 g body wt, mg |
|---|---|---|---|---|---|---|
| Controls, ovariectomized | 15 | 27.8 ± 5.8 | 64.8 | 1.805 ± .387 | 25.6 | .706 ± .151 |
| 1.0 μg EB* | 11 | 26.6 ± 3.9 | 102.3 | 2.720 ± .406 | 30.1 | .902 ± .135 |
| *Idem* + 3 mg progesterone | 12 | 26.4 ± 3.3 | 170.6 | 4.528 ± .564 | 34.6 | 1.304 ± .163 |
| " + 4 μg Ant. pituitary | 10 | 38.4 ± 5.3 | 171.3 | 6.578 ± .912 | 35.5 | 1.853 ± .257 |
| " + " initial residue | 9 | 36.0 ± 5.4 | 137.4 | 4.941 ± .737 | 34.1 | 1.451 ± .216 |
| " + .01 mg Fraction B | 9 | 38.4 ± 4.7 | 127.9 | 4.912 ± .607 | 35.1 | 1.403 ± .173 |
| " + " " A | 11 | 31.6 ± 5.7 | 112.7 | 3.564 ± .672 | 32.6 | 1.162 ± .206 |
| 18-day pregnant† | 15 | 57.0 ± 1.7 | 125.6 | 7.007 ± .281 | 38.5 | 1.832 ± .021 |

* Estradiol benzoate.

† Brookreson and Turner(8).

mammogenic potency of AP powder, initial residue and mammogenic fractions A and B, equivalent amounts of these fractions were injected along with .75 μg estradiol benzoate into groups of ovariectomized female mice for period of 19 days. Mammary glands were removed and growth determined by DNA measurement (Table II).

*Results.* Estrogen and progesterone have been shown to promote extensive lobule-alveolar mammary gland growth in intact albino mice(7). Good growth as determined by DNA and whole mount technics was produced by 1 mg progesterone plus 0.75 μg estradiol benzoate per day for 10 days (Table I). The highly purified lactogenic preparation produced approximately comparable results when 0.4 mg was injected per day with estrogen. Calculated relative potency was 1.59, indicating that this preparation is slightly more potent than progesterone. Mammogenic fraction A produced good development when 0.01 mg was injected with estrogen daily for 10 days. The mean relative potency was 223.3, indicating much greater mammary gland stimulating potency than either highly purified lactogen or progesterone.

Results of 19-day injection of single doses of various fractions into ovariectomized female mice (Table II) show that estrogen plus crude AP powder produced growth comparable to that noticed in the 18-day pregnant primiparous mouse(8), which was notably better than growth produced by estrogen and progesterone, initial residue or either of mammogenic fractions.

While it required 4.0 mg initial residue to produce mammary gland growth equivalent to that produced by 3.0 mg progesterone, only 0.01 mg fraction A or fraction B were required to produce equivalent amounts of growth.

*Discussion.* Methods have been described for extraction and concentration of fractions from the anterior pituitary gland which, in intact male albino mice or ovariectomized female mice, exhibits marked mammogenic properties. That crude AP powder produced better growth than progesterone, initial residue or either of the mammogenic fractions could be explained on the basis that other hormones injected tended to equalize amount of circulating AP hormones in individual animals, thus permitting mammary glands to reach maximum development under stimulation by mammogenic hormone.

Existence of a separate specific mammogenic hormone has been doubted due to the presence of mammogenic activity in lactogenic extracts of highest potency. However, the fact that 0.01 mg of mammogenic extract produced lobule-alveolar growth approximately equal to that produced by 0.4 mg of lactogen indicates a maximum contamination of the latter with 0.71% of mammogen. This would indicate that mammogen is extracted to a slight extent by methods of lactogen preparation.

*Summary.* It has been shown that appreciable quantities of mammogenic factor are left in initial residue of anterior pituitary gland after extraction of the known hormones by usual technics. This factor was extractable by either a monosodium-disodium phos-

phate buffer solution or a saline solution. The active factor precipitated from the phosphate buffer system along with phosphate salts after addition of ethanol and butanol and adjusting the pH to 12.5. Phosphate salts were subsequently removed by dialysis, during which mammogenic hormone precipitated out of solution. Active factor was precipitated from saline solution by saturation of solution with NaCl and chilling. Resulting light grey, amorphous powder obtained by phosphate extraction was 223 times more potent in promoting mammary gland growth than progesterone, and contained negligible, if any, lactogenic activity. A highly purified lactogenic preparation assayed for mammogenic activity at the same time was only 1.59 times as potent as progesterone. On this basis the lactogen need contain no more than 0.71% of mammogen to produce the biological response observed.

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## Effect of Corticotropin and Growth Hormone on Allantoin Synthesis and Excretion in the Dog.* (25878)

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The effect of corticotropin on excretion of end products of purine metabolism seems to vary with species. In dogs urinary output of allantoin is increased by massive doses of corticotropin, presumably as a result of accelerated excretion as well as increased allantoin synthesis(1). On the other hand, corticotropin apparently does not increase allantoin synthesis in the rat, and the renal tubule has been suggested as a possible site of action of hormone on allantoin excretion(2). Likewise uricosuria in human subjects after administration of corticotropin has been attributed to increased renal clearance of uric acid rather than increased synthesis(3). A recent investigation of effects of corticotropin and growth hormone on utilization of $N^{15}$ from individual sources(4) afforded an opportunity to secure data on incorporation of the isotope, ingested as labeled glycine or ammonium citrate, into urinary allantoin of normal dogs, during control experiments, and after administration of corticotropin or growth hormone. Experiments with growth hormone seemed relevant because of its importance in nitrogen metabolism in general, and in view of reports of its positive action on mitotic activity and synthesis of nucleic acids(5,6).

*Materials and methods.* Two adult mongrel bitches were used as experimental animals. Details regarding care, diet, source of hormones, and preparation of labeled compounds have been described(4). Corticotropin was given subcutaneously in 2 doses of 10 units each (9:00 a.m. and 3:00 p.m.) for 3 successive days. Growth hormone, in doses of 5 mg dissolved in 1 ml of normal saline, was administered subcutaneously on 4 consecutive days. The labeled compound was fed with the morning ration on second day of corticotropin treatment and on third day of growth

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