Effects of Anterior Pituitary Preparations on Mammary Gland Growth in Mouse.* (26787)

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Previous comparisons of lactogenic and mammogenic potencies of various pituitary preparations have indicated extreme differences in relative amounts of these hormones when assayed in mice(1). Recent studies have indicated that it is possible to extract fractions with fair mammogenic activity when injected with estradiol benzoate (EB) in the ovariectomized mouse, but possessing little or no lactogenic activity(2). Present report deals with mammogenic effectiveness of a number of crude and purified pituitary extracts, including one newer mammogenic fraction extracted by improved techniques.

Materials and methods. Purified FSH, lactogen and growth hormone (GH) were obtained from the Endocrinology Study Section, N.I.H. Anterior pituitary powder (AP) was obtained by washing frozen bovine anterior pituitary slices several times with cold acetone, followed by ether extraction. Resulting dry, fat-free slices were then ground to a fine powder in a Wiley Mill. Initial residue (IR) was prepared as described previously(2). A concentrated mammogenic preparation (mammogen "C") extracted by stirring one part initial residue twice with 15 parts (by weight) distilled water at approximately 29°C for 2-3 hours, centrifuging and saving clear supernatants. Supernatants were pooled and made up to 60% by addition of 95% ethanol. After adjusting pH to 12-13 by addition of 2N NaOH, solution was chilled by placing in deep freeze (−10°C) overnight. A fine precipitate formed either immediately (during chilling) or on standing thereafter at room temperature for several days. A Whatman No. 50 filter paper over a Buchner funnel was used to recover the precipitate, after which it was dried by washing with acetone. Resulting precipitate was crystalline, white, apparently salt-free, and readily soluble in distilled H_2O. Wilhelmi preparations XIX-36-A, XIX-36-B, XIX-36-C, XIX-34-C1 were prepared as follows (3). XIX-36-A represented dialyzed, lyophilized water extract of whole ground fresh bovine anterior pituitary lobes at pH 5.5. Principal component reported was FSH plus small amount of LH. XIX-36-B was prepared from residue of XIX-36-A resuspended in 0.1 M ammonium sulfate, pH 4, reextracted, dialyzed and lyophilized. Fraction was rich in TSH and LH. XIX-36-C was prepared from residue of XIX-36-B, resuspended in 0.25 M ammonium sulfate, pH 7.5, reextracted, dialyzed and lyophilized. Fraction rich in GH. XIX-34-C1 was final residue, and contained primarily lactogenic hormone.

Groups of mature female ovariectomized Webster-Swiss mice received subcutaneous injections beginning day 14 post-operatively. EB and progesterone (P) were dissolved in olive oil carrier in concentrations such that each animal received 0.1 ml oil carrier daily for 19 days. Pituitary preparations were suspended in a water carrier and injected separately from the synergizing dose of 1 μg EB injected daily.

On day 20, animals were sacrificed and 7 mammary glands of each mouse were taken for desoxyribosenucleic acid (DNA) determination as described previously(4).

Results. Crude bovine AP, when injected with EB, produced greatest mammary gland growth of any preparations in dose injected but did not produce growth equivalent to pregnancy as measured by total DNA (Table I). IR produced a greater mean total DNA than either EB alone or EB plus P, but did not differ significantly from either. Mammogen "C" produced significantly

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TABLE I. Anterior Pituitary Preparations on Ovariectomized Mouse Mammary Gland Growth.

Treatment	No. of mice	Body wt mean, g	DFFT,* mg	DNA, μg/mg DFFT	Total DNA, mg	DNA/100 g BW, mg
Castrate control	10	33.9	73.3	27.7	2.03	6.00 ± .48
EB, 1 μg	10	30.1	85.1	26.5	2.26	7.29 ± .38
EB, 1 μg + P, 3 mg	9	30.4	92.2	26.8	2.47	8.43 ± .35
18-days pregnant†	28	37.4	123.5	52.0	6.22	16.90 ± .69
EB, 1 μg + AP, 4 mg	10	32.2	103.3	33.9	3.51	10.90 ±1.08
Idem + IR, 4 mg	9	29.5	92.5	28.1	2.60	8.80 ± .72
" + GH, 2 mg	9	33.4	96.6	21.7	2.10	6.27 ± .40
" + FSH, 2 mg	9	30.1	72.1	29.0	2.09	6.95 ± .84
" + lactogen, 1 mg	9	33.1	99.5	27.3	2.72	8.22 ± .61
" + mammogen "C," .02 mg	10	33.3	98.3	29.7	2.92	8.76 ± .34
" + XIX-34-C1, 4 mg	11	36.0	73.6	37.8	2.78	7.93 ± .33
" + XIX-36-A, 4 mg	9	29.6	80.2	25.4	2.04	6.89 ± .47
" + XIX-36-B, 4 mg	8	34.2	77.8	31.9	2.48	7.25 ± .37
" + XIX-36-C, 4 mg	11	33.0	92.7	26.3	2.43	7.38 ± .32

* Dry, fat-free tissue. † Data from (6).

greater amounts of total DNA than did EB alone ($P < .01$) but only produced slightly greater mean total DNA than EB plus P. Lactogenic preparation plus EB produced greater mean DNA than either EB alone or EB plus P, although significant difference was marked by high variation in this group. GH, FSH and Wilhelmi preparations were generally ineffective in production of mammary gland growth with the exception of XIX-36-C1, containing lactogen. Total DNA produced by this preparation plus EB approached significance ($P < .08$) compared to DNA produced by injection of EB alone.

Whole mount observation indicated lobule-alveolar development in mice treated with EB plus P, and EB plus AP, IR, lactogenic preparation, mammogen "C," and XIX-34-C1.

Discussion. Previous studies in mice(2) have indicated that IR, obtained from acetone dried bovine AP after known hormones are removed produced significant mammary gland growth in mouse, when injected with synergizing dose of EB. Present study confirms these findings, and points to newer method of extraction of pituitary mammogen from IR. Mammogen "C," which produced lobule-alveolar growth when injected in very small quantity (.02 mg) with a synergizing dose of EB, had relative mammogen potency not dissimilar to mammogen "A" and "B" (2), but was apparently soluble in distilled water and salt-free (indicating less partial denaturation or contamination as in previous preparations). On basis of these data, it would appear that at least one pituitary mammogen effective in mouse is left in pituitary residue after extraction of known pituitary hormones.

Purified FSH would not be expected to produce mammary gland growth in the ovariectomized animal, except by reason of possible contamination by a pituitary mammogen. In present study, no significant lobule-alveolar growth was obtained when large amounts of crude FSH extract (Wilhelmi preparation XIX-36-A) or more purified FSH were injected into ovariectomized mouse with synergizing dose of EB, indicating mammogen is not extracted by technics employed in extraction of FSH.

Crude and purified preparations of growth hormone, when injected with EB, produced no lobule-alveolar growth, confirming previous studies using hypophysectomized C_3H/He Crgl mice(5). Higher doses should have produced noticeable mammary gland development if contamination of these fractions by mammogenic factor existed.

Summary. Mammogenic potencies of a number of crude and purified anterior pituitary fractions were determined by estimation of mammary gland desoxyribosenucleic acid (DNA) in treated ovariectomized mice. Crude bovine AP, initial residue (IR) obtained by depleting AP of known hormones,

a newly developed mammogen extracted from IR (mammogen "C") and lactogenic preparation produced lobule-alveolar development. Extracted mammogen "C" was white, crystalline, apparently salt-free and water soluble. Crude and purified FSH and growth hormone exhibited little or no mammogenic effects when injected with a synergizing dose of estradiol benzoate in ovariectomized mouse.

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Ingestion of Latex Particles by Chicken Fibroblasts in Tissue Culture.* (26788)

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The ingestion of foreign particles by certain cell types both in intact tissue and in tissue culture has been studied extensively and generally referred to as phagocytosis. Cells of the phagocytic type include mammalian granular leucocytes and those of mesoblastic origin commonly called macrophages. These cells not only can ingest bacteria, cell debris and protozoa but also may ingest smaller particles of a colloidal size, this latter phenomenon being called *ultraphagocytosis* or *colloidopexy*. The precise mechanisms of the phagocytic process are not entirely clear and some variations in the manner by which particles are taken in by cells have been described. Thus, in some cases particles appear in cell vacuoles and in others such vacuoles are not seen. Pinocytosis, or "cell drinking," is a second method by which the cell may take in material from its environment. Initially described by Lewis as a mechanism by which fluid enters cells(1), the studies of Mast and Doyle(2), Holter and Marshall(3) and Chapman-Andresen and Holter(4) indicate various materials may enter the cell in this manner. Palade has described a vesicular component of endothelial cells which functions in the pinocytotic process(5) and De Robertis and Bennett(6) have described a similar component for nervous tissue cells. Ferritin molecules are ingested by endothelial cells by the pinocytotic process as reported by Wissig(7), and small (0.014 μ) colloidal gold particles have been reported to be taken in by HeLa cells *in vitro* by Harford *et al.*(8).

No electron microscopic studies have been published of ingestion of foreign inert particles by tissue cells of the fibroblastic type. The present study presents results in which cell uptake of polystyrene latex particles having a diameter of 0.264 μ is demonstrated in tissue cultures of chicken fibroblasts examined by electron microscopy.

Materials and methods. Polystyrene latex suspensions (PSL). Polystyrene latex (PSL) was obtained from Dow Chemical Corp., Midland, Mich., run No. LS-057-A. This lot had a particle size of 0.264 μ and a standard deviation of 0.0060 μ in 577 measurements. Stock suspension of PSL was made in distilled water. PSL content of this suspension determined by direct sedimentation with technics described previously(9,10) was found to be 2.3×10^9 PSL particles/ml. PSL was then diluted further in tissue culture media to obtain the final concentration of inoculum.

Chicken fibroblast cultures. Chicken fibroblast cell suspensions were prepared by trypsinizing tissue of 9-10 day old chicken embryos. These cells were placed in large Rous or T-60 flasks and growth observed

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