Effect of Insulin and Cortisol on Organ Cultures of Adult Mouse Mammary Gland.* (24995)

JOEL J. ELIAS† (Introduced by Howard A. Bern)
Dept. of Zoology and Cancer Research Genetics Laboratory, University of California, Berkeley

Effects of hormones on organ cultures of adult mouse mammary gland are being investigated in this laboratory. Previous reports (1,2) concerned responses of mammary explants after addition of various ovarian, adrenal, and pituitary hormones to a defined medium. This report describes the effects of adding insulin and cortisol.

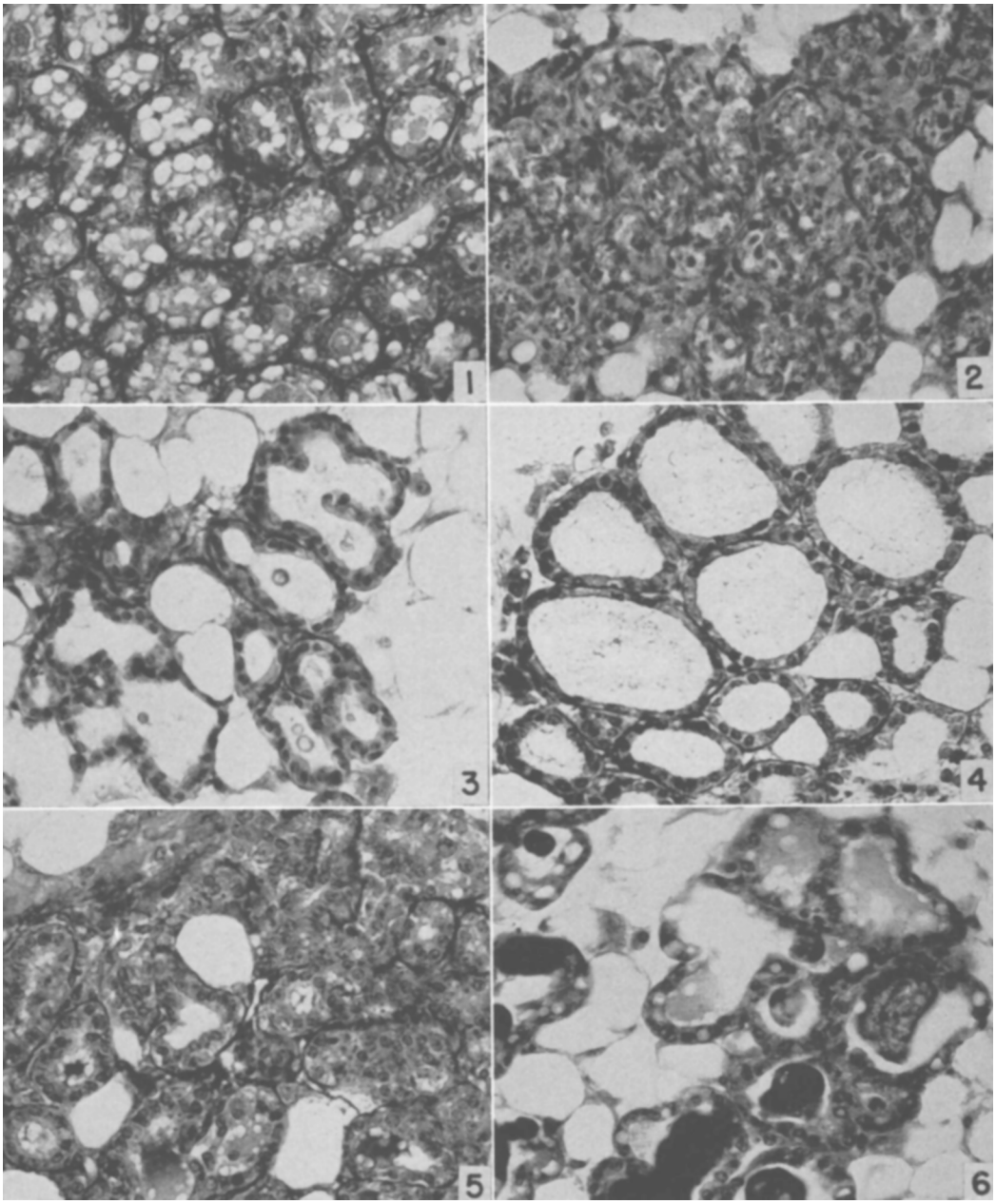
Materials and methods. The experimental procedures used herein have been described (1,2). All tissues were derived from "high mammary tumor" (agent-bearing) C3H/He Crgl female mice. Prelactating explants were taken from mammary glands between 14th and 18th days of pregnancy (Fig. 1). Multiparous females, both with and without spontaneous mammary tumors, provided hyperplastic alveolar mammary nodules(3) for cultivation. Most explants were about 1 x 1 x 0.5 mm in size. The tissues were cultured for 5 days at 37°C, using floating lens paper method of organ culture(4). After cultivation, explants were fixed in Bouin's fluid, and serial sections of paraffin-embedded material stained with modified Masson's trichrome stain (containing iron hematoxylin, acid fuchsin, Biebrich scarlet, and aniline blue). The defined culture medium was "199"(5) supplemented with cortisol and insulin, alone and in combination. The crystalline Zn-insulin preparation used (Eli Lilly #535664) had 25 units of activity/mg. It was dissolved in "199" to produce a concentration of 140 μg/ml in final medium. The non-esterified cortisol preparation was first dissolved in 100% ethanol, then added to "199" to provide a concentration of 8 μg/ml of cortisol and 0.5% ethanol in final medium. *Normal histology.* The prelactating mammary gland from 14th to 18th day of pregnancy consisted of well developed lobules of alveoli showing histologic evidence of secretory activity (Fig. 1). The alveolar cells possessed large intracellular vacuoles, and lumina of moderately enlarged alveoli contained secretory material composed of a stainable granular material and extruded vacuolar contents (lipids). Hyperplastic alveolar nodules, in general, had an histologic appearance similar to that of prelactating lobules(6), but occurred in otherwise inactive glands.

Results. A total of 72 prelactating explants derived from 12 animals, and 26 hyperplastic nodules from 10 animals, were used. Since prelactating lobules and hyperplastic alveolar nodules responded similarly, the results are presented together. Tissues cultured in "199" alone, or in "199" containing added cortisol, underwent extensive alveolar degeneration after 5-day culture period (Fig. 2). Addition to "199" of a combination of cortisol + insulin resulted in alveolar maintenance in all explants during the 5-day culture period. The cuboidal alveolar cells appeared healthy, but contained no intracellular vacuoles. Alveoli were generally distended (Fig. 3), often markedly so (Fig. 4). The lumina of most alveoli showed little or no stainable material, but a few contained some stainable secretion. The material in the lumina of most of the distended alveoli was not sudanophilic.

Addition of insulin alone to the "199" also improved survival of explants (Fig. 5), but not so well as those cultured with both cortisol and insulin. On the other hand, Trowell(7) apparently obtained good maintenance of rat mammary explants using his synthetic medium (containing insulin) and a high oxygen gas phase. He further reports "progressive reduction in secretion content of cells," but details are not given, and the accompanying Figure shows a highly vacuolated alveolar epithelium.

Appearance of alveoli following cultivation in cortisol + insulin-supplemented medium

* Aided by research grants from Am. Cancer Soc.
† Present address: Dept. of Anatomy, School of Med., Univ. of California, San Francisco.



All Figures are of 7 μ thick sections stained with modified Masson's stain. 270× magnification.

FIG. 1. Normal prelactating mammary gland of C3H/He Crgl mouse at about 15-16th day of pregnancy. Note large intracellular vacuoles, secretory material in lumina of alveoli.

FIG. 2. prelactating explant after 5 days cultivation in "199" with added cortisol, 8 μg/ml, showing degeneration of alveoli.

FIG. 3. Prelactating explant after 5 days cultivation in "199" with added cortisol + insulin, 8 + 140 μg/ml. Note loss of intracellular vacuoles, distension of alveoli, unstained contents of alveolar lumina.

FIG. 4. Prelactating explant, treated as in Fig. 3. Note extreme distension of alveoli.

FIG. 5. Hyperplastic nodule explant after 5 days cultivation in "199" with added insulin, 140 μg/ml. Note loss of alveolar organization in area in upper right part of Figure. Some alveoli have *extra*cellular vacuoles and stainable material in their lumina.

FIG. 6. Prelactating explant after 5 days cultivation in "199" with added cortisol + mammotropin, 8 + 140 μg/ml. Compare to Fig. 3 and 4.

differs from that seen after addition of cortisol + mammotropin to the culture medium (1,2). Cortisol + mammotropin-treated alveoli contain many intracellular vacuoles and a dense, darkly-stained secretion in alveolar lumina (Fig. 6), in contrast to loss of intracellular vacuoles and the unstained nature of luminal contents following cortisol + insulin treatment. Thus, there is more than one hormonal combination that will maintain prelactating and nodular alveoli in culture, but there are differences in the resulting histologic picture.

Summary. Cortisol plus insulin, and to some extent insulin alone, maintained normal and hyperplastic mouse mammary lobules in organ culture in a defined medium.

I am grateful to Drs. H. A. Bern and K. B. DeOme for suggestions, and to Victor Duran for photography.

1. Elias, J. J., *Science,* 1957, v126, 842.
2. Elias, J. J., Rivera, E., *Cancer Research,* in press, 1959.
3. Bern, H. A., DeOme, K. B., Alfert, M., Pitelka, D. R., *Second Internat. Symp. on Mammary Cancer,* Perugia, 1958, 565.
4. Chen, J. M., *Exp. Cell. Res.,* 1954, v7, 518.
5. Morgan, J. F., Morton, H. J., Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.
6. Harkness, M. N., Bern, H. A., Alfert, M., Goldstein, N. O., *J. Nat. Cancer Inst.,* 1957, v19, 1023.
7. Trowell, O. A., *Exp. Cell Res.,* 1959, v16, 118.

Received May 18, 1959. P.S.E.B.M., 1959, v101.

Prevention by Intravenous Injection of Antigen and Antibody of Passive Arthus Reaction to Unrelated Immune System. (24996)

OTTO G. BIER AND MARIA SIQUEIRA (Introduced by Z. Ovary)
Depts. of Microbiology, Escola Paulista de Medicina and of Immunology, Inst. Biológico, São Paulo, Brazil

Previous work has shown that intravenous injection of antigen followed by its specific antibody resulted in marked decrease of passive cutaneous anaphylactic (PCA) response to an unrelated immune system in the rat(1). The possible dependence of this suppressive effect upon adequate levels of circulating complement (C′) has been suggested(2), since intensity of PCA paralleled *in vivo* fixation of C′ under a variety of experimental conditions. The present report concerns observations along similar lines in relation to direct Arthus reactions passively induced in guinea pigs.

Materials and methods. Antigens and antisera. The following antigen preparations were employed: 1. Bovine serum albumin (BSA), crystallized, obtained from Pentex, Kankakee, Ill., used without further purification. 2. *Chicken egg albumin* (Ea), recrystallized 5 times, prepared by method of Kekwick and Cannan(3). 3. *Rabbit anti-BS,* lots 23, 24, 27 and Pool I, '58, containing 0.479, 1.080, 0.263, and 0.362 AbN/per ml respectively. 4. *Rabbit anti-Ea,* lots 11, 15, and 17, containing 0.746, 1.412, and 1.197 mg AbN/per ml respectively. Antibody content of antisera was determined by quantitative precipitin method described in reference 4. *Complement titrations.* Serum samples for C′ titrations were obtained from blood (1-2 ml) drawn by cardiac puncture. Sera were clarified by centrifugation at 0°C and stored at −20°C until assayed. Estimations of hemolytic activity were carried out as described (5). *Hematologic determinations.** 2 ml samples of blood were obtained by cardiac puncture with silicone-coated syringe and needle and collected into siliconed vial containing 0.025 ml of 10% solution of sodium versenate (EDTA). Leucocyte counts were performed as usual. For platelet counts the method of Feissly and Ludin, modified by Rosenfeld(6) was adopted. Blood was diluted 1:200 in siliconed red cell pipette using

* The authors are indebted to Drs. M. Lindenberg and P. Azevedo for carrying out hematologic determinations. The help of Dr. G. Rosenfeld is also gratefully acknowledged.