more rapid means of attempting to isolate measles virus as well as studies on adaptation of the virus to other tissues.

The observations that CF and HA-I titers were comparable in measles sera from simian, rabbit, guinea pig, and human (the latter small in number), and that no CF or HA-I antibodies were found in a larger number of measles negative sera would indicate that hemagglutination-inhibition may be useful in evaluation of antigenic responses in man and animals, assay of virus pools, and standardization of hyperimmune sera.

Summary. Evidence has been presented to show that concentrated measles virus in presence of monkey red blood cells will demonstrate the phenomenon of hemagglutination. Baboon kidney propagated measles virus yielded higher levels of hemagglutinin than measles virus grown in other primate tissue. Chick tissue propagated measles virus did not demonstrate hemagglutination. Specific inhibition of hemagglutination was demonstrated by rabbit and guinea pig antisera prepared against measles virus in our laboratory and in another laboratory, by naturally occurring simian measles antisera, by human gamma globulin and by human convalescent measles antisera. Inhibition was not observed when measles CF negative simian sera, measles acute human sera and a number of viral antisera were tested.

ADDENDUM: After this paper was submitted for publication, an article entitled "Hemagglutination and Hemagglutination-Inhibition with Measles Virus" by Leon Rosen appeared in *Virology*, 1961, v13, 139. The work reported herein is in complete agreement with his observations.

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Experimental Growth of Mammary Gland in Male and Female Mice.* (26404)

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Estrogen (E.B.) and progesterone (P) (1,2) or relaxin(3) have been shown to synergize in stimulating lobule-alveolar growth in male and female mice in short-time experiments. Recently, normal growth of mammary glands of mice pregnant 18 days was determined using total DNA (desoxyribonucleic acid) as an index of growth(4). Levels of E.B. and P. which produce maximum mammary gland growth in ovariectomized rats(5) have been reported. The present study is concerned with levels of E.B. and P. which stimulate maximum growth of the mammary gland of male and ovariectomized female mice during period of 19 days.

Materials and Methods. Mature male and female Webster-Swiss mice were maintained on standard laboratory feed at constant temperature of 78±1°F. Ovariectomized mice received subcutaneous injections beginning day 14 postoperatively. Male mice were pretreated 28 days with 1.23 mg diethylstilbestrol per kg ground lab feed to insure good duct development before injection(6). E.B. and P. were dissolved in olive oil carrier at

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TABLE I. Experimental Growth of Mammary Gland in Male and Female Mice.

Sex	Treatment	No. of mice	DFFT,* mg	DNA/mg of DFFT, μg	Total DNA, mg	Final mean body wt, g	Total DNA/100 g final body wt, mg
♀	Intact virgins	28	43.3	41.2 ± 1.6	1.76 ± .07[1]	29.5	5.93 ± .16
	33-day castrates	12	53.8	37.5 ± 2.9	1.95 ± .17	32.3	6.01 ± .30
	Sham-operated controls	7	45.7	33.6 ± 2.8	1.52 ± .10	27.3	5.69 ± .49
	E.B., 1 μg P., 1 mg	29	71.8	35.4 ± 1.2	2.59 ± .14[2]	30.6	8.46 ± .36
	E.B., 1 μg P., 2 mg	25	65.1	38.1 ± 1.0	2.55 ± .13[3]	30.2	8.48 ± .32
	E.B., 1 μg P., 3 mg	27	78.7	40.7 ± 1.1	3.20 ± .19[4]	32.4	9.73 ± .43
	E.B., 1 μg P., 4 mg	15	78.4	39.6 ± 1.6	3.15 ± .33	34.1	9.05 ± .62
	E.B., 1 μg P., 5 mg	16	67.9	38.9 ± 1.2	2.65 ± .22	31.4	8.64 ± .47
	18-days pregnant	28	123.5	52.0 ± 1.6	6.22 ± .37	37.4§	16.90 ± .69
♂ †	Controls‡	20			1.20 ± .35[5]		
	E.B., 1 μg P., 1 mg	10	67.7	27.1 ± .9	1.82 ± .12[6]	34.6	5.27 ± .26
	E.B., 1 μg P., 2 mg	8	100.1	29.4 ± 2.0	3.12 ± .45[7]	39.6	7.70 ± .87
	E.B., 1 μg P., 3 mg	10	94.4	34.6 ± 1.6	3.10 ± .35[8]	38.3	8.17 ± .76
	E.B., 1 μg P., 4 mg	9	69.6	40.2 ± 1.7	2.77 ± .16[9]	39.9	7.02 ± .41

* DFFT, dry fat-free tissue.
† Pre-treated 28 days with 1.23 mg diethylstilbestrol/kg feed.
‡ From unpublished laboratory data.
§ Corrected for wt of fetuses.

Student's "t" probability: [1-2] .01; [5-6] .01; [4-2, 3] .05; [6-7, 8, 9] .01

concentrations of 1 μg E.B. and graded increments of P (1-5 mg) per 0.1 ml. Each animal received 0.1 ml carrier per day for 19 days. On day 20, animals were sacrificed and 7 mammary glands of each mouse were taken for DNA determinations as outlined by Brookreson and Turner(4). Remaining 3 glands were used for whole-mount observations.

Results. Statistical analysis showed experimental groups had total DNA levels significantly higher ($P<.01$) than appropriate controls (Table 1). Ovariectomized female groups receiving 1 μg E.B. and 3 mg P. were significantly higher ($P<.05$) in total DNA than groups receiving 1 or 2 mg P. No statistical differences were shown among female groups receiving 3 mg P. and more, while in male mice 2 mg to 4 mg P. showed no differences. However, when DNA was corrected for variations in body weight, 1 μg E.B. and 3 mg P. were optimal in males also.

Comparing control groups of intact virgin, ovariectomized, and sham-ovariectomized mice revealed castrates exhibiting higher levels of total DNA than virgin groups, while sham removal of ovaries resulted in lower DNA values than intact virgins. Neither of these differences was significant, however. On a total DNA basis, development of mammary glands in ovariectomized female group with highest DNA was 51.5% of 18-day pregnant controls and in male group 50.2%. When body weights were equalized, treated female group with highest DNA was 57.6% of 18-day pregnant controls and male group was 48.3%.

Whole-mount observations in both male and female mice indicated lobule-alveolar development in experimental groups most extensive in animals receiving 1 μg E.B. and 3 mg P.

Frequency distributions of DNA of virgin controls, ovariectomized females receiving 1

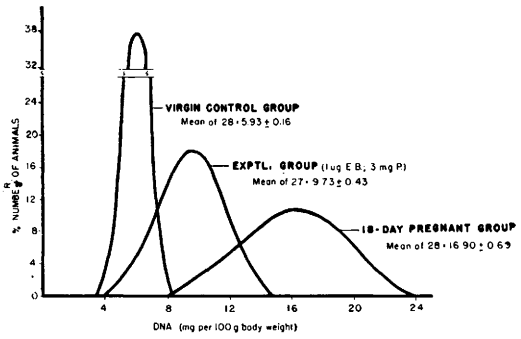

FIG. 1. Frequency distribution of DNA of mice mammary glands (7 of 10 glands). Pregnant mice on day 18 show mean DNA of 16.9 mg/100 g body wt with range from 8 to 24. Optimal level of EB and P stimulated mean DNA of 9.75 mg with range of 4 to 15 or only 57.6% of pregnant mice.

μg E.B. and 3 mg P. for 19 days, and pregnant group were not significantly different from normal distribution as shown by chi-square test (Fig. 1).

Discussion. Previous studies in rats(5) indicated that a level of 1 μg E.B. and 2 mg P. for 19 days developed mammary glands of ovariectomized females comparable to those of pregnant controls; levels of P. above 2 mg did not enhance or inhibit further growth. The present study indicates 1 μg E.B. and 3 mg P. for 19 days is optimal in mice. Since 2 mg P. and 5 mg P. did not produce as much growth, ratio of E.B. and P. in mice appears to be more critical in mice than in rats. In contrast to results in rats, experimental growth comparable to pregnancy was not reached in this study. Total DNA values of 3.20 $\pm$ 0.19 mg and 3.10 $\pm$ 0.35 mg for ovariectomized females and pre-treated males, respectively, were highest of experimental groups compared to 6.22 $\pm$ 0.37 mg for 18-day pregnant controls. DNA values per 100 g body weight were 9.73 $\pm$ 0.43 mg and 8.17 $\pm$ 0.76 mg for females and males, respectively, while pregnant controls reached 16.90 $\pm$ 0.69 mg. Thus, in experimental animals, mammary gland growth per unit of body weight in females was 57.6% and in males 48.3% of pregnant controls.

In comparing mammary growth of rats with mice, total DNA per 100 g body weight may be used. Moon *et al.*(5) reported a value of 7.63 $\pm$ 0.40 mg DNA per 100 g body weight. This includes 6 posterior glands only. Present study using 4 posterior and 3 anterior glands in mice shows a value of 16.90 $\pm$ 0.69 mg DNA per 100 g body weight. If each gland has approximately the same amount of DNA, then total DNA per 100 g would be 15.26 mg in rats and 24.15 mg in mice. Relatively larger mammary glands plus higher metabolic rate partially explain higher requirements of hormone per unit of body weight in mice compared to rats.

Experiments with E.B. and anterior pituitary extracts (APE)(7) in mice indicated that mammary gland growth comparable to pregnancy could be attained with 1 μg E.B., 3 mg P. and 0.4 mg APE. Such evidence leads to several explanations for inability of E.B. and P. to develop glands equal to pregnancy: (1) E.B. and P. in amounts used may not be stimulating adequate production and release of hormones from anterior pituitary; (2) placenta may play a role in mammary growth of mice while not doing so in rats. Some evidence to support the latter view has been presented(8,9,10). Placental factor may be similar to anterior pituitary hormone(s) or it may be a stimulant on AP synthesis and release. The third possibility for inability of experimental growth to equal that of pregnancy is greater sensitivity of mice to E.B. and P. ratio changes during normal pregnancy. It is known that ratios change throughout pregnancy. Various ratio changes have been attempted without success to date, however.

Summary. 1. Maximum growth of the lobule-alveolar system of mammary gland, as measured by total DNA, in ovariectomized female and estrogen-primed male mice (to develop duct system) was observed when 1 μg estradiol benzoate and 3 mg progesterone were administered daily for 19 days. (2) Maximum total DNA experimentally stimulated in female mice, on an equal weight basis, was only 57.6% of the DNA observed in normal mice pregnant 18 days. (3) Corresponding value for males was 48.3%. (4) These data suggest that a hormone of placental origin may be involved in mammary gland growth other than ovarian hormones.

(5) On an equal body weight and gland basis, the mammary glands of mice at end of pregnancy contain approximately 150% as much DNA as do rats.

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Active Immunization of Mice Against *Candida albicans.** (26405)

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Despite scanty unequivocal evidence, the possible beneficial role of immunity in human candidiasis has been suggested by clinical experience (*e.g.*, 1, 2) and in one case of pulmonary candidiasis, the use of rabbit antiserum was reported to be efficacious(3). Experimentally, immunologic studies with *C. albicans* have been largely confined to rabbits. Hurd and Drake(4) gave such animals repeated intravenous injections of heat-killed suspensions of a strain described as highly pathogenic for them. When agglutinin titers reached 1:2560 the vaccinated animals, as well as a control group, were given a dose of *C. albicans* defined as "one ml of sufficient concentration as to bring death on the seventh day." The authors concluded that the vaccination was not only ineffective but possibly harmful. Winner(5) found that normal rabbits possessed a high level of natural agglutinins but that actively or passively induced agglutinins did not protect the animals from an inoculum of *C. albicans* lethal for controls.

Since little immunologic work with *C. albicans* in mice was encountered in the literature and since with mice the use of larger groups would be facilitated, this problem was investigated by challenge of such animals which had been previously vaccinated with variously prepared materials, including sonically ruptured cells.

Materials and methods. *C. albicans*, strain 266, of human origin was used throughout this study. The culture was maintained on slants consisting of 1% casamino acids, 1% yeast extract, 2% glucose and 2% agar (CYE agar). Growth less than a week old was used to inoculate 150 ml of CYE broth in 500 ml Erlenmeyer flasks which were incubated on a rotary shaker at 37°C for 24-36 hours. The concentration of yeast particles was first estimated by direct microscopic counts using a hemacytometer and the viables were determined by the pour plate method.

Cells of *C. albicans* were washed and resuspended in saline so as to contain 2×10^8 particles per ml. From this stock, 3 different vaccines were prepared: (a) viable cells; (b) cells killed by exposure to merthiolate for 48 hours, then rewashed with saline; (c) cells killed by subjecting them to sonic vibrations in a 10 kc Raytheon oscillator for nearly 7 hours, the temperature of which was controlled by chilling to approximately 6°C.

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