been fed 0.6% 2-acetylaminofluorene. In this study 3 rats or 25% in each of 3 groups had tumors of the external ear at death. All 9 were squamous cell carcinoma.

Adenocarcinomas of the mammary gland were found in 2 rats that received 1.4% tryptophan and in 2 rats that received 0.8% indole.

Cancers of the urinary bladder were present in rats of all 4 groups. They were observed in 75% of the rats that received 1.4% DL-tryptophan; 58% of the rats that received 1.0% indole acetic acid; and in 83% of the rats of the 2 groups that received indole.

Previously bladder cancers were observed in 100 and 92%, respectively, of the rats that received 1.4 and 4.3% DL-tryptophan added to a tryptophane-free casein hydrolysate but were not found in rats on a 26% casein diet or in rats on a synthetic diet deficient in tryptophan that survived as long or longer than the rats that received added tryptophan or indole. The induced cancers were classified as papillary (Fig. 5) or squamous cell carcinoma (Fig. 6) or a combination of both types. In many instances the cancerous growth filled and distended the lumen of the bladder. From these results it is apparent that indole is as effective as tryptophan in the initiation of these neoplasms.

Summary. 1. Fischer line 344 female rats were fed 2-acetylaminofluorene in synthetic diets containing 25% casein and supplements of 1.4% DL-tryptophan; 1.0% indole acetic acid; 0.8% indole and 1.6% indole. Liver cancers developed in 91% of the rats of the first group and in 83% of the rats of the other 3 groups. 2. The majority of the liver neoplasms in the rats that received 1.4% DL-tryptophan and 1.0% indole acetic acid were malignant hepatoma and lung metastases were present in 63% and 90% respectively. 3. The majority of the liver neoplasms in the rats that received 0.8% and 1.6% indole were cholangioma or adenocarcinoma and lung metastases from these tumors were identified in 38 and 12%, respectively. 4. Cancers of the urinary bladder were observed in 75% of the rats that received 1.4% DL-tryptophan; 58% of the rats that received indole acetic acid and in 83% of the rats that received indole. Indole appeared to be as effective as dietary tryptophane in the initiation of the bladder neoplasms. 5. The cancers of the urinary bladder were papillary or squamous cell carcinoma.

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Effect of Age at Ovariectomy on Mammary Gland Development in C3H/He Crgl Mice.* (24259)

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It is well recognized that in certain strains of susceptible mice, including the C3H strain (1,2), mammary tumors develop despite ovariectomy. The hormonal stimulus for genesis of these tumors has been ascribed to the adrenal cortex which, in several strains, undergoes a tumorous change leading to so-called nodular hyperplasia or to carcinoma(1-6).

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TABLE I. Degree of Alveolar Development of Mammary Glands of Virgin C3H/He Crgl Mice Ovariectomized at Various Ages.

Age at ovariectomy (mo)	No. of mice	Mean age at death & range (mo)	No. and (%) of mice showing various degrees of mammary alveolar development*						No. of mammary nodules per mouse†
			3	2	1	±	N	0	
Controls	53	15 (8–26)	12(23)	26 (49)	15 (28) (1 no T)‡	—	—	—	32.1 ± 3.9
2	8	23 (23)	—	2 (26) (1 no T)	3 (37) (1 no T)	3 (37) (3 no T)	—	—	6.4 ± 1.8
3	15	17 (13–23)	—	2 (13)	3 (20)	4 (27)	4 (27)	2 (13) (2 no T)	14.9 ± 4.7
4	19	15 (9–26)	—	—	—	2 (10)	14 (74) (1 no T)	3 (16) (1 no T)	11.5 ± 2.9
5	21	13 (8–30)	—	1 (5)	—	2 (9) (2 no T)	16 (77) (3 no T)	2 (9)	10.3 ± 2.3
6.5	29	16 (12–27)	—	—	—	—	24 (83) (2 no T)	5 (17) (2 no T)	11.2 ± 2.1
8.5	11	17 (11–21)	—	—	—	—	10 (91) (1 no T)	1 (9) (1 no T)	14.7 ± 3.5

* Rating scheme for mammary gland alveolar development: 0 = completely devoid of alveoli and nodules; N = devoid of normal alveoli, but hyperplastic nodules present on otherwise bare ducts (as in Fig. 3 and 4); ± = occasional alveolar buds and isolated alveoli; 1 = isolated alveoli and small clusters; 2 = many alveoli and small clusters (as in Fig. 2); 3 = definite lobuloalveolar development (as in Fig. 1).

† Mean ± stand. error of mean.

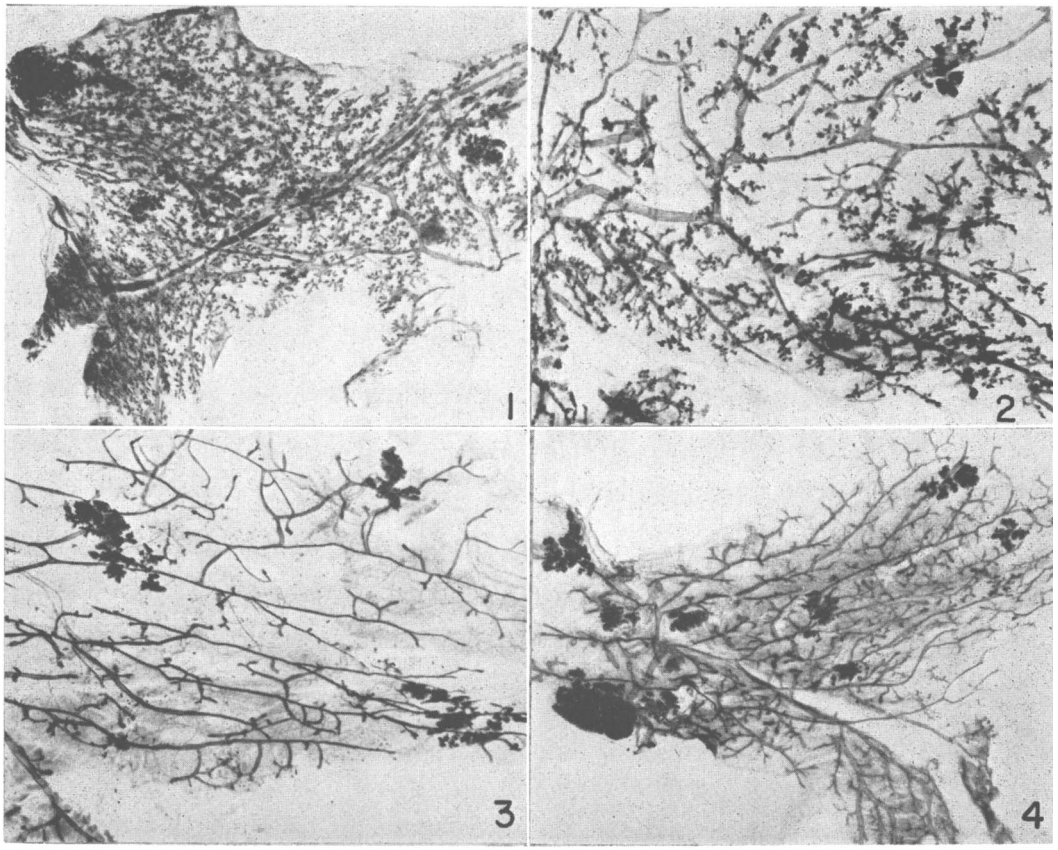
‡ "no T" = No. of mice in group without gross or microscopic tumors.

The mammary glands of mice ovariectomized before 2 months of age have been examined in certain strains. The C3H(2) and NH(4) strains showed less ductal and alveolar development than the non-ovariectomized controls; however, hyperplastic nodules were present in all groups. In the ce strain (3) the majority of the animals exhibited ductal development as the only form of mammary gland development; however, about 25% of the animals over 12 months old showed some alveolar development. In this strain no hyperplastic nodules were observed.

The present investigation was undertaken to determine whether ovariectomy performed at different ages would produce differences in state of stimulation of the mammary gland of our C3H/He strain at the time of mammary tumor development.

Materials and methods. A total of 148 virgin female mice of the C3H/He Crgl strain was used in this experiment. The experimental groups consisted of 15 animals bilaterally ovariectomized at 3 months of age, 19 at 4 months, 21 at 5 months, 29 at 6.5 months, and 11 at 8.5 months. Fifty-three animals were maintained as unoperated virgin controls. In most cases the animals were maintained until palpable mammary tumors appeared, at which time they were sacrificed. Twelve percent of the experimental animals died before showing tumors, and an additional 4% failed to develop tumors and were sacrificed between 23 and 30 months of age (nontumorous mice are indicated by "no T" in Table I). In addition, 8 animals serving as part of another experiment are included in Table I. This group was bilaterally ovariectomized at 2 months of age and sacrificed at 23 months of age.

At autopsy, the mammary glands were prepared as whole-mounts and stained with hematoxylin. The number of hyperplastic nodules and presence of mammary tumors were recorded. The state of alveolar development of the mammary gland was evaluated according to the rating scheme indicated in Table I. It is to be emphasized that judgment of degree of alveolar development was made without regard to extent of elaboration of the duct system and hence without regard to total mass of the gland.



All photographs are of whole-mounts of a No. 3 mammary gland stained with hematoxylin. × 5. All mice possessed palpable tumors.

FIG. 1. Control showing considerable lobuloalveolar development and several hyperplastic nodules (13 mo old at death).

FIG. 2. Ovariectomized at 2 mo showing many alveoli and small clusters as well as hyperplastic nodules (23 mo old at death).

FIG. 3. Ovariectomized at 5 months showing hyperplastic nodules on otherwise bare ducts as the only form of alveolar development (16 mo old at death).

FIG. 4. Ovariectomized at 6.5 months showing hyperplastic nodules, bare ducts, and a small tumor (15 mo old at death).

Results. Nodular hyperplasia of the adrenal cortex was evident in all experimental animals. At tumor age, the mammary gland ducts of the control animals were well developed and possessed many thin branches, in addition to showing active duct growth as indicated by terminal buds and "club ends." There was appreciable alveolar development in the control group varying from glands showing diffuse alveolar development with few lobules of alveoli to glands showing definite lobuloalveolar development (Fig. 1). Hyperplastic nodules were present in the mammary glands of all of the control animals, averaging 32 per animal, approximately 3 times the average number (12) seen in all the experimental groups.

The mammary ducts of the experimental groups showed considerably less branching than the controls (Fig. 1 and 2). Degree of branching was usually more extensive in animals castrated at later ages than those castrated at an early age (Figs. 2 and 4). In the groups castrated at 2, 3, and 4 months of age, wide ducts with short, thick branches predominated. Evidence of active duct growth was observed in some members of all experimental groups.

The mammary glands of animals ovariectomized at 2 months of age had fairly extensive alveolar development ranging from localized development of alveolar buds to large numbers of alveoli and many lobules. The average picture was that of alveoli throughout the glands with some lobule formation (Fig. 2). Uteri from these mice were large and showed cystic glands.

Ovariectomy at 3 months of age resulted in mammary glands with a wide range of alveolar development, *i.e.*, no alveolar development to large numbers of alveoli with some lobule formation. In 27% of the animals the only form of alveolar development was that of hyperplastic nodules on otherwise bare ducts.

Ovariectomy at 4 months resulted in a larger percentage (74%) of animals possessing hyperplastic nodules as the only form of alveolar development, with a small number showing either slight or no alveolar development.

Ovariectomy at 5 months resulted in approximately the same picture (Fig. 3) as that of the 4-month group, but included one animal with fairly extensive alveolar development. This exceptional animal possessed very large adrenals (totaling 24 mg) and a cystic uterus.

83% (24 mice) of the mice ovariectomized at 6.5 months showed glands containing hyperplastic nodules on otherwise bare ducts as the only representation of alveolar development (Fig. 4). Seventeen percent (5 mice) of this group possessed glands with neither alveoli nor nodules, although 3 of these mice had palpable tumors. Ovariectomy at 8.5 months resulted in essentially the same pattern as that of the 6.5-month castrate group. Uterine development in these two older groups was similar to that seen in the non-operated controls.

Within a single experimental or control group, there was no correlation between age at death (generally the same as tumor age) of the individual animal and degree of mammary gland development.

Discussion. Nandi(7) has determined for the C3H/He Crgl mouse that the minimum hormonal combination necessary for lobuloalveolar development of the mammary gland is estrogen, progesterone, and somatotropin *or* mammotropin. Relating these findings to the present investigation, it would appear that the nodular hyperplastic adrenal, at least in mice ovariectomized at 2 and 3 months of age, elaborates estrogen-like and progesterone-like substances in sufficient quantities to account for the observed lobuloalveolar development. In the groups ovariectomized at late ages (6.5 and 8.5 months), the nodular hyperplastic adrenal cortex contributes to a hormonal environment that is sufficient for induction and/or maintenance of hyperplastic alveolar nodules of the mammary gland but not of normal alveolar development. Thus, the steroid hormonal requirements for nodule formation, as opposed to generalized alveolar development, can be defined in terms of the secretions of the transformed adrenal cortex of a mouse ovariectomized late in life. This latter adrenal appears to be less efficient in replacing the ovary than is the adrenal from a mouse ovariectomized at an earlier age, judged both by mammary development and by uterine size.

Summary. C3H/He Crgl virgin female mice were ovariectomized at approximately monthly intervals between 2 and 8.5 months, and the mammary glands were examined at the time of mammary tumor development. Normal alveolar development of the mammary gland was greatest in animals ovariectomized at an early age and less in animals ovariectomized at later ages. In mice ovariectomized at 6.5 and 8.5 months, normal alveolar development was absent, the sole alveolar development being in the form of hyperplastic nodules. The secretory products of the transformed adrenal in the older mice thus appear to be adequate for development of hyperplastic nodules, but not for normal alveoli.

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Differences in Inositol Requirements of Several Strains of HeLa, Conjunctival and Amnion Cells.* (24260)

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A minimal growth medium consisting of 27 factors capable of supporting the propagation of several strains of human cells for at least several months had been described by Eagle (1). Eagle and his associates(2) also reported that meso-inositol was an additional essential growth factor and that the requirement of the HeLa cell for inositol was irregular and was dependent on method of dialysis. Geyer and Chang(3) found that the HeLa and the conjunctival cells would show advanced degenerative changes after 4-7 days in Eagle's minimal growth medium, that addition of inositol prevented the appearance of cell degeneration, and that the HeLa cell regularly required inositol. Since the minimal growth medium described by Eagle is an important advance in the methodology of human cell culture, these conflicting reports prompted us to explore the basis of this irregularity. Among the factors studied are differences between strains of a cell, difference between stable laboratory cell lines and primary cell explants, contamination of cell culture by the pleuropneumonia-like organisms, and spontaneous appearance of nutritional variants and the extent of dialysis of serum. The results of these studies form the basis of this report.

Materials and methods. The methods were those described earlier(4). The minimal growth medium for determination of inositol requirement of the various strains of human cells consisted of 10% dialyzed human or horse serum in Eagles basal medium(1) plus inositol at concentration of 10 to 20 μM. The withdrawal of inositol resulted in advanced degeneration of the inositol-requiring strains within 5 to 10 days. Total inositol content of serum was analyzed, using *Saccharomyces cerevisiae* as assay organism(5).

Results. Differences in HeLa strains from several laboratories. The inositol requirement of several strains of HeLa cells, kindly furnished by other investigators, was determined. The strain maintained in the author's laboratory since 1954 invariably degenerated in the minimal growth medium without inositol. The strain obtained from Dr. B. Roizman, Johns Hopkins University, was found to propagate at approximately the same rate in the minimal growth medium with or without inositol for 14 days (Table I). Several other strains were found contaminated with a pleuropneumonia-like organism and were observed only for 7 days in the presence of the contaminant; 4 degenerated completely in medium without inositol while one gave inconclusive results.

Difference in conjunctival strains maintained in different growth media. The stock culture has been found on numerous occasions to degenerate in the assay medium without inositol(3). A subculture maintained in a 0.1 mM glucose medium (10% dialyzed serum in Eagle basal medium with the concentration of glucose reduced to 0.1 mM and with addition of inositol to a cencentration of 10-20 μM) for about 6 months failed to show similar changes. It showed a definite though small net increase in cell number during the first 2 weeks in the assay medium without inositol, and has since been propagating for at least 2 months (Table I).

Difference between a stable strain and fresh explants of amnion cells. The FL strain of

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