the second 24 hour urine collection. This patient had serious kidney damage which may have accounted for the delay in excretion. No attempt was made to determine the amount of diethylstilbestrol excreted in the feces.

Finally, the material obtained from the Venning procedures was assayed qualitatively for estrogenic activity using the castrated female guinea pig as the test animal. Opening of the vaginal canal by dissolution of the vaginal membrane was taken as the index of estrogenic activity. It was found that only those samples which showed an increased pregnanediol titer with the Venning method over the free pregnanediol method exhibited estrogenic potency during the period of observation.

Summary and conclusions. When diethylstilbestrol is administered to a woman during pregnancy, much of this material appears in the urine as a glucuronide. In the Venning method for the assay of urinary pregnanediol, all of the glucuronides are included in the final determination. Thus diethylstilbestrol glucuronide as well as pregnanediol glucuronide appears in the result obtained. The apparent rise in the urinary pregnanediol values obtained by the Venning method may be due to the ingested diethylstilbestrol which is conjugated and eliminated in the urine. The figures do not indicate an increased production of progesterone and resultant increased output of urinary pregnanediol.

Increasing amounts of diethylstilbestrol are being used in the treatment of pregnancy complications. In most instances the theory behind this therapeutic measure is that this estrogen stimulates steroid production. If urinary pregnanediol is to be used as a measure of increased steroid metabolism the value of diethylstilbestrol is open to question. It is possible that diethylstilbestrol exerts a favorable influence on placental circulation or on early placental development. However, there is no evidence that it results in an increased production of progesterone if urinary pregnanediol is to be regarded as an index of progesterone metabolism.

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Age Factor in Estrogen-Induced Breast Cancers of Mice.*

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In male mice of strains C3H and D, the age at the beginning of the treatment was one of the factors which determined the susceptibility of these animals to estrogen-induced breast cancers[1] and leukemias.[2-4] The breast cancer rate was higher in mice injected with estrogenic hormone before or about the onset of sexual maturity than in those receiving the hormone from the age of 4 to 6 months on. In female mice similarly treated, this age effect was much less conspicuous or lacking. According to Loeb,[1b] these findings suggest the following interpretation: (1) In younger mice, the breast tissue itself might be more susceptible to growth stimulation than in older animals. If this is the case, however, the lack of an age effect in females would be difficult to explain. Still, such an age factor might be present in female mice also, but it might be obscured or neutralized by the periodic stimulation of the mammary gland by the intrinsic estrogenic hormone produced in

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1 (a) Loeb, L., *Harvey Lectures*, 1941, **36**, 228; (b) Loeb, L., *Biol. Symposia*, 1945, **11**, 197; (c) Loeb, L., Suntzeff, V., Burns, E. L., and Schenken, I. R., *Arch. Pathol.*, 1944, **38**, 52.

2 Murphy, J. B., *Cancer Res.*, 1944, **4**, 622.

3 Silberberg, M., and Silberberg, R., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 347.

4 Law, L. N., *J. Nat. Inst. Canc.*, 1947, **8**, 157.

the female during the estrus cycle. (2) The male sex hormone might have exerted an antagonistic effect on the carcinogenic action of the estrogenic hormone.[5-8]

The present experiments were carried out in order to obtain further information concerning the role of the age factor in estrogen-induced mammary cancers.

Material and methods. One hundred and twenty-three male mice of the closely inbred strain C3H, raised in our laboratory, and kept on a standard diet of Purina Laboratory Chow and water were used. Sixty-five animals were castrated at the age of 3 to 4 weeks. Twenty-three castrates (Group I) and 26 animals with intact testicles (Group II) received subcutaneous injections of 0.03 mg (200 Rat Units) of alpha estradiol benzoate† in sesame oil once a week for 5 months starting at the age of one month. The remaining 42 castrates (Group III) and 32 mice with intact testicles (Group IV) were injected with the same amount of hormone for the same length of time, but the treatment was begun at the age of 4 months. The animals were inspected at weekly intervals for the appearance of tumors and leukemia. Dead animals were examined for gross lesions. Tumor-bearing mice or animals which looked sick were killed. The tumor and 3 or 4 mammary glands, pieces of internal organs and some bones were removed and saved for histological studies. These findings will be reported at a later date.

Observations. The results are summarized in Table I. The first breast tumors were noted at the age of 7 months, and, therefore, mice living to this age and beyond are listed as animals "reaching the tumor age". These animals were distributed, as follows:

Group I: Castrates receiving the hormone from 4 weeks of age on: 18.

TABLE I.

Experimental group	Total No. of animals	No. of animals reaching tumor age	Age at death of animals reaching tumor age Range	Age at death of animals reaching tumor age Mean (mo.)	Animals with breast cancers No.	Animals with breast cancers %	Age at appearance of tumors Range	Age at appearance of tumors Mean (mo.)
I. Male castrates receiving hormone at 4 wk	23	18	7-18	12.9	8	44.4	7-15	9.7
II. Males with intact testicles receiving hormone at 4 wk	26	19	7-17	12.8	3	15.8	8-14	10.7
III. Male castrates receiving hormone at 4 mo.	42	40	7-19	13.0	12	30.0	8-19	12.8
IV. Males with intact testicles receiving hormone at 4 mo.	32	25	7-20	10.8	1	4.0	—	17.0

[5] Lacassagne, A., and Raynaud, A., *Compt. Rend. Soc. Biol.*, 1939, **131**, 186.

[6] Nathanson, I. T., and Andervont, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 421.

[7] Gardner, W. U., *Cancer Res.*, 1946, **6**, 493.

[8] Jones, E. E., *Cancer Res.*, 1941, **1**, 787.

† We are indebted to the Schering Corporation for the generous supply of Progynon-B.

Group II: Controls receiving the hormone from 4 weeks of age on: 19.

Group III: Castrates receiving the hormone from 4 months of age: 40.

Group IV: Controls receiving the hormone from 4 months of age on: 25.

In males with intact testicles injected with alpha estradiol benzoate for 5 months from the age of one month on (Group II), the cancer rate was 15.8%; the tumors appeared at a mean age of 10.7 months. In males with intact testicles treated similarly but receiving the hormone from the age of 4 months on (Group IV), the cancer incidence was 4%; the single tumor observed developed at the age of 17 months. Thus, in the younger control group, the estrogen-induced neoplasms were almost 4 times more numerous, and they appeared 6.3 months earlier than in the older control group. These results are essentially in agreement with the findings obtained by Loeb and his co-workers.[1c]

In males castrated at the age of 3 to 4 weeks and injected immediately with alpha estradiol benzoate for 5 months (Group I), the incidence of tumors was 44.4%; the neoplasms appeared at a mean age of 9.7 months. Orchidectomy thus raised the tumor rate in this age group about threefold and accelerated the appearance of the cancers by one month. Of males castrated at the age of 3 to 4 weeks but receiving the estrogenic hormone from the age of 4 months on (Group III), 30% developed mammary cancers as compared with 44.4% in the group injected at an earlier period of life, and the tumors were noted at a mean age of 12.8 months. Thus, in the older age group, castration increased the incidence of estrogen-induced breast cancers 7½ times and advanced the time of their appearance by about 4.2 months over that seen in mice with intact testicles.

Discussion. Orchidectomy performed before the onset of sexual maturity raised the incidence of estrogen-induced breast cancers. This increase was observed not only if the hormonal treatment was begun immediately after castration[9] but also, if the injections were started as late as 3 months after removal of the testicles. However, the results obtained in these 2 age groups differed in degree: Castrates injected from the age of one month on, showed a cancer rate about 50% higher than those in which the hormonal treatment was begun at the age of 4 months (44.4% in castrates of Group I as compared with 30% in castrates of Group III). These two groups of castrates thus showed variations in the incidence of estrogen-induced breast cancers similar to those occurring in the corresponding mice with intact testicles. However, whereas in the latter animals the tumor rate of the two age groups differed as much as 300%, the difference in injected castrates of the two age groups amounted to about 50%. This latter difference in the cancer rate of both age groups thus occurred in the absence of the testicles. It can, therefore, not be attributed to any inhibiting effect of the sex glands but it must be caused by certain extratesticular factors. It would be premature to define more precisely the nature of this age factor. It is presumably located in the mammary gland itself and constitutes a loss of responsiveness to estrogenic stimulation with advancing age, an interpretation which would be in agreement with the suggestion of Loeb.[1b]

A decrease of susceptibility may manifest itself not only in a lowered cancer incidence but also in a prolongation of the latent period of tumor development. In our present experiments, the latent period was apparently not influenced by the age of the castrates at the beginning of the hormonal treatment: In the younger castrates (Group I), the estrogen-induced breast cancers appeared 3.1 months earlier than in the older castrates (Group III), the mean tumor age being 9.7 months in the younger and 12.8 months in the older group. This difference in time corresponds to the 3 months' delay of the administration of the estradiol. This result may be correlated to the comparatively small difference in the tumor incidence of the two groups. On the other hand, in younger animals with intact testicles (Group II), estrogen-induced breast cancers appeared 6.3 months earlier

[9] Miller, E. W., and Pybus, F. C., *J. Pathol. and Bact.*, 1942, **54**, 155.

than in the older ones (Group IV), the mean tumor age being in Group II 10.7 months, and in Group IV 17 months. Three months of this interval can be accounted for by the 3 months' delay of the hormonal treatment in the older group. However, the additional 3.3 months' difference has to be attributed to conditions of the experiment. Although the absolute number of tumors observed in these two age groups is small, the increase in the latent periods is in agreement with the decrease in the tumor incidence. It is, therefore, not unlikely that both are governed by similar factors.

Summary. Male mice of strain C3H castrated at the age of 3 to 4 weeks and injected with alpha estradiol benzoate immediately after castration develop a larger number of breast cancers than males castrated at the same age but injected from the age of 4 months on. Thus, an age factor operates in the susceptibility to estrogen-induced mammary cancers in male mice. This age factor is independent of the testicle but acts synergistically with it.

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Effect of Insulin and Glucose Upon Survival Time of Eviscerated Rats.

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The data of these experiments show that the administration of glucose with insulin to the eviscerated rat permits longer survival times than the administration of glucose alone.

Methods. Male rats of the Sprague-Dawley strain were fed Friskies Dog Cubes. At a weight of 185 to 205 g, the inferior vena cava was ligated between the liver and the kidneys in order to cause the development of a collateral circulation. Asepsis was preserved in this operation. When the animals reached a weight of 250 ± 2 g they were anesthetized (intraperitoneal injection of 18 mg of cyclopentenylallyl-barbituric acid sodium) and eviscerated by the method of Ingle and Griffith.[1] Hemostasis was attained by applying a gelatin sponge (Gelfoam, Upjohn) with thrombin to the stumps of the oesophagus, colon, ligated vessels and between the muscle and the skin when the incisions were closed. The animals were not fasted prior to operation. Intravenous infusions of solutions of glucose (C.P. Dextrose, Merck) in 0.9% saline with and without added insulin (Crystalline zinc insulin, Lilly) were made by continuous injection machines which delivered fluid into the saphenous vein of the right hind leg at the rate of 20 cc per 24 hours. The glucose loads used in this experiment represent amounts which sustain the average level of blood glucose at approximately normal values during a period of 24 hours. Temperature was constant at 26.5 ± 0.5°C.

The time at which the heart of the rat stopped beating was determined by use of a heartbeat amplifier (Model A, Upjohn) which was designed to amplify the D.C. potential generated by the heart beat to actuate a 6-point recording mechanism (Leeds and Northrop Micromax S.) This apparatus gives a permanent visual record of an all-or-none response of the amplifier to the beating heart for 6 animals simultaneously.

In Experiment 1, 21 eviscerated rats were given a glucose load of 4 mg per 100 g of rat per hour (4/100/h). The average survival was 1748 minutes ± 113 (standard deviation of the average). Glucose loads of 4/100/h without insulin and 40/100/h with insulin are each able to sustain approximately normal levels of blood glucose during 24 hours

[1] Ingle, D. J., and Griffith, J. Q., Chapter 16, The rat in laboratory investigation. J. B. Lippincott Co., Philadelphia, 1942.