Experiments with the shore crab, *Carcinus maenas*, which can penetrate far into brackish water, but not actually live in fresh water, have shown essentially similar, but less powerful mechanisms, taking up at least K and Na and Cl, Br and CNS. There can be no doubt that the ion absorbing mechanisms have been independently developed in many different forms, and we have a rather primitive stage in such development in the two crabs just mentioned.

The cells doing the work have not been definitely located so far. In the frog they are situated in the skin. In the freshwater fishes special experiments have shown them to be located in the gills, and there is some evidence to show that they can be acted upon through the nervous system. In many arthropods special groups of cells readily take up silver from silver nitrate and there is some evidence, though scarcely conclusive, for identifying these with the cation-absorbing apparatus.

Machinery for selective and active ion absorption—active in the sense that energy is expended to move ions against a concentration gradient—is very widely distributed throughout the animal kingdom although poorly developed in strictly marine invertebrates. It is present in most kidneys and has recently been demonstrated by Visscher[1] in the intestinal wall of mammals. All such mechanisms are probably derived from properties inherent in living cells as such. They may and probably do differ in many important respects. What I wish to submit is that the most highly specialized types are most likely to furnish clues by which to find out how they accomplish the trick.

[1] A detailed bibliography is given in my book, *The Osmotic Regulation in Aquatic Animals*, Cambridge University Press, 1939.

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*MAMMARY TUMOR DEVELOPMENT IN MICE OVARIECTOMIZED AT BIRTH**

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Lathrop and Loeb[1] observed that castration of female mice below the age of six months led to a marked decrease in mammary cancer incidence, and an increase in the cancer age. Castration at 3 to 4 months completely, or almost completely, prevented the appearance of carcinoma of the breast.[2] Murray[3, 4] found 17.1 per cent mammary gland tumors in 200 mice castrated 28 to 35 days of age, while 207 normal but non-breeding females gave an incidence of 11.1 per cent. Cori[5] found that castration of mice between 15 and 22 days of age entirely prevented the occurrence of spontaneous adenocarcinoma of the breast while the control of breeding mice showed a tumor incidence of 78.5 per cent.

The present experiment was an attempt to demonstrate further the influence of ovarian hormonal factors on mammary tumor development. The Jackson Laboratory dba strain of mice, in which there is 50.4 per cent mammary tumors in virgin females, was used.[†,6]

Eighty-two mice were anesthetized by chilling and were completely ovariectomized within 24 hours after birth. Of these, 22 mice or 26.8 per cent developed mammary tumors at an average age of 522 days. In 102 control mice in which one ovary had been similarly removed, 40 animals or 39.2 per cent developed mammary tumors at an average age of 461 days.

Since there have been a number of reports indicating ovarian regeneration in mice[7] a careful check was made for the presence or absence of ovarian tissue in all animals at autopsy. All suspicious nodules were carefully sectioned and examined. Especially careful search was made for ovarian regeneration in all animals which had developed mammary tumors and in many of them histological examination of serial sections of all tissue near the former ovarian site proved the absence of all ovarian tissue.

The appearance of mammary tumors in animals in which the ovarian influence had been completely removed throughout their entire lifetime was unexpected, the general opinion being that prepuberty castration reduced the rate to a negligible one. This led to further search for the cause of the unusually high mammary tumor incidence.

At six months the uterus was thin, thread-like and showed marked atrophy. In those animals which were killed at a later age, the uterus was enlarged and hypertrophied. Observation of vaginal smears and of microscopic sections of vaginal epithelium showed estrous cycles to occur in several of the mice examined beyond the age of one year.

Whole mounts of mammary glands at six months of age showed little growth to have taken place. Whole mounts of the glands beyond one year of age showed extensive duct development, while the presence of end buds gave evidence of active growth.

It was noted that the adrenal glands in the completely ovariectomized animals were in all cases enlarged and had yellowish nodules which gave the gland an uneven contour. The degree of the abnormality increased with the age of the animal. Similar gross abnormalities were never found in normal animals, but have been observed in a few castrate male mice examined by us.

In almost all the completely ovariectomized animals both adrenals, parts of the mammary gland and uterus were sectioned and examined microscopically. Both adrenals of all these mice contained areas of nodular hyperplasia involving and partly replacing all three zones of the cortex.

The nodules were composed of large fat-containing, and often pigmented, cells which in arrangement resembled the structure of the zona glomerulosa. These areas were not encapsulated. In some of the older animals, the medulla was pushed to the opposite side, due to the large size of the nodule. In three mice malignant tumors of the adrenal cortex were found.

The mammary glands of the older ovariectomized animals showed well-developed and extensive duct systems. In many, groups of alveoli were also present. In several mice the ducts and alveoli contained secretion. Most of the mammary gland tumors were adenocarcinomas, while a few could be more properly described as carcinoma simplex.

The uterus of the older animals contained dilated and in some cases cystic, endometrial glands. A very characteristic occurrence was a peculiar hyaline change of the connective tissue fibers of the subepithelial uterine mucosa.

Conclusions.—The possibility is suggested that: 1. The removal of both ovaries at 24 hours after birth allowed, in some manner, the nodular hyperplasia of the adrenal cortex; 2. The prolonged action of the hyperplastic adrenal probably stimulated the development of the mammary glands and the uterus in the older animals, thereby simulating or replacing ovarian activity.

Further studies are in progress.

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† This, however, probably should not be considered an absolutely accurate figure for the stock at the present time as tumor incidence different from that reported by Murray has recently been recorded in some substrains of this stock.

[1] Lathrop, A. E. C., and Loeb, L., *Jour. Cancer Res.*, **1,** 1 (1916).

[2] Loeb, L., *Jour. Med. Res.*, **40,** 477 (1919).

[3] Murray, W. S., *Science*, **46,** 600 (1927).

[4] Murray, W. S., *Jour. Cancer Res.*, **12,** 18 (1928).

[5] Cori, C. F., *Jour. Exper. Med.*, **45,** 983 (1927).

[6] Murray, W. S., and Little, C. C., *Genetics*, **20,** 446 (1935).

[7] For a review see Pencharz, *Jour. Exper. Zoöl.*, **54,** 319 (1929).