eral cases within three or four days of the time of castration. In no case, however, has the mammary gland been as thick as that obtained at the end of pregnancy, the thickest lactating gland obtained by this means being about one third as thick as glands obtained at parturition or a few days before.

No attempt has been made to assay the pituitaries in these rabbits to see what effect the oestrin may have had on them, but it is probable that the effect on the corpora is an indirect one, since many observers have shown that oestrin alters the gonadotropic activity of the pituitary.

These findings may explain the persistence of the corpora lutea during pregnancy in the rabbit. We have assumed, as many others working in the field of reproduction undoubtedly have also, that during pregnancy the placenta elaborates a gonadotropic hormone which, either directly or indirectly through the pituitary, causes the corpora to persist. This assumption is not based on direct evidence from the rabbit but rather by analogy because of the finding of pituitary-like substances in human pregnancy urine and placenta. In the light of these experiments it is much more likely that in the rabbit the placenta elaborates oestrin and that this rather than a placental gonadotropic hormone causes the corpora to persist.

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SOME POSSIBLE EFFECTS OF NURSING ON THE MAMMARY GLAND TUMOR INCIDENCE IN MICE[1]

FOLLOWING the publication[2] by the staff of the Jackson Memorial Laboratory (1933) on the extrachromosomal influence in the etiology of breast tumors, several experiments were designed in an attempt to determine the basis of such an effect. In this note the writer presents a preliminary report on the foster-nursing of the young cast by females of a high mammary gland tumor line by females of a low tumor stock and its possible effects on the incidence of that type of tumor.

Three litters of mice from the inbred A strain of mice, which has a mammary gland tumor incidence of 88 per cent.,[3] were fostered by females of the X stock (Strong's CBA race). The breast tumor incidence in the latter strain is approximately 10 per cent. The young were removed from their A stock mothers as soon as noticed—none were more than twenty-four hours old.

In the three litters of fostered A stock mice were nine females. They were used as breeders as well as forty of their progeny. Hence, the mice were subjected to all the irritation factors considered essential for the development of breast tumors in individuals having such an inherited constitution.

Of the nine A stock females fostered by CBA stock females, three developed mammary gland tumors, four had primary lung tumors and two died non-tumorous. Among 40 of their progeny which were not fostered, there were observed 12 with breast tumors only, two with breast and pulmonary cancers, 13 with primary lung tumors and 13 died non-tumorous. The proportion for each group was 30.6 per cent., 4.1 per cent., 34.7 per cent. and 30.6 per cent., respectively. The average age at death or observation of the various classes was: breast cancer, 12 months; lung cancer, 17 months; and non-cancer, 15 months.

Ten of the 13 progeny of fostered females which had breast cancer developed similar growths as compared with four of the 24 progeny from fostered females which had lung tumors. The respective proportions were 77 per cent. and 17 per cent.

The three fostered litters were descended from one subline of the A stock. All had from 16 to 18 successive generations of mammary gland tumors in their direct ancestry. Prior to the birth of the litters 78 mice had been observed, of which 92.3 per cent. had developed breast tumors. In later generations 219 mice, exclusive of the fostered females and their progeny, have died from various causes. The proportion developing mammary carcinoma was 88.1 per cent.

While the number of animals used in the preliminary work has been small, a larger group of females fostered by C_{57} Black stock mice are giving observations which are indicative of similar results. Should further study demonstrate that the incidence of mammary gland tumors in mice may be affected by nursing, an explanation may be offered for the so-called extrachromosomal influence as a cause in the development of this type of neoplasm.

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BONE ASH IN PREVENTION AND HEALING OF EXPERIMENTAL RAT RICKETS

THE very extensive use of rickets curative technique in judging the effects of vitamin D in both rats and human subjects, where the conclusions are based on subjective estimates of the degree of healing, makes it desirable to supplement the information, if possible, with more objective measurements.

The ash content of the normal rat femur (stock diet)

[1] Preliminary report.
[2] Staff, Jackson Memorial Laboratory, SCIENCE, 78: 465, 1933.
[3] J. J. Bittner, *Amer. Jour. Cancer*, 25: 791, 1935.

at 7 weeks of age is about 56 per cent. A rat of similar age having been on a rachitogenic (Steenbock) diet for 3 weeks has a femur ash which varies somewhat with rat strain, season, etc. In the experiments reported here the mean value was 30 per cent., ranging from 24 to 34 per cent. When ample Vitamin D was given during the three weeks on the rickets-producing diet, the bone ash did not rise above a mean of 50 per cent. (49 to 52 per cent. in groups of about 10 animals); with added phosphate, however, the bone ash values clustered very closely about the normal 56 per cent. The 50 per cent. bone ash level was attained with 0.5 U.S.P. unit of D per day. Several times higher Vitamin D intake produced no further increase. Dose response curves over a significant range have been worked out.

Ten-day curative experiments carried out as Vitamin D assays (U.S.P. technique) gave the following results. A definite fairly wide continuous line of recalcification (++ on our scale) raised the bone ash to no higher than a mean value of 34 per cent., with about the same variation as applied to the 30 per cent. value for negative controls. Effects of this magnitude are commonly produced by 0.2 to 0.3 units of D per day. Distinctly visible recalcification but less than the ++ healing gave extremely variable bone ash values and very poor correlation with the visual picture. In several groups no increase whatever was shown in bone ash when mean values were compared. Fifty or a hundred times the dose which produced a ++ healing did not raise the bone ash above 40 per cent., although the silver stained bone showed the entire previously rachitic area filled with dense newly formed calcified trabeculae.

It is indicated therefore that in curative experiments a definite effect noted visually may correspond to but a small fraction of the healing process, and that what is considered very substantial healing effects raise the bone ash through only about one fifth of the interval between the rachitic and the full Vitamin D prevention levels. One hundred times this dose raises the bone ash half way towards the prevention level. Any further increase requires more time.

Besides these planned experiments, observations on more than 400 bones from assay studies, mostly on cod liver oil concentrates but also including other forms of Vitamin D, verify this picture. It is not surprising to find that the variation in bone ash values increases, the further they are removed from normal.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

PREPARATION OF METHIONINE-FREE NATURAL LEUCINE

IN a recent note in SCIENCE, Mueller[1] pointed out that commercial l-leucine is contaminated with methionine. The presence of a sulfur-containing impurity was recognized by Emil Fischer[2] in 1900. Unsuccessful attempts to purify natural l-leucine have included: the amino-acid ester method, repeated recrystallization from water, electrodialysis, precipitation of methionine as mercury complex and recrystallization from acidified butyl alcohol. Recrystallization of the formylated amino-acid has now been found, however, to yield a sulfur-free leucine without appreciable racemization.

Mixtures of l-leucine samples containing 2.6, 5.5 and 8.0 per cent. methionine were formylated by the methods of Fischer and Warburg[3] and of Steiger.[4] Two recrystallizations from water rendered the formyl-leucine practically sulfur-free, as determined by combustion sulfur analysis. For purposes of precise calorimetry, these samples were recrystallized from water four more times. The formyl-leucine was then decomposed by refluxing one hour with ten cc of 10 per cent. HBr per gm of formyl-leucine, and most of the excess acid removed by evaporation *in vacuo*. After solution of the residue in water, the evaporation was twice repeated. The final residue was dissolved in a little water, neutralized with conc. NH_4OH, chilled, and the crystals were filtered off. The leucine was washed with alcohol until halide-free, and finally recrystallized from water.

The yield of leucine from original crude material, after six recrystallizations of formyl body, was 50 per cent. The product was sulfur-free and ash-free. The specific rotation, in 20 per cent. HCl, checked that of l-leucine obtained by synthesis and resolution.

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1 J. H. Mueller, SCIENCE, 81: 50–51, 1935.
2 E. Fischer, *Ber. chem. Ges.*, 33: 2372, 1900.
3 E. Fischer and O. Warburg, *Ber. chem. Ges.*, 38: 3998, 1906.
4 R. E. Steiger, *Jour. Biol. Chem.*, 86: 695, 1930.

A DIFFERENT PRINCIPLE IN THE CONTINUOUS RENEWAL OF CULTURE SOLUTION

DURING the past fifteen years numerous pieces of apparatus have appeared that were planned to deliver culture solutions at a uniform rate. Several of these employ the principle of dripping from a nearly constant level in order that the rate of drip be uniform.