pelves, but no formation of concrement. This question is receiving further attention.

The uroliths consisting of the acetyl derivative of sulfapyridine permit penetration by X-rays. However, it has been observed that calcium can be deposited about these concrements which act as a nucleus in which case the shell may become X-ray opaque.

10442 P

Production of Mammary Carcinomas in Male Mice With a Single Implantation of Oestrone.

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Lacassagne[1] produced tumors in male mice of the R III strain, in which 70% of the females develop spontaneous mammary carcinomas, by the weekly injection of 300 I.U. of oestrone benzoate in oil. The tumors were found to develop sooner in males so treated than they occur spontaneously in females of the same strain. These findings have been repeated by Burrows,[2] by Bonser,[3] by Cramer and Horning,[4] and by Gardner, Smith, Allen and Strong.[5] Lacassagne[6] used oestrone, oestradiol, equiline, and equilinine, all hormones being injected weekly in oily solution. Burrows painted his animals twice weekly with oestrone, .01% in benzene; Bonser used oestrone benzoate in olive oil as did Gardner, *et al.*, while Cramer and Horning used oestrone in chloroform applied to the skin twice weekly.

Recently Deanesly and Parkes[7] have shown that prolonged effects may be obtained by the implantation of crystals of oestrone under the skin. A capon so treated with a 3 mg crystal of oestrone maintained the hen coloration of the breast feathers for over 3 months. It seemed that this method might be made use of to repeat the experiments of Lacassagne while decreasing the labor of weekly injec-

1 Lacassagne, A., *Compt. rend. Acad. de sc.*, 1932, **195**, 630.

2 Burrows, H., *Am. J. Cancer*, 1935, **24**, 613.

3 Bonser, G. M., *J. Path. and Bact.*, 1935, **41**, 217; 1936, **42**, 169.

4 Cramer, W., and Horning, E. S., *Lancet*, 1936, **1**, 247.

5 Gardner, W. U., Smith, G. M., Allen, E., and Strong, L. C., *Arch. Path.*, 1936, **21**, 265.

6 Lacassagne, A., *Bull. de l'assoc. fr. p. l'etude du cancer*, 1938, **27**, 1.

7 Deanesly, R., and Parkes, A. S., *Proc. Roy. Soc., B*, 1937, **124**, 279.

tions and removing any confusing results due to the application of such large quantities of solvent.

In order to determine whether prolonged action of oestrone could be obtained in mice by the implantation of oestrone crystals 7 stock females were selected and vaginal smears taken for a week to show that they were all ovulating normally. At the end of this time crystals of oestrone (obtained from the Schering Corporation through the kindness of Dr. Schwenk) weighing 0.68 mg to 1.06 mg recrystallized from ethyl alcohol were introduced beneath the skin of the abdomen of each animal with a trocar. Each animal received one crystal. Daily smears were taken and showed all the animals to be in constant oestrus until the day of death. Four animals died of pyometra at the end of 6 weeks. One was accidentally killed at the end of 3 months. One died with pyometra after 5 months and the last at 8½ months. The latter showed uterine horns so distended with fluid as to make the animal appear almost circular in outline.

Having demonstrated that single crystals of oestrone would produce constant estrus in female animals for at least 8 months, young male mice of the R III strain in which 70% of the females develop mammary tumors were selected at approximately 10 days of age and injected with crystals of oestrone beneath the skin in the pectoral region. A fine spinal puncture needle was found to make a very satisfactory trocar for this procedure. Twenty-one animals were so treated. The amounts of oestrone used in each animal varied from .06 mg (600 I.U.) to .2 mg (2000 I.U.). Sixteen of the animals died before 100 days, all showing signs of urinary obstruction (distended bladder, hydronephrosis, hydroureter) from prostatic enlargement and keratinization. Two animals are living to date (5 months) with no signs of oestrinism. In these animals the minute crystals of oestrin probably came out unnoticed with the needle. Of the remaining 3, one died at 6½ months without tumor and 2 developed large mammary carcinomas.

The first of these occurred in a male mouse injected with 0.1 mg of oestrone 4 months and 26 days before the appearance of the tumor in the right inguinal region. The mouse was killed 3 weeks after the tumor was first seen, at which time 2 smaller tumors were found in the axillary region. The tumor was transplanted to 6 male mice of the same strain and grew in all. Gross preparations of the breast tissue showed tremendous development (both duct growth and acinar proliferation) of the non-cancerous portions of the breast. The bladder was filled with phosphate crystals and there was bilateral hydronephrosis. The seminal vesicles were greatly enlarged and

weighed 404 mg. The pituitary weighed 2.3 mg, about twice the normal weight in this strain. The adrenals showed the brown degeneration described by Cramer[8] in animals treated with large amounts of oestrin.

The second tumor occurred in a mouse injected 5 months before with a single crystal of oestrone. This animal also showed a distended bladder and bilateral hydronephrosis but in contrast to the first animal the seminal vesicles were atrophied. There was a very marked enlargement of the pituitary to at least 5 times normal size. Histological sections showed this to be more a diffuse hypertrophy rather than an adenoma, all 3 types of cells lying scattered throughout the gland. The adrenal showed a less degree of brown degeneration than occurred in the first mouse. The breast tumor grew progressively in one of 2 animals into which it was transplanted, confirming the histological diagnosis of adenocarcinoma.

Fourteen virgin littermate females have shown as yet no spontaneous mammary tumors at 3 to 7 months of age. The treatment received by the 2 males developing tumors not only induced the formation of carcinomas but produced them sooner than any such tumors occurred spontaneously in their sisters. Mammary carcinoma does not occur spontaneously in male mice.

Since this article was submitted for publication, 13 additional male mice similarly treated have developed mammary tumors.

10443 P

Possible Effects of Vitamin K on Prothrombin and Clotting Time in Newly-born Infants.

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Three deaths from hemorrhagic disease of the newly-born occurring recently on the pediatric service of the University of Virginia Hospital prompted studies to determine the normal prothrombin level in a group of newly-borns with the hope that some agent could be found which might materially effect prothrombin levels. Very early in this investigation 2 cases with abnormally high prothrombin

[8] Cramer, W., and Horning, E. S., *J. Path. and Bact.*, 1937, **44**, 633.