as failing to provide evidence for the tumor toxin, toxohormone.

Summary. Extracts of bacteria-free tumor and normal tissues were prepared by methods commonly used to obtain toxohormone and tested by measuring the lowering of plasma iron concentration in rats. The toxohormone extracts of both tumor and normal tissues were active. The plasma iron decrease produced by the extracts could be altered by the methods used in handling the tissue both before extraction and in the final extract. These results provide further evidence that tissue autolysis and procedures employed in extraction and drying can markedly alter the final activity obtained from both tumor and normal tissues. Further proof would, therefore, seem to be required before the synthesis of a tumor toxin, toxohormone, by tumor tissue can be established.

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Received Oct. 23, 1967. P.S.E.B.M., 1968, Vol. 127.

Maturation Changes of Vaginal Epithelium in Pregnant Rats (32761)

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The use of the exfoliative vaginal cytology to evaluate functions of ovaries dates back more than 100 years (6). Later Loch (1) in 1909 described the vaginal epithelium of various animals. Stockard *et al.* in 1917 (2) demonstrated the existence of a typical estrous cycle in guinea pigs, and Allen (4) in 1922, published a monograph entitled "the Oestrus Cycle in the Mouse." The extensive studies of Long and Evans in 1922 (3) on the estrous cycle in rat proved that the cycle is characterized by regular, periodic, coordinated histological changes in the epithelium of the uterus and vagina.

Since no previous work has been done on the maturation changes of vaginal epithelium of the pregnant rat, we have explored this field and submit a report on our observations.

Materials and Methods. Twelve young adult female albino rats of the Walter Reed-Carworth Farm strain were mated. They were placed in four groups of three each. To avoid too frequent handling of the pregnant animals, on day 1 vaginal smears were

obtained from animals of group 1, on day 2 from group 2, etc., until day 5 when smears were again taken from group 1. This procedure continued daily throughout gestation, thus giving us a series of vaginal smears on each day of pregnancy.

Smears were spread on a microscopic glass slide, fixed immediately in ether-absolute alcohol (50:50) for at least 20 min and then stained with cresylecht violet. We used the same cytological criteria for maturation of vaginal epithelium as Frost (7) in his studies of clinical cytology namely, a differential count of three major types of cells, i.e., parabasal (P), intermediate (I), and superficial (S). We made such a count on 100 cells from each smear of each animal. We thus examined 300 cells for each day of pregnancy.

The following phenomena were investigated: 1. Maturation index (MI) which is the changing ratio level of maturation of the three major types of exfoliating stratified squamous epithelial cells under the influence of the animal's sex hormones. Thus 2/42/56 represents the MI 2% P, 42% of I, and 56% of S cells. A shift to the left denotes less mature cells and shift to right indicates more mature cells.

2. General cytology of the smear, i.e., clumping of the cells, folding of cytoplasm, presence of mucus and leukocytes.

Results. In Table I the MI of the differential cell count is summarized.

From this data a marked and regular fluctuation in the maturation index of the parabasal cells in rat was observed as shown in Fig. 1.

For the first 6 days of gestation the vaginal smears showed a moderate amount of clumping of cells, folding of cytoplasm, and presence of mucus and leukocytes. On day 7, however, there was no clumping and only slight folding of cells, a slight amount of mucus, and few leukocytes (Fig. 3). On day 8 and 9 all criteria were maximum (Fig. 4) and remained high to day 13. On day 14, except the presence of some mucus, there was a sudden drop in the other features of the cells (Fig. 5). From day 14 to term, there was gradual increase in all cytological criteria. These observations are summarized in Table II.

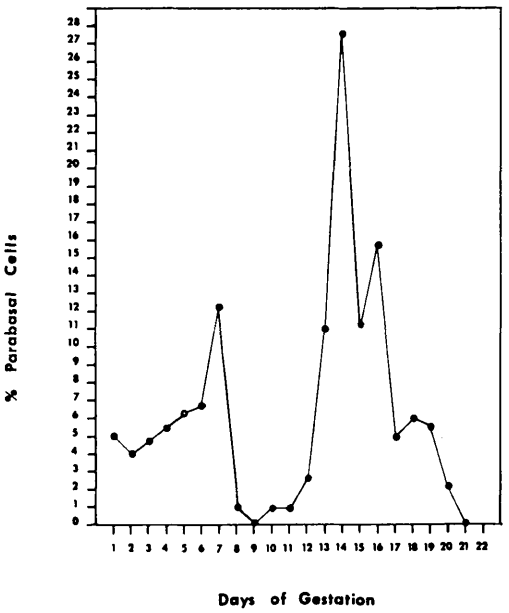

FIG. 1. The MI of parabasal cells during gestation. Day 7 attachment of placenta. Day 14 maximal development of placenta.

Discussion. It is well known that under the influence of estrogens, all layers of vaginal epithelium proliferate and become thickened.

TABLE I. Maturation Index of Vaginal Epithelial Cells during Gestation.

Day	MI P/I/S (%)	Day	MI P/I/S (%)	Day	MI P/I/S (%)
1	5.0/50.0/45.0	8	1.0/47.0/50.3	15	11.3/49.0/39.7
2	4.0/34.0/62.0	9	0.0/33.6/66.9	16	15.7/46.0/38.3
3	4.7/46.8/46.8	10	1.0/65.0/34.0	17	5.0/44.0/51.0
4	5.5/42.1/51.2	11	1.0/47.0/52.0	18	6.0/32.3/61.7
5	6.3/37.3/55.6	12	2.7/52.1/45.2	19	5.7/38.3/56.0
6	6.6/51.0/42.3	13	11.0/52.0/37.0	20	2.3/59.0/38.7
7	12.2/45.0/42.8	14	27.5/48.0/24.5	21	0.0/15.2/84.8

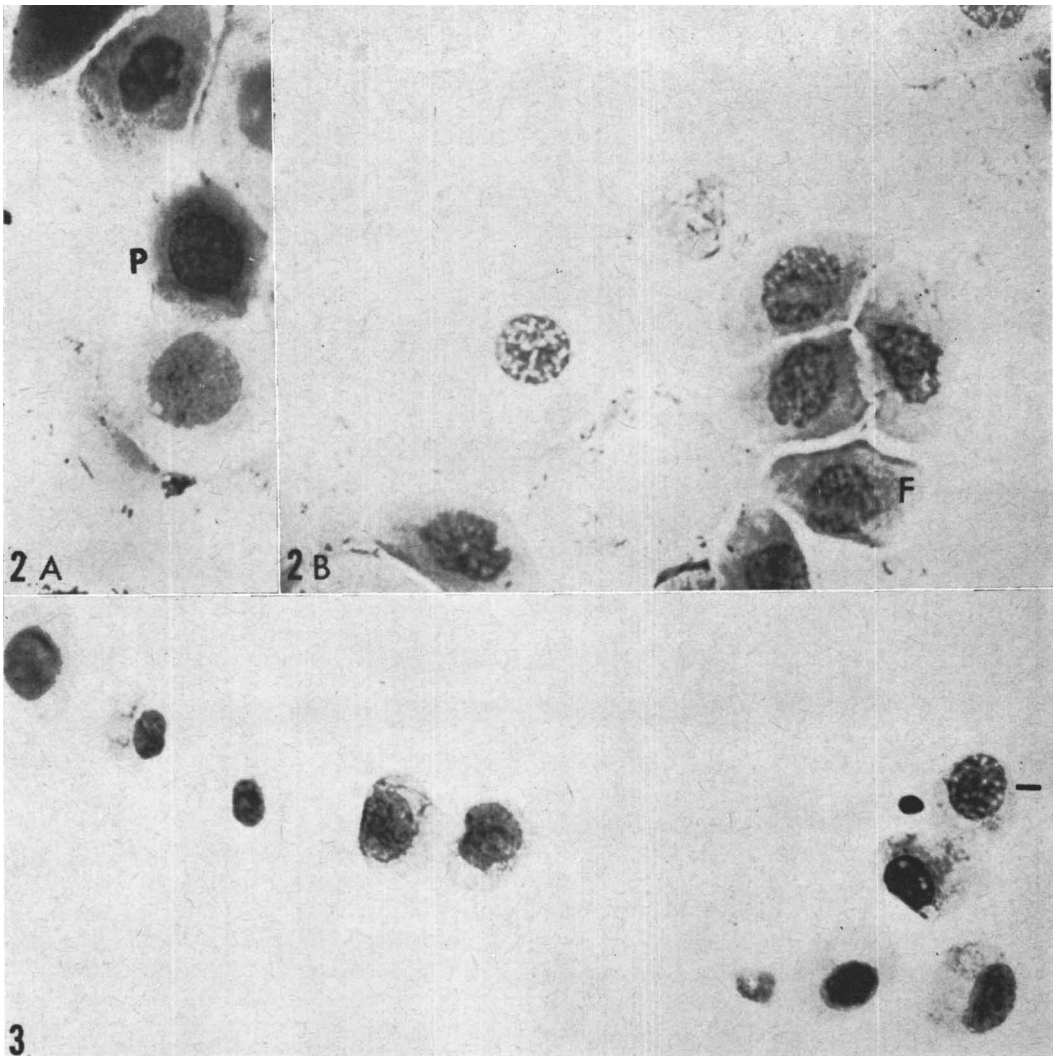

FIG. 2. No mucus, some clumping of cells (++), few leukocytes (±), folding of cell membrane (++) as seen as an uneven cell outline (F) × 455. Parabasal cells: Cell is spherical, cytoplasm dark staining, viscus, and nonfolding cell membrane. Nucleus is plump with marked regular chromatin pattern (Fig. 2). Intermediate cells: Cytoplasm is uniformly light staining, and wafer-thin, cell membrane usually folded. Nucleus is plump and vesicular retaining a definite chromatin pattern (Fig. 3). Superficial cells: Cytoplasm is wafer-thin, frequently folded, very light staining. The nucleus is hyperchromatic and has lost the chromatin pattern (Fig. 5).

FIG. 3. Day 7: Slight mucus (+), no clumping of cells, few cell membranes folded (+), and occasional leukocytes (±); × 455.

Thus the intermediate and superficial cells become most numerous on the surface of the epithelium with a resultant shift to the right of the MI. If the epithelium has been primed with estrogen, cytolysis occurs and the background of the cell becomes coagulated with mucus, with an abundance of leukocytes and cellular debris. On the other hand, with progestrone administration maturation progresses through the intermediate cell stage and the MI shifts towards the midzone and the cells do not tend to clump or stick. In the human female immediately after conception, the MI shifts towards midzone, i.e., 0/70/30

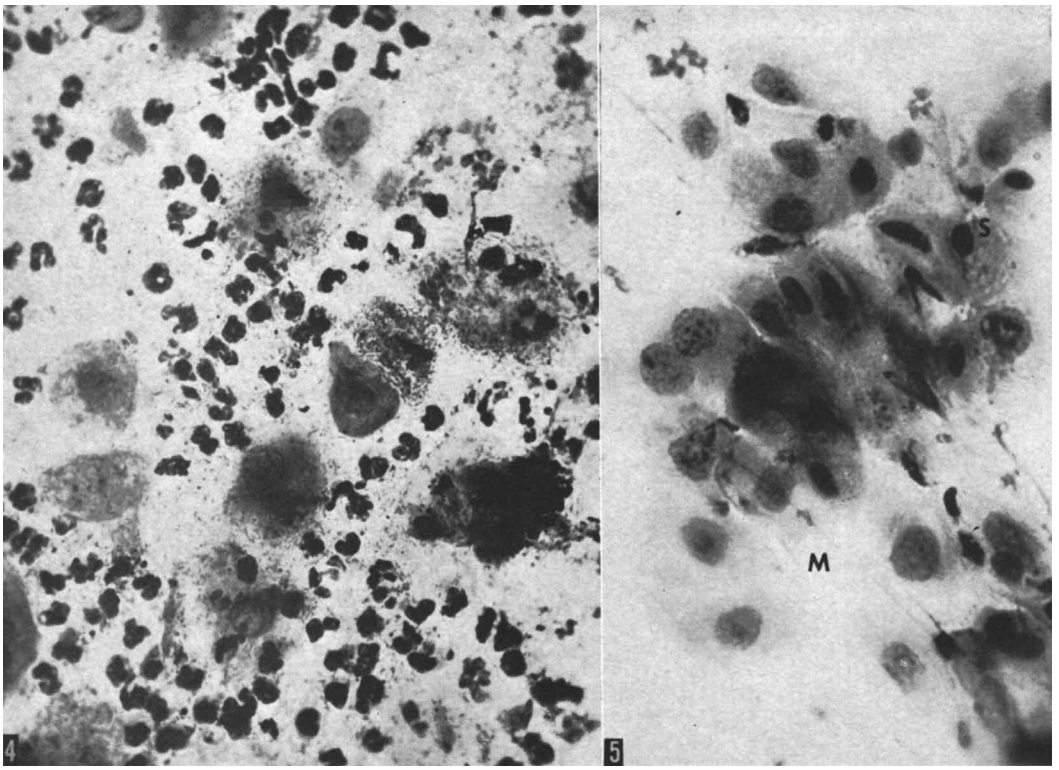

FIG. 4. Day. 9: Considerable mucus and clumping of cells (+++), cell membranes folded (+++), leukocytes abundant (++++) × 455.

FIG. 5. Day 14: Considerable mucus and (++) some clumping of cells (+), slight folding of cell membranes (±), and few leukocytes (±) × 455.

(Frost 7), and does not alter its direction but continues its midzone climb to 0/95/5 until delivery when it suddenly plunges to the left from 0/95/5 to 100/0/0. Unlike the human female the pregnant rat shows 2 distinct different phenomena, (i) regular fluctuations in the MI of the parabasal cells (Fig. 1 and Table II) and (ii) variations in smear cytology, as noted in Table II, show opposite values to the MI curve in our experiment. (Fig. 1).

The following observations may perhaps explain the conflicting values: (i) In the rat there are four kinds of corpora lutea, those of ovulation, copulation, pregnancy, and lactation (3). (ii) On day 7 attachment of the placenta takes place (5). (iii) Day 13 is maximal development of placenta (8). Cytological vaginal smear changes on day 7 and 14 of pregnancy in (Figs. 3, 5 and Table II) show a marked reduction in the amount or number of the cytological criteria. This suggests a progesterone-like pattern, which is further supported by the fact that an additional quantity of progesterone is being released by the implanted placenta on day 7 and by the maximally developed placenta on day 13. The increase in debris and leukocytes (Fig. 4), which we noted on days 8 and 9, could be caused by the implantation of the placenta, as suggested by Krehbiel (5).

TABLE II. General Cytological Criteria of Vaginal Smears during Gestation.

Day	Mucus	Clumping of cells	Folding of cells	Leukocytes	
1–6	±	++	++	±	(Fig. 2)
7	+	—	+	±	(Fig. 3)
8–9	+++	+++	+++	++++	(Fig. 4)
14	++	+	±	±	(Fig. 5)
21	+++	+++	+++	+++	

High concentration MI of parabasal cells on above dates (Fig. I) resembles horomonal withdrawal pattern, which could be caused by the four different corpora luteal complex. This is further supported by our observation that in 6 animals which, in spite of several matings, never became pregnant, and an autopsy showed small ovaries with no maturing follicles. They also had a high count of P 36%.

Summary. Studies on the effects of estrogen–progestrone levels on the MI of the squamous epithelium of vagina of pregnant rats were made. We observed fluctuations in MI for different periods of gestation as well as variations in cytological criteria which occurred especially on days 7 and 14. The MI shifts and marked alterations of the cytological criteria coincides on the same gestational days.

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Received Oct. 23, 1967. P.S.E.B.M., 1968, Vol. 127.

Biological Separation of Adenovirus T and V Antigen Synthesis in KB Cells* (32762)

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Sero-epidemiological studies of the relationship of the oncogenic DNA viruses to human cancer are plausible based on the occurrence of virus specific nonvirion T antigens in tumor cells and the occurrence of specific antibody in animals bearing such tumors (1).

We have explored several methods of producing T antigens for the human adenoviruses with the aim of devising a single procedure which would prove applicable to both non-oncogenic and oncogenic viruses. The desired result is a T antigen preparation, free of viral structural antigens, of sufficient titer to test sera obtained from carefully matched cancer patients and controls. While there are multiple possible sources of antigen and a variety of possible approaches to this problem, this paper will be restricted to experiments involving the lytic interaction of adenoviruses in KB cells.

* This investigation was supported by contract number PH43-65-634 from the National Cancer Institute, National Institutes of Health, Bethesda, Maryland. Tumor antisera were prepared under contract number PH43-64-1169 of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, by Mr. T. Beddow. Complement fixation tests were carried out under the supervision of Mr. M. Faulkner.

Materials and Methods. Virus. Adenovirus types 7 (Gomen) and 12 (Huie), obtained from the American Type Culture Collection and the Trailer Facility of the Laboratory of Viral Diseases, National Institutes of Health, respectively, were passed several times in human embryonic kidney (HEK) cells prior to preparation of standard pools in this tissue. Pools at the third to fifth and second to fourth passages of adenoviruses 7 and 12, respectively, with infectivity titers in HEK cells (2) of $10^{7.5}$ to $10^{8.5}$ and $10^{7.2}$ to $10^{7.7}$ $TCID_{50}$ per ml, were used in these studies. Virus pools and all T antigen preparations were tested and found negative for adeno-associated viral (AAV) antigens (3).

Complement fixation (CF) tests. The microtiter procedure using 1.8 exact units of