cated that PAM reacted with a component or components of blood and was then not available for color reaction with NBP. That the colored material was formed with biologically active PAM was indicated by experiments with mice and dogs (unpublished) in which PAM was dissolved in animal's blood and reinjected at intervals. White blood cell count depression decreased with decrease of colored material formed.

The reaction of PAM, HN2, and U-8344 with blood in concentrations and at conditions comparable to those in the perfusion system as measured by color reaction is given in Fig. 2. These results show that PAM and HN2, 2 agents currently used in perfusions, react with blood at different rates and persist in blood for different periods of time, while U-8344 reacts at a rate similar to that of PAM.

Loss of color reactive PAM from blood of a perfusion system may be attributed to PAM which reacted with blood of the system and with tissues of perfused part plus amount lost *via* leakage into the general circulation (Fig. 3). The loss due to reaction with blood was rapid for about 15 minutes, then proceeded at a steady rate. In a perfusion system when PAM reacted with both blood and tissues the initial loss was greater, but the general pattern was maintained when leakage was less than 5%. When leakage was of the order of 25% the pattern changed. The color reaction 5 minutes after addition of PAM to the system (5 minutes are required for thorough mixing) corresponds to less than total amount injected. Therefore, when percentage of color reactive PAM lost from the perfusion system was calculated from residual percentage (Fig. 3,V) it was actually greater than the 70% indicated by the graph.

Summary. A procedure for estimation of PAM and related alkylating agents in blood by its reaction with NBP is given. This procedure is applicable for study of the reaction with and persistence in blood of these agents *in vitro* and in perfusion systems.

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Epithelial Changes in Vaginal Isografts in Male Mice.* (25932)

W. E. GIBBS AND H. C. BROWNING
University of Texas Dental Branch, Houston

Vaginal isografts in female mice respond normally to both intrinsic and extrinsic estrogens, although response is less regular than that of the intact organ(1). Similar isografts in male mice show cornification in response to extrinsic estrogens and mucification in response to intrinsic androgens(2,3). In castrated male mice bearing ovarian transplants, cornification of vaginal grafts occurred but cyclic activity was not demonstrated. In females, similarly prepared, however, cyclic changes have been described(3). Ovarian isografts in castrated female mice or rats show cyclic formation of vesicular and luteinized follicles but do not do so in intact or castrated males(4,5). The following report concerns our observations on periods of estrus in vaginal epithelium transplanted to castrated male mice bearing ovarian isografts.

Material and methods. Intact vaginas, removed from 16- to 20-day-old strain A female mice, were transplanted into 6 groups of hosts, each consisting of 8 male strain A mice 2 to 4 months old (Table I). Three of these groups (I,II,III) were castrated at the same time and 2 of them (I,II) received bilateral intraocular isografts of one-eighth of an adult ovary from mice 2 to 4 months old, 5 to 6 weeks later. Identical ovarian transplants were made into one (IV) of the 3 non-castrated groups (IV, V, VI). All vaginal grafts were fistulated so that smears of exfoliated

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TABLE I. Mean Diameters of Vaginal Isografts before and after Estrogen Administration (If Any) to Castrated and Intact Male Mice with or without Ovarian Transplants.

Exp. group	Vaginal diameter before estrogen, mm	Wk of single estrogen dose admin.	Vaginal diameter after estrogen, mm
I Cast.; ovary	9		
II *Idem*	9	12	9
III Cast.	5	14, 15, 16	7
IV Intact; ovary	3	10, 11	6
V Intact	6		
VI "	6	8, 9, 10, 11, 12	9

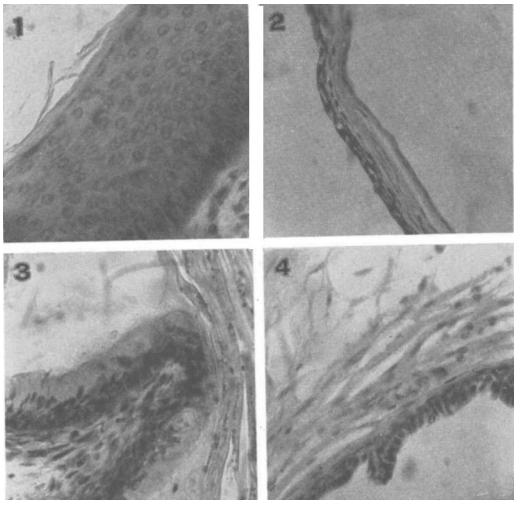
cells could be obtained (by lavage); such smears were examined for 8 to 14 consecutive days upon 3 to 5 occasions, between 11th and 30th weeks after vaginal transplantation. Twenty months after original experiment, experimental groups I, III, IV and V were duplicated and vaginal smears taken at same intervals for 17 to 56 consecutive days. At beginning of each series of examinations contents of the fistulated grafts were washed with buffered sterile saline. Representative vaginal grafts were removed for histological preparation in 19th or 20th week. All animals, except those in Groups I and V, were given subcutaneously from 1 to 5 weekly standard doses of 0.025 mg of estradiol dipropionate in sesame oil. This was done not only to determine effects of extrinsic estrogen upon size of vaginal grafts but to enlarge size of graft to facilitate preparation of satisfactory smears. The last dose of estrogen was administered during the week before grafts were prepared for taking vaginal smears, except in Group III where the last dose was given during week that preparations for smears were made.

Results. Ovarian transplants. In castrated males (I, II) the intraocular ovarian transplants, by the end of first month, occupied approximately half of the anterior chambers. Thereafter, rate of growth was retarded and, by the end of week 12, transplants occupied 70 to 90% of anterior chambers. They exhibited vesicular, cystic and hemorrhagic follicles, but, as hitherto reported(5), did not develop corpora lutea. In intact males (IV), intraocular transplants attained their maximal growth by end of first month, when they occupied 20 to 30% of anterior chambers. They, too, showed vesicular follicles regularly, and hemorrhagic follicles occasionally.

Vaginal transplant growth. By end of 12th week, vaginal transplants had attained mean diameters shown in Table I. Estrogen administration did not affect transplant size in castrated males bearing ovarian transplants but did cause moderate enlargement in castrated and intact males and marked enlargement in intact males bearing ovarian transplants. All vaginal transplants diminished in size during daily smearing procedures. Subsequently, with healing of fistulas prepared to allow smear sampling, vaginal grafts in castrated males bearing ovarian transplants (I, II) continued to dilate, reaching a diameter of over 4 cm by end of one year.

Vaginal histology and exfoliation. Castrated males with ovarian transplants (I) had proestrous smears during weeks 11 and 12. In some animals one or 2 phases of estrus (with diestrus between them) occurred following weeks 15 and 16; in others a proestrous smear was found at the same period but similar estrous phases occurred following weeks 19 and 20. From 8 to 14 days elapsed between beginning of 2 successive estrous states; full vaginal estrus itself occupied 2 to 6 days. In either case, smears had returned to proestrous (or occasionally the diestrous) phase a month later and remained so until week 30 when daily smearing was discontinued. In animals (II) that received estrogen at week 12, smears were of the estrous type at weeks 13 and 14 but reverted to proestrus and showed 1 or 2 estrous phases following weeks 15 and 16. Estrogen, in a single standard dose, was given to duplicate group I of castrated males with ovarian transplants after their reversion to proestrus or diestrus following single or repeated estrous phases. A single long period of estrus (22 to 28 days from one proestrus to the next; 1 to 17 days of full vaginal estrus) followed this administration with reversion to continuous proestrous state.

Histologically, vaginal transplants in these groups showed a typically stratified squamous epithelium with 10 to 12 layers of cells (Fig. 1) and a graft content of keratinized layers



Vaginal isografts in various types of male hosts, × 218.

FIG. 1. Castrated male bearing ovarian transplants.

FIG. 2. Castrated male without ovarian transplants.

FIG. 3. Intact male bearing ovarian transplants.

FIG. 4. " " without " " .

with some epithelial cells and leucocytes.

Castrated males without ovarian transplants (III) had a diestrous smear (although a number of epithelial cells were occasionally present) from week 19 to 24. Estrogen was administered at time of initial smearing (weeks 15 to 16) when smears disclosed a late proestrous phase. Estrogen was administered to the duplicate group, after continuous diestrus was established, at week 19, as a single standard dose. Vaginal transplants responded with a single long period of estrus (16 to 28 days from diestrus to diestrus; 8 to 20 days of full vaginal estrus), then reverted to continuous diestrus.

Histologically, the vaginal epithelium consisted of one or 2 layers of cuboidal cells (Fig. 2) with graft content of leucocytes and epithelial cells.

Intact males with ovarian transplants (IV) had a diestrous smear (except for a few animals in metestrus from the previous estrogen) at all times. Histologically the vaginal epithelium showed 2 to 4 layers of cuboidal cells (Fig. 3), the outermost being strongly mucified with occasional infiltrated leucocytes, and a graft content of epithelial cells and necrotic leucocytes.

Intact males without ovarian transplants (V) had diestrous smears, often with numbers of epithelial cells, at all examinations. Similar hosts (VI) which had received 5 preliminary doses of estrogen showed an estrous or late proestrous smear at initial examination. The duplicate group received a single dose of estrogen after establishment of a continuous diestrous smear and responded to it with a single long period of estrus, followed by reversion to diestrus.

Histologically, vaginal epithelium consisted of 2 to 4 layers of somewhat flattened cuboidal cells with occasional areas of mucification (Fig. 4). The graft contents were scanty epithelial cells with leucocytes.

Discussion. Changes in vaginal smears have not been described by other authors in castrated males with ovarian grafts(3) or in hypophysectomized males with grafted male or female pituitaries(6). However, examinations were made at one or 2 months after grafting; estrous phases in our animals were absent until 4th or 5th month after grafting. Repeated cyclic activity occurs in comparably treated females within one month. Presumably, periodicity of pituitary output of FSH, with its stimulation of estrogen secretion by transplanted ovarian tissue to a level sufficient to induce vaginal cornification, is not established in the male castrate for a relatively long period, then is only ephemeral.

The virtual absence of growth of vaginal grafts in intact males with ovarian transplants without estrogen administration, together with their histological structure, in comparison to that in intact or castrated males without ovaries, illustrates the antagonism between estrogenic and androgenic action on vaginal mucosa previously described(2,3,7,8). The relatively great amount of vaginal growth, and type of vaginal histology, in castrated males with ovarian transplants was due, presumably, to the higher output of estrogen by ovarian grafts in these hosts under the influence of post-castrational elevation of pituitary FSH output.

Summary. 1. Isografts of intact immature

vaginas grew maximally in castrated male mice with ovarian transplants and minimally in intact males with similar grafts. Growth was intermediate in castrated or intact males without ovarian grafts. 2. Vaginal grafts showed one or 2 estrous phases in castrated males with ovarian grafts between 4 and 5 months after grafting but otherwise remained in late proestrus. Such activity did not occur in castrated males without ovarian transplants or in intact males with or without ovarian transplants, vaginal grafts in these hosts remaining in diestrus.

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Time Response of Lipid Changes in Cockerels after Single Dose of Estradiol Cyclopentylpropionate. (25933)

K. KOWALEWSKI

McEachern Cancer Research Lab. and Dept. of Surgery, University of Alberta, Edmonton, Canada

Estrogen-induced hyperlipemia and atherosclerosis in cockerels has been described by several investigators(1,2,3,4). More recently Caldwell and Suydam(5) have studied atherogenic effect of estradiol cyclopentylpropionate (ECP) in young birds and noted significant changes in concentration of serum lipids and polysaccharides. Purpose was to study the effect of a single dose of ECP on the lipid pattern in 3-week-old cockerels. Cholesterol and phospholipids of serum, liver and aorta were determined daily, during a 7-day period, following a dose of 5 mg of ECP, to evaluate time response of cockerels to this estrogen.

Methods. Cockerels of White-Rock Broiler strain used for this experiment were obtained from a commercial hatchery when 8 days old. They were kept in thermostatic brooders and received a routine chicken starter with tap water as desired. Hormone was injected when birds were 3 weeks old. Estradiol cyclopentylpropionate (Upjohn Co.) was given intramuscularly as a single dose of 5 mg, dissolved in 0.2 ml of corn oil/bird. Beginning the next day after injection and every day for 7 days, a group of cockerels was anaesthetized, killed by decapitation and the blood collected. Untreated birds, killed on various days of this 7-day period, were considered as controls. Livers and aortas were dissected and weighed. Serum, livers and aortas were studied for lipid content. Total cholesterol was determined following the procedure of Abell *et al.*(6). For study of lipid phosphate the samples were extracted with alcohol-ether and analysis carried out following the method of Zilversmit and Davis(7). Mean values for treated and control groups were compared by the Wilcoxon Rank Test and significance levels were expressed in per cent(8).

Results. Body weight of cockerels was 226 g (200-252) at day of injection, and 387 g (343-452) at end of experiment. Injection was well tolerated and no inhibition of appetite or growth resulted. The results of study of lipids in serum, liver and aorta are presented in Tables I and II. Early and significant rise of both cholesterol and phospholipids was noted in serum and in liver after ECP. Changes in aorta were slower to appear, but significant increase of both studied lipids occurred between the 4th and 7th days, after injection of hormone.

Discussion. Significant increase of lipids