individual kidneys and livers of rats of lots III and IV as evidenced by the lack of blue coloration with $SbCl_3$ reagent. Petroleum ether extracts of livers and of kidneys from these lots were pooled in order to obtain a greater concentration of vitamin per colorimeter tube. These showed only a very slight blue coloration. From consideration of the data of Moore and Sharman(10) it is evident that very small amounts of vit. A should be present in the tissues concerned at 0.75 γ per day level of supplementation.

It would appear, on the basis of these data, that aureomycin does not function in the absorption of vit. A from the tract. Whether this is true also for the B vitamins requires additional experimental work.

Summary. 1. Growing male albino rats were fed vit. A ester at levels of 0, 0.75, and 7.5 γ/rat/day. Comparisons were made at these levels of vit. A intake with and without aureomycin added to the diet at the rate of 100 mg/kg. 2. Statistically significant differences were found in weight gains and food intakes between groups receiving different levels of vit. A. 3. Statistically significant differences were not found in weight gains and food intakes between groups receiving antibiotic and their controls at the various levels of intake of vit. A, except on vit. A-free diets. Here weight gains and food intake of the control animals were significantly greater than for animals receiving antibiotics. 4. At a level of intake of vit. A of 7.5 γ/day no significant difference of liver storage was found upon aureomycin supplementation. Kidney storage of aureomycin supplemented animals was significantly less than that of control animals. Under conditions of this experiment at the 7.5 γ/day level of supplementation of vit. A significantly greater storage of vit. A occurred in the liver than in the kidneys. 5. No effect of sparing of vit. A by aureomycin was demonstrated, but on vit. A-free diets aureomycin appeared to enhance the deficiency syndrome.

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Acid Phosphatase in Human Female Genital Tract, A Histochemical and Biochemical Study.* (20260)

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In the course of histochemical studies of the normal and malignant human cervix uteri on the possible relationship of alkaline phosphatase activity at pH 7.4 and 9.0, with various phosphorylated derivatives, to glycogen deposition, surprisingly slight activity of the cervical epithelium was a consistent finding.

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Because of reports by Gomori(1,2) on marked phosphamidase activity in this tissue in the malignant state at pH 5.6, we studied histochemically the acid phosphatase activity. The enzyme activity was found in the normal cervical epithelium, in the vagina, endocervical glands, glands and stroma of the endometrium, mucosa of the fallopian tube, and, to a much lesser extent, the ovary. Supporting biochemical data were also obtained and so far, at least, no obvious contradictions between the two independent methods of study have appeared, though precise correlation and quantitation have not as yet been attempted.

Materials and methods. Tissues for histochemical studies were treated as previously described(3). Fragments of tissue, preferably less than 1 cc, were frozen in melting isopentane and stored at —30°C until sectioned. Sections were cut at 15 μ in a Linderstrom-Lang type cryostat and appressed to slides without benefit of adhesive. Incubation periods ranged from 10 minutes to 48 hours. The incubating medium was essentially as recommended by Gomori(4): 0.05 M CH_3COON_a; 0.01 M phosphorylated substrate; and 0.004 $(CH_3COO)_2Pb$. pH was adjusted with concentrated HCl. Using the principle of Friedenwald and coworkers(5), the solution was saturated with PO_4^{---} ions with the aid of concentrated Na_3PO_4. As already reported, 0.01 M NaF proved to be a very effective inhibitor of this reaction. *Biochemical studies,* using homogenate technics, were also performed on tissues of the female genital tract. Tissues, homogenized at concentrations of 1-5% in distilled H_2O, were used as enzyme source. Lower concentrations of tissue were necessitated by paucity of material in such cases as the vaginal and cervical epithelium and fallopian tube mucosa, which were obtained by scraping the luminal surface of these organs. The homogenizer was chilled in an ice-water bath during maceration and in storage prior to actual running of the test. Various buffer or buffer combinations and two substrates were employed in the biochemical studies with the homogenates. In the acid range (pH 2.2 to 8.0) a simple CH_3COON_a (0.028 M) buffer (adjusted with HCl) appeared superior to one with added veronal. In the alkaline range (9.0 to 10.6 or 11.0) veronal alone (0.028 M) appeared satisfactory. Disodium phenylphosphate (0.005 M) was used as a substrate besides sodium glycerophosphate (0.01 or 0.02 M), since it has been reported to be more readily hydrolyzed than the latter (*i.e.* glycerophosphate). As a rule the homogenate (1-5%) was introduced as 0.2 ml of tissue suspension into 2 ml of substrate-buffer mixture. The reaction was terminated by the addition of 0.5 ml of 20% C Cl_3COOH. Inorganic PO_4^{---} assays, as a measure of enzyme activity, were performed by the method of Fiske and Subbarow(6) on 1 ml aliquots of supernatant from centrifuged reaction mixtures. Controls consisted of tubes (a) lacking only substrate, (b) lacking only homogenate, (c) lacking both, and (d) to which TCA was added prior to incubation period.

Results. Biochemical. Except for the sample of endocervical tissue, the amount of alkaline phosphatase activity has been a fifth of the acid variety, or even less. In order to characterize more closely the acid phosphatase activity encountered, assays for phosphatase activity were run at various pH levels from 2.2 to 11.0, usually in steps of 1 pH unit, and in the presence of some selected inhibitors, which, from the work of Abul-Fadl and King(7) among others, could be expected to be informative. Because endometrium was available to us in greater quantities than any of the other tissues studied, most of these experiments were performed with this tissue. Rat liver and human prostate (benign adenoma) were assayed, using identical technics in independent and parallel runs for comparative purposes.

pH. The acid phosphatase activity of the female genital tract has a pH optimum around 5. The peak for this tissue, as for other tissues simultaneously investigated, does not appear sharp. Considerable activity has been encountered at pH 4, or even a little lower (3.4), and marked inorganic phosphate release obtains at as high as pH 7 or slightly above (Fig. 1).

Inhibitors. Like others, we have found that 0.001 M NaF gives 100% inhibition of this enzymic activity (Fig. 1). Tartrate (0.02 M)

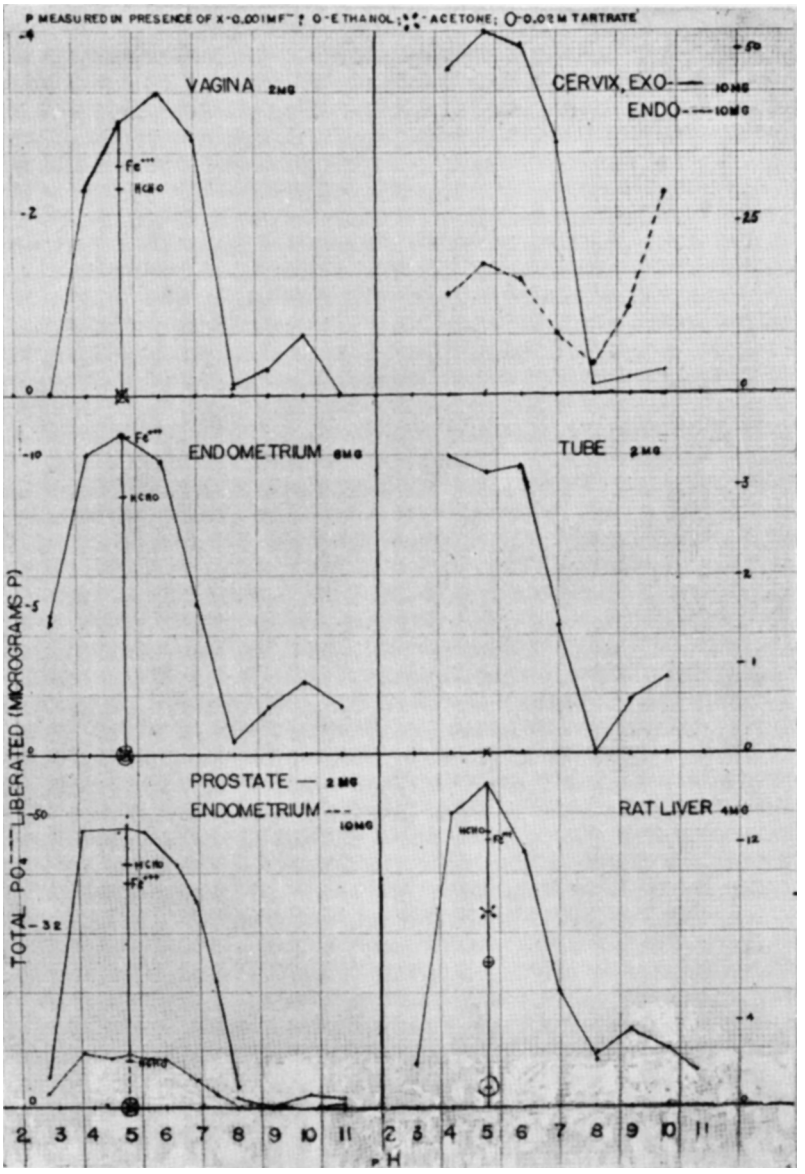

FIG. 1. Six curves, showing variation in total PO_4^- liberated (expressed as μg P) by homogenized tissues from various linings of the female genital tract and from human prostate, and rat liver, at various pH levels in acid and alkaline range. The level of activity in presence of various inhibitors at pH 5 is indicated by horizontal marks on ordinates at pH 5. Amount of tissue (wet wt) per tube in each reaction mixture is indicated with each graph and ordinates vary with each graph as indicated. HCHO employed at 0.5%. Fe^{+++} employed at 0.001 M.

likewise has been found to give about this much inhibition. This has been found to depend to some extent on the substrate used. With disodium phenyl phosphate, tartrate inhibition in endometrium was not nearly as marked as with sodium glycerophosphate. Both alcohol and acetone, when incubated at 2/5 volumes concentration preliminary to substrate-buffer addition(7) were found to be very potent inhibitors of acid phosphatase activity in the female genital tract, but only about half as effective against rat liver phosphatase. This finding is in partial agreement with Abul-Fadl and King(7) who report

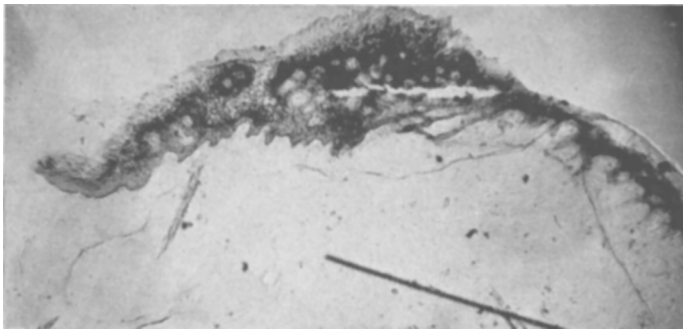

FIG. 2. Section of wall of vagina of human (× 25), showing marked activity in epithelium and stroma quite inert. Pointer is on stroma. Incubated with glycerophosphate for 2 hr at pH 5.

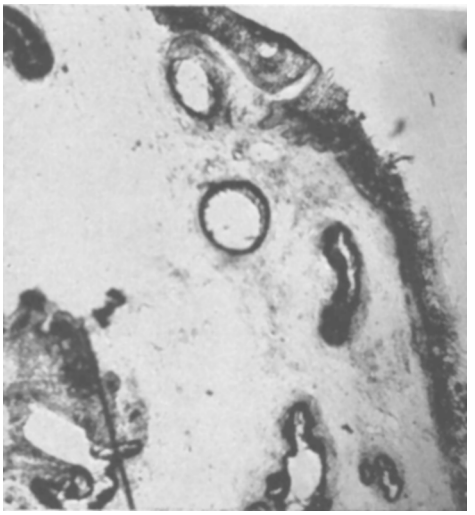

FIG. 3. Section of biopsy of human cervix uteri (× 25), showing marked activity in epithelium and very active endocervical glands. Tip of pointer is on basal layer of a short segment of epithelium. Incubated as above.

human liver less sensitive to alcohol and acetone inhibition than prostatic acid phosphatases. Fe^{+++} in the form of $Fe(NO_3)_3 \cdot 9H_2O$ (0.001 M) and 0.5% HCHO caused only slight inhibition of acid phosphatase activity.

Histochemical. There is a high level of acid phosphatase activity in the epithelial layers of the vagina and cervix (Fig. 2 and 3), but a much lower alkaline phosphatase activity. The same is true for the endometrium. All data given below refer to results obtained with glycerophosphate as substrate unless otherwise indicated.

Vagina. To date we have examined sections of the anterior and posterior wall of the vagina from one patient (Fig. 2). The basal layer of the epithelium does not stand out from the rest of the epithelium as much as it does in the exo-cervix. As in the latter, the zone of greatest activity lies 3 to several cell layers removed from the stroma, and toward the lumen of the organ, activity gradually becomes almost nil. In this case there seems to be an appreciable amount of nuclear activity, relatively more so than in the cervix.

Cervix. The epithelium of the pars vaginalis of the cervix reacts like the epithelium of the vagina. In general it is much more reactive than the stroma with the notable exception of the endocervical glands, which are intensely reactive (Fig. 3). The reaction in the epithelium is primarily cytoplasmic, though slight to rather strong nuclear activity has been noted on occasion. The reaction is by no means of uniform intensity across the thickness of the epithelium. There is an accentuation of deposition in the basal epithelial layer as compared to the stroma, and to a lesser extent as compared to the parabasal layers (Fig. 3). A zone of maximum phosphatase activity occurs at a region 2 to several cell layers removed from the basal layer. This zone of maximum activity does not extend entirely to the luminal border, the intensity of deposition as a rule diminishes gradually to about nothing as this border is approached, or even before the peripheral cell layer is reached. In this region of intense activity the deposition around the cell periphery is quite pronounced, particularly since the central area of these squamous cells has taken on a void-like appearance with the dissolution of contained glycogen. The epithelial cells of the endo-

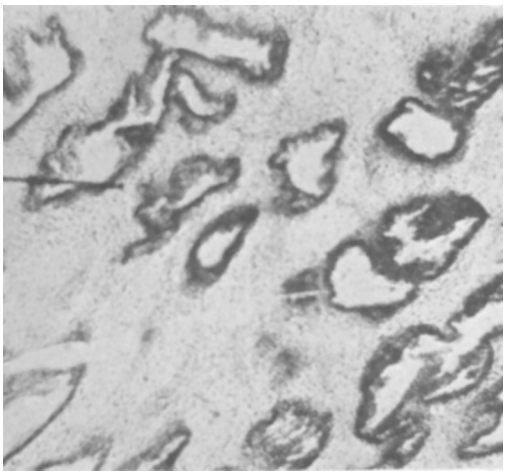

FIG. 4. Section of endometrium of human (× 75) in luteal phase of menstrual cycle (17 day), showing very active glandular epithelium and much less active stroma. Incubation in glycerophosphate at pH 5, 1 hr.

cervical glands show as a rule much greater activity in the free border area, although frequently the entire cell contains a large deposit. There is slight activity in the glandular lumen itself and in the periglandular tissue. Whereas the foregoing statements apply more specifically to what obtains when glycerophosphate is the substrate, the picture with other substrates employed differs noticeably. Thus with glucose-6-phosphate an approximately reciprocal relation is encountered. With this substrate the cervical epithelium is much more inactive and the stroma in some cases considerably more active. The pattern of activity with glucose-l-phosphate as a substrate is, however, similar to that of glycerophosphate but lower. Activity with fructose-6-phosphate resembles that with glucose-6-phosphate but appears to be lower in the endocervical glands. Activity with adenosine-triphosphate was very low, in contrast to results of our studies in the alkaline range. Overnight incubation was necessary in order to obtain even slight reactions. Under these conditions some activity was noted in the cervical epithelium but not in the stroma.

Endometrium. We have had an opportunity to study acid phosphatase in the endometrium of women at different times in the menstrual cycle. Activity was greater in the glands than in the stroma; and both were higher than in other tissues (Fig. 4). Activity appears in both the nucleus and cytoplasm. In the glandular epithelium there is a slight to pronounced intensification of cytoplasmic free border activity. In contrast to our own and other studies with alkaline glycerophosphatase, there is an absence of activity in the vascular system. With glucose-6-phosphate, the one endometrium examined was again quite active; however, as in the case of the cervix, with shorter incubation time of 1-2

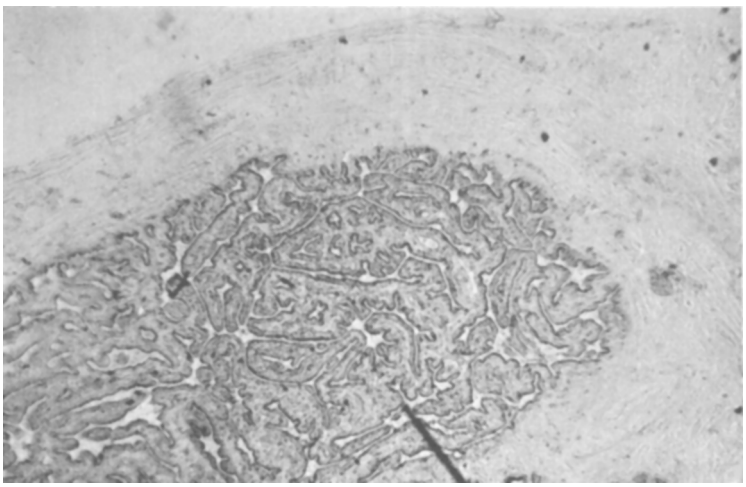

FIG. 5. Section of Fallopian tube of human (× 25) showing moderately active mucosa with activity concentrated primarily at or near free border of epithelial cells and rather inactive muscularis. Incubated as in Fig. 4.

Fig. 2-5 are from sections of fresh frozen tissues.

hours, the glandular epithelium shows a lower activity than the stroma.

Fallopian tube. The epithelial layer surrounding the lumen shows by far the greatest activity. It appears to be nuclear as well as cytoplasmic, but in the latter primarily in the free border of the cell (Fig. 5).

Discussion and summary. Biochemical and histochemical methods have demonstrated acid phosphatase activity in the vagina, exo- and endo-cervix, the endometrium, and mucosa of the fallopian tube. The enzyme is similar to that in the human prostate but the activity per mg wet weight is lower.

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Effect of Aureomycin in Rats with Chronic Alloxan Diabetes.* (20261)

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Clinical observations on several patients with severe diabetes showed that during treatment with aureomycin a marked improvement of the diabetic condition took place. Since no obvious infection was established by clinical and laboratory means in some of these patients, the impression was gained that aureomycin may have a direct favourable effect on the diabetes. These observations prompted us to study the effect of aureomycin on experimental diabetes in the rat.

Material and methods. Adult male rats weighing 130-160 g were used. Diabetes was induced by a single subcutaneous injection of 230 mg alloxan per kg body weight. Following this, half a unit of PZI was given daily subcutaneously for 7 days. Forty-four rats which were diabetic for 6 weeks were put in special metabolic cages for the following 2 weeks; the urine was collected every 48 hours, measured and examined for sugar, the daily *ad libitum* food intake was measured. The ration consisted of a mixture of ground wholewheat, ground alfalfa, bran, skimmed milk powder, salts and contained 21% proteins. Once a week the rats were weighed. Following the period of 2 weeks the diabetic rats were divided into 2 subgroups, one consisting of 25 rats (subgroup I) was given aureomycin in a dosage of 20 mg per 100 g food for 28 days, and 19 diabetic rats (subgroup II) were not given aureomycin. Nineteen normal non-diabetic male rats of the same stock and age served as control for the diabetic rats, and were given the same ration. Of these, 11 rats (subgroup III) were given the same food plus aureomycin in the same dosage as the diabetic rats, while 9 rats (subgroup IV) were not given aureomycin. In another series of experiments 8 rats which had alloxan diabetes for 6 months were given 40 mg aureomycin per 100 g food for 10 days, and 60 mg per 100 mg food for 6 days. Urinary sugar was determined daily and the bodyweight was measured once a week. *Sugar* in the urine was examined by Benedict's quantitative method.

Results. As shown in Table I the average gain in weight in the 19 diabetic rats of the first series of experiments (subgroup II) which did not receive aureomycin was 9.6 g during 28 days. During this period the average amount of sugar excreted in the urine was 3.75 g per day per animal. The average food intake in these animals amounted to 22 g per day. The average gain in weight in the 25