a single injection. Immediately after an injection there is also an increase in colloidal osmotic pressure with a return of it to normal later. The fall in pressure is paralleled by an increase in serum proteins and a decrease in serum acacia.

The acacia is progressively deposited in the liver as shown by periodic biopsies, gradual increase in liver size and tissue analyses. In one animal the liver was 5 times normal size. Of the 756 g injected, 444.9 g (58.8%) were recovered from the liver, 105.4 g (13.9%) from the muscle, 41.1 g (5.4%) from the serum, and 11.4 g from other sources. This left a balance of 153.2 g for excretion in the urine.

The blood is cleared fairly rapidly of acacia, largely through storage. The mobilization from storage is small but definite, as shown by its presence (1%) in the serum one year after last injection. The recession of serum proteins with the addition of acacia to the blood and their return with the storage of acacia indicate a reciprocal functional relationship between the two. The rise of the colloidal osmotic pressure with the injection of acacia and its fall with storage of acacia may now receive a valid explanation. Obviously the adjustments are directed towards the maintenance of a constant osmotic pressure.

Hence, under the experimental conditions cited, the diminution of the serum proteins is not to be regarded as an index of failure of the proteogenic function of the liver, but as due to a displacement of the proteins by the acacia.

10242

Effect of Testosterone Propionate on Glycogen Content of Human Vaginal Smears.

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As part of an investigation into the subject of the hormonal control of the glycogen present in the vaginal mucosa, it was felt desirable to determine whether glycogen could be demonstrated in human vaginal smears. This has been accomplished by the following method:

Technic of Glycogen Stain. Patients are instructed to take a plain water douche the evening before the smears are taken. A

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speculum is inserted into the vagina and the cervix and fornices are wiped clean. The speculum is then withdrawn, at the same time sweeping off the secretion from the anterior wall with the posterior blade of the speculum. The secretion on the blade is then diluted with an equal part of normal saline and spread on a glass slide. Fixation is obtained by drying in air. A modification of the Best carmine† stain (which is used in the Department of Pathology of the Mt. Sinai Hospital to demonstrate glycogen in tissues) was adapted for the smears.‡

1. Stain with hematoxylin—5 min.
2. Rinse in cold water.
3. Stain with carmine solution—15 min.

Stock carmine is made as follows:

Carmine	1
Pot. chloride	2.5
Pot. carbonate	.5
Distilled water	50

Mix and boil 1 minute until solution turns a deep red. Cool at room temperature. Add 10 cc of ammonium hydroxide. Keep over night in the ice box before using.

Carmine solution for use—prepared fresh.

Stock solution, filtered	10 cc
Ammon. hydroxide	15
Absolute methyl alcohol	10

4. Differentiate in

Absolute ethyl alcohol	16
" methyl "	8
Distilled water	20

Immerse 4-5 times

5. Absolute alcohol—2 immersions
6. Xylol—2 immersions
7. Mount with balsam

The glycogen appears as deep red granules in the cytoplasm. In some cells the granules are coarse, while in others they are so fine that the cytoplasm is diffusely pink. The nuclei stain blue. This stain also reveals distinctly the morphologic characteristics of the cellular elements in the smear.

With the aid of this method, studies are being carried on to determine the glycogen content of the vaginal smears in hypo-ovarian states, in menopause, in castrates and in senile states. At present we wish to report on the effect of testosterone propionate on the glycogen content of the smears of normally cyclical women.

† *Pathological Technique,* Mallory and Wright, 8th Edition, 1924.

‡ We wish gratefully to acknowledge the helpful advice of Drs. Paul Klemperer and Sadao Ottani in the adaptation of the glycogen stain to the vaginal smear.

Smears taken throughout the menstrual cycle (except during the period of actual menstruation) in women with regular cycles show slight variations in glycogen content which are difficult to interpret. In some women the smears are uniformly lower in glycogen content than in others. This phase of the problem will be reported on later. For the investigation reported here 2 young women, in whom abundant glycogen had been demonstrated during a preceding cycle, were given testosterone propionate to determine its effect on the glycogen content of the epithelial cells. The following is a protocol of Case I:

Patient N.F. Age 25. Regular 28-day cycle. Preliminary smears showed abundant glycogen. Testosterone propionate injections were started on the 1st day of the cycle and continued to the 18th day. Single doses varying from 25-100 mg were given intramuscularly every other day until a total of 700 mg was given. No changes were noted in the smear until after the 9th day when 500 mg had been given. On this day, the smear contained a scattering of small, oval epithelial cells (atrophy cells—"deep cells" of Papanicolaou and Shorr[4, 8]) abundantly supplied, however, with glycogen. From this day the vaginal smears showed gradual disappearance of the squamous epithelial cells containing the glycogen and their replacement by the atrophy cells. After a few days, the atrophy cells grew progressively smaller in size, fewer in number and poorer in glycogen content. A further significant feature was the appearance of leucocytes in increasing numbers. From the 18th to the 25th day of the cycle, the vaginal smear consisted of a predominance of leucocytes with a scattering of very small atrophy cells. Squamous epithelial cells and glycogen were absent in the smears during this period. (The menstrual period which was expected at the 28th day did not appear.) Signs of beginning restoration to normal began to appear after the 25th day of the cycle. Small, round epithelial cells showing traces of glycogen began to appear among the leucocytes. During the next 10 days, rapid progressive changes were manifested both in the morphologic characteristics as well as in the glycogen content from day to day. There was a rapid increase in the number of round and oval epithelial cells containing glycogen and a parallel decrease in the number of leucocytes. Within a few days these atrophy cells were replaced by epithelial cells which appeared to grow larger from day to day and contained increasing amounts of glycogen. On the 35th day, the smear consisted of large, clearly-defined, squamous epithelial cells abundantly supplied with glycogen. No atrophy cells and no leucocytes were present. This status per-

[8] Papanicolaou, G. N., Ripley, H. S., and Shorr, E., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 689.

sisted until the 54th day of the cycle when the patient began what appeared to be a normal period lasting 4 days. Essentially similar changes were noted in the second case.

Summary. A method of demonstrating simultaneously the presence of glycogen and the morphologic characteristics of the desquamated cellular elements of the vaginal mucosa is presented. The glycogen in the desquamated vaginal epithelial cells of normally menstruating women can be made to disappear by administering adequate amounts of testosterone propionate. The disappearance of the glycogen is apparently dependent upon the production of atrophic changes in the vaginal mucosa since the first effect of the testosterone propionate is the disappearance of the squamous epithelial cells and their replacement by cells from the deeper layers of the mucous membrane. Coincident with this change in the size of the cells, the glycogen begins to decrease steadily and finally vanishes completely. Restoration of the glycogen in the smears parallels closely the reappearance of the normal vaginal epithelium.

The question arises as to the mechanism of this regression in the smear and the disappearance of the glycogen. There is experimental evidence indicating that androgens negate the biologic effect of estrogens.[1-3] It is, therefore, conceivable that the testosterone propionate in the cases reported here inactivated the estrogen formed in the individual and, as a result, atrophy of the vaginal mucosa occurred with loss of cornification and consequent disappearance of glycogen. It seems likely, however, that the regressive changes induced in the smear are also the end results of inhibition of the gonadotropic hormone formation of the hypophysis resulting in suppression of the follicular ovarian cycle. That the testosterone propionate probably inhibits the hypophysis is suggested by the following observations: (a) the excessive gonadotropic hormone excretion in a human female castrate can be suppressed with testosterone propionate;[5] (b) ovulation can be similarly inhibited in monkeys;[6] (c) menstruation can be inhibited and the estrogen and progesterone effects in the endometrium of cyclical human females can be suppressed by administering adequate amounts of testosterone propionate.[7]

1 Ihrke, I. A., and D'Amour, F. E., *Am. J. Physiol.*, 1931, **96**, 289.

2 Robson, J. M., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 49.

3 Browman, L. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 205.

4 Papanicolaou, G. N., and Shorr, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 585.

5 Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 488.

6 Zuckerman, S., *Lancet*, 1937, **2**, 676.

7 Gaines, J. A., Salmon, U. J., and Geist, S. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 779.