tained at significantly lower levels than control leukemics and in some cases disappear entirely from the peripheral blood. (3) Hemoglobin values remain at higher levels in the urethane-treated animals throughout treatment. (4) Local subcutaneous growth of the tumor mass is retarded. Some infiltration of lymphoblasts into spleen, thymus and lymph nodes occurs, however this is not of the characteristic massive and diffuse type. Lymphoblast destruction is evident. (5) A statistically significant greater life expectancy in urethane-treated leukemics is obtained.

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Comparison of Rates of Penetration of Unwashed and Washed Spermatozoa in Cervical Mucus.*

W. T. POMMERENKE AND ELLENMAE VIERGIVER. (Introduced by Henry A. Blair.)

From the Department of Obstetrics and Gynecology, The University of Rochester School of Medicine and Dentistry.

The evidence at hand indicates that the secretion of cervical mucus is under hormonal control.[1-4] It is most abundant when ovulation is believed to occur, *i.e.* at midinterval in the usual 28-day cycle.[5-7] At this time the mucus possesses its lowest viscosity and cellularity,[8] highest water content,[9] and is well supplied with glycogen and other potential reducing substances.[9] *In vitro* experiments show that it is during midcycle that the cervical mucus is most readily penetrable by spermatozoa,[6,8] probably due to the physical and chemical properties that prevail at this time. MacLeod has made the observation that human spermatozoa require glucose or a like monosaccharide for their metabolism and prolonged activity.[10] The fresh semen itself contains some 300 mg per 100 cc of reducing substance expressed as glucose.[11,12] Since spermatozoa probably carry with them little of this extracellular nutriment, they may find in their new environment the necessary substrate for their subsequent metabolism. Enzymes may play an important rôle in the utilization by the spermatozoa of the substances in cervical mucus, but this conjecture constitutes a separate study.

This report is concerned with an endeavor to ascertain the effect of washing of the spermatozoa on their ability to penetrate a column of cervical mucus *in vitro*.

Material and Methods. Subjects: Healthy young women with normal menstrual cycles and pelvic findings supplied the mucus which was collected from the cervical canal by aspiration.[8] Semen specimens were obtained from healthy young donors by manual stimulation. In the evaluation of these specimens consideration was given to the count and

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1 Guttmacher, A. F., and Shettles, L. B., *Human Fertil.*, 1940, **5**, 4.

2 Bennett, H. G., Jr., *Am. J. Obst. and Gynec.*, 1942, **44**, 296.

3 Abarbanel, A. R., *Trans. Am. Soc. for Study of Sterility*, 1946.

4 Pommerenke, W. T., and Viergiver, E., *J. Clin. Endocrin.*, 1946, **6**, 99.

5 Séguy, J., and Simonnet, H., *Gynéc. et obst.*, 1933, **28**, 657.

6 Lamar, J. K., Shettles, L. B., and Delfs, E., *Am. J. Phys.*, 1940, **129**, 234.

7 Viergiver, E., and Pommerenke, W. T., *Am. J. Obst. and Gynec.*, 1944, **48**, 321.

8 Viergiver, E., and Pommerenke, W. T., *Am. J. Obst. and Gynec.*, 1946, **51**, 192.

9 Viergiver, E., and Pommerenke, W. T., *Am. J. Obst. and Gynec.*, in press.

10 MacLeod, J., *Endocrinology*, 1941, **29**, 583

11 Hotchkiss, R. S., Brunner, E. K., and Grenly, P., *Am. J. M. Sci.*, 1938, **196**, 362.

12 Huggins, C. B., and Johnson, A. A., *Am. J. Physiol.*, 1933, **103**, 574.

TABLE I.
Rate of Penetration of Unwashed and Washed Spermatozoa in Cervical Mucus.

	No. of specimens	Range of rate of penetration, mm/min	Avg rate of penetration, mm/min
Preovulatory phase			
Unwashed	8	0.0-0.7	0.3
Washed	5	0.0-0.4	0.2
Ovulatory phase			
Ascending			
Unwashed	13	0.6-2.3	1.3
Washed	10	0.5-1.9	1.0
Peak			
Unwashed	6	1.3-2.0	1.7
Washed	10	1.0-2.7	2.0
Descending			
Unwashed	5	0.2-1.8	0.8
Washed	3	0.7-1.4	1.0
Postovulatory phase			
Unwashed	7	0.0-1.5	0.5
Washed	6	0.0-0.6	0.3

morphology.

Preparation of Suspensions of Washed Spermatozoa. One volume of semen and 5 volumes of Ringer's solution were mixed and then centrifuged at approximately 1500 r.p.m. for 10 minutes. The packed spermatozoa were then resuspended in fresh Ringer's solution, the final mixture being brought up to the original volume of semen used. Observations on the counts and morphology were also made on these suspensions. The washing and centrifugation of the spermatozoa resulted in some diminution in the number of motile forms and also in the destruction of some of the cells.

Penetrability. The rate of pentrability was determined by the method of Lamar, Shettles and Delfs.[6] A column of mucus followed by a column of fresh semen or of washed spermatozoa suspended in Ringer's solution was drawn into a capillary tube, leaving a small bubble between the 2 media to serve as a marker. These tubes were then placed on glass slides and covered with mineral oil to reduce refraction for observation under the microscope. By means of a stop watch and a calibrated mechanical stage the rate at which the spermatozoa invaded the cervical mucus was determined.

Results. The mucous cycles were arbitrarily divided into 3 phases: preovulatory, ovulatory, and postovulatory, the ovulatory phase consisting of those days in midcycle during which the amount of mucus was greatly increased and the cellularity decreased. In the normal cycle the amount of mucus increases for 2 to 3 days in the beginning of the ovulatory phase, reaches a peak which is maintained from 1 to 2 days, and then decreases for 2 to 3 days before reaching the postovulatory level.[7,8] Since it has been shown that the rate of penetrability of spermatozoa also follows this general pattern,[6,8] the ovulatory phase was further subdivided into the ascending side, the peak, and the descending side of the curve.

Reference to Table I shows that the rates of penetration of the unwashed spermatoza,[6,8] are in accord with previous observations.[6,8] It can likewise be seen that washing and centrifuging of the spermatozoa did not appear to modify appreciably their ability to invade the mucus. In some tests the spermatozoa, both unwashed and washed, were entirely stopped in their attempt to penetrate the cervical mucus obtained during the pre- and postovulatory phases. However, the average rate of penetration varied from 0.2 to 0.5 mm/min. The highest rate of penetration, *i.e.* from 1.7 to 2.0 mm/min., occurred in those specimens of mucus collected during the ovulatory phase, with some diminution in rate as the collections of mucus departed on either side from the peak.

In these studies measurements of the rate

of penetration were begun promptly after the spermatozoa were brought into contact with the column of mucus. In many cases the spermatozoa on finding the mucus medium particularly favorable, *i.e.* during the ovulatory phase, could be observed to pass through the entire length of the mucus column, an average distance of 25 mm, without apparent deceleration. However, in the pre- and post-ovulatory phases of the cycle when the mucus was more viscid and cellular, the entire distance to which they could penetrate averaged only 1.4 mm. There was no apparent difference between the distances penetrated by the unwashed and the washed spermatozoa.

In all, 42 semen specimens coming from 4 donors were used. These specimens ranged in volume from 0.6 to 4.2 cc and in count from 34 million to 262 million per cc. We were unable, in these studies, to correlate the degree of penetrability of the mucus with the concentration of spermatozoa, either unwashed or washed.

Summary. This study indicates that washing human spermatozoa with Ringer's solution and resuspending the washed spermatozoa in this solution does not impair their ability to penetrate a column of cervical mucus *in vitro*.

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Uneven Distribution of Glycogen in the Liver.*

G. GOMORI AND M. G. GOLDNER.

From the Department of Medicine, University of Chicago.

A strikingly uneven distribution of glycogen in the liver of rabbits was found in the course of experiments in which an attempt was made to follow with biopsies the variation in liver glycogen under various conditions. Irregularities in the intracellular ("glycogen flight") and intralobular[1] distribution of glycogen have been known for some time. Recently, Deane, Nesbett and Hastings[2] have found significant differences in the glycogen content of the two lobes. Since it is obvious that such observations tend to limit the value of liver biopsies in judging the metabolic state of the organ, a short report appears to be warranted.

Experimental. Rabbits were fasted for 18 to 24 hr and laparotomized under sodium pentothal anesthesia. On gross inspection no difference in the appearance of the lobes of the liver was noted. Simultaneous wedge shaped specimens weighing 1000 to 1500 mg were removed from different parts but usually from the same lobe of the liver. Where biopsies were taken at intervals Gelfoam proved very helpful in the control of bleeding. Subsequent biopsies were taken from sites far away from that of the previous resection.

The present report is concerned only with variations observed in simultaneous biopsies. The specimens were cut into 3 portions. The middle slice was fixed promptly in chilled 90% alcohol or Bouin's fluid, embedded, and the sections stained with Best's carmine, Bauer-Feulgen's method[3] and a silver stain.[4] The two lateral portions were weighed and dropped immediately in 30% KOH for the chemical determination of glycogen according to Good, Kramer and Somogyi;[5] the glucose

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1 Edlund, Y., and Holmgren, *H.*, *Z. f. mikr-anat. Forsch.*, 1940, **47**, 467.

2 Deane, H. W., Nesbett, F. B., and Hastings, A. Baird, PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 401.

3 Bauer, H., *Z. f. mikr.-anat. Forsch.*, 1933, **33**, 143.

4 Gomori, G., *Am. J. Clin. Path.*, 1946, **10**, 177.