The other 2 with double lumen anastomosis remained in perfect condition and were sacrificed 43 days after the starting of the injections. No evidence of erosions or ulcers were found in the stomach, duodenum or jejunum.

Protocols. Dog No. 1, weight 30 lb. Operation December, 1946. Gastrojejunostomy with a double lumen jejunojejunal anastomosis. April 7, 1947 histamine beeswax, 30 mg daily. April 29, 1947 dog sacrificed. Shallow round jejunal ulcer, 1 cm in diameter with a pin-point penetration.

Dog No. 3, weight 26 lb. Operation, January 16, 1947. Gastroenterostomy with a double lumen jejunojejunal anastomosis. April 7, 1947, daily injections of 30 mg of histamine beeswax. May 20, 1947 dog sacrificed. No ulcers or erosions found.

Dog No. 4, operation, January 22, 1947. Devine exclusion procedure with a double lumen gastrojejunal anastomosis. April 7, 1947, daily injections of 30 mg of histamine beeswax. May 20, 1947, dog sacrificed. No ulcers or erosions found.

Normal dog No. 1, weight 22 lb. May 12, 1947, daily injections of histamine beeswax. May 20, 1947, dog sacrificed. Shallow ulcers about 1 x 1 cm with induration on the lesser curvature of the pylorus.

Normal dog No. 2, weight 18 lb. May 12, 1947, daily injections of histamine beeswax. April 29, 1947, dog sacrificed. Several small ulcers and erosions in the mucosa of the transverse stomach. Two large ulcers of the first part of the duodenum and one shallow ulcer 4 cm distal to the pylorus.

16018

Effect of Urethane on a Transplantable Acute Lymphoid Leukemia.*

L. W. LAW. (Introduced by C. C. Little.)
(With the technical assistance of Lester E. Bunker, Jr.)
From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

It has been shown that urethane produces a pronounced reduction in blood leukocytes and in immature myeloid cells in both the human and the mouse in cases of myeloid leukemia.[1,2] In addition definite palliative effects have been reported for human beings.[1] The response in human lymphoid leukemias was less pronounced and more variable than in myeloid leukemias. Recently,[3] the response to urethane of spontaneous lymphoid leukemias in the mouse has been studied and the following encouraging effects were obtained: (1) a pronounced fall in blood leukocytes to or below normal levels and maintenance of these levels with continued therapy (2) a marked reduction in the number of immature cells in the circulating blood. (3) a temporary stabilizing effect on hemoglobin levels. (4) a pronounced diminution in size of subcutaneous lymph nodes, spleen and thymus and (5) a significantly greater life expectancy for urethane-treated cases.

A study of the effect of urethane on several acute and chronic lymphoid and myeloid transplantable leukemias in the mouse has been made. This paper summarizes the response of a transplantable acute lymphoid leukemia, line L825, which arose spontaneously in ♀ 79374 (6 months of age) of the C58 inbred leukemic strain of mice. The leukemic

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TABLE I.
Periodic Peripheral Blood Counts of Urethane-Treated and Untreated C58 Mice Inoculated with Acute Lymphoid Leukemia, Line L825.*

	Blood counts	7 days				10 days				12 days			
	Hb—g %	Total leucocytes	Lymphocytes	Neutrophils	Lymphoblasts	Hb—g %	Total leucocytes	Lymphocytes	Neutrophils	Lymphoblasts	Hb—g %	Total leucocytes	Lymphocytes	Neutrophils	Lymphoblasts
Urethane†	15.7	5985	45	51	3.6	13.1	6550	30	66	1.6‡	9.1	7970	37	57	4.0‡
Controls	13.6	28950	48	41	10	9.2	76280	23	37	35	5.3	125750	18	20	58

* Means based upon 9 experimental and 9 control animals except at 12-day period where only 2 control animals were alive for blood studies. Same mice used throughout. Blood obtained from tail vein.
† Dosage: 0.75 mg per g body weight per day.
‡ No lymphoblasts found in blood smears of 4 experimental animals.

animals used were of the 32nd, 33rd and 34th transfer generations of this leukemia and were from 5 to 6 weeks of age.

Leukemia line L825 forms a local tumor following subcutaneous transfer and produces massive and diffuse infiltration into the lymph nodes, spleen, liver and thymus in the terminal stages leading to death of mice in 9 to 10 days. The leukocyte count is elevated after 4 to 5 days and lymphoblasts appear at this time. In terminal stages leukocyte counts of 200,000 (90% lymphoblasts) have been encountered.

Urethane was administered daily in the following dosages to 4 separate groups of mice beginning injections 24 to 48 hours after transfer of leukemic cells: 0.75 mg per g body weight, 0.45 mg, 0.25 mg, and 0.15 mg per g body weight. A 6% aqueous solution was given intraperitoneally. Significant palliative effects noted in the experimental series treated with 0.75 mg per g body weight are reported herein.

The blood leukocyte count of urethane-treated leukemics was maintained at levels below normal throughout the period of inoculations of 0.75 mg urethane per g of body weight, whereas the untreated leukemic animals showed a progressive leukocytosis to a mean leukocyte count of 125,750 at 12 days. The absolute lymphoblast count of urethane treated leukemias was 7%, 0.3% and 0.4% of the absolute lymphoblast count of untreated leukemics at 7, 10 and 12 days respectively following transfer of leukemic cells. At 12 days following treatment the mean absolute number of malignant lymphoblasts in urethane treated mice was 318.8 compared with 72935 in untreated leukemics (Table I).

It has been observed in chronic myeloid leukemias in humans that hemoglobin values rise or remain stationary in the majority of cases. No deleterious effects on the red cell series was observed.[1] In our urethane-treated leukemics the hemoglobin levels did not rise nor were they maintained for any length of time. However there were significantly higher values at each blood count period as compared with untreated controls. When the hemoglobin values fell to 5 g %, death ensued shortly

TABLE II.
Effect of Urethane on Infiltration of Leukemic Cells of Lymphoid Leukemia, Line L825, in Various Tissues of C58 Strain Mice.

Exp.	No. animals	Tissue weights in mg/25 g of final body weight* Subcutaneous mass	Spleen	Liver	Subcutaneous lymph nodes‡
Controls	16	1234 ± 93.3†	540.6 ± 41.7	2291.8 ± 158	217.5 ± 13.6
Urethane§	16	230.9 ± 47.6	72.5 ± 6.9	828.8 ± 39.3	62.1 ± 7.3
Difference of means		1003.1 ± 104.7	464.1 ± 42.3	1463 ± 162.7	155.4 ± 15.4
P values		<0.01	<0.01	<0.01	<0.01

* Urethane causes a slight decrease in body weight after many inoculations of sub-anesthetic doses. The means of tissue weights of control and urethane series are weighted equally by using body weights at death of animals.

† Means and standard errors.

‡ Including paired inguinal, axillary, and cervical lymph nodes.

§ Dosage: 0.75 mg per g body weight per day.

thereafter.

Following urethane treatment there resulted a conspicuous decrease in circulating lymphocytes and a corresponding increase in polymorphonuclear leukocytes. This change was evident after 3 daily injections. In view of the fact that this phenomenon occurs in lymphoid as well as in myeloid leukemia the explanation offered for the depression of white blood-cell counts of myeloid leukemias following urethane therapy does not seem tenable.[4]

All urethane-treated mice responded as above described. In 4 mice at the 10 and at the 12 day periods no malignant lymphoblasts could be found in blood smears.

A significantly greater life expectancy was obtained in the urethane-treated (0.75 mg per g daily) series. These experimental animals lived 17.53 ± 0.63 days (25 animals) as compared with 10.37 ± 0.26 days (45 animals) for the controls. The difference of the means of the groups is 7.16 ± 0.69 days where $P<0.01$. On the other hand leukemic mice given smaller doses of urethane did not live longer than untreated leukemics.

A definite retardation of the growth of the subcutaneous mass was evident at 5 days after transplantation of leukemic cells in the urethane series. All urethane-treated mice were negative at this time whereas control leukemic mice showed definite nodules. At 7 days the mean tumor size of urethane-treated animals was 0.98 cm (sum of 2 axes) and of control animals 3.2 cm. There was a profound difference in tumor weights obtained at death of the animals. (Table II). Infiltration into the liver, spleen and subcutaneous lymph nodes as determined by organ weights and by histological study was slight in the urethane-treated series. Urethane-treated mice showed a mean loss in weight of nearly 2 g after 14-15 days of daily injections. It is evident that the loss of weight *per se* in the experimental animals producing some splenic and lymphoid atrophy did not result in the significantly lower organ weights for the following reasons: (1) At 10-12 days following initiation of urethane-therapy, at which time there was no loss in body weight, massive and diffuse infiltration into spleen and subcutaneous lymph nodes was not conspicuous. (2) Organ weights in urethane-treated leukemics were not significantly different from similar organ weights of normal C58 mice of the same age and body weight. (3) It is evident from histological studies that cell destruction of infiltrating lymphoblasts is occurring. Complete histological studies will be given later.

Summary. Urethane, administered intraperitoneally in subanesthetic dose, 0.75 mg per g of body weight per day in leukemic mice transplanted with leukemic cells of the 32nd to 34th transfer generations of an acute lymphoid leukemia, produces the following profound palliative effects: (1) The blood leukocytes are maintained at leukopenic levels. (2) The immature lymphoblasts are main-

[4] Kirchbaum, A., and Lu, C. S., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 62.

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.

16019

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

* Aided by a grant from the Ortho Research Foundation, Raritan, N.J.

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