Summary. Using a fat test meal containing a radioactive labeled fat, a study has been carried out in dogs to assess the rate and total amount of radioactive labeled fat entering the blood in normal dogs and in dogs with exclusion of bile and pancreatic juice during a 6-hour period after administration of the meal. These studies indicate that bile plays a minor role and pancreatic juice a major role in fat digestion and absorption. This study suggests the use of a similar test for clinical use in evaluating pancreatic function in suspected pancreatic disease.

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Received November 18, 1955. P.S.E.B.M., 1955, v90.

Experimental Hydro-uteri (Hydrometra) in Rodents and Some Factors Determining Their Formation.* (22148)

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In mice of the English Silver Strain, and occasionally in other strains there occurs a condition of imperforate vagina which leads to a marked distention of uteri with accumulation of fluid up to 15 ml or more per mouse. Yet normal function of the uterus is preserved, since such animals become pregnant if the membrane occluding the vagina is removed(1). Existence of huge hydro-uteri in the presence of imperforate vaginas suggested that simple ligation of the cervix in rodents might likewise induce formation of hydro-uteri. This new technic not only provides a large uterine receptacle for tumor transplants but also permits the collection of large amounts of uterine secretions in small animals.

This paper reports studies on production of hydro-uteri in mice, rats and hamsters and on hormonal and other factors which play a role in development of experimental hydro-uterus.

Methods. The cervix of the uterus was ligated under nembutal anesthesia through a supra-pubic incision in 456 mice, in a smaller number of rats (Wistar) and Syrian hamsters. The variables for this study of effects on development of hydro-uteri were strain of mouse, endocrine status and time elapsing between uterine cervix ligation and autopsy. The strains of mice and types of animals used are shown in Tables I and II. Eighty-four mature white Swiss mice, 40 DBA/1 and 40 C57BL/10J mice were castrated and their cervices ligated (Table I). Forty-seven immature intact white Swiss mice underwent

* Aided by grants from Damon Runyon Memorial Fund for Cancer Research, New York, American Cancer Society, Inc. Mass. Division, and institutional grant of American Cancer Society, New York City. M. S. Grossman is a medical student, Washington U., St. Louis, Mo.

TABLE I. Hydro-uterus Formation in Immature and in Castrated Animals.

	No. of animals	Strain	Age, mo	Duration of ligation (D)	% hydro-uteri		
Intact immature animals	47	Wh. Sw. Tufts	½–1	13–28	21		
Castrated mature animals	44	Wh. Sw. Tufts	3–8	15–60	40		
					Castrate control	Testosterone (.6 mg)	Estradiol (12 γ)
Castrated mature animals	40 10*	Wh. Sw. RJB stock	2½	21	0	0	30
	40 10*	DBA/1 RJB stock	2½	21	0	0	20
	40 10*	C57BL/10J	2½	21	0	0	20

* In each category.

TABLE II. Hydro-uterus Formation in Intact Mature Animals.

	No. of animals	Strain	Age (mo)	Duration of ligation (D)	% hydro-uteri
Mice	163	Wh. Sw. Tufts	3–12	21–180	33–(66)
	51	BBF1	3– 7	21–190	59–(90)
	12	C57BR/a	6	24	33
	35	Wh. Sw. RBJ stock	2½	22	20
	42	C57BL/H-2d	2½	42	29
	26	DBA/1 RBJ stock	3	30	35
Rats	10	Wistar rats	4	22	30
Hamsters	1	Golden Nugget	2	35	Positive

cervical ligation. One to 2 weeks later, castrates received injections subcutaneously as follows: *controls,* 0.5 ml sesame oil; *estrogen treated animals,* 1 γ estradiol daily; *testosterone treated animals,* 0.1 mg testosterone propionate every other day for 12 days. Twelve days later these animals were sacrificed. The effect of length of time after ligation of the cervix was studied in mature intact animals by performing exploratory laparotomies at varying intervals (up to 12 months) after cervical ligation. At the end of the experiments the animals were sacrificed and histological studies made of the uteri. The fluid which accumulated in the uteri was harvested and analyzed† for the following constituents by the indicated methods: for proteins(2), carbohydrates(3,4), hexosamine(5) and hexuronic acid content(6). Bacteriological and cytological studies were also carried out. For purposes of comparison fluid from the hydro-uterus of a white Swiss mouse with imperforate vagina, ascitic fluid from Ehrlich's ascites tumor growing in white Swiss mice and from a foreign body ascites provoked by intraperitoneal injection of Walker (rat) Sarcoma cells into the abdomen of mice was similarly analyzed.

Results. The data obtained are summarized in Tables I, II and III.

Hydro-uterus formation in intact mature animals. In Table II approximately one-third of all animals which had undergone ligation of their uterine cervix developed distended uterine horns containing at least 0.5 ml of fluid 21 days after ligation. It is not clear why the remaining two-thirds of these

† We are indebted to Drs. W. H. Fishman and J. Nisselbaum for these analyses.

TABLE III. Chemical Composition of Various Hydro-Uterine and Ascitic Fluids.

Fluid pools studied	Protein content(2) (mg/ml) Ppt'd by $HClO_4$	Protein content(2) (mg/ml) Ppt'd by phospho-tungstate	Carbohydrates in the phosphotungstate precipitate: Carbohydrate content (3,4) (mg/ml) expressed as half mannose, half galactose	Hexosamine(5) content (mg/ml)	Hexuronic acid(6) content (mg/ml)
HF* from WSM No. 4 7 mo after uterine ligation	trace	12.2	3.8	2.5	.15–.25
HF from WSM No. 4 11 mo after uterine ligation	trace	5.2	1.6	.77	.4 –.5
HF from mouse with imperforate vagina	30.0 (approx. values; total ls 63.5 mg/ml)	33.5	2.8	3.6	
AF from Ehrlich Ascites tumor	52.2	2.9	.24	1.2	0
AF from ♂ WSM with Walker tumor abdom.	34.2	1.5	.13	.6	.17

* HF = Hydrouterine fluid; WSM = White Swiss mouse; AF = Ascitic fluid.

animals fail to accumulate fluid in distended horns and show reasonably normal uterine horns in spite of cervical ligation. The possibility that this is merely a result of chance variations in the surgical technic can not be ruled out. Simultaneous ligation of the 2 oviducts in 136 mice and 7 rats did not increase significantly the frequency of hydro-uteri. The most important single factor favoring development of hydro-uteri is time, since in 2 groups of 12 animals, studied for 6 months, from 66 to 90% showed large hydro-uteri (Fig. 1). Fluid continued to accumulate rapidly in spite of periodic emptying of the hydro-uterus by aspiration of the fluid by needle puncture.

The tendency to formation of hydro-uterus following cervical ligation is not clearly determined by genetic factors since non-inbred white Swiss mice (Tufts), inbred white Swiss mice (Jackson, C57Br/a, C57BL/H-2d and DBA/1 mice all behaved essentially in the same way. It appears, however, that the BBFl mice tend to have a greater incidence of hydro-uteri under otherwise similar experimental conditions. Ligation of the cervices of rats produced the same results as in mice. A hydro-uterus was also obtained in a hamster.

Chemical analysis of various hydro-uterine fluids yielded results shown in Table III. It appears that the protein content (precipitated by $HClO_4$) of hydrouterine fluid in experimental hydro-uteri is considerably lower than that in ascites fluids and that the mucopolysaccharide content as reflected by the phosphotungstate precipitable proteins and carbohydrates of the phosphotungstate precipitate is higher. In fluid collecting in the uterus with imperforate vagina the protein content is higher than in ascites fluid. Bacteriological studies showed that all fluids studied were sterile.

Studies on the effects of hormonal factors are shown in Table II. Within the same period of time immature white Swiss mice showed the same incidence of hydro-uteri as mature intact animals. Castrated white Swiss mice observed for the same length of time (21 days) had no hydro-uteri, but after 60 days formed as many or more hydro-uteri as did intact controls (Tables I and II).

In other strains of mice, namely in inbred white Swiss mice, DBA/1 and C57BL/10J mice, castration prevented formation of hydro-uteri but they formed again when estradiol was given. However, it was found that if the castrated, untreated animals were again examined 2 or 3 months after ligation, many had formed hydro-uteri. Castration merely slows development of hydro-uteri but does not prevent it. Testosterone did not prevent or alter the retarding effect of castration, but

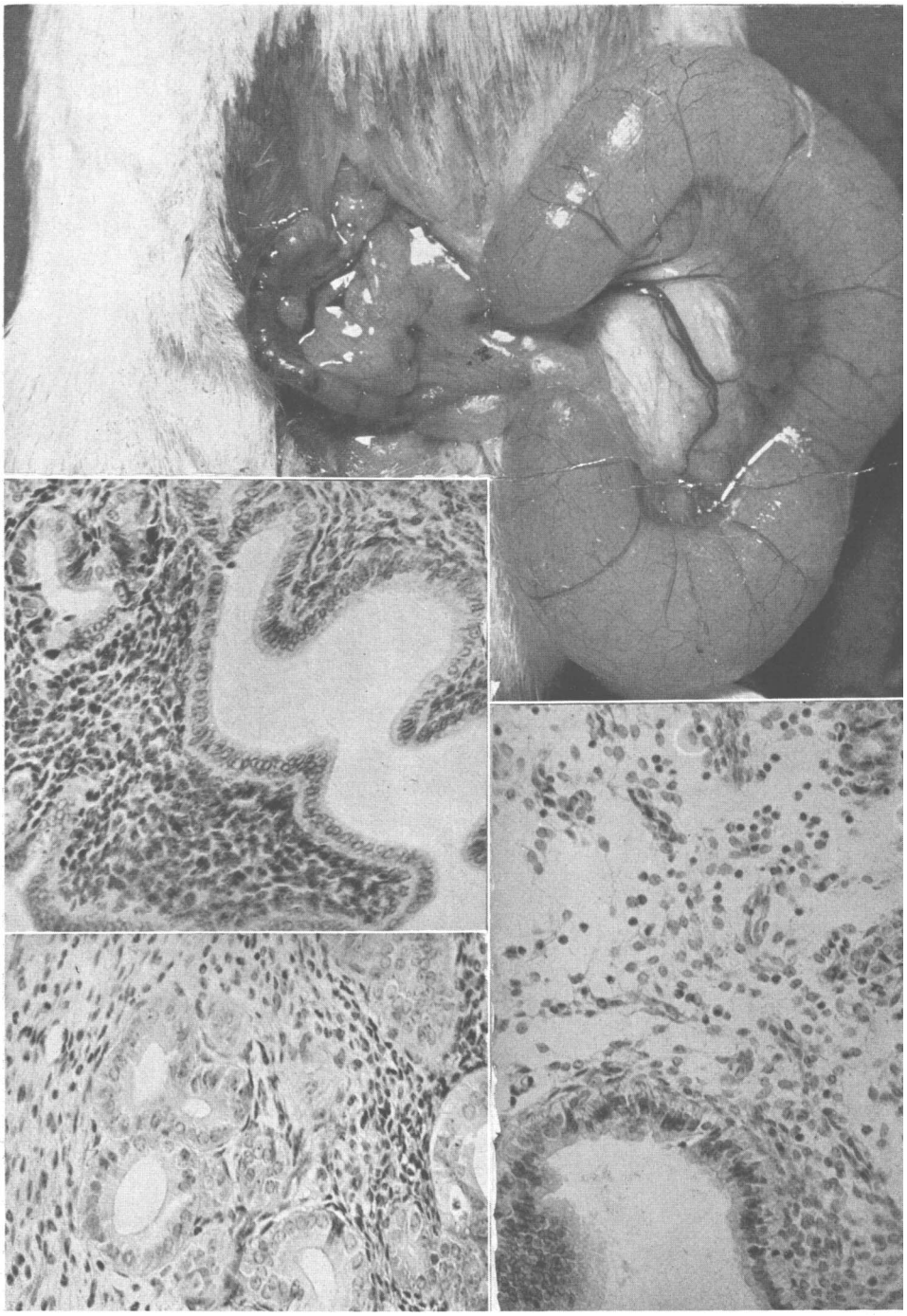

FIG. 1. Top: Appearance of unilateral hydrouterus in a white Swiss mouse, 9 mo of age, 8 mo after ligation of left uterine horn, near cervix. Compare size of hydrouterus with that of non-ligated horn. Left center: Microphotograph of hydrouterus in normal control animal, 21 days after ligation. Left bottom: Microphotograph of hydrouterus in estrogen treated castrate, 21 days after ligation (same magnification). Right bottom: Microphotograph of hydrouterus in testosterone treated animal, 21 days after ligation (same magnification).

estradiol given to castrates restored the rate of formation of the hydro-uterus.

Histological examination of uteri after cervical ligation showed that the wall of the uterus, the endometrium and the endometrial glands react normally to stimulation by estrogens (Fig. 1). Testosterone produced atrophy of the glandular elements of the uterus and edema of the uterine wall with rarification of its cellular elements (Fig. 1). In all instances studied histologically there was complete absence of any inflammatory reaction in the uterine wall.

Discussion. Ligation of the cervix of mice, rats and hamsters leads to accumulation of a fluid in the uterus which can be harvested by uterine puncture and which keeps accumulating after each consecutive tap. As much as 100 ml of hydro-uterine fluid has been obtained from a single mouse during a year's time . The hydro-uteri provide an ideal site for tumor growth(7).

Endocrine imbalance produced by castration retards formation of hydro-uteri but does not prevent it, and administration of estrogen restores the rate of accumulation of intrauterine fluid to normal. In immature mice the incidence of formation of hydro-uteri is reduced to about one-half of that seen in normal mature animals of the same strain.

Characteristics of hydrouterine fluid are different from those found in ascitic fluid, since the protein content is low and carbohydrate is high. This feature of hydrouterine fluid is compatible with the characteristics one would expect from a collection of endometrial secretions. If the collecting fluid is allowed to accumulate for several months, the relative proportions of its main components do not change but their concentrations are lowered, indicating some dilution taking place with time. It is believed that the experimental production of hydro-uteri in rodents constitutes a new method for the collection of endometrial secretions.

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Received November 10, 1955. P.S.E.B.M., 1955, v90.

Estimation of Bromide on Fingertip Blood in Bromide Intoxication. (22149)

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On several occasions it has been necessary to follow bromide levels of serum of small infants after bromide administration either therapeutically or accidentally. The method usually employed (bromide as gold bromide) requires amounts of serum greater than is conveniently obtainable from capillary blood. This paper describes a procedure practical for fingertip blood. The gold bromide procedure is used except that readings are taken in ultraviolet where a higher absorption peak exists for gold bromide.

Method. Reagents and materials. The contents of a one g vial of gold chloride ($AuCl_3 \cdot HCl \cdot 3H_2O$) 0.25%, is washed into distilled water and made up to 400 ml. Trichloracetic Acid: 20 g is made up to 100 ml with distilled water. Carbon Tetrachloride: Analytical reagent grade. Bromide Standard (100 mg/100 ml): 641 mg of sodium bromide (dried overnight in vacuum desiccator) is made up to 500 ml with distilled water. Screw Cap Tubes: Kimble #45066, 16 x 125 mm, fitted with screw caps, teflon lined, are rinsed with dilute nitric acid, distilled water, and dried.