made indicated that 90 to 95% of cells in exudates from both species were "large mononuclear" macrophages. Rat exudates, however, contained 4 to 6% mast cells, absent in guinea pig exudates. The significance of mast cells in antibacterial activity noted was not determined in the work reported here.

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Hyaluronate-Inactive Spreading Factor of Murine Endometrial Secretions (Uterone).* (24456)

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The spread of indicators such as india ink or hemoglobin in the dermis of test animals is increased by 2 types of so-called spreading factors(1). The first type is hyaluronate-active and includes hyaluronidase and other substances which act by depolymerization of hyaluronic acid gel; the second type is the hyaluronate-inactive spreading factors which do not exhibit this activity *in vitro* or in dead skin. Several mechanisms have been proposed to account for the spreading effect of hyaluronate-inactive spreading factors. These are: a) liberation of bound hyaluronidase from skin(2); b) action through alteration of another component of the dermal barrier, different from hyaluronic acid(3); or c) production of inflammatory exudate with subsequent edema(4).

This paper deals with observations made during studies on biologic activities of endometrial secretions (uterone) obtained by cervical ligation in mice(5), revealing that crude uterone enhances intradermal spread of india ink. Experiments were designed to quantitate the spreading effect of uterone and to elucidate its mechanism.

Methods. Animals. Mature male and female mice of RFM and BALB/c strains, 2-3 months of age, were kept in animal rooms of Jackson Memorial Laboratory and fed tap water and purina chow *ad libitum. Materials.* Uterone was obtained from mice of the same strain as that of test animals. Before its use in experiments with BALB/c mice, uterone was lyophilized and reconstituted with sterile Ringer's solution to concentrations varying from one-third to 9 times that of the original uterone. On completion of each experiment, sterility of uterone was ascertained by culture on blood agar plates to establish absence of extraneous bacterial spreading factors. India ink was diluted with Ringer's solution 1:3 and 1:7. Hyaluronidase was commercial preparation No. 214004 (Wydase, Wyeth), diluted in Ringer's solution to the desired concentration. Control serum, known to contain no spreading factor(3), was obtained from RFM mice and used fresh. *Procedures.* Spreading of india ink was determined by measuring area of spread of intradermally injected india ink, using a tuberculin syringe and gauge 27 needle to introduce the substance to be tested

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TABLE I. Comparison in RFM Males of Surface Areas of Ink Spots Resulting from Intradermal Spread of India Ink Suspended in Mouse Serum (RFM), Uterone (RFM) and Various Concentrations of Wydase.

			Wydase			
	Serum	RFM uterone	6 units	12 units	50 units	100 units
Mean spread, cm^2	1.14 (1.02–1.40)	1.87 (1.49–2.12)	1.55 (.92–2.16)	2.86 (2.60–3.08)	3.40 (3.24–3.63)	3.90 (3.30–4.62)

( ) = Range.

together with india ink into a shaved area on flank of the mouse. Total injected volume was 0.1 ml. The animals were killed and skinned 12 hours after injection. The skin containing the ink bleb was placed on a transilluminated glass plate, subcutaneous tissue facing the observer, and its borders were traced on a sheet of transparent "Lucolite 11-6." The traced plastic areas were cut out and weighed. Conversion of weights thus obtained into area of spread was made by dividing weights of traced plastic areas by weight of plastic area measuring exactly 1 cm^2. 1. *Spreading effect of commercial hyaluronidase* was determined in 27 RFM males divided into 6 groups receiving india ink marker containing 6U, 12U, 50U, and 100U of hyaluronidase. The spread obtained was compared with that resulting from serum or Ringer's solution. 2. *Spreading effect of RFM uterone* was measured in 5 RFM males receiving india ink and fresh RFM uterone and was compared with results obtained in 6 RFM mice receiving india ink and serum. 3. *Spreading effect of BALB/c uterone* (Lyophilized) was measured in 34 BALB/c mice (19 males, 15 females). These animals were divided into 4 groups (females) and 5 groups (males) and given india ink marker and uterone made of the following concentrations: original concentration (2.43 g of solid/100 ml), and approximately one-third, one-half, 2 and 9 times the original concentration. Results were compared with the spread obtained in 3 males and 3 females with india ink and serum. 4. *Spreading effect of BALB/c uterone and commercial hyaluronidase combined.* Eight BALB/c males were given india ink and 6 units of hyaluronidase dissolved in lyophilized BALB/c uterone reconstituted to its original concentration. 5. *Spreading effect of BALB/c uterone in dead BALB/c skin.* India ink marker and BALB/c uterone reconstituted to 5 times its original concentration was injected into BALB/c skin one hour after killing the animal, and compared with spreading of india ink containing Ringer's solution and 15U of commercial hyaluronidase. The skin was fixed in formaldehyde 30 minutes later. 6. *Spreading of india ink in rabbit skin in relation to time as influenced by hyaluronidase, mouse uterone and ovomucoid.* Rabbits were used because they are the standard assay animal for spreading factor, and permit numerous spots to be studied and biopsied in the same animal in rapid succession. Flanks of male rabbits were shaved and india ink containing the following materials was injected: a. 30 units of commercial hyaluronidase; b. 15 units of commercial hyaluronidase; c. BALB/c mouse uterone 1.3 times original concentration or d. BALB/c mouse uterone, 9 times original concentration; e. ovomucoid, 2.5 g/100 ml; f. saline. The resulting spots were traced on plastic sheets placed over the skin at intervals of 1′, 2′, 5′, 10′, 20′, 60′, 90′, and 18 hours. Another rabbit was treated in a similar fashion, except that no india ink was used. In this animal 3 injections were made for each substance and biopsies were taken 5 minutes, 20 minutes, and 24 hours after injection. These tissues were fixed in formaldehyde and sections were prepared and stained with hematoxylin-eosin. A similar experiment was done in Swiss mice using uterone obtained from the same strain. 7. *In vitro assay of uterone for depolymerization of hyaluronic acid.* The viscosity reduction method described by J. Madinaveitia and T. H. H. Quibell(6) was followed using vitreous body hyaluronic acid as a substrate and an Oswald viscometer Kimble, size No. 200.

Results are shown in Tables I and II and Figs. 1 and 2. The results indicate that commercial Wydase enhances the spreading of india ink in the dermis of mice much as it

TABLE II. Comparison in BALB/c Males and Females of Surface Areas of Ink Spots Resulting from Intradermal Spread of India Ink Suspended in Mouse Serum (RFM), Uterone (Lyophilized BALB/c Reconstituted to Varying Concentrations) and the Same Uterone Reconstituted to Original Concentration Combined with 6 Units Wydase.

		BALB/c uterone					BALB/c uterone (1.1 N) + 6 u Wydase
	Serum	.3 N	.6 N	1.1 N	2.2 N	9.0 N	
Mean spread in males, cm²	1.24 (1.16–1.31)	1.00 (.91–1.10)	1.16 (1.08–1.20)	1.57 (1.41–1.73)	1.63 (1.27–2.10)	2.47 (2.30–2.73)	2.68 (2.03–3.40)
Mean spread in females, cm²	.95 (.75–1.09)	1.40 (1.30–1.46)	1.15 (.91–1.29)	1.70 (1.63–1.74)	1.69 (1.31–1.91)		

( ) = Range.

does in the rabbit which has previously been used as a test animal to measure spreading factors. The intradermal injection of murine uterone from BALB/c and RFM mice enhances spreading of india ink in BALB/c and RFM mice (Fig. 1 and Table I and II). BALB/c uterone potentiates spreading effect of Wydase in BALB/c male mice. The same BALB/c uterone which had these spreading effects *in vivo* did not enhance spreading when injected into dead BALB/c skin. Viscosimetric studies showed that BALB/c uterone does not depolymerize hyaluronic acid *in vitro*.

In rabbits, Wydase caused a rapid initial enhancement of spreading of india ink, whereas ovomucoid and uterone resulted in a much slower progressive enhancement of spread (Fig. 2). Histologic study in rabbits and mice revealed that this was accompanied by edema and inflammation of dermis while no such changes were seen with Wydase.

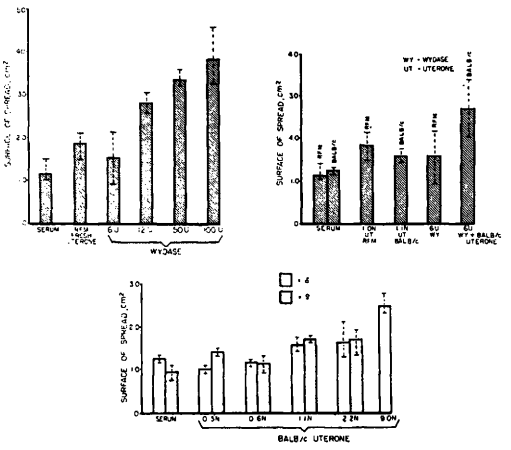

FIG. 1. Bar graph expressing avg area of spread of india ink (and range) following intradermal inj. of substances listed on abscissa.

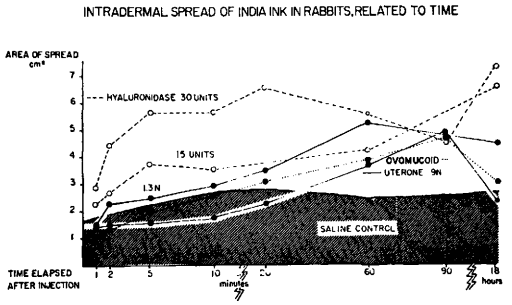

FIG. 2. Comparison of development of spreading area related to time following inj. of saline, hyaluronidase, ovomucoid and uterone with india ink marker into dermis of rabbit. Note rapid spreading of hyaluronidase during first minutes compared to slow and progressive spread caused by ovomucoid and uterone. Also note shrinkage of spreading area at end of experiment in case of uterone and ovomucoid.

Discussion. Murine uterone was shown to enhance spread of intradermal india ink and to potentiate the spreading effect of commercial hyaluronidase. This appears to be caused not by hyaluronidase activity, but by some other mechanism. Histologic observations made in rabbits and mice indicate that Hechter's explanation of edema and inflammation as hyaluronate-inactive spreading mechanisms applies to this situation (4). Potentiation of Wydase by uterone could be explained by increase of intradermal pressure caused by inflammation with ensuing wider diffusion of the enzyme and therefore a greater spread. The hyaluronate-inactive nature of uterone spreading factor is considered to be all the more likely since ovomucoid, a mucoprotein which shares many physical and chemical properties of uterone, causes in the rabbit an enhancement of spreading of india ink comparable to that observed with uterone. It may be noted here, as previously described

by Hechter, that curves relating spreading to time are of 2 distinct types, one with rapid onset and S-shaped slope for the enzymatic spreading factor, the other typical for hyaluronate-inactive spreading factor with slowly ascending, progressively mounting slope. In addition, we noted that the area of ink spot retracts after 24 hours where inflammation and edema were instrumental in causing spreading. This type of curve was obtained repeatedly at various dosages of uterone and with several experiments done at different times by different observers. Uterone does not exert its spreading effect in dead skin, where inflammation and edema no longer occur but where enzymatic activity of injected hyaluronidase still is effective.

Summary. A spreading factor is contained in uterone which may be classified as a hyaluronate-inactive spreading factor exerting its effect through inflammation and edema. The nature of the component of uterone responsible for provoking inflammation remains to be determined and may well be a mucoprotein.

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Question of Purity of Erythropoietic Factor Concentrates.* (24457)

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There is now much evidence for a circulating erythropoietic factor and attempts have been made to concentrate it. Active material in plasma of rabbits made anemic with phenylhydrazine can be precipitated by ammonium sulfate and has been associated with a mucoprotein fraction(1,2,3). We prepared ethanol precipitates from anemic plasma according to Grant *et al.*(4)† and found them similar to concentrates made with ammonium sulfate. The stimulating factor found in urine of certain anemic patients is probably of the same nature as that found in anemic animals(5). This report compares certain properties and potencies of various active preparations made in 4 different ways.

Materials and methods. One preparation was from human urine(6),‡ the rest from plasma of anemic rabbits. These also were compared to a preparation made from plasma of normal rabbits. Starting material (PF) for some fractions was the filtrate obtained after boiling plasma at pH 5.5 for a few minutes(7). It contained only about 2% of plasma protein. We have shown that injections of unboiled plasma provoke immunological reactions which interfere with tests lasting 10 days or 2 weeks. After boiling there is no interference(3). The different preparations compared were: 1. PF from rabbits made anemic with phenylhydrazine was precipitated with 1.5 volumes of absolute ethanol. After 2 hours at 5°C the precipitate was removed in the centrifuge and an equal volume of absolute ethanol was added to supernatant. After 16 hours at 5°C it was centrifuged, the supernatant was poured off and the precipitate dried in a desiccator(4). About 25% of the protein and all erythropoietic activity of anemic PF was recovered in this fraction. In one case the yield was about 350 mg/l of

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