say methods to a scale requiring 1/500th to 1/1000th the sample ordinarily used is described with specific application to the measurement of biotin and pantothenate in tissue sections.

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Antimycobacterial Activity of Extracts of Rat Peritoneal Cells.* (24455)

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Lurie concluded (a) that acquired immunity to tuberculosis is merely enhanced natural resistance, and modes of action of the 2 phenomena are probably the same(1,2) and (b) that altered physiological reactivity of mononuclear phagocytes of the immunized animal is the most important single factor in resistance to the disease(3). These conclusions have gained considerable support. It appeared possible therefore that comparative studies of some physiological activities of mononuclear cells from animals of differing susceptibility to this disease might contribute to a better understanding of mechanisms of restriction of intracellular mycobacterial growth. As a part of such studies, antimycobacterial activities of extracts of cells of induced peritoneal exudates from the highly susceptible guinea pig and the very resistant rat have been investigated. It seems desirable to report preliminary results of these experiments.

Materials and methods. Male albino guinea pigs of the "English" breed weighing 800 to 1200 g each, and 2 strains of male albino rats of 250 to 400 g weight were used in groups of 8 to 18 animals for each experiment. Exudate induced by intraperitoneal injection of glycogen(4) was collected on sixth day, at time of monocyte-type cell predominance, by washing peritoneal cavities with large quantities of isotonic 0.005 M Tris buffer, pH 7.4. Fluid was collected by gravity from living guinea pigs, and from rats by exposing peritoneal cavities immediately following their death from sharp head blows. Siliconed glassware or lusteroid or polyethylene containers were used for handling all cell suspensions. About 1 to 2.5 l of pooled cell suspension was obtained from each group of animals. The number of cells was determined by hemocytometer for preliminary standardization. Cells were concentrated in 20 to 40 ml of isotonic Tris buffer in a refrigerated centrifuge. Concentrated cell suspensions were freed from contaminating erythrocytes, if any, by recentrifuging and suspending 10 minutes in hypotonic NaCl solution, which was then made isotonic by addition of an equal volume of hypertonic NaCl solution(5). Total and differential cell counts were made and the suspension recentrifuged; thus cells were washed twice after removal from animals. The button of cells was evenly suspended in glass-distilled water to make concentrations of about 60 to 70 x 10^6 cells/ml and suspensions were subjected to sonic vibration in Raytheon

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TABLE 1. Antimycobacterial Activity of Mononuclear Cell Extracts. Figures refer to average number of colonies in 4 weeks from replicate tests of 0.02 ml of filtrate-bacteria mixtures. Test organism: *Mycobacterium tuberculosis*, var. *hominis*, Strain H37Ra.

		Hours of contact with bacteria:							
		0	1	24	48	0	1	24	48
Exp. 1:		Rat cell extract (Wistar)				H_2O			
Filtr.-bact. mix.:	Undil.	>250	>250	250 approx.	0 (6 repl.)* 1 (1 plate)* 2 (1 ")*	>250	>250	>250	1+ (8 repl.)
	Dil. 1:5	67	60	52 (5 repl.)	0 (9 repl.)*	59	55	68	98 (14 ")
		No. mammalian cells, 3000 No. bacterial cells, 1							
Exp. 2:		Rat cell extract (CFN)				H_2O			
Filtr.-bact. mix.:	Undil.	>300	>300	20 (5 repl.)*	0 (4 repl.)*	>300	>300	>300	1+
	Dil. 1:5	122	107	5 (7 ")*	0 (6 ")*	114	111	103	155 (9 repl.)
		No. mammalian cells, 1200 No. bacterial cells, 1							
Exp. 3:		Guinea pig cell extract				H_2O			
Filtr.-bact. mix.:	Undil.	>150	>150	>150	>150	>150	>150	>150	>150
	Dil. 1:5	32	34	36	34 (9 repl.)	32	33	30	44 (16 repl.)
		No. mammalian cells, 5000 No. bacterial cells, 1							

* No increase in No. of colonies after 6 weeks' incubation.

9 Kc sonic oscillator until microscopic examination revealed only granules with no whole cells (5 to 10 minutes). Glass-distilled water was then added to make suspensions correspond to 45 to 55 x 10^6 cells/ml, sediment was removed by 1 hour's centrifuging at 20,000 g and 1.5 to 2.5 ml of supernate was sterilized by filtration through a millipore (MF) pad using the Swinney adapter. A corresponding quantity of glass-distilled water was similarly filtered as control for each experiment. Antibacterial tests were performed as follows: Test organisms: *Mycobacterium tuberculosis* var. *hominis*, strain H37Ra. Five-day cultures in Proskauer and Beck type medium containing bovine serum albumin and Tween 80® for dispersed growth, were centrifuged lightly to remove clumped bacteria. The top layer of supernatant was diluted 1:200 in 0.005M phosphate buffer, pH 6.8, containing albumin and Tween 80®. Diluted culture was added to cell extract or water filtrate in 1 to 4 parts. Immediately, 0.02 ml amounts of each mixture were cultured in replicate on coagulated egg medium. Filtrate-bacteria mixtures were then diluted 1:5 in phosphate buffer-Tween 80®-albumin and similarly cultured in replicate. When surface of medium was dry, plates were sealed in polyethylene envelopes or saran wrap and incubated for growth. Replicate cultures of filtrate-bacteria mixtures were repeated after 1, 24 and 48 hours at 37°C. Colonies were counted at intervals after 2 to 6 weeks' incubation.

Results. It is evident from Table I that many tubercle bacilli were rendered non-cultivable after 24 hours' contact with extract of normal rat cells and that 48 hours' contact resulted in practically complete inhibition. In contrast, 48 hours' incubation in normal guinea pig cell or distilled water filtrate had no effect; the number of bacteria in water controls appeared to increase slightly.

Extract of a relatively large number of mammalian cells was employed per single bacterial cell. Rat cell filtrate effectively inhibiting bacteria in a ratio of 1200 to 1, lost its inhibitory effect under our conditions when diluted 10 times prior to addition of bacteria.

Differential counts (Giemsa stained, at least 500 cells counted) revealed that 90 to 95% of cells from both animal species were "large mononuclear" cells. Suspensions from rats, however, were characterized by 4 to 6% large granular basophilic mast cells, absent in guinea pig cell preparations. The latter more

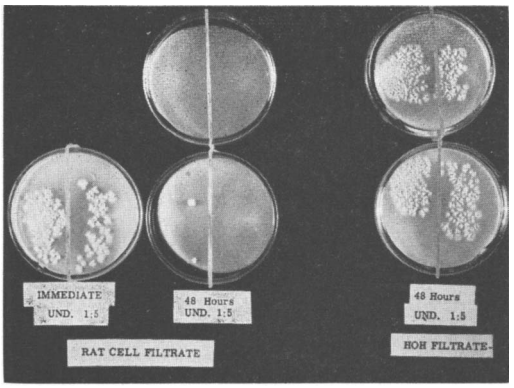

FIG. 1.

characteristically contained a comparatively larger percentage of eosinophilic-staining macrophages than those from rats.

Analyses for protein content by biuret and microkjeldahl methods indicated that soluble cell material (*i.e.*, supernate of centrifuged lysates) from rat cell preparations had somewhat less protein (corresponding in different groups of rats to approximately 40 mg/ 1×10^9 cells of original suspensions) than guinea pig cell preparations (about 50 mg 1×10^9 cells). Membrane filtration for sterilization resulted in only a slight decrease in protein content of extracts of cells from both species.

Discussion. Most previous attempts to obtain antibacterial substances from leukocytes have been made with polymorphonuclear cells (see Skarnes and Watson(6) for review). However in a report concerned mainly with extracts of normal rat spleen, Bloom, Hudgins and Cummings(7) briefly described tuberculostatic activity of cells of peritoneal exudates obtained from rats 48 hours after injection of mineral oil. These exudates contained "large numbers of monocytes." The decrease in number of viable mycobacteria reported by these investigators appeared to be of the same order as that obtained in our experiments.

Rich stated that it had never been demonstrated that extracts of mononuclear phagocytes had a deleterious effect on survival or multiplication of tubercle bacilli(8). Although with certain bacteria it is not difficult to demonstrate a weak *in vitro* bactericidal effect of phagocytes by using concentrated extracts from masses of phagocytes, he believes that such activity is of little significance in killing of ingested bacteria *in vivo*. This view may be essentially correct since it appears likely that a combination of effects, rather than a single agent, is responsible for inhibition of intracellular bacteria by macrophages. One factor in a complex interreaction might be a growth-inhibiting chemical. Hirsch, for example, stated that at least 3 potentially bactericidal substances appear to be present in polymorphonuclear cytoplasm: acid, lysozyme and phagocytin, present information suggesting that these agents may act synergistically(9). The possibility of this renders the disproportion between numbers of mammalian and bacterial cells of less significance. Further work may indicate that amount of extract used in the present experiments is sufficient for a greater number of bacteria. Optimum conditions for maximum activity of this property of rat cell extracts have not yet been established. Hirsch has shown that number of bacteria present does not strikingly affect activity of phagocytin on enteric bacteria.

The main cytological difference between rat and guinea pig peritoneal exudates, *i.e.*, mast cells in the former, induces speculation concerning the source of the antibacterial property of these preparations. Should it prove to be the mast cell, the significance of this activity in destruction of intracellular bacilli by macrophages might be questioned. However it has been suggested that mast cells are merely polysaccharide-laden macrophages (Higginbotham and Dougherty, quoted by Padawer(10)). In this connection, it is interesting that many antibacterial substances extracted from tissues are of the nature of basic proteins or basic peptides(6).

Summary and conclusions. 1) Membrane-filtered water-soluble constituents of sonic-disrupted cells from peritoneal exudates of 2 strains of albino rats exert a definite inhibitory effect on cultivability of *Mycobacterium tuberculosis*, strain H37Ra, in phosphate buffer at pH 6.8 on 24 hours' contact at 37°C, inhibition being almost complete after 48 hours incubation. 2) Similarly treated extracts of cells from peritoneal exudates of normal guinea pigs standardized in the same manner have no effect. 3) Differential cell counts of preparations from which extracts were

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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