mal protein present between the alpha 1 and alpha 2 globulins in the serum electrophoretic pattern of male rats following treatment with trypan blue. It is plausible that a causal relationship exists between the alteration of proteins in the maternal serum and yolk sac fluid and embryonic malformations.

Other observations have shown that trypan blue can compete for protein binding sites in plasma(7). Therefore, it would not be unreasonable to presume that Niagara blue 2B could act in a similar manner, and thus compete with trypan blue for a protein binding site. If this is true, it could explain the interference of Niagara blue 2B with the lethal and teratogenic activity of trypan blue as described here.

Summary. The results demonstrate that the non-teratogenic disazo dye, Niagara blue 2B, can afford some measure of protection to the embryo against the lethal and teratogenic action of trypan blue when injected prior to trypan blue in Sherman rats, over a selected range in dye concentration. This protection was not complete.

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Interaction of Lysates of Histiocytes and Tubercle Bacilli.* (27316)

HAJIME JAMES AKIYAMA† AND JACOB FONG
Department of Bacteriology, University of California, Berkeley

Studies of the interaction of tubercle bacillus and phagocyte have resulted in a large body of evidence which suggested that, under certain circumstances, phagocytes may exhibit antibacterial activity, either independently or in conjunction with humoral factors (1,2). Although these observations have indicated suppression of growth of tubercle bacilli by phagocytic cells derived from various animals, the underlying mechanisms still remain to be elucidated.

In view of various suggestions(3-5) that cellular enzymes or other native, biologically active intracellular substances may contribute toward the antimycobacterial activity of these cells, the present investigation was undertaken to determine whether elements derived from disrupted histiocytes would in some manner exert a deleterious effect on the tubercle bacillus.

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† U.S.P.H.S. Predoctoral Fellow (1959-1961).

Materials and methods. Histiocytes were obtained from the peritoneal cavity of adult normal or BCG-immunized rabbits or guinea pigs which had been injected 5 days previously with sterile Klearol using methods previously described(6). Immunized animals used as sources of cells or serum had received 2 to 3 subcutaneous injections of BCG over a period of 2 to 3 months. Each immunizing dose consisted of approximately 5×10^8 viable organisms which had been cultivated on Sauton's potato plug medium for 3 weeks. Only animals giving a positive tuberculin test (0.2 ml of 1:100 dil. of O.T., Eli Lilly Co., Indianapolis) were used as donors of immune histiocytes.

Lysates of histiocytes were prepared in the following manner: A suspension of washed cells of known concentration was sedimented by centrifugation at 250 *g* for 10 minutes; the sedimented cells were resuspended to a predetermined volume with sterile, chilled distilled water, mixed thoroughly by pipet-

ting, and immediately lyophilized. When needed, the material was rehydrated with a known volume of Tyrode's solution, mixed thoroughly, and centrifuged at 850 *g* for 30 minutes to remove cell stroma. With this treatment, no intact cells were seen in the sediment. The supernate, which constituted the cell lysate, was slightly opalescent, had a pH range of 6.5 to 6.8 and was not further neutralized. The lysate was usually adjusted to an equivalent cell concentration of 1×10^7 cells per milliliter.

To determine the effects of preparations of lysates of histiocytes on the tubercle bacillus, one-week-old Tween-albumin (Difco, Detroit) cultures of the organism (H37Ra, H37Rv and BCG strains of *Mycobacterium tuberculosis* originally obtained from the Phipps Institute, Philadelphia) were added to various nutrient and non-nutrient fluids (the particular fluid used is shown in Table I) containing dilutions of histiocytic lysate. The lysate (as equivalent cell concentration) to bacterium ratio in these mixtures was adjusted to approximately 10:1. These mixtures were incubated at 37°C and samples withdrawn at various times for determination of viable bacilli. This determination was performed by making serial 5-fold dilutions in Tween-albumin liquid medium and replicate samples inoculated on glycerol blood agar medium utilizing the "drop method" described by Tarshis and Elberg(7).

Results. Incubation of tubercle bacilli (H37Rv and BCG) for 1 to 7 days at 37°C in media (Tween-albumin, Tyrode's solution, 50% normal or anti-BCG serum in Tyrode's solution) containing histiocytic lysates prepared from normal or BCG-immunized rabbits or guinea pigs had no demonstrable effect on the acid-fast staining characteristic, neutral red binding capacity or viability of bacilli, as indicated by comparison of lysate-treated and untreated bacilli (results not shown). The cytotoxic activity of these lysate-treated organisms for normal histiocytes in Mackaness tissue culture chambers was also determined according to a method previously described(6) and no discernible loss in ability to cause degeneration of *in vitro* cultures of histiocytes was found.

When the above experiments were repeated with modification of the experimental procedure by omission or substitution of certain constituents of the standard Tween-albumin medium, a marked bactericidal activity of histiocytic lysate was observed (Table I, upper half). Thus, while incubation of bacilli in lysate-Tween-albumin medium had no effect upon the bacilli, in the absence of albumin a marked reduction of viable bacilli occurred. Normal serum, like albumin, also prevented killing of the bacilli. Incubation of bacilli in a lysate-base medium (no Tween or albumin) was without deleterious effect upon the bacilli although very little growth occurred in this medium. The use of Tyrode's solution also resulted in killing of bacilli when lysate and Tween were present together, but not in the absence of either component. These results therefore indicated that in these experiments Tween 80 was a necessary component in the inactivation of tubercle bacilli by histiocytic lysate. The fact that killing occurred in Tyrode's solution would suggest that resting bacilli as well as actively growing bacilli were susceptible to lysate-Tween action. Since no differences were noted between normal and immune histiocytic lysate, only the results for normal histiocytic lysate are shown in Table I.

In an attempt to characterize further the mechanism of inhibition by lysate-Tween mixtures, various other experiments were made. These results (not shown) indicated that the activity of histiocytic lysate preparations was not dependent upon lysozyme activity. While the tuberculocidal activity of the lysate-Tween mixture was lost upon heating of the lysate preparation for 10 minutes at 70 to 100°C, it was retained if the lysate-Tween mixture was kept at 37°C for 24 to 48 hours prior to heating. These results indicated that probably some heat stable component produced by lysate-Tween interaction was responsible for the observed tuberculocidal effect. This conclusion was substantiated by analysis of the lysate-Tween system; when this system was subjected to ether extraction(8) and the extracted material analyzed by paper chromatography(9), the presence of oleic acid, presumably arising

TABLE I. Inhibitory Effect of Lysate-Tween Mixture and Lysate-Lipid Mixtures upon Tubercle Bacilli.

Type of medium used*	Log No. viable bacilli/ml Hr after incubation at 37°C 0	8	48	120	168
Lysate in Tween-albumin medium†	5.2	5.3	6.1	6.8	8.0
Tween-albumin medium (no lysate)	"	"	"	7.2	8.4
Lysate in Tween medium (no albumin)	5.2	5.3	3.8	<1.6	<1.6
Tween medium (no albumin or lysate)	5.1	"	5.9	6.8	8.0
Lysate in serum-Tween medium (no albumin)‡	5.6	—	—	—	7.0
Serum-Tween medium (no albumin or lysate)	"	—	—	—	7.3
Lysate in base medium (no Tween or albumin)	5.3	5.3	5.2	5.6	5.9
Base medium (no Tween, albumin or lysate)	5.2	5.4	5.5	"	"
Lysate in 0.02% Tween-Tyrode's solution	6.2	—	—	—	3.5
0.02% Tween-Tyrode's solution (no lysate)	"	—	—	—	6.4
Lysate in Tyrode's solution	6.2	—	—	—	5.9
Tyrode's solution (no lysate)	"	—	—	—	6.3
Lysate in serum lipid-base medium§	6.9	—	—	—	5.3
Serum lipid-base medium (no lysate)	6.4	—	—	—	8.6
Lysate in lipemic serum-base medium‖	6.2	—	—	—	1.6
Lipemic serum-base medium (no lysate)	6.0	—	—	—	7.0

* Dubos broth base medium (Difco, Detroit) for incubation of H37Rv with 10% bovine serum albumin and/or 0.02% Tween 80 added in specified cases.

† Normal rabbit histiocytic lysate (equivalent cell conc. of 1×10^7 cells/ml) was diluted 1:10 in each medium. In the case of lipemic serum, the lysate was diluted 1:5 in the medium. Final volume of the mixture was 10 ml in all cases. In control media, Tyrode's solution was added in place of lysate.

‡ Serum-Tween medium: Normal rabbit serum was diluted 1:20 in Tween medium.

§ Serum lipid medium: Total extractable chloroform-soluble, acetone insoluble lipid (phospholipid) from 10 ml of normal rabbit serum was resuspended to 10 ml in base medium. Lipid prepared by slight modification of procedure of Patnode(11) for extraction of phospholipid from rabbit lung tissues.

‖ Lipemic serum medium: Lipemic rabbit serum [from rabbits inj. with Triton A20 according to the procedures of Kellner *et al.*(10)] was diluted 1:10 in base medium.

— indicates sample not tested.

Similar results were obtained with the H37Ra and the BCG strains of tubercle bacillus but no inhibitory effect was observed with *Escherichia coli* and *Staphylococcus aureus* (results not shown in table).

from breakdown of Tween by lysate, was demonstrated.

In view of the probable presence of lipase or esterase activity in histiocytic lysate, as suggested by the above findings, it seemed of interest to determine whether tuberculocidal activity might also be demonstrated when histiocytic lysate was mixed with normal tissue components. For this purpose, either crude chloroform-soluble, acetone-insoluble serum lipids (phospholipids) or lipemic serum obtained from Triton injected animals (10) was substituted for Tween. The results (lower half of Table I) indicated a similar tuberculocidal activity. The more pronounced activity noted for lipemic serum was probably a reflection of the higher content of lipids in this system.

Discussion. The present investigation, which was undertaken to determine the effects of histiocytic extracts of normal and BCG-immunized animals upon the physical structure and physiological activities of tubercle bacilli, failed to demonstrate any direct action upon the organisms despite the presence of lipase in these preparations. It is realized, however, that the criteria adopted for measurement in these studies may not have been sufficiently sensitive to detect subtle modifications of the organism.

The demonstration of a tuberculocidal activity of histiocytic lysates in the presence of either a synthetic lipid ester or lipid components of serum seemed of interest in view of the reported sensitivity of the tubercle bacillus to minute concentrations of unsaturated

fatty acids(12), and various reports alluding to the correlation between lipase activity in serum, organs and tissues(13,14) with the resistance of host species or organs to tuberculous infection. Although it would be premature to suggest that such a postulated lipase-lipid mechanism may be involved in the suppression of tubercle bacilli by histiocytes, the results reported here are suggestive and appear worthy of more intensive investigation.

Summary. Cell lysates derived from peritoneal histiocytes of normal and BCG-immunized rabbits or guinea pigs had no demonstrable direct effect on the morphology, neutral red binding capacity, viability or cytotoxic activity of virulent and attenuated strains of *Mycobacterium tuberculosis*. Histiocytic lysate in combination with Tween 80 was found to be bactericidal for strains of tubercle bacilli but not for a strain of *Escherichia coli* or a strain of *Staphylococcus aureus*. The mechanism of this activity appeared to be due to the enzymatic degradation of Tween 80 by histiocytic lipases with the formation of oleic acid. A similar antimycobacterial activity was manifested in mixtures of histiocytic lysates with crude lipid extracts of normal rabbit serum and with lipemic rabbit serum. No difference in activity was found between cell lysates prepared from histiocytes of normal and BCG-immunized rabbits or guinea pigs.

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An Intracellular Parasite Resembling a Microsporidian Associated with Ascites in Swiss Mice. (27317)

JOHN B. NELSON
The Rockefeller Institute, New York City

Morris *et al.*(1) described an enzootic disease of mice characterized by ascites, hepatosplenomegaly, and the presence of an intracellular protozoan-like agent. A seemingly identical disease, previously unreported, has been maintained in our laboratory for a period of years by serial passage in Swiss mice. Some additional properties of the associated agent, brought out by our studies, are presented.

Methods. Swiss W mice of a commercial subline, which had shown an atypical response to certain carcinogens, were sent to us for examination in 1953. The mice were autopsied with findings that were clearly indicative of chronic respiratory disease. Splenic enlargement, which is not an accompaniment of that condition, was also observed. A saline suspension of ground spleens from 3 of the animals was injected intraperitoneally in Swiss weanlings from the random-bred mouse colony of the Rockefeller Institute. At autopsy on the 21st day these mice likewise showed enlarged spleens.

Separate passages with suspensions of pooled spleens and undiluted ascitic fluids, which first appeared during the second transfer, were maintained thereafter in groups of