antibiotic Mycostatin§ at certain concentrations. This substance was added to a few tubes in some of the preliminary experiments in an effort to suppress mold contaminants. In concentrations of 12.5 or 25 units per ml molds were suppressed but few or no snails hatched; at 5 units per ml a small proportion of snails hatched but molds were not uniformly suppressed. It is of interest that a very low concentration of Mycostatin is rapidly lethal to adult *A. glabratus*(9).

The development of a technic for securing newly-hatched, bacteriologically sterile, *A. glabratus* should permit a variety of additional studies. For example, the metabolic characteristics and nutritional requirements of the young snails can be investigated in the absence of micro-organisms or in the presence only of selected micro-organisms. Furthermore, snail tissues free of contamination can now be secured for *in vitro* cultivation. Finally, 'germ-free' snails maintained under sterile conditions should provide an ideal tool for screening and studying microbial or viral agents which might be of value in biological control. Aspects of these problems are now under investigation.

Summary. Details of a technic are presented by means of which newly-hatched, bacteriologically sterile snails (*Australorbis glabratus*) can be secured for study. Snail eggs containing mature embryos are separated manually from the egg-mass, disinfected externally with dilute sodium hypochlorite, and isolated in sealed tubes containing a suitable salt solution and antibiotics. Using this procedure, 90-100% hatch occurs within 3-4 days of treatment, and tests have shown that only a negligible proportion are bacteriologically contaminated. Details of maintenance, behavior, and longevity of these snails are discussed.

I am indebted to Dr. E. H. Michelson for maintaining the colony of *A. glabratus* from which the eggs used in these studies were derived; to Dr. John H. Hanks, Harvard Medical School, for suggestions regarding the composition of HS; and to Miss Sarah E. Dwight for technical assistance.

§ Squibb brand of Nystatin. Kindly supplied by E. R. Squibb and Sons, New Brunswick, N. J.

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Observations on *in vitro* Behavior of Altered Mononuclear Phagocytes Following Exposure to Tubercle Bacilli.* (23434)

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Although cellular response to infection with the tubercle bacillus has been extensively investigated, there is considerable difference of opinion as to the role of phagocytes in tubercular infections(1,2,3). Mononuclear phagocytes have been proposed as the main factors of resistance to tuberculosis infection(4,5). Vorwald and co-workers(6) indicate that BCG will produce fatal cavitary disease in silicotic guinea pigs. Dubos(3) pointed out

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that disease in the silicotic individual infected with BCG develops because silicosis changes the physicochemical environment of the tissues. These changes allow bacillary growth in excess of that obtained in normal tissues. The effect of various forms of silica on motility and metabolism of phagocytes has been described by Vorwald and Delahant(7), and Gardner(8). However, adequate descriptions of the incidence of phagocytosis of tubercle bacilli by cells "blocked" or altered through ingestion of various particles, including silica, are not available. In the present paper, observations of the behavior of "blocked" or altered mononuclear phagocytes toward tubercular infections by virulent and attenuated strains are described. "Blockade" of the monocytes was accomplished with trypan blue, silicia, carbon, coal dust and cement particles.

Materials and methods. Female albino guinea pigs, weighing between 300 and 400 g were used. All animals were tuberculin negative. A chemotactic agent, 10 ml of a 1% starch solution, was injected by the intraperitoneal route into each animal. "Blockade" of the mononuclear phagocytes was accomplished by intraperitoneal injections of 10 ml of a 0.5% solution of trypan blue, 8% solution India ink (Higgins liquid solution), 5% silica suspension, 1% coal dust suspension (Bituminous-West Kentucky Vein) or a 2% suspension of Portland cement on the 4th and 5th day after injection of the starch solution. The blocking agents were injected into groups of at least 20 animals with equal numbers receiving injections of normal saline. Trypan blue and India ink were colloidal solutions while silica, coal dust and cement suspensions contained particles of less than 43 μ size as determined by a standard sieve.

Exudate was collected from all experimental animals on the 6th day according to the method described by Suter(5). Suspensions of the strains of tubercle bacilli* used, were obtained by differential centrifugation of 8-10-day-old Tween-albumin cultures. Bacterial numbers were estimated turbidimetrically through the use of a Bausch and Lomb spectrophotometer. The bacterial suspension was diluted with Tween-albumin medium so that 5 ml contained a final concentration of approximately 2 x 10^6 viable units of tubercle bacilli.

After addition of fresh homologous serum in a final concentration of 2.5% to the exudate, 8 ml of the exudate was mixed with 2 ml of the standardized bacterial suspension. The experimental series consisted of "blocked" and "non-blocked" monocytes plus H37Rv or BCG. Other cultures of monocytes were also maintained in the absence of tubercle bacilli. The mixtures were pipetted in 0.5 ml amounts onto 7/8 inch square coverslips in sealed partitioned Petri dishes and incubated at 37°C. Coverslip cultures were selected at 1, 2, 4, 8, 16 and 24-hour intervals and stained by the Ziehl-Neelson method. A phagocytic index was obtained by determining the percentage of mononuclear phagocytes containing tubercle bacilli in each of 200 cultures. In all instances, a minimum of 100 cells in 2 or more diameters of a coverslip culture were counted.

Results. Comparison of the phagocytosis of *Mycobacterium tuberculosis* strain H37Rv by unaltered and altered mononuclear phagocytes of guinea pig exudate is presented in Fig. 1. Although observations were made at 8, 16, and 24 hours, no significant difference in degree of phagocytosis was noted. For this reason no results beyond 4 hours are reported. These results show that all agents tested significantly inhibited phagocytosis of the test organism. Minimal phagocytosis was observed in the presence of carbon particles. Although phagocytosis in the presence of trypan blue was greater than that observed with other "blocking agents," it was significantly lower than that observed in the "non-blocked" or unaltered cells.

Observations on the phagocytosis of *Mycobacterium tuberculosis* strain BCG by altered and unaltered monocytes are presented in Fig. 2. Although phagocytosis was decreased in the presence of all agents tested, it was greater than that observed with the same

* H37Rv was obtained from the Trudeau Laboratory, Saranac Lake, N. Y., BCG was obtained from the Henry Phipps Institute, Philadelphia, Pa.

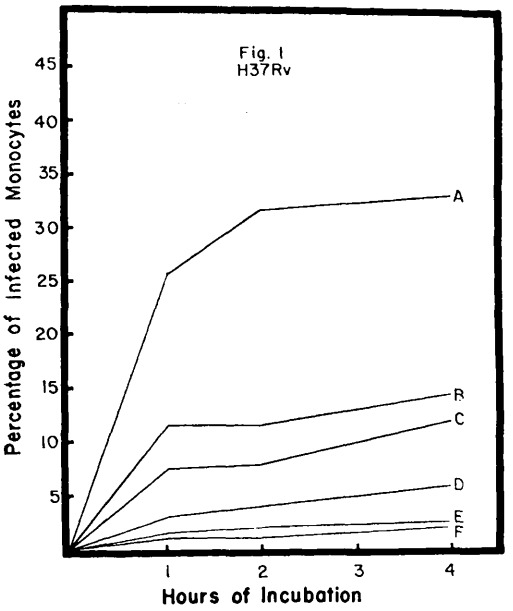

FIG. 1. Phagocytosis of H37Rv by altered and unaltered mononuclear phagocytes. A—Monocytes with no altering agent; B—monocytes with trypan blue; C—monocytes with cement; D—monocytes with silica; E—monocytes with coal dust; F—monocytes with India ink.

agents in presence of the virulent organism. The pattern of relative effectiveness of "blocking agents" observed in the experiment with the virulent organism was apparent in this experiment with the BCG strain.

Discussion. Although the phagocytic cells were subjected to 2 days of *in vivo* exposure to the various altering agents, the ingestion of particles varied. It is recognized that the starch solution, which was used as a chemotactic agent, might have contributed to some extent to the "blockade" of the phagocytes, as well as stimulating new cell activity. This additional effect of starch solution does not appreciably alter interpretation of results since the same quantity was used to produce all the peritoneal exudates and all cells were counted. Some of the altering agents like cement and silica may cause toxic effects at high concentrations which may affect new cell activity or blockade(9). However, in the concentrations used here, percentage uptake did not vary significantly from uptake when relatively non-toxic particles like carbon particles in water (India ink) were used for altering. Prior to 24 hours there was no evidence of intracellular multiplication as Mackaness pointed out(1). Therefore, it is felt that these responses represent phagocytosis without subsequent multiplication.

Our observations indicate that ingestion of various particles significantly decreased the ability of mononuclear cells to phagocytize both attenuated and virulent strains of the tubercle bacillus. There was a higher average uptake of BCG than H37Rv by both altered and unaltered monocytes which may be due to the tendency of attenuated forms to be more readily phagocytized than their virulent forms. Other investigators(6,8,10) have shown that phagocytic ingestion of dust particles resulted in altered activities of these cells, but the effect on ability to phagocytize has not been described. Kato and Gozsy(11) have recently demonstrated that low concentrations of histamine and varying concentrations of antihistaminics inhibit phagocytosis. The mechanism of these observations does not appear readily apparent, but is not likely related to the results described here.

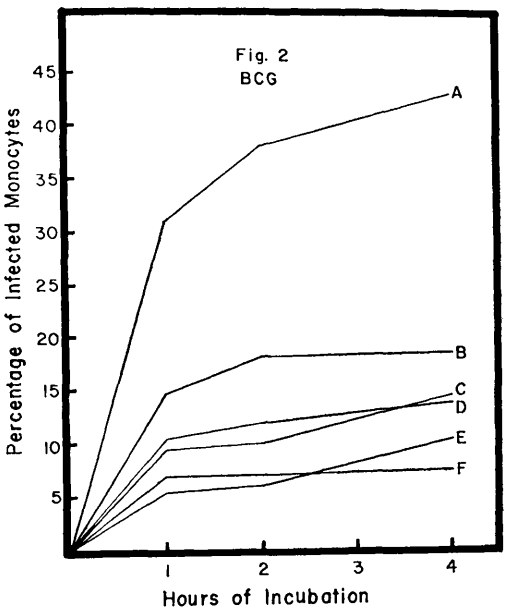

FIG. 2. Phagocytosis of BCG by altered and unaltered mononuclear phagocytes. A—Monocytes with no altering agent; B—monocytes with trypan blue; C—monocytes with cement; D—monocytes with silica; E—monocytes with coal dust; F—monocytes with India ink.

Several reports(6-10,12-14) suggest various reasons for the disseminated nature of tuberculosis in silicotic individuals. Although somewhat limited, these results may suggest that the phenomenon of "blockade" of phagocytic cells could be of importance in helping explain the spread of tuberculosis in individuals exposed to extensive dust inhalation.

Summary. Mononuclear phagocytes which have been altered by ingestion of various particles, take up tubercle bacilli less readily than the same type cell in an unaltered state. This decreased phagocytic activity is observed with both attenuated and virulent tubercle bacilli.

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Tissue Culture Studies on Arbor Viruses of the Japanese B-West Nile Complex.* (23435)

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In October, 1955, a tissue culture section was established at the Virus Research Centre (VRC), Poona, India, with a view to developing tissue culture methods for the study of arthropod-borne (arbor) viruses in India. This communication presents some of the initial results obtained with viruses of the Japanese B-West Nile complex of the B Group of arbor viruses in monkey kidney epithelial cell and chick embryo cell tissue culture. Similar attempts with the Egypt 101 strain of West Nile virus in HeLa, KB, HELL, Detroit 6 (1,2,3,4) and human amnion and chicken kidney cells were unsuccessful in demonstrating significant cytopathogenic effects.

Materials and methods. Viruses: The viruses used in this study were the Egypt 101 strain of West Nile, the P4230 strain of West Nile isolated from the blood of an investigator at the VRC who became ill, presumably from a laboratory infection with West Nile virus(5), the Nakayama strain of Japanese B and the Tamil Nad virus recently isolated from *Culex vishnui* mosquitoes in South India and found to be serologically related to both West Nile and Japanese B(6). Details of the viruses inoculated are given in Table I.

Tissue culture technics: The two types of cell cultures used in this work were *Macacus rhesus* monkey kidney epithelial cells and chick embryo cells. The procedure for preparation of monkey kidney cells was that de-

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