12088

Mobility Velocities of Histiocytes from Normal and from Sensitized Guinea Pigs.*†

MORTON C. KAHN.

From the Department of Public Health and Preventive Medicine, Cornell University Medical College, New York City.

In a previous communication[1] it was shown that the electrophoretic mobility velocities of rough and of smooth avian *Mycobacterium tuberculosis* of the A-I and N strains varied considerably. The organisms from the smooth colonies were considerably more virulent and gave rise to a more rapid velocity across the electrical field. The results of the present communication indicate that the velocities of histiocytes (tissue phagocytes, macrophages, monocytic cells) obtained from guinea pigs sensitized with human *Mycobacterium tuberculosis* of the H37 strain and those from normal animals also differ, the sensitized animals revealing the reverse of that obtained with the smooth type of avian organisms, namely a slower rate on the whole.

The histiocytes were obtained by injecting each animal intraperitoneally with a sterile suspension of aleuronat (500 mg in 15 cc of Ringer's solution) and about 72 hours later one-half of this dose was injected in the same region. Eighteen hours after the second injection the animal was sacrificed by a blow on the back of the neck and the peritoneal cavity opened under sterile precautions. Twenty-five cc of a 25% solution of sterile sucrose solution was then introduced into the cavity by employing a syringe with a piece of glass tubing attached by means of rubber tubing. The animal was gently tilted from side to side to insure thorough mixing of the fluids. Sucrose solution was employed in this experiment because it is non-electrolytic, prevented clotting, and, moreover, hardened the cells so that they did not degenerate under subsequent manipulation. A series of experiments indicated that sucrose in the concentration above mentioned was optimal for the purpose at hand. The exudate and sucrose solution mixture was then centrifuged in a Bolaget Winkle angle centrifuge for some 20 minutes at a speed of about 3000 r.p.m., which was sufficient to deposit the vast majority

* This study is part of a group investigation being carried on in coöperation with the Medical Research Committee of the National Tuberculosis Association.

† With the technical assistance of Ann Sonnentheil.

1 Kahn, M. C., and Schwarzkopf, H., *Am. Rev. Tuberc.*, 1931, **23**, 45.

of the cells. This process was repeated 3 times, adding fresh 25% sucrose solution and thoroughly mixing the cells after each centrifuging. In order to remove as much electrolyte as possible the cells were then washed 4 times by centrifuging employing sterile distilled water.

Prior to these manipulations, vital neutral red preparations were made in order to determine the percentage of histiocytes present in each exudate. The counts varied from 85 to 100%. The exudates of only 3 of the guinea pigs employed gave a count below 95%.

The apparatus and technic for determining the mobility velocities has been previously described.[1, 2] A high dry lens was employed (8 mm 0.50, × 21). All readings were gathered from observations made in the lower level of the apparatus, as this zone was found to be the more constant. The apparatus was filled with 5 separate samples from each peritoneal exudate and only 12 readings were taken from each one. The lower zone level could not be calculated individually for each set-up as the time necessary for this procedure would have permitted many of the histiocytes to sink to the bottom of the glass chamber; therefore, the number of revolutions of the fine adjustment of the microscope was computed several times for each of the 5 glass chambers employed and an average thus arrived at. There was only a negligible variation and constant results were obtained in calibration.

Ten guinea pigs were injected with 0.01 mg of living *Mycobacterium tuberculosis* of the H37 strain. The suspension of organisms was made according to Corper's method by sterilely lifting the growth membrane from Long's liquid medium on to pieces of sterile filter paper, thus allowing the filter paper to absorb as much of the liquid as possible. This partially dried membrane was transferred to a sterile mortar and triturated with about 15 drops of 0.5% sodium taurocholate solution until the mass was reduced to a homogeneous pasty consistence. Ten cc of sterile distilled water was then gradually added and grinding continued further. The suspension was allowed to settle in the mortar and the supernatant fluid pipetted into a test tube and the organisms were allowed to sediment further. The top portion of this fluid containing mycobacteria was transferred to another test tube and exactly one cc of this was pipetted to a sterile weighed watch crystal, evaporated to dryness over a hot water bath and weighed. The suspension was then diluted with sterile physiological saline solution until each cc contained 0.01 mg of organisms. Injections into the guinea pigs were made subcutaneously and sen-

[2] Falk, I. S., and Jensen, L. B., *J. Bact.*, 1928, **15**, 367.

sitization was proven by applying the tuberculin test with purified protein derivative. Nine animals reacted positively and from these a total of 500 histiocytes were tested.

Histiocytes in the sensitized normal animals were called out in the same fashion as already described for the normal animals, and the count as made by the vital neutral red method revealed about the same percentage of cells for each animal. The exudates from 20 normal guinea pigs were employed involving a total of 954 histiocytes.

For both experiments the source of current was two 45-volt Standard Layer-bilt Radio "B" batteries connected in series. This produced 90 volts as elicited by the voltmeter prior to each experiment.

TABLE I.

Velocity Statistics of Histiocytes from Normal and from Sensitized Guinea Pigs.

	Normal sec	Sensitized sec
Range	2.0 to 7.0	2.4 to 6.6
Mean	3.82	4.32
Median	3.80	4.20
Lower Quartile	4.4 to 7.0	5.0 to 6.6
Upper "	2.0 to 3.2	2.4 to 3.6
Avg Dev. from Mean	0.758	0.788
Possible Range of Mean	3.77 to 3.87	4.25 to 4.39
Standard Error	0.024	0.035

Results. The velocities of the histiocytes obtained from both the normal and sensitized animals were measured across an electrical field which measured 360 micra. As far as the 954 cells from the normal guinea pigs are concerned, the velocities ranged from 2.0 to 7.0 seconds. The mean of the speed was found to be 3.82 seconds and the median 3.8 seconds. The lower quartile ranged from 4.4 to 4.0 seconds, while the upper quartile range was from 2.0 to 3.2 seconds. The average deviation from the mean was found to be 0.7581 seconds. The standard error was calculated as 0.0245. The possible range of the mean velocities was 3.77 to 3.87 seconds.

In comparison it is interesting to note the data gathered for the 499 histiocytes from those animals sensitized with *Mycobacterium tuberculosis.* These velocities ranged from 2.4 to 6.6 seconds. The mean was found to be 4.32 seconds and the median, 4.2 seconds. The range of the lower quartile was 5.0 to 6.6 seconds and that of the upper quartile 2.4 to 3.6 seconds. The average deviation from the mean was 0.7884 seconds and the standard error 0.0353. The possible range of the mean velocities of these histiocytes obtained

from the sensitized guinea pigs was found to be 4.25 to 4.39 seconds.

Summary. With the technic described the mobility velocities of histiocytes obtained from normal guinea pigs were compared with those obtained from guinea pigs sensitized with the H37 strain of living *Mycobacterium tuberculosis.* The cells of the infected animals revealed a trend toward a slower rate. Statistically the difference obtained is significant as the range of the mean of the two in no point coincides. These findings may indicate a difference in surface charge.

12089 P

Growth of Louse-Borne Relapsing Fever Spirochetes in Chick Embryo.

KUO-CHING CHEN. (Introduced by Samuel H. Zia.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

It is well known that important biological differences exist between the tick-borne and louse-borne relapsing fever spirochetes. With the former, infection can readily be produced in a large variety of laboratory animals which together with the tick, serve as means of keeping the strain in the laboratory. On the other hand, few animals were found to be susceptible to the louse-borne type, and the lice cannot be used to maintain the spirochetes. Although Meleney[1] was able to produce infection and relapses in squirrels and chipmunks with a Chinese louse-borne strain of relapsing fever spirochetes, splenectomy of these animals has to be resorted to in order to assure complete success. It is, therefore, of more than academic interest to determine if the louse-borne strains can also be grown and passed in the embryonic chick as is possible for the tick-borne spirochetes,[2, 3, 4] and the preliminary results are herewith communicated.

Blood samples were obtained from 7 patients with louse-borne

1 Meleney, H. E., *J. Exp. Med.*, 1928, **48**, 65.

2 Oag, R. K., *J. Path. and Bact.*, 1939, **49**, 339.

3 Chabaud, A., *Bull. Soc. Path. Exot.*, 1939, **32**, 483.

4 Bohls, S. W., Irons, J. V., and Shazo, T. D., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 375.