squamous ependyma of the sacular wall of the infundibulum is secreting hormones. This places the responsibility for vasopressin secretion at this age upon the cells of the hypophyseal floor which are columnar and have considerable cytoplasm.

It is interesting that pituicytes, most frequently given the function of secretion, are thought to be derived from the ependyma as is demonstrated in the bird by Griffiths.[4] It is also interesting that the hypophyseal floor is essentially a modified ependyma. In mid-larval life, both in the normal hypophysis and in functional transplants, the processes of the cells of the hypophyseal floor can be seen. Certain cells appear to be detaching themselves and may be developing pituicytes. Further experimental and cytological studies are in progress upon the development and function of this region.

12087

Bacteriostatic Properties of Histiocytes Toward *Mycobacterium tuberculosis* as Determined by the Single Cell Method.*†

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The fate of *Mycobacterium tuberculosis* within the phagocytic cell has been a subject of great interest to numerous investigators. Cunningham, Sabin and their coworkers[1] believed that the monocyte and clasmatocyte differed in structure, the former becoming changed into the epithelioid cell and in view of its inability to destroy the tubercle bacilli, the organism lived as a parasite within the transformed cell. Sabin and Doan[2] found clasmatocytes from tuberculous rabbits filled with acid-fast debris and concluded that this organism is as freely fragmented by the clasmatocyte as are other ingested particles.

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4 Griffiths, M., *Endocrinology*, 1940, **26**, 1032.

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† This study is part of a group investigation being conducted in conjunction with the Medical Research Committee of the National Tuberculosis Association.

1 Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 231.

2 Sabin, F. R., and Doan, C. A., *J. Exp. Med.*, 1927, **46**, 627.

Borquist and Rowe[3] found no evidence to indicate fragmentation of the organisms by the cell even though some of the mycobacteria had been ingested for many days. They suggested that the longevity of the epithelioid cell rather than the multiplication of the mycobacterium therein accounted for the greater content of organisms as compared with the clasmatocyte. In this connection it is interesting to note that no fragmentation of the phagocytized organism was observed in the present study. Lurie[4] has recently indicated that monocytic cells from sensitized animals are more active in their ability to ingest carbon particles, tubercle bacilli and staphylococci than those cells obtained from normal animals. His experiment was performed *in vitro*.

These investigators arrived at their conclusions by observing microscopically the number and morphology of the organisms ingested by the cell under consideration. This leaves open the important question of the actual effect of phagocytosis upon the viability of *Mycobacterium tuberculosis* after having been engulfed *in vivo*.

Experimental. The phagocyte herein employed will be termed histiocytes (clasmatocytes, tissue macrophage, mononuclear cell). It seems inappropriate to use the term "monocyte" as the exact origin of that cell is at present unknown. In view of the fact that the so-called milky spots are the site of origin of many histiocytes, one would expect that the phagocytic cells found in the peritoneal fluid would be of a histiocytic nature.

The histiocytes were called out by injecting a sterile suspension of 500 mg of aleuronate in 15 cc of Ringer's solution into the peritoneal cavity of normal guinea pigs weighing about 500 g. After 72 hours a heavy homogeneous suspension of *Mycobacterium tuberculosis* was introduced into the same locality. Two strains were employed, namely the H37 and the more virulent H9096 which is also of human origin. All phagocytosis occurred *in vivo*. After 24 hours had elapsed the animal was killed by a sharp blow on the neck and the peritoneal cavity opened under sterile precautions. The fluid was washed out by introducing 15 cc of sterile heparin in Ringer's solution (0.1 g heparin in 200 cc of solution previously heated to 60 degrees for 15 minutes and filtered through a tested Berkefeld candle). Prior tests have indicated that heparin in far stronger concentrations has no effect on the viability of *Mycobacterium tuberculosis*. By employing the neutral red technic, vital stained prepa-

[3] Borquist, M., and Rowe, C., *Am. Rev. Tubere.*, 1931, **24**, 2.

[4] Lurie, M. B., *J. Exp. Med.*, 1939, **69**, 579.

rations were made to determine the number of histiocytes present. Only one of the guinea pigs revealed less than 90%, the average being 93.9%. Films of the peritoneal fluid were then stained by the Ziehl-Neelsen method to ascertain the number of histiocytes which had engulfed the *Mycobacterium tuberculosis.* This range varied from 7% to 78%, the median being 32.5% and the average 34.8%. In one instance 78% of the cells had engulfed the organisms. Fifty percent of the peritoneal exudates revealed a phagocytosis rate of from 24% to 44%. Twenty-five percent of the fluids were found to have 45% to 78% of the cells engulfing organisms, while another 25% revealed a phagocytic index of from 7% to 23%.

By employing the single cell technic previously described[5, 6, 7] the histiocytes were isolated singly in microdroplets of Ringer's-heparin solution slightly tinted with neutral red. The cells were thoroughly washed by sucking up into a micropipette the fluid of the microdroplet surrounding the cell and by applying fresh fluid until no extracellular particles were visible under the oil immersion lens. As a further control, to make certain that the histiocyte had been washed free of unengulfed tubercle bacilli, the last wash fluids applied to the cells just prior to planting, were collected in a single micropipette from all of the microdroplets containing isolated histiocytes, and planted upon suitable medium. Finally, to determine that the technic and reagents employed did not affect the viability of the mycobacteria, a small droplet of the peritoneal fluid from each animal containing free organisms, was also planted.

The washed histiocytes were then collected in a fresh micropipette and planted upon slants of Corper's egg medium.

To determine whether the technic and method employed would support the growth of very few *Mycobacterium tuberculosis,* small numbers of organisms were isolated in microdroplets and counted under the oil immersion lens. The microdroplets were minute enough to be included within the circumference of the lens, and shallow, so that all parts could be focused upon. Moreover, the double micromanipulator was utilized so that one micropipette was in readiness to pick up the counted organisms, just as soon as the bacteria had been isolated by the other. Plants were made upon 111 slants of Corper's medium, and upon 98 of Petroff's. Thirty-two, or 28.8% of the slants of Corper's medium, on which from 2 to 33

5 Kahn, M. C., *Am. Rev. Tuberc.*, 1929, **20**, 150.

6 Kahn, M. C., and Schwarzkopf, H., *J. Bact.*, 1933, **25**, 157.

7 Hotopp, M., and Kahn, M. C., *J. Infect. Dis.*, 1936, **58**, 324.

organisms were seeded, revealed growth, the average number of organisms for each tube was 13. From 2 to 28 organisms were seeded on 98 slants of Petroff's medium, the average number for each being 16.9. Eight of these tubes revealed growth or 8.2%. A similar series was made employing Petragnani medium, the results in our hands being somewhat better than those obtained on Petroff's. When from 25 to 100 tubercle bacilli were planted on Corper's medium, growth was obtained in 48% of the 52 tubes utilized.

This control did not favor the hypothetical bacteriostatic properties of the histiocyte as almost always more than one mycobacterium was observed in the cell, and clumps were frequently encountered. Also it is much easier to pick up a large body such as a histiocyte with the micropipette, and relatively few were lost in the micromanipulation, as must have been the case with the mycobacteria.

Experiments were performed 24 and 48 hours after the tubercle bacilli had been phagocytized. In other words, one plant was made upon the day upon which the guinea pig was sacrificed. The residual peritoneal fluid then being kept over night in the incubator at 37°C, and worked upon the day following.

Results. The following is a brief summary of this problem which has been under investigation for nearly 4 years. In the beginning of the experiment, washed histiocytes from guinea pigs were planted on Petroff's medium involving a total of 4398 cells, 1128 of which had phagocytized *Mycobacterium tuberculosis.* In view of the fact that the control experiments indicated Corper's egg medium to be so much more sensitive when small numbers of unengulfed bacteria were planted, the results obtained on Petroff's medium are considered invalid.

Employing Corper's egg medium, the peritoneal exudates of 32 guinea pigs were used and 4198 washed histiocytes were planted on 101 tubes of medium. The total number of histiocytes planted which had engulfed *Mycobacterium tuberculosis* was 1513 or 34.8% of those planted. The average number of histiocytes planted per tube of medium was 39.3.

It was found that the median percentage of cells engulfing tubercle bacilli was 32.5. The theoretical average number of histiocytes containing mycobacterium was 14 per tube of medium. Fifty per cent of these tubes ranged from 9 to 19 microörganisms each, 25% ranged from 20 to 46, and 25% contained from 2 to 8 tubercle bacilli per tube.

Of the entire 101 tubes planted with histiocytes containing microorganisms only 2 slants or 2% of the total revealed growth; one of

these tubes received a minimum of 36 ingested organisms while the other was seeded with a minimum of 20. Although these tubes received inoculations with numbers of bacteria that were above the average for the control (13 organisms), it must be taken into consideration that during the experiment one tube of medium received a minimum of 36 engulfed tubercle bacilli, 3 tubes received at least 40 organisms each and one was inoculated with at least 46 intracellular mycobacteria. None of these cultures gave rise to growth.

The mycobacteria contained within 3327 histiocytes were counted to determine the average number of organisms per cell. Twenty-eight percent of these histiocytes had engulfed clumps which were very conservatively estimated as 5 bacteria per clump. The number of *Mycobacterium tuberculosis* engulfed by the other 2311 histiocytes ranged from one to 10, the average being 3.5. Therefore, it will be observed that the results presented are of interest because in each case the conclusions are based upon counting only one organism per histiocyte, according to the phagocytic index.

After each day's work the wash waters left in all of the microdroplets which had contained histiocytes were collected in a micropipette and planted upon tubes of Corper's medium. This was done to test for the presence of unengulfed bacteria. Of the 101 tubes employed for such purpose, all remained sterile. Contrasted with this we find that of 32 tubes of media inoculated with a small droplet of unmanipulated peritoneal fluid, 31 revealed growth.

Summary. Under the experimental conditions described, it would seem that the histiocytes of normal guinea pigs are in many instances capable of exerting at least bacteriostatic properties toward engulfed *Mycobacterium tuberculosis* of the human variety when phagocytosis occurs *in vivo*. Of 101 tubes of Corper's egg medium seeded with a total of 4190 histiocytes, 1513 or 34.8% which had engulfed the mycobacteria, growth was obtained in only 2 instances, or 2%. When comparably small numbers of free *Mycobacterium tuberculosis* were inoculated upon the same medium, growth was obtained in 28.8% of the tubes employed. As no fragmentation of the bacteria within the histiocytes was observed, the bacteriostatic action exerted may be possibly explained as due to a disturbance in the metabolism of the engulfed *Mycobacterium tuberculosis*.