CURRENT LITERATURE

It is intended that the current literature shall be dealt with in this department. It is a function of the Contributing Editors to provide abstracts of all articles published in their territories, but when necessary such material from other sources is used when procurable.

Special Section

The Lysosomes

Here follow several brief abstracts of available articles, all but one from the Rockefeller Institute in New York and the other from the Mayo Clinic, that deal with the lysosome elements of the leucocytes (the granules of the neutrophils and eosinophils), and of the macrophages. One of the last two is of particular interest because of the number and excellence of the electron micrographs showing the effects of phagocytosis (of zymogen particles), and the last is of special interest because it deals with the ultracytology of experimental infections with acid-fast bacilli. These "collateral" abstracts are intended to supplement, by specific observations, one of the editorials and a review that appear in this issue of The Journal.—H.W.W.

Cohn, Z. A. and Hirsch, J. G. The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leucocytes. J. Exper. Med. **112** (1960) 983-1004.

This report is the first of the series by this group of workers in which the granules, the major particulate element of the polymorphonuclear leucocytes, were recognized to be of lysosome character. The earlier studies [*J. Exper. Med.* **110** (1959) 419-443; **111** (1960) 667-687; and **111** (1960) 689-704] were of other features and functions, the last of these dealing with the effects of bacterial lipopolysaccharide endotoxins. The granules obtained from peritoneal exudates were isolated and investigated with respect to, among other things, the hydrolytic enzymes they contain. After separation, they were disrupted (by acid, or saponin, or repeated freezing and thawing) and studied chemically for their various constituents. After sectioning the granule pellets obtained by centrifuging, electron microscopy showed the particles to be analogous to the lysosomes of the rat liver described by de Duve. One of the electron micrograms shows the granules in the pellet, the other in the leucocyte; in both instances they vary considerably in size, shape, and density. In the pellet section most of them are homogeneous, but some appear as more or less empty rings some of which are broken; it is in these forms that the enclosing membrane is most clearly demonstrated.

Hirsch, J. G. Cinemicrophotographic observations on granule lysis in polymorphonuclear leucocytes during phagocytosis. J. Exper. Med. **116** (1962) 827-833.

This is a particularly interesting report of work in which human, rabbit, and chicken leucocytes were used; the leucocyte granules of the last species are large enough to permit detailed study by the light microscope. Phase contrast was used, and the pictures were taken at the rate of 10 per second; rupture of a granule may occur in 1/10th second or less. The hypothesis adopted is that there is fusion of the membrane of the neighboring granule with that surrounding the ingested body (i.e., the invaginated portion of the cell wall) and discharge of the granule content directly into the phagocytic vacuole [called by some the phagosome]. The granules are said to "resemble

closely" the lysosomes of de Duve. [The 7 plates of photographs showing sequences (usually 8 or 9 shots on each) are fascinating.]

ARCHER, G. T. and HIRSCH, J. G. Isolation of granules from eosinophil leucocytes and study of their enzyme content. J. Exper. Med. **118** (1963) 277-285.

Eosinophils were studied in the same way that polymorphonuclear leucocytes had been, with respect to the functions of their particularly large granules. Those obtained from horse blood (as pictured) are huge, and only about 40 per cell; those of the rat eosinophils are relatively tiny, 250 per cell. In neither kind does this structure resemble, on electron microscopy, that shown by the granules of human eosinophils, in which there is an unexplained crystalline structure in a homogeneous matrix. The enzymes were found to differ from those of the neutrophil granules in having a high content of peroxidase (bound to the insoluble residue of the granules), and in the absence of the bacteriolytic agents lysozyme and phagocytin. It is concluded that eosinophil granules are lysosome-like structures. (Also [*J. Exper. Med* **118** (1963) 287-293] that eosinophils are phagocytic cells, and that any special functions that distinguish them from other phagocytes remain to be discovered.)

COHN, Z. A. and WIENER, E. The particulate hydrolases of macrophages. I. Comparative enzymology, isolation and properties. J. Exper. Med. **118** (1963) 991-1008.

Rabbit macrophages were used in this study, which was prompted by previous work with leucocytes and by that of de Duve on rat-liver particles. The macrophages were of three varieties: peritoneal, normal alveolar, and alveolar after stimulation by dead BCG. The last were by far the most abundant, most varied in size and morphology, and evidently the most interesting. Variations apparently depended upon environment and stimulus. Some of the enzymes were most abundant in the particles from the BCG-induced cells; they were least in the peritoneal cells. The particles within the cells took up neutral red avidly, and retained it strongly after separation of the particles as long as they were intact. (Mitochondria, when present, were evidenced by cytochrome oxidase; they were not stained by neutral red.) The macrophage particles lack any bactericidal material. Besides "primary lysosomes," many of the granules may represent "phagosomes" or "residual bodies." Electron micrograms of BCG-induced macrophages demonstrate the great heterogeneity of the granule structures, including myelin bodies resulting from undigestible substance [probably, according to Bessis, fatty material].

COHN, Z. A. and WIENER, E. The particulate hydrolases of macrophages. II. Biochemical and morphological response to particle ingestion. J. Exper. Med. **118** (1963) 1009-1019.

This report is concerned with the fate of the organelles in BCG-induced alveolar macrophages (previously described) following ingestion of heat-killed microorganisms (*Escherichia coli* and *Staphylococcus albus*), and also zymosan particles, under various conditions. For example, in one experiment, immediately after addition of *E. coli* to a suspension of the macrophages, almost all of the enzymes were in the hydrolase particles, but after 90 minutes about 60% of the total activity was in the soluble phase (because of break-down of the membranes of the particles). Morphologically, after ingestion of zymosan by neutral red-stained macrophages, in which the color is confined to the hydrolase particles, the color was transferred from those particles and preferentially localized in the vacuole around the phagocytosed zymosan particles; ultimately it disappeared from them, leaving the macrophages degranulated. There was also a loss of acid phosphatase-positive granules after phagocytosis. These findings are consistent with the concept of granule lysis as a concomitant of the phagocytic event. Apparently the granule contents are liberated, not into the general cytoplasm, but

into the vicinity of the phagocytic vacuole that surrounds all phagocytized material. It must be assumed that, in the homogenization of the cell, these phagosome-lysosome complexes are ruptured with the liberation of soluble enzymes. Once a foreign substance has been phagocytosed, its fate may depend upon its susceptibility to the macrophage enzymes. Undigestible materials, e.g., carbon particles, are retained indefinitely; and thus may be explained certain features of the lesions of tuberculosis and leprosy.

ZUCKER-FRANKLIN, D. and HIRSCH, J. G. Electron microscope studies on the degranulation of rabbit peritoneal leukocytes. J. Exper. Med. **120** (1964) 569-575.

A further study of the degranulation process, mostly in neutrophils, with respect to phagocytosis of zymosan, under which conditions three types of granules are seen. In the fusion of the granule with the phagosome the membranes of the two bodies fuse and maintain continuity, thus preventing the enzymes from gaining access to the cytoplasm. The substance of the granule at first retains its rounded form, but later it becomes confluent and eventually surrounds the phagocytosed organism. There must be a process, the reverse of phagocytosis, for the discharge of potentially injurious material from the cells without disruption of the cell membrane. This process, which the authors call "exoplasmosis" [certainly a more elegant term than "defecation" as used by de Duve] is not seen in normal cells, i.e., those without phagocytosis. [The electron micrograms that illustrate this article are particularly excellent and well worth study.]

MERCKX, J. J., BROWN, JR., A. L. and KARLSON, A. G. An electron-microscopic study of experimental infections with acid-fast bacilli. American Rev. Resp. Dis. **89** (1964) 485-496.

The bacilli used were mainly *M. tuberculosis* (H37Rv) and the nonchromogenic Battey bacillus (B41); each of them injected intravenously into 52 mice. Of each group, 2 animals were killed at almost daily intervals until the 16th day, and at progressively longer intervals thereafter. In the electron micrographs of ultra-thin tissue sections, it was not possible to distinguish between these bacilli, or between them and others also used. In the lung lesions of the H37Rv mice, clearly identifiable organisms were not seen until the 31st day, although in impression smears from the fresh lesions, and in night blue-stained thick sections of the plastic blocks, bacilli were to be seen with the light microscope from the outset. [The failure of earlier demonstration in the ultra-thin sections is not discussed.] The bacilli as seen were surrounded, usually singly but sometimes in multiples, by an electron-transparent zone enclosed in a thin membrane, as has been described for other mycobacteria, including *M. leprae*. Some—most numerous in the B41 animals—were embedded in what were taken to be lysosomes, probably of the same nature as the "electron-opaque bodies" described in leprosy lesions. One kind of inclusion (the "X-body") was found only in pulmonary lesions. It was composed of a loosely laminated, peculiarly fragmented, osmiophilic material, with no resemblance to the "onion-like bodies" described by Nishiura in leprosy lesions. A second type of inclusion, the "Y-body," was smaller, more homogeneous, frequently vacuolated, and less opaque to electrons. In the terminal stage of the infection they were found extracellularly in large numbers. These structures, and certain others, may all be lysosomes in different states of activity. Finally, late in the infection—most often in the B41 mice—there began to appear, bare in the cytoplasm and not within "bags," bacilli that contained "spherules." *In vivo* these are apparently released by rupture of the bacilli, some then becoming embedded in opaque bodies "(lysosomes?)." Spherules may be homogeneous or finely granular, or may contain solid little electron-opaque granules, or filaments that appear as irregular skeins. They were particularly prominent in regressing lesions in the liver and spleen. *In vitro* with the B41 organisms (in Proskauer-Beck liquid medium) spherules develop very quickly, within 24 hours, and later the cultures may show only these forms.—H.W.W.