dogs with corresponding values for basal and histamine-stimulated specimens revealed that outputs of pepsin for the mucinous secretion collected during the first 2 hours after IAA application were considerably higher (at least 2-fold) than for these other types of secretion. This observation may be significant in regard to the problem of whether histamine acts as a stimulus to pepsin as well as to acid secretion, or whether the enzyme is merely "washed out" by the parietal fluid. A study of enzyme response to histamine injected simultaneously with topical application of IAA may supply important evidence toward solving this problem.

1. Hollander, F., *Am. J. Physiol.*, 1956, v187, 231.
2. ———, *Nature*, 1958, v181, 847.
3. Hollander, F., Weinstein, V. A., *Fed. Proc.*, 1956, v15, 95.
4. Hollander, F., *J. Biol. Chem.*, 1934, v104, 33.
5. Hollander, F., Lauber, F. U., Stein, J., *Am. J. Physiol.*, 1947, v149, 724.

Received Sept. 12, 1960. P.S.E.B.M., 1960, v105.

Ultrastructural Abnormalities In Whipple's Disease.* (26126)

ALAN S. COHEN, ELIHU M. SCHIMMEL, PETER R. HOLT AND KURT J. ISSELBACHER
Departments of Medicine, Harvard Medical School and Massachusetts General Hospital

Whipple's Disease has been recognized with increasing frequency as one of the clinical entities associated with intestinal malabsorption. The underlying etiology has remained obscure but the histopathology of this disorder has been defined more accurately as a result of a variety of technical advances. Whipple(1) noted a prominence of fatty deposits in extracellular spaces as well as within tissue mononuclear cells. He also found many macrophages with foamy cytoplasm which did not stain for fat. Black-Schaffer subsequently demonstrated that these latter cells stain strongly with the periodic acid-Schiff (PAS) stain(2). His observations have been amply confirmed, and the occurrence of these PAS positive cells in the connective tissue throughout the body has been appreciated(3,4,5).

The introduction of peroral biopsy instruments(6,7) has made possible the histologic examination of normal and diseased small intestinal mucosa unaltered by post-mortem autolysis. This technic also permits tissue to be obtained rapidly enough for fixation and preservation of the fine structure for electron microscopic studies. This is a preliminary communication of light and electron microscopic findings in an intestinal biopsy from a patient with previously proven Whipple's Disease.

Methods. A fasting jejunal biopsy was obtained by means of a Crosby capsule from a 65-year-old male with known Whipple's Disease.† Portions of the specimen were fixed within one minute after biopsy in buffered osmium tetroxide(8) and in Dalton's solution (9), then dehydrated and embedded according to standard procedures(10).

Portions of the biopsy were also fixed in formalin for light microscopy. Sections of the osmium fixed as well as formalin fixed material were stained with the periodic acid-Schiff reagent. Thin sections were examined in an RCA-EMU 2B electron microscope at original magnifications of 1200 to 10,000 ×.

Results. By light microscopy the lamina propria of the biopsy specimen was found to contain a dense population of macrophages filled with PAS positive granules (Fig. 1). The granules appeared to be smaller in the cells adjacent to the lining of the epithelial

* This is publication No. 284 of Robert W. Lovett Memorial Unit for the Study of Crippling Disease. Supported by grants from Nat. Inst. of Arthritis and Metab. Dis., Nat. Inst. Health, U.S.P.H.S.

† This patient was Case 46112, Case Records of Mass. Gen. Hosp., *New England J. Med.*, 1960, v262, 572.

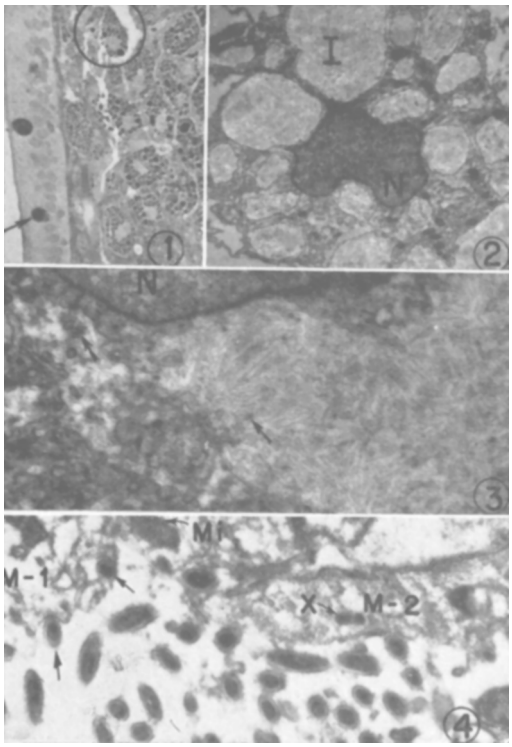

FIG. 1. Periodic acid-Schiff (PAS) stain of osmium material obtained by peroral intestinal biopsy. The lamina propria is filled with macrophages that contain numerous PAS positive granules. Mucus secreting cells in epithelium (arrows) also are strongly PAS positive. One macrophage, comparable to that in the circle, is seen in Fig. 2. ×225.

FIG. 2. Electron micrograph of a macrophage which, by light microscopy, was loaded with PAS positive granules. The large pale inclusions (I) indent nucleus (N) and fill cell cytoplasm. Within these inclusions are fine membranous structures. ×2800.

FIG. 3. Electron micrograph of a macrophage inclusion body adjacent to nucleus (N). Within the inclusion are a series of membranes, vesicles and dense granules (arrows). ×9000.

FIG. 4. Electron micrograph of cell borders of 2 adjacent macrophages (M-1 and M-2) that were located immediately subjacent to epithelium. Ovoid bodies (arrows) with outer limiting membranes, central dense areas enclosing a small pale core impinge upon cytoplasm. At the arrow at extreme left an ovoid body is seen to indent the cell membrane, while that to its right is almost completely enclosed by 2 arms of cytoplasm. A mitochondrion (M1) is seen in one macrophage and a dense body (X) without an outer limiting membrane can be observed in cytoplasm of the second macrophage. ×10,800.

cells than in those lying deeper in the lamina propria.

Successive thick and thin sections cut on the Porter-Blum microtome allowed identification in the electron microscope of the PAS positive inclusions of light microscopy. These inclusions were complex and varied in structure. In general, they consisted of series of closely packed membranes, granules, and vesicles (Fig. 2). The membrane-containing inclusions varied in size, and though they usually appeared to be discrete and separate, in several areas possible interconnecting channels were visualized. The majority of the large inclusions contained short curved membranes. The small granules were sometimes seen along the membranes and in other instances were formed in small clusters. Vesicles and tubules were also present (Fig. 3). Other less finite structures, some dense and some amorphous appeared in these large inclusion bodies.

Along the edges of the macrophages, rare microvilli were observed. Dense round, oval and rod shaped bodies were seen in close apposition to these cells, especially, but not solely near the epithelial lining. These dense bodies had a discrete structure with an outer limiting membrane, a less dense "jacket," and inner dark body with a pale core (Fig. 4). In several sections these bodies indented the cell border, were surrounded by cytoplasm, and finally appeared singly or in groups as fully "phagocytized" particles. Their average outermost diameter measured 250 mμ.

The "brush border" of the epithelial cells of the biopsy specimen had normal appearing microvilli, whose average length was 750 mμ and diameter was 100 mμ.

Discussion. In Whipple's disease the principal changes recognized in the small intestinal mucosa consist of dilation of lymphatic spaces, and accumulation of foamy macrophages in the lamina propria. Since the occasional lipid-filled macrophages are rather non-specific, it is the non-lipid, PAS positive material which is considered characteristic of the disease.

The present observation that PAS positive material represents a complex membrane system is of interest in the light of what is known of the biochemistry of the PAS reaction and previous electron microscopic observations on other PAS positive material. It

was first believed that the positive PAS reaction of the macrophage indicated the certain presence of glycoprotein. However, since this reaction depends upon oxidation of 1,2 glycol groups (as in carbohydrates) to form aldehydes, not only glycoprotein, but other substances containing unsaturated lipids, phospholipids, etc. would be expected to react. Thus the PAS reaction *per se* does not provide positive identification of the reacting material(11).

A number of electron microscopic observations have also demonstrated the diversity of PAS positive material. For example, PAS positive mucin in the epithelial cells of the intestinal border have a grey, homogeneous and structureless appearance, while large round PAS positive bodies of fetal kidney contain both amorphous material and concentric lamellar structures(12). In the present study, the large PAS positive inclusion body seemed to contain a variety of forms, *i.e.*, large and small granules, vesicles and membranes, the last predominating.

Although macrophage fine structure has occasionally been commented on, few detailed observations are available. It has, however, been observed that phagocytized material of many different types contains laminated structures ("myelin figures") when viewed in the electron microscope(13,14,15,16). However, it appears that the present large inclusion bodies with their sinuous membranes and granules are a unique finding in this patient with Whipple's disease. It will be important to determine whether these inclusions represent abnormal products of the metabolism of the cell itself or are the result of ingestion of an unusual substance.

The nature of the dense bodies observed at the macrophage cell border is unknown. Their increased numbers near the epithelium are suggestive of material being absorbed from the intestine and engulfed by the macrophages. Although the fasting state of the patient does not rule this out, the particles do not have the characteristics of absorbed fat as described by Palay(17,18). Although virus particles and inclusion bodies have been observed with dimensions and appearance similar to the present dense bodies(19,20), no conclusion concerning the possibility that these particles represent a virus can be drawn from the electron microscopic data presented. Further investigations are being carried out.

In several preivous studies of intestinal mucosa in patients with malabsorption due to non-tropical sprue, abnormally short microvilli have been observed by electron microscopy(21,22). It is interesting that in this patient with Whipple's disease and malabsorption, the microvilli in the samples studied were of normal dimensions, suggesting that malabsorption may occur in presence of normal microvilli.

Summary. A fasting jejunal biopsy specimen, obtained from a patient with Whipple's disease, was studied by light and electron microscopic technics. Periodic acid-Schiff positive inclusions in the macrophages were found to have a complex ultrastructure consisting of a series of membranes, vesicles and granules. Dense bodies of unknown nature were observed in close approximation to the macrophage cell border, and within its substance. The virus-like character of these particles has been discussed.

1. Whipple, G. H., *Bull. Johns Hopkins Hosp.*, 1907, v18, 382.

2. Black-Schaffer, B., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 225.

3. Jones, C. M., Benson, J. A., Roque, A. L., *New England J. Med.*, 1953, v248, 665.

4. Puite, R. H., Tesluk, H., *Am. J. Med.*, 1955, v19, 383.

5. Sieracki, J. C., *Arch. Path.*, 1958, v66, 464.

6. Shiner, M., *Lancet*, 1956, v1, 17.

7. Crosby, W. H., Kugler, H. W., *Am. J. Digest. Dis.*, 1957, v2, 236.

8. Palade, G. E., *J. Exp. Med.*, 1952, v95, 285.

9. Dalton, A. J., *Anat. Rec.*, 1955, v121, 281.

10. Farquhar, M. G., *Lab. Invest.*, 1956, v5, 317.

11. Pearse, A. G. E., *Histochemistry*, 2nd Edit., 1960, Little, Brown and Co., Boston.

12. Clark, S. L., Jr., *J. Biophys. and Biochem. Cyt.*, 1957, v3, 349.

13. Policard, A., Bessis, M., Breton-Gorius, *J. Exp. Cell. Res.*, 1957, v13, 184.

14. Schulz, H., *Beitr. Path. Anat.*, 1958, v119, 71.

15. Karrer, H. E., *J. Biophys. and Biochem. Cytol.*, 1958, v4, 693.

16. ———, *ibid.*, 1960, v7, 357.

17. Palay, S. L., Karlin, L. J., *J. Biochem. and*

Biophys. Cytol., 1959, v5, 363.

18. ———, *ibid.*, 1959, v5, 373.

19. Burnet, F. M., Stanley, W. M., *The Viruses*, 1959, v3, Academic Press, New York.

20. Luse, S. A., Smith, M. G., *Ann. N. Y. Acad. Sci.*, 1959, v81, 133.

21. Zetterqvist, H., Hendrix, T. R., *Bull. Johns Hopkins Hosp.*, 1960, v106, 240.

22. Hartman, R. S., Butterworth, C. E., Jr., Hartman, R. E., Crosby, W. H., Shirai, A., *Gastroenterology*, 1960, v38, 506.

Received Sept. 21, 1960. P.S.E.B.M., 1960, v105.

Influence of Total Body X-irradiation on Tumor Induction by Parotid Tumor Agent in Adult Mice. (26127)

L. W. LAW AND C. J. DAWE

Laboratories of Biology and Pathology, National Cancer Institute, N.I.H., Bethesda, Md.

The parotid tumor agent, (PTA, polyoma virus), has certain limitations of action in several parameters, such as species, age, mouse strain, and tissue susceptibilities and type of response by different cells or by cells of the same morphogenetic lineage infected at different levels in their development. Differences in cell sensitivity and specificity of response to PTA have been discussed(1).

In a previous study(2), sensitivity in certain strains of mice to PTA was demonstrable through the first 14 days *post partum* without appreciable lengthening of the latent period. Beyond this age the response has not been adequately studied. Preliminary data from our laboratory indicated that sensitivity was increased in 1 to 2-month-old mice given whole-body X-radiation prior to injection of virus. The present report concerns the response of adult mice of several age groups to PTA, influence of whole-body X-irradiation on this response and a histologic comparison of the tumors and lesions in mice inoculated as adult or as newborns.

Methods and materials. Mice. C3Hf/$_{BiLw}$ mice from our pedigreed colony were used. This strain was obtained directly from Dr. J. J. Bittner in 1955 and has been inbred continuously since that time, brother x sister. Frequency and types of neoplasms in a sample of mice of this subline have been reported (2). Parotid tumors within the breeding colony or following acute whole-body-X-irradiation have been observed only very rarely (3), despite a high frequency of serologic convertors(4). Age of these mice at radiation and injection of virus is given in the results.

Virus. PTA has been passaged in the continuous tissue culture line derived from a lymphocytic leukemia, designated P-388D_1. The origin of virus and cell strain have been described(2,5). Source of virus was the tenth serial passage obtained by collecting tissue culture fluid at time of maximum CPE. This material had been stored at −70°C for 8 months prior to use here. The HA titre of the 10-fold diluted material, used for intravenous inoculations in adult mice was 1:40. Intravenous injection of 0.5 ml of virus was accomplished in Groups 1 and 2 (Tables I and II) through the lateral tail vein. This represented an inoculum of $10^{4.9}$ adult mouse ID_{50}/mouse as determined by the MAP test(6). Although HA titre and mouse ID_{50} are relatively low, this preparation has proved to be highly oncogenic and primarily thymotrophic, inducing thymic epithelial tumors in nearly all mice inoculated at birth (7).

The adult C3Hf/$_{BiLw}$ mice in the third group, (Table IV) received either tissue culture explants of salivary gland tissue or tissue culture fluid plus cell scrapings from these cultures. Explants of salivary gland tissue were exposed to tenth passage P-388 D_1- passaged PTA prior to explanting as gelatin-sponge-matrix cultures as previously described(8). Transfers of explants or tissue culture fluids were made on the 48th day. (See(8) for morphologic changes observed *in vitro* in these intact fragments of salivary