The Ingestion of Proteins and Colloidal Materials by Columnar Absorptive Cells of the Small Intestine in Suckling Rats and Mice*

By SAM L. CLARK, Jr., ‡ M.D.

(From the Department of Anatomy, Washington University School of Medicine, St. Louis)

PLATES 14 TO 21

(Received for publication, August 11, 1958)

ABSTRACT

Proteins and colloidal materials, administered orally to suckling rats and mice, were ingested by columnar absorptive cells of the jejunum and ileum, but not of the duodenum. Bovine gamma globulin and ovalbumin were identified in the apical cytoplasm by staining with fluorescent antibody; trypan blue, Evans blue, saccharated iron oxide, and colloidal gold were detected intracellularly by their color, specific staining, and appearance in the electron microscope. Each substance was segregated in membrane-enclosed vacuoles, apparently part of a system of potentially interconnecting vacuoles and tubules in the apical cytoplasm which is continuous in places with the apical cell membrane. We postulate that ingestion of foreign materials was accomplished by pinocytosis, that is, by invagination of the apical cell membrane to form vacuoles containing material from the intestinal lumen.

Approximately 18 days after birth columnar absorptive cells lost the ability to ingest proteins and colloids, and no longer contained large vacuoles and numerous tubules. At this age rats and mice lose the ability to absorb antibodies from the intestine in an immunologically intact form, and we conclude that cellular ingestion is part of the mechanism of absorption of intact proteins in suckling animals. Particulate fat apparently is absorbed in both newborn and adult animals by micropinocytosis. Thus adult animals may not have lost the capacity for pinocytosis, but rather have become selective as to what substances provoke it.

Cortisone acetate, administered subcutaneously to rats 8 to 10 days old alters the columnar absorptive cells within 72 hours so that they resemble the cells in adult animals and no longer ingest proteins.

Mammals too young to synthesize antibodies acquire passive immunity either during gestation by the transfer of maternal antibodies across the fetal membranes, or after birth by the intestinal absorption of milk globulins (reviewed by Brambell, Hemmings, and Henderson, 1951). Rabbits, guinea pigs, and man apparently receive maternal antibodies only before birth, whereas domestic ungulates obtain them exclusively by absorption through the small intestine during the first 24 to 48 hours after birth (Deutsch and Smith, 1957). The route of absorption from the small intestine is by way of the intestinal lymphatics and thoracic duct, from which antibodies may be recovered within 1 or 2 hours after oral administration. Antibodies do not appear to enter the portal blood directly (Comline, Roberts, and Titchen, 1951). Dogs, rats, and mice receive maternal antibodies both before and after birth, but the greater part of the transfer occurs in the intestine during the first 2 to 3 weeks after birth. Suckling rats and mice absorb not only maternal antibodies, but, in experimental situations, globulins derived from a variety of species. However, these heterogenous globulins are not absorbed so completely as homogenous globulins, and they interfere with

* This investigation was supported, in part, by Grant RG3784 from the National Institutes of Health, United States Public Health Service.

‡ Lowell M. Palmer Senior Fellow in Anatomy.

the absorption of homogenous globulins when the two are fed simultaneously (Halliday, 1957; Morris, 1957; Brambell, Halliday, and Morris, 1958). Approximately 3 weeks after birth, the rat intestine loses the ability to absorb intact proteins (Halliday, 1956).

The columnar absorptive cells of the small intestine in suckling calves, lambs, piglets, and kittens contain large droplets with the staining characteristics of colostrum during the period when antibody absorption occurs (Smith, 1925; Comline, Roberts, and Titchen, 1951; Comline, Pomeroy, and Titchen, 1953; Hill and Hardy, 1956). Similar material can be found in the intestinal lumen and in the lacteals. The droplets disappear at the age at which absorption of antibody ceases, hence it has been concluded that droplet formation is a stage in the absorption of intact protein by the intestine. Von Möllendorff (1924) has demonstrated that a variety of acid dyes accumulate as large inclusions in the epithelial cells of the jejunum and ileum of young rats when administered either enterally or parenterally, whereas they produce only a few small inclusions when administered to rats more than 3 weeks old. Thus the ability of newborn animals to absorb intact proteins is correlated with a capacity for ingestion of proteins and vital dyes by the columnar absorptive cells of the small intestine.

Moog (1953) has demonstrated that the alkaline phosphatase activity of the small intestine is relatively low in newborn mice, and rises to adult levels between 2 and 3 weeks after birth. She was able to cause a precocious elevation of activity by the administration of adrenal cortical hormones to mice during the 2nd week after birth. Halliday (1958) has reported that adrenal cortical hormone also will produce precocious cessation of antibody absorption in suckling rats and mice.

We have investigated the response of the mucosa of the small intestine in rats and mice to the oral administration of several proteins and colloidal materials, using fluorescent antibody staining to locate the proteins within the cells, and the high resolution of the electron microscope to determine the relationships of these adventitious materials to cell organelles. Changes in structure and responsiveness of the mucosa with age and after the administration of cortisone have been studied in an effort to elucidate the cellular mechanism by which, in adult animals, selective exclusion of large molecules and colloids is achieved.

Materials and Methods

More than 150 albino rats and 150 Swiss albino mice, bred in the laboratory and fed Purina dog chow, were used in these experiments. The proteins, which were administered to rats 2 to 27 days of age, included purified bovine gamma globulin (fraction II), crystalline bovine albumen, and crystalline ovalbumin, all prepared by Nutritional Biochemicals Corporation; as well as fresh preparations of rat and rabbit plasma and rat whole blood, prevented from coagulating by heparin. The colloidal materials, which were administered to both rats and mice, included India ink, trypan blue, Evans blue, saccharated iron oxide, and colloidal gold (aurcoloid-Abbott, a saline suspension of Au^{198} which had been stored until it was no longer radioactive). All of these materials, suspended in 0.9 per cent sodium chloride solution at concentrations of 1 to 5 per cent, were administered in volumes of ¼ to 1 ml., depending upon the size of the animal, by passing fine polyethylene tubing down the esophagus without anesthesia. It was found useful to separate suckling animals from their mothers for several hours prior to intubation to clear the intestine of milk. One-half to forty-eight hours after intubation the animals were decapitated and short lengths of small intestine obtained for study. Duodenum was obtained approximately 1 cm. from the pylorus, jejunum from the midpoint of the small intestine, and ileum from the distal quarter of the small intestine.

For electron microscopy, the tissues were fixed approximately 1 hour at room temperature in buffered 1 per cent osmium tetroxide (Palade, 1952) containing 3.5 per cent sucrose, dehydrated rapidly in graded ethyl alcohol-water mixtures, imbedded in a 6:1 mixture of butyl and methyl methacrylate polymerized with benzoyl peroxide at 60°C., sectioned with glass knives in a Porter-Blum microtome (Porter and Blum, 1953), and examined in an RCA model EMU 2E electron microscope with a compensated objective lens and a 40 micron objective aperture. Sections 1 to 4 microns thick, prepared in the same way, were examined by phase contrast microscopy.

For staining with fluorescent antibody, the tissues were freeze-dried, imbedded in butyl methacrylate *in vacuo*, polymerized as described above, sectioned at 2 to 3 microns using glass knives in a Porter-Blum microtome, transferred to glass slides by floating on water, dried at 37°C., and immersed in ethylene dichloride for 1 hour to remove the plastic prior to staining. Sections prepared in this manner were used also for histological staining.

Fluorescent antibodies against bovine gamma globulin and ovalbumin, generously provided by Dr. Jack Davies, were prepared by injecting rabbits subcutaneously with saline suspensions of the proteins emulsified with the adjuvant recommended by Moloney and Coval (1955). After several weekly injections of

100 mg. of antigen, serum was obtained by heart puncture and a globulin fraction prepared by precipitation with half-saturated ammonium sulfate and dialysis against 0.9 per cent sodium chloride solution. The globulin fraction was conjugated with fluorescein isocyanate according to the method of Coons and Kaplan (1950), and absorbed with rabbit liver powder to prevent non-specific staining (Coons, Leduc, and Connolly, 1955). Sections of freeze-dried tissue were stained with a drop of fluorescent antibody solution for 15 minutes in a humid atmosphere, washed for 10 minutes in neutral 0.9 per cent sodium chloride solution, and mounted in glycerin adjusted to pH 7. Fluorescence microscopy was performed using a mercury vapor arc as light source (Scopicon, Inc., Chauncey, New York), a cardioid condenser, and the filters recommended by Coons and Kaplan (1950). The fluorescent staining obtained was judged to be specific because it could be prevented by prior absorption of the antibody preparation with antigen, and it did not occur unless the antibody used for staining corresponded to the antigen administered to the animal.

OBSERVATIONS

The proteins and colloidal materials used in these experiments, when administered by stomach tube to suckling rats and mice, appeared in vacuoles in the supranuclear cytoplasm of columnar absorptive cells of the small intestine. Animals more than 18 days old failed to develop such inclusions, and did not respond in any detectable way to the materials administered. No part of the intestinal mucosa other than the columnar absorptive cells was observed to respond to proteins and colloids in suckling animals, and our observations of goblet cells, Paneth cells, argentaffin cells, and the chief cells of the crypts corroborate those of other investigators (Palay, 1958; Hally, 1958; Christie, 1955). In newborn animals the lamina propria appeared to be devoid of plasma cells, which did not appear until at least 3 weeks after birth.

The columnar absorptive cells of the small intestine in adult animals appeared to us, by both light and electron microscopy, to fit the descriptions of previous investigators (Macklin and Macklin, 1932; Dalton, 1951, Weiss, 1955; Zetterqvist, 1956; Sjöstrand and Zetterqvist, 1957), and we have detected no differences between the cells of rats and mice (Figs. 1 to 4). The striated cuticular border consists of closely packed, uniform microvilli which are projections of the apical cell membrane. It covers a subcuticular zone lacking the usual cytoplasmic organelles but containing irregular strands of a homogeneous material which extend into the microvilli of the striated border. This region is traversed by small tubular membranes which occasionally are seen to be continuous with the apical cell membrane. One or two terminal bars mark the junction between subcuticular zones of adjacent cells and form an apparently continuous barrier between the intestinal lumen and the intercellular space. The apical cytoplasm contains filamentous mitochondria, strands of ergastoplasm, and small membranous vesicles devoid of ergastoplasmic granules. The vesicles may contain lipide droplets identifiable by their osmiophilia. Small vacuoles and dense bodies containing parallel arrays of membranes are found occasionally. A characteristic Golgi complex occupies the supranuclear region and frequently contains lipide droplets within its membranous sacs. There is a basal cytoplasmic region which contains mitochondria and ergastoplasm, as well as occasional lipide droplets. The space between adjacent epithelial cells varies in appearance. The lateral cell membranes may be folded and interlocked with those of adjacent cells, or the intercellular space may be dilated and extend from the terminal bars to the thin but continuous basement membrane upon which the epithelium rests. Lipide droplets frequently fill the intercellular space as well as the extracellular spaces of the lamina propria and the lumens of the lacteals. Both Weiss (1955) and Palay and Karlin (1956) concluded that particulate fat, in the process of absorption, passes through the apical cytoplasm of the columnar absorptive cells, the intercellular spaces of the epithelium, and the extracellular spaces of the lamina propria to reach the lacteals. During its passage through the columnar absorptive cells it is enclosed in membrane-bound vesicles.

The undifferentiated chief cells of the crypts differentiate into columnar absorptive cells at the bases of the villi. The structural changes which may be observed there in continuous transition include an increase in the alkaline phosphatase activity of the striated cuticular border, an increase in the length and concentration of its microvilli, an increase in the number and apparent length of mitochondria in the apical cytoplasm, the advent of intracellular and extracellular fat droplets, and the development of a supranuclear Golgi complex. These changes are related inversely to the presence of mitotic figures in the epithelium.

The columnar absorptive cells of the small intestine in suckling rats and mice differed in structure from those of adult animals and differed from one region of the small intestine to another (Figs. 5 to 7). In suckling animals a greater number of small tubules traversed the subcuticular zone, there were numerous vacuoles and large dense inclusions distinct from lipide in the apical cytoplasm, and the Golgi complex was poorly developed. The vacuoles and dense inclusions usually were separated from the cytoplasm by an encircling membrane, and frequently contained complex arrangements of parallel membranes. The tubules in the subcuticular zone, together with vacuoles and dense inclusions in the apical cytoplasm occurred with increasing frequency in successively distal portions of the small intestine, and in this respect, were related inversely to the presence of particulate fat within the cells and intercellular spaces. Macroscopically the upper part of the small intestine was milky in color, whereas the lower portion was yellow or brownish, as noted by von Möllendorff (1924). The cells of the duodenum contained numerous lipide droplets but few vacuoles or dense inclusions (Fig. 5); those of the jejunum contained a moderate number of all three types of inclusions (Fig. 6), and the cells of the ileum were almost devoid of fat, but contained large vacuoles which filled the apical cytoplasm and deformed the nuclei (Fig. 7). In addition, lipide and membranous nuclear inclusions, to be reported elsewhere (Luse, Clark, and Davies, in preparation) were found in the columnar absorptive cells of the duodenum and jejunum (Fig. 23). Subcuticular tubules, vacuoles, and dense inclusions were rare in the crypts and bases of the villi. All of these features were most prominent during the 1st week after birth and diminished gradually with advancing age until approximately 18 days after birth, when all levels of the small intestine assumed the uniform appearance found in adult animals. The jejunum, because of its intermediate morphology, was the principal object of study in these experiments.

When a foreign protein or colloidal material was administered to suckling rats or mice, the columnar absorptive cells of the jejunum became crowded with vacuoles in which the material administered could be identified. These inclusions appeared within 1 hour after the material was administered, grew in size and number, and within 3 hours filled the apical cytoplasm, rivalling in size those large vacuoles normally found in the ileum (Figs. 8 and 9). Bovine gamma globulin and ovalbumin were identified intracellularly by staining with fluorescent antibodies (Fig. 11). Evans blue and trypan blue produced blue droplets visible in sections of freeze-dried tissue. Colloidal gold could be identified as very dense granules by electron microscopy (Fig. 16). Saccharated iron oxide was stained with the Prussian blue reaction in paraffin sections of formalin-fixed tissue prepared by the method of Tirmann-Schmelzer (Lillie, 1954). India ink was identified presumptively as a dense granular material in large vacuoles (Fig. 14), and bovine albumen, rabbit and rat serum and rat whole blood, although not identified specifically, produced large vacuoles similar to those described above. The vacuoles produced by saccharated iron oxide were particularly large, often stretching the cells beyond normal size (Fig. 15). The preparation used contains approximately 95 per cent free sucrose, and solutions of sucrose alone, both isotonic and hypertonic with plasma, produced similar very large vacuoles, whereas distilled water (Fig. 23) and solutions of glucose and sodium chloride produced vacuoles of only moderate size. Among the colloidal materials used, only Evans blue appeared to be absorbed beyond the epithelium of the small intestine. It produced vital staining of the entire body when administered orally to suckling rats and mice, but was not absorbed by animals more than 18 days old.

In all of these experiments, inclusions took the form of multiple small vacuoles in the apical cytoplasm and single large vacuoles in the supranuclear region. As seen in the electron microscope, the vacuoles usually were surrounded by single membranes and contained a granular material, as well as complex membranous structures (Figs. 18, 23 to 26). Vacuoles frequently were seen closely apposed and mutually deformed, and occasionally the membrane of one vacuole was ballooned into an adjacent vacuole as if the two might fuse (Fig. 17). The apical cell membrane, tubules in the subcuticular region, and vacuoles in the apical cytoplasm all appeared to be interconnected in places, and in addition, large indentations of the apical cell membrane were found forming vacuoles in the apical cytoplasm which opened directly into the lumen of the intestine (Figs. 6, 7, 19 to 22). Perhaps the vacuoles containing foreign materials were formed by invagination of the apical cell membrane in a process related to pinocytosis.

Bovine gamma globulin stainable with fluorescent antibody was found in intracellular droplets in the ileum as well as in the jejunum after its administration to suckling rats, but did not appear in the duodenum. Although large granular inclusions visible by both light and electron microscopy persisted in the jejunum for at least 48 hours following the administration of bovine gamma globulin, material stainable with fluorescent antibody had disappeared by 24 hours after administration. Sections of liver did not stain with fluorescent antibody within 4 hours after protein administration but material could be stained in the mesenteric lymph node, both in the lymphatic ducts and as intracellular droplets in the body of the node. Occasionally faint staining was seen in the lacteals of the villi as well. These observations confirm the conclusions of Comline and his colleagues (1951, 1953), and Hill and Hardy (1956) that intact proteins are absorbed by way of the lymphatics in suckling animals.

The ingestion of materials administered by stomach tube varied with the age of the animal (Figs. 12, 13, 2 to 4). During the 1st week after birth, bovine gamma globulin produced such a profusion of vacuoles and tubules in the apical cytoplasm and subcuticular zone of the columnar absorptive cells of the jejunum that it was difficult to distinguish their separate outlines. With advancing age the response gradually diminished in intensity until 18 days after birth, when the administration of proteins or colloidal materials no longer produced vacuoles or evidence of ingested material within the cells, even in experiments in which protein was injected directly into the lumen of the small intestine. The vital dyes, in similar fashion, produced deep staining of the jejunum and ileum in newborn animals, progressively less staining in successively older animals, and no staining at all in animals more than 18 days old.

Cortisone acetate (Merck and Co., Inc., Rahway, New Jersey), injected subcutaneously into rats 8 to 10 days old, in doses of 0.03 to 0.06 mg. per gm. body weight, altered the morphology of the columnar absorptive cells of the small intestine so that they were indistinguishable from the cells of adult animals and no longer formed vacuoles or dense inclusions in response to the oral administration of bovine gamma globulin (Figs. 10, 27, 28). This effect of cortisone appeared between 48 and 72 hours after injection and persisted at least 6 days after a single injection. The effect was sometimes incomplete in that in some animals a few cells near the tips of the villi remained juvenile in appearance and developed inclusions containing protein stainable with fluorescent antibody when observed 72 hours after cortisone injection. Six days after injection all the responses observed appeared to be complete. Cortisone injected into 5 and 6 day old animals produced an effect in some but not in others.

Thus in respect to age, level in the small intestine, and treatment with cortisone, the ability of columnar absorptive cells to ingest proteins and colloids was found to be correlated with the presence of subcuticular tubules, vacuoles, and membranous inclusions. It is also correlated with the reported ability of suckling animals to absorb antibodies.

DISCUSSION

Von Möllendorff (1924) demonstrated that the columnar absorptive cells of the lower small intestine in suckling rats ingest acid dyes. In the present study, using both suckling rats and suckling mice, the list of colloidal materials which can be ingested was extended to include a variety of proteins as well as insoluble particles such as carbon, iron oxide, and colloidal gold. The largest individual particles ingested were colloidal gold, which reached a size of approximately 175 A.

By electron microscopy the ingested colloids and proteins were found within a system of vacuoles and dense inclusions in the apical cytoplasm of the columnar absorptive cells of the jejunum and ileum. The membranes which surrounded these vacuoles and inclusions resembled the cell membrane in appearance and were continuous in places with tubules in the subcuticular cytoplasm which in turn have been found to be continuous with the apical cell membrane. Sites of grosser indentation of the apical cell membrane were observed also, as well as appearances which could be interpreted as incipient fusion of vacuoles. We interpret these observations with the hypothesis that in suckling animals the columnar absorptive cells ingest proteins and colloidal materials by invagination of the apical cell membrane, that is, by pinocytosis. The processes of fusion and dehydration of pinocytotic vacuoles which have been observed in tissue culture might be used to explain the formation of the large supranuclear vacuoles and dense inclusions observed in these experiments. Excess membrane derived from the

fusion and dehydration of vacuoles might be the source of the complex arrays of parallel membranes found associated with these inclusions. The combination of a microvillous cell border, subcuticular tubules, apical vacuoles, and dense inclusions is characteristic not only of intestinal cells in suckling animals, but of the visceral epithelium of the mammalian yolk sac (Dempsey, 1953) and the proximal tubular epithelium of the kidney (Rhodin, 1954; Clark, 1957). Both the yolk sac and the proximal tubular epithelium ingest vital dyes and proteins. Therefore the features listed above may represent a specialization of cell structure for the ingestion of particulate or macromolecular substances.

The ingestion of particulate material is characteristic of certain cells in primitive organisms, such as the endodermal epithelium in embryonic vertebrates and adult invertebrates (Patzelt, 1936). It appears to serve a nutritive function when coupled with intracellular digestion. Perhaps the ingestion of particulate materials by columnar absorptive cells in newborn animals is a primitive mechanism which provides nourishment at an age when the glandular apparatus for producing digestion within the lumen of the intestine has not matured (Hill, 1956). Ingestion by cells of the small intestine may also be important in providing the young animal with specific macromolecules. Macromolecules acquired from the environment may serve the immature organism not only in protecting it from infection by passive immunity, but also by contributing in specific ways to its growth and development (Schechtman, 1956).

Halliday (1957), Morris (1957), and Brambell, Halliday, and Morris (1958), have demonstrated that the absorption of antibodies by suckling animals is selective, in that homogenous antibodies are absorbed more completely than heterogenous ones; whereas ingestion by columnar absorptive cells appears to be an unselective mechanism for taking in whatever is in the environment. However, ingested materials are enclosed by membranes, and remain, in a sense, extracytoplasmic. If this process is indeed a part of protein absorption, the selective barrier may be in the membranes which separate inclusions and cytoplasm. Properties which could determine the ability of a substance to penetrate such a barrier, might include, in addition to solubility, particle size and charge, specific affinity between the substance and the membrane, and its susceptibility to intracellular digestion.

Approximately 18 days after birth, at the age when rats and mice lose the ability to absorb intact proteins, the columnar absorptive cells of the small intestine lose the capacity to ingest proteins and colloids, and the subcuticular tubules, vacuoles, and inclusions taken as evidence of pinocytosis in younger animals disappear almost completely. This change probably does not depend upon aging of individual cells, because the lifespan of columnar absorptive cells appears to be approximately 48 hours, at least in adult animals (Leblond, Everett, and Simmons, 1957; Hughes *et al.*, 1958). Thus several generations of cells presumably have originated in the crypts, migrated out along the villi, and fallen off into the intestinal lumen during the 18 days since birth without, during their lifespan, losing the ability to ingest particles. Hill (1956) has suggested that changes in the pH and enzyme content of the stomach and intestine which occur at the age when protein absorption ceases lead to digestion of protein before it reaches the columnar absorptive cells. However, the ingestion of indigestible colloids terminated also at 18 days after birth and injection of protein directly into the lumen of the jejunum failed to produce signs of ingestion in animals more than 3 weeks old. Therefore if changes in the contents of the intestinal lumen prevent ingestion, it is probably through direct effects on the activity of the columnar absorptive cells.

Moog (1953) has demonstrated that adrenal cortical hormones will produce a precocious increase in the alkaline phosphatase activity of the duodenum in suckling mice, causing it to reach adult levels several days early (see also Moog and Thomas, 1955), and Halliday (1958) has reported a similar effect of adrenal cortical hormones in terminating the intestinal absorption of antibodies at an early age. We have observed that cortisone acetate, when administered to rats 8 to 10 days old, altered the columnar absorptive cells to resemble those of adult animals and prevented the ingestion of protein. In Moog's experience and in the present study, the effects of a single injection of hormone required 2 to 3 days to appear and were irreversible for a period longer than the lifespan of a single generation of columnar absorptive cells. Cells near the tips of the villi, which presumably were older than more proximal

cells at the time of injection, did not always respond to cortisone. These observations are consistent with the interpretation that cortisone acts by influencing cellular differentiation soon after mitosis. In Moog's experiments mice failed to respond to adrenocorticotrophic hormone before 12 days of age, although her animals and our own responded to cortisone as early as 1 week after birth. Animals less than about 1 week of age failed to respond to cortisone. Therefore it appears that there is a sequence in postnatal development in the rat and mouse, in which the intestine acquires a new responsiveness to adrenal cortical hormones during the 1st week after birth, and the adrenal cortex matures during the 2nd week after birth, both events together producing functional maturation of the small intestine.

Subcuticular tubules, small vacuoles, and dense inclusions do not disappear entirely from the columnar absorptive cells of adult rats and mice. Furthermore, we agree with Palay and Karlin (1956) who interpret the morphological evidence of fat absorption as an indication that particulate fat enters the columnar absorptive cells by a process akin to pinocytosis and is disgorged into the intercellular spaces by a reversal of the same mechanism. Thus pinocytosis, at least on a small scale, may occur in the columnar absorptive cells throughout life, and accordingly, digestion may not be exclusively extracellular. The failure to ingest colloids may represent, then, not a loss of the capacity for pinocytosis, but the development of discrimination by the cell membrane concerning what substances stimulate pinocytosis. Bennett (1956) has suggested that such a mechanism operates through specific affinities between the cell membrane and materials in the environment, and Tompkins (1953), Chapman-Andresen and Prescott (1956), and Chapman-Andresen (1957) have demonstrated that environmental protein may evoke pinocytosis in amebae and mammalian cells. In suckling animals, fat is ingested in the upper small intestine and protein in the lower small intestine. Perhaps this phenomenon depends upon a greater affinity of the cell membrane for fat than for protein, leading to the preferential ingestion of dietary fat as long as it is available. If so, fasting should facilitate the ingestion of protein in the duodenum.

Suckling animals, in absorbing proteins, absorb potentially antigenic materials, but the possibility of hypersensitivity apparently is avoided by the inability of the suckling animal to synthesize gamma globulins (Halliday and Kekwick, 1957). Plasma cells appear and gamma globulin synthesis begins in the rat and mouse more than 3 weeks after birth, when absorption of antigenic proteins from the intestine has ceased. In view of the work of Medawar and his collaborators (reviewed by Medawar, 1956) on the phenomenon of immunological tolerance in young animals, it is possible that the coincidence described above is more than fortuitous and represents a general change in the way the cells of the organism react to antigenic substances.

BIBLIOGRAPHY

Bennett, H. S., The concepts of membrane flow and membrane vesiculation as mechanisms for active transport and ion pumping, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 99.

Brambell, F. W. R., Halliday, R., and Morris, I. G., Interference by human and bovine serum and serum protein fractions with the absorption of antibodies by suckling rats and mice, *Proc. Roy. Soc. London, Series B*, 1958, **149,** 1.

Brambell, F. W. R., Hemmings, W. A., and Henderson, M., Antibodies and Embryos, London, University of London, The Athlone Press, 1951, 31.

Chapman-Andresen, C., Some observations on pinocytosis in leukocytes, *Exp. Cell Research*, 1957, **12,** 397.

Chapman-Andresen, C., and Prescott, D. M., Studies on pinocytosis in the amoebae *Chaos chaos* and *Amoeba proteus*, *Compt.-rend. trav. Lab. Carlsberg., Series Chim.*, 1956, **30,** 57.

Christie, A. C., A study of the Kultschitzky (argentaffin) cell with the electron-microscope, after fixation by osmium tetroxide, *Quart. J. Micr. Sc.*, 1955, **96,** 295.

Clark, S. L., Jr., Cellular differentiation in the kidneys of newborn mice studied with the electron microscope, *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 349.

Comline, R. S., Pomeroy, R. W., and Titchen, D. A., Histological changes in the intestine during colostrum absorption, *J. Physiol.*, 1953, **122,** 6 P.

Comline, R. S., Roberts, H. E., and Titchen, D. A., Histological changes in the epithelium of the small intestine during protein absorption in the newborn animal, *Nature*, 1951, **168,** 84.

Coons, A. H., and Kaplan, M. H., Localization of antigen in tissue cells. II. Improvements in a method for the detection of antigen by means of fluorescent antibody, *J. Exp. Med.*, 1950, **91,** 1.

Coons, A. H., Leduc, E. H., and Connolly, J. M., Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit, *J. Exp. Med.*, 1955, **102,** 49.

Dalton, A. J., Electron micrography of epithelial cells of the gastro-intestinal tract and pancreas, *Am. J. Anat.*, 1951, **89,** 109.

Dempsey, E. W., Electron microscopy of the visceral yolk-sac epithelium of the guinea pig, *Am. J. Anat.*, 1953, **93,** 331.

Deutsch, H. F., and Smith, V. R., Intestinal permeability to proteins in the newborn herbivore, *Am. J. Physiol.*, 1957, **191,** 271.

Halliday, R., The termination of the capacity of young rats to absorb antibody from the milk, *Proc. Roy. Soc. London, Series B*, 1956, **145,** 179.

Halliday, R., The absorption of antibody from immune sera and from mixtures of sera by the gut of the young rat, *Proc. Roy. Soc. London, Series B*, 1957, **148,** 92.

Halliday, R., The increase in alkaline phosphatase activity of the duodenum and decrease in absorption of antibodies by the gut induced in young rats by deoxycorticosterone acetate, *J. Physiol.*, 1958, **140,** 44 P.

Halliday, R., and Kekwick, R. A., Electrophoretic analysis of the sera of young rats, *Proc. Roy. Soc. London, Series B*, 1957, **146,** 431.

Hally, A. D., The fine structure of the Paneth cell, *J. Anat.*, 1958, **92,** 268.

Hill, K. J., Gastric development and antibody transference in the lamb, with some observations on the rat and guinea pig, *Quart. J. Exp. Physiol.*, 1956, **41,** 421.

Hill, K. J., and Hardy, W. S., Histological and histochemical observations on the intestinal cells of lambs and kids absorbing colostrum, *Nature*, 1956, **178,** 1353.

Hughes, W. L., Bond, V. P., Brecher, G., Cronkite, E. P., Painter, R. B., Quastler, H., and Sherman, F. G., Cellular proliferation in the mouse as revealed by autoradiography with tritiated thymidine, *Proc. Nat. Acad. Sc.*, 1958, **44,** 476.

Leblond, C. P., Everett, N. B., and Simmons, B., Sites of protein synthesis as shown by radioautography after administration of S^{35}-labelled methionine, *Am. J. Anat.*, 1957, **101,** 225.

Lillie, R. D., Histopathologic Technic and Practical Histochemistry, New York, The Blakiston Company, Inc., 1954, 242.

Macklin, C. C., and Macklin, M. T., The intestinal epithelium, *in* Special Cytology, (E. V. Cowdry, editor), New York, Paul B. Hoeber, Inc., 1932, **1,** 231.

Medawar, P. B., Introductory remarks, *in* A Discussion on Immunological Tolerance, *Proc. Roy. Soc. London, Series B*, 1956, **146,** 1.

Möllendorff, von, W., Ueber die Anteilnahme des Darmepithels an der Verarbeitung enteral und parenteral zugefuhrter saurer Farbstoffe, *Münch. med. Woch.*, 1924, **71,** 569.

Moloney, P. J., and Coval, M., Antigenicity of insulin: diabetes induced by specific antibodies, *Biochem. J.*, 1955, **59,** 179.

Moog, F., The functional differentiation of the small intestine. III. The influence of the pituitary-adrenal system on the differentiation of phosphatase in the duodenum of the suckling mouse, *J. Exp. Zool.*, 1953, **124,** 329.

Moog, F., and Thomas, E. R., The influence of various adrenal and gonadal steroids on the accumulation of alkaline phosphatase in the duodenum of the suckling mouse, *Endocrinology*, 1955, **56,** 187.

Morris, I. G., The effect of heterologous sera on the uptake of rabbit antibody from the gut of young mice, *Proc. Roy. Soc. London, Series B*, 1957, **148,** 84.

Palade, G. E., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **95,** 285.

Palay, S. L., The morphology of secretion, *in* Frontiers in Cytology, (S. L. Palay, editor), New Haven, Yale University Press, 1958, 305.

Palay, S. L., and Karlin, L., Absorption of fat by jejunal epithelium in the rat, *Anat. Rec.*, 1956, **124,** 343.

Patzelt, V., Der Darm, *in* Handbuch der Mikroskopischen Anatomie des Menschen, (W. von Möllendorff, editor), Berlin, Julius Springer, 1936, **V3,** 1.

Porter, K. R., and Blum, J., A study in microtomy for electron microscopy, *Anat. Rec.*, 1953, **117,** 685.

Rhodin, J., Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney, Karolinska Institutet, Stockholm, Aktiebolaget Godvil, 1954, 1.

Schechtman, A. M., Uptake and transfer of macromolecules by cells with special reference to growth and development, *Internat. Rev. Cytol.*, 1956, **5,** 303.

Sjöstrand, F. S., and Zetterqvist, H., Functional changes of the free cell surface membrane of the intestinal absorbing cell, *in* Electron Microscopy, Proceedings of the Stockholm Conference, September, 1956, (F. S. Sjöstrand and J. Rhodin, editors), New York, Academic Press, Inc., 1957, 150.

Smith, T., Hydropic stages in the intestinal epithelium of new-born calves, *J. Exp. Med.*, 1925, **41,** 81.

Tompkins, E. H., Resting reticulo-endothelial cells and their "fluid vacuoles": reactions to potassium, tonicity and albumin, *Bull. Johns Hopkins Hosp.*, 1953, **92,** 79.

Weiss, J. M., The role of the Golgi complex in fat absorption as studied with the electron microscope with observations on the cytology of duodenal absorptive cells, *J. Exp. Med.*, 1955, **102,** 775.

Zetterqvist, H., The ultrastructural organization of the columnar absorbing cells of the mouse jejunum, Karolinska Institutet, Stockholm, Aktiebolaget Godvil, 1956.

Explanation of Plates

All electron micrographs represent thin sections of villi from the small intestines of rats and mice, fixed for 1 hour in 1 per cent osmium tetroxide buffered to pH 7.2 and containing 3.5 per cent sucrose, imbedded in methacrylate, sectioned with glass knives, and examined in an RCA EMU 2E electron microscope. The drawings, made by Mrs. Sidney F. Velick, represent 2 or 3 micron sections of tissues prepared in the same manner and examined by phase contrast microscopy. Fluorescence photomicrographs were taken on Eastman Kodak plus-X 35 mm. film, using a mercury vapor arc as light source and a microscope equipped with a cardioid condenser and filters for observing fluorescence to near-ultraviolet light.

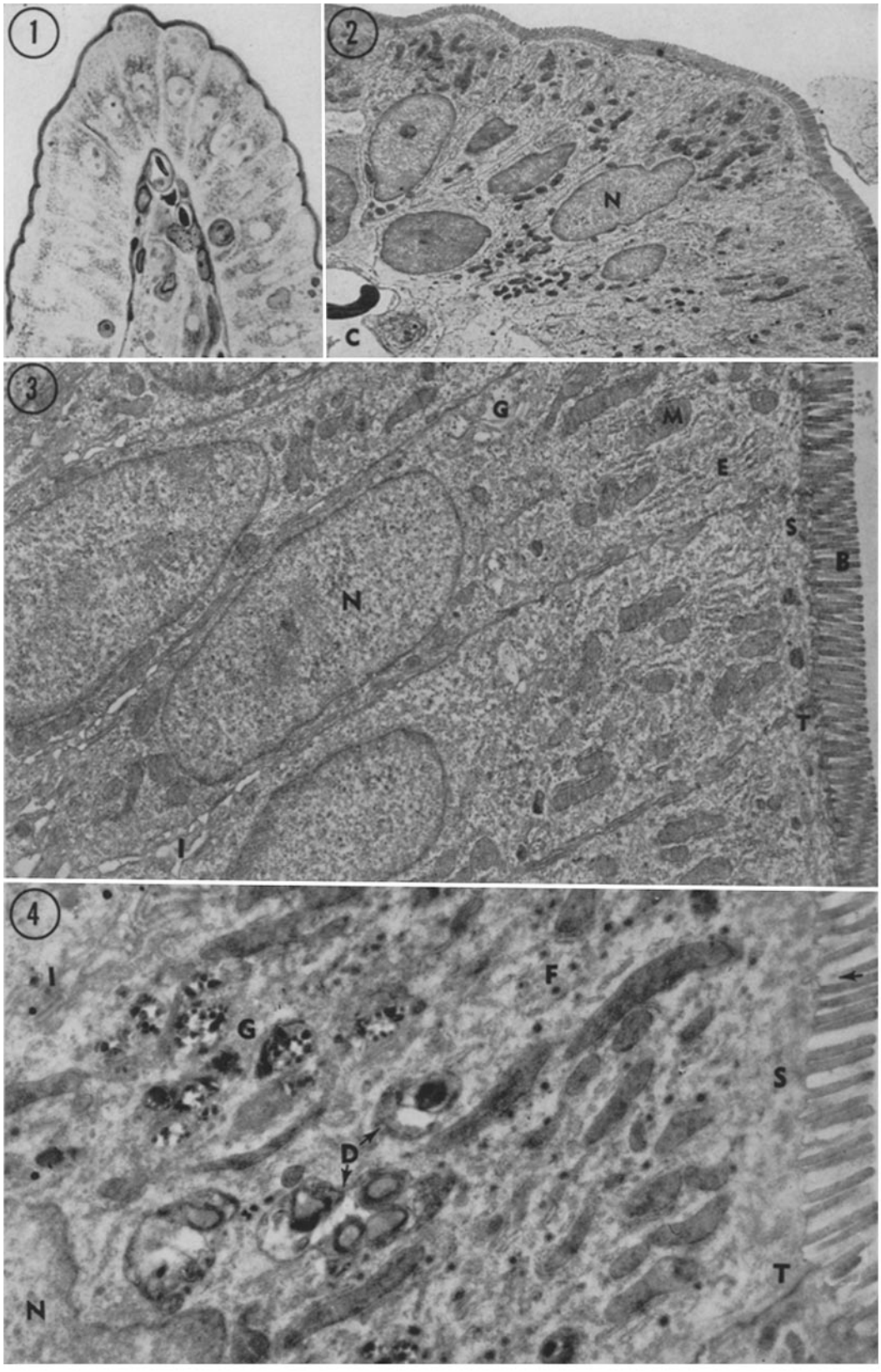
Plate 14

Fig. 1. A drawing of a villus from the upper small intestine of an adult rat. The columnar absorptive cells are uniform in size and shape and contain no large vacuoles or inclusions. × 1,000.

Fig. 2. An electron micrograph of a jejunal villus from a 21 day old rat given Evans blue orally 20 hours prior to death. No evidence of ingestion of Evans blue was obtained by light or electron microscopy. The cells are uniform in appearance and contain no large vacuoles or inclusions; a nucleus (*N*) is identified. *C*: a capillary containing an erythrocyte in the lamina propria of the villus. × 2600.

Fig. 3. Portions of several columnar absorptive cells from the jejunum of a 24 day old rat given Evans blue orally 20 hours prior to death. There was no evidence of ingestion of Evans blue by light or electron microscopy. Features typical of columnar absorptive cells in adult animals which may be observed in this electron micrograph include a striated cuticular border consisting of uniform microvilli (*B*), a subcuticular region free of the usual cytoplasmic organelles (*S*), filamentous mitochondria (*M*), and strands of ergastoplasm (*E*) filling the apical cytoplasm, and a Golgi complex (*G*) containing closely apposed and mutually deformed membranous sacs in the supranuclear region. The nucleus (*N*) is regular in shape. The lateral cell membranes are joined to those of adjacent cells by terminal bars (*T*) in the subcuticular region and are apposed, folded, and interlocked throughout the apical region. Basal to the nuclei an intercellular space (*I*) separates adjacent cells. × 6500.

Fig. 4. A portion of a jejunal villus from a 23 day old rat which had received bovine gamma globulin 20 hours prior to death. Fluorescent antibody staining did not reveal any intercellular gamma globulin. At the higher magnification of this electron micrograph the character of the subcuticular zone (*S*) is more clearly shown than in Fig. 3. In addition to an irregularly arranged amorphous material of intermediate density, this region contains only a few membranous structures which are tubular or vesicular in shape. The arrow points to an indentation of the apical cell membrane between the bases of adjacent microvilli which forms a small cleft or tubule extending into the subcuticular cytoplasm. In the apical cytoplasm, in addition to mitochondria and ergastoplasm, there are small dense or osmiophilic droplets of uniform size identified presumptively as lipide (*F*). Each is surrounded by a membrane which may be seen more easily at higher magnification, and similar droplets lie within the sacs of the Golgi complex (*G*). The intercellular space (*I*), although largely obliterated by apposition of adjacent cell membranes, contains a few lipide droplets. In addition to a characteristic Golgi complex, the supranuclear region contains several dense bodies (*D*) characterized by complex arrays of concentric membranes similar to those illustrated in Fig. 24. These structures are rare in the columnar absorptive cells of adult animals. *T*: terminal bar. *N*: nucleus. × 16,000.



(Clark: Ingestion by intestinal cells)

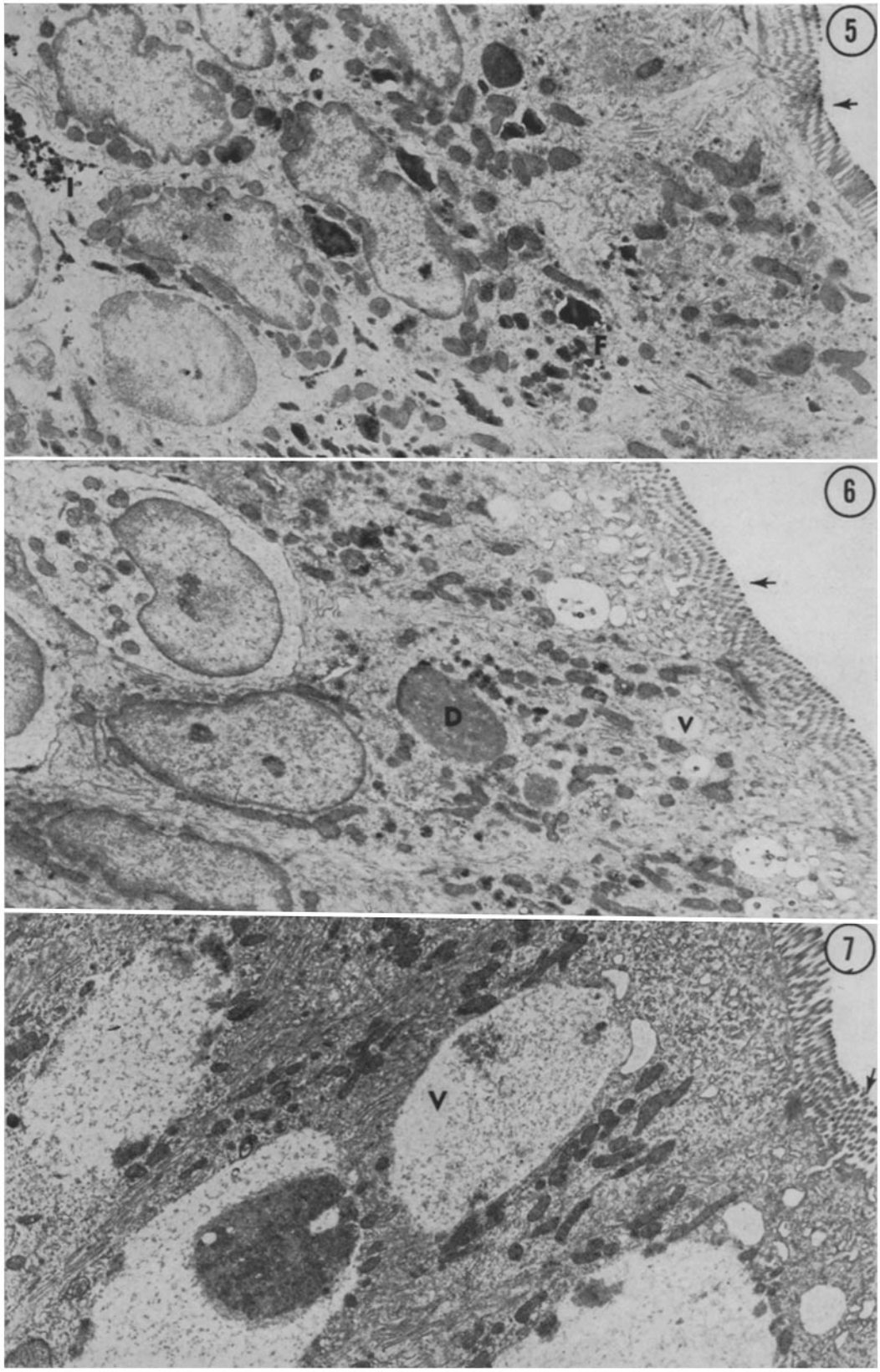
PLATE 15

FIGS. 5 to 7 are electron micrographs of duodenum, jejunum, and ileum, respectively, all taken from the same 5 day old rat which had been isolated from its mother for 5 hours prior to death and given nothing by mouth.

FIG. 5. The columnar absorptive cells in the duodenum resemble those of adult animals. A few small indentations of the apical cell membrane extend into the subcuticular cytoplasm (arrow), and there are lipide droplets (*F*) in the apical cytoplasm, Golgi region, and intercellular spaces (*I*). Few vacuoles or dense bodies are to be seen. × 6,000.

FIG. 6. In the columnar absorptive cells of the jejunum there are larger and more numerous indentations of the apical cell membrane which extend through the subcuticular region (arrow). The subcuticular region is crowded with small vacuoles and tubules, and larger vacuoles (*V*) and dense bodies (*D*) occur in the apical cytoplasm. Conversely, lipide droplets are less numerous than in the duodenum. The pale cell in the upper left corner of the picture probably is a lymphocyte which has migrated into the epithelium. × 6000.

FIG. 7. Columnar absorptive cells in the ileum possess a broad subcuticular region traversed by numerous tubules, vacuoles, and indentations of the apical cell membrane (arrow). The apical cytoplasm is almost filled by arge single vacuoles (*V*) containing dense bodies and a granular material. × 6000.



(Clark: Ingestion by intestinal cells)

PLATE 16

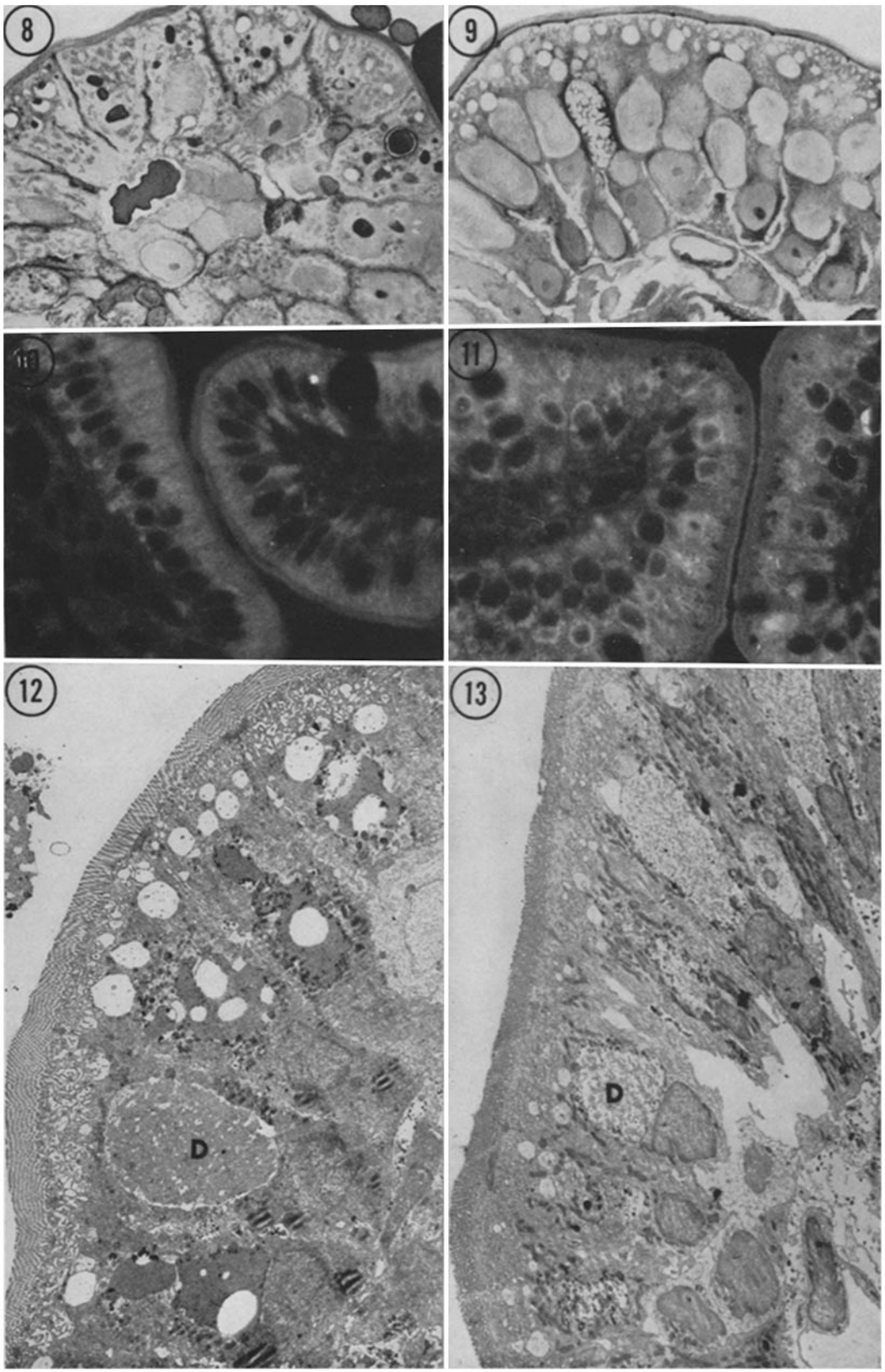
FIG. 8. A drawing of a villus from the upper small intestine of a 2 day old mouse killed without previous treatment. There is lipide in the lumen of the intestine, in the apical cytoplasm of the columnar absorptive cells, in the intercellular spaces, and in the lamina propria. In the lower right hand part of the picture there is a lipide nuclear inclusion. There are also small vacuoles in the apical cytoplasm of the columnar absorptive cells. × 1000

FIG. 9. A drawing of a jejunal villus from a 2 day old rat given bovine gamma globulin 4 hours prior to death. Each columnar absorptive cell contains a large single supranuclear inclusion of moderate density and numerous apical vacuoles. There is a goblet cell just to the left of the center of the picture. × 1000.

FIGS. 10 and 11 are photographs of jejunal villi which had been freeze-dried, stained simultaneously with fluorescent antibody against bovine gamma globulin, and examined by fluorescence microscopy. Fig. 10 represents tissue taken from a 13 day old rat 3 days after it had received a subcutaneous injection of 0.03 mg. of cortisone acetate per gm. of body weight and 4 hours after it had received bovine gamma globulin by mouth. All of the fluorescence observed in this photograph was the faint bluish autofluorescence of the cells, and the only place in which the bright green fluorescence of the antibody could be found was in the intestinal lumen. Fig. 11 represents tissue taken from a 12 day old rat which had received no cortisone, but had been given bovine gamma globulin orally at the same time as the animal of Fig. 10. In addition to blue autofluorescence there was bright green fluorescence characteristic of fluorescent antibody in small droplets in the apical cytoplasm and about the periphery of large supranuclear vacuoles in the columnar absorptive cells. In places, the striated cuticular border also stained with the fluorescent antibody, and there was a faint green fluorescence throughout the cells and intracellular spaces of the villi. Both figures: × 600.

FIG. 12. An electron micrograph of a jejunal villus taken from a 3 day old rat 28 hours after it had received bovine gamma globulin by mouth. The subcuticular regions of the columnar absorptive cells are filled and expanded by numerous tortuous tubules and vacuoles, and the apical cytoplasm is filled with irregular dense bodies (*D*). × 3,000.

FIG. 13. This jejunal villus was taken from a 14 day old rat 2 hours after it had received bovine gamma globulin orally. There are numerous subcuticular tubules, apical vacuoles, and large dense supranuclear bodies (*D*), but the reaction to protein administration was not so violent as in the younger animal of Fig. 12. This difference was found to be consistent with age regardless of the time elapsed after the administration of protein. × 3000.



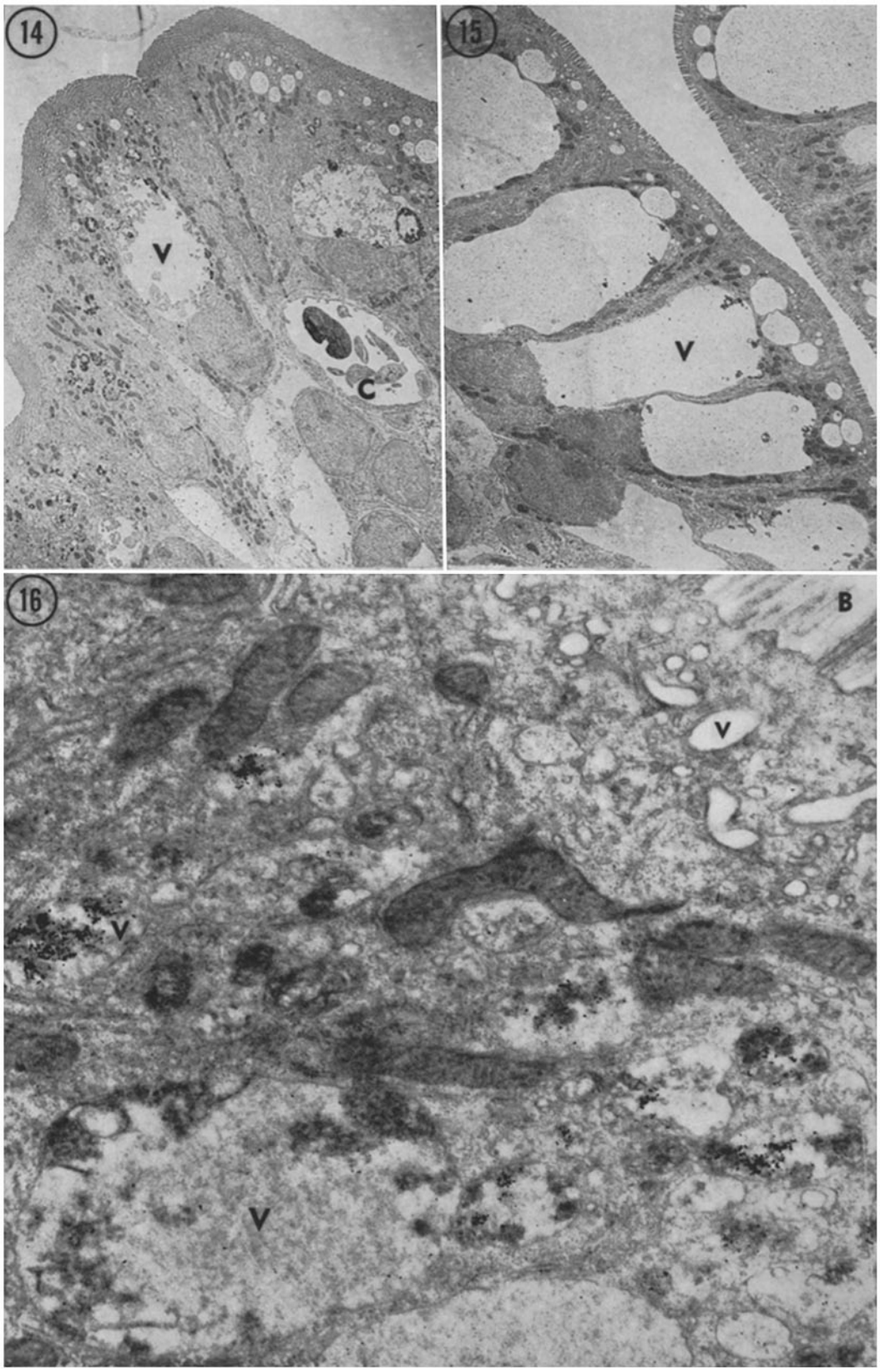
(Clark: Ingestion by intestinal cells)

PLATE 17

FIG. 14. A jejunal villus taken from a 3 day old mouse 6 hours after the oral administration of India ink. The numerous apical vacuoles and large single supranuclear vacuoles (*V*) contain both a homogeneous material of moderate density and a very dense granular material which probably represents agglutinated carbon particles. *C*: capillary. × 2400.

FIG. 15. Jejunal villi taken from a 6 day old mouse 1½ hours after the oral administration of saccharated iron oxide. Both small and large vacuoles (*V*) contain groups of small very dense particles not resolved at this magnification, corresponding in location to material which stains for iron with the Prussian blue reaction in formalin-fixed tissue. The preparation of iron used contained approximately 95 per cent sucrose, and sucrose alone was found to produce large vacuoles. × 2400.

FIG. 16. Columnar absorptive cells from the jejunum of a 3 day old mouse killed 6 hours after the oral administration of colloidal gold. The small, uniform, dense particles of gold lie within membrane-enclosed vacuoles (*V*) in the apical cytoplasm. *B*: striated cuticular border. × 24,000.

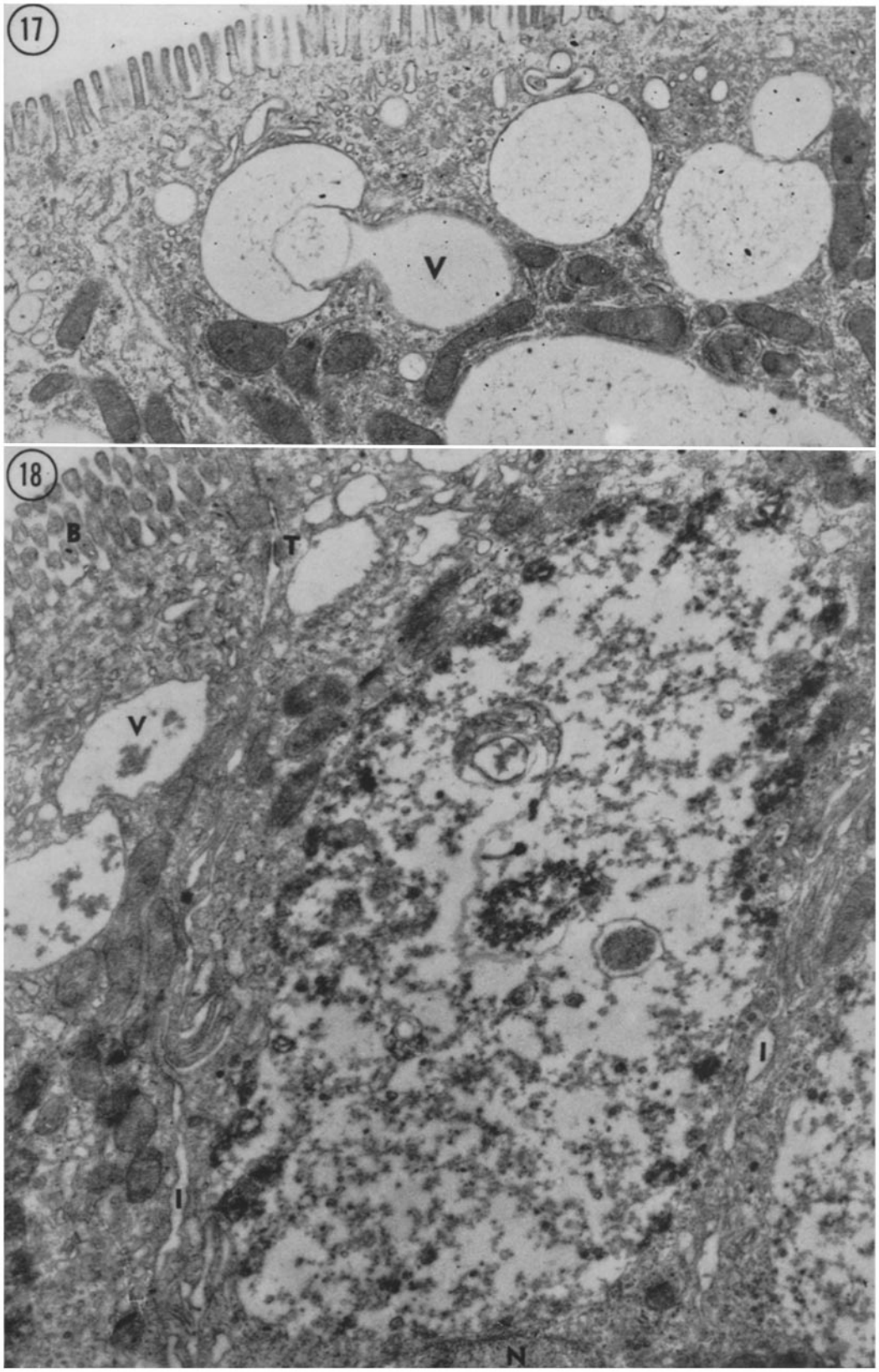


(Clark: Ingestion by intestinal cells)

PLATE 18

FIG. 17. The apical portion of two columnar absorptive cells from the jejunum of a 6 day old mouse killed 1½ hours after receiving saccharated iron oxide by mouth. One vacuole (*V*) is ballooned into its neighbor as if the two might fuse. × 12,000.

FIG. 18. Columnar absorptive cells from the jejunum of a 14 day old rat given bovine gamma globulin 1 hour prior to death. A large vacuole surrounded by a single membrane almost completely fills the apical cytoplasm of the central cell which is separated from adjacent cells by narrow intercellular spaces (*I, I*). Part of a similar vacuole is seen in the cell to the right. The central vacuole contains a granular material more concentrated at the periphery and a variety of complex membranous structures. The smaller vacuoles (*V*) appear to be continuous in places with small tubules of the subcuticular region. *B*: striated cuticular border. *T*: terminal bar. *N*: nucleus. × 19,000.



(Clark: Ingestion by intestinal cells)

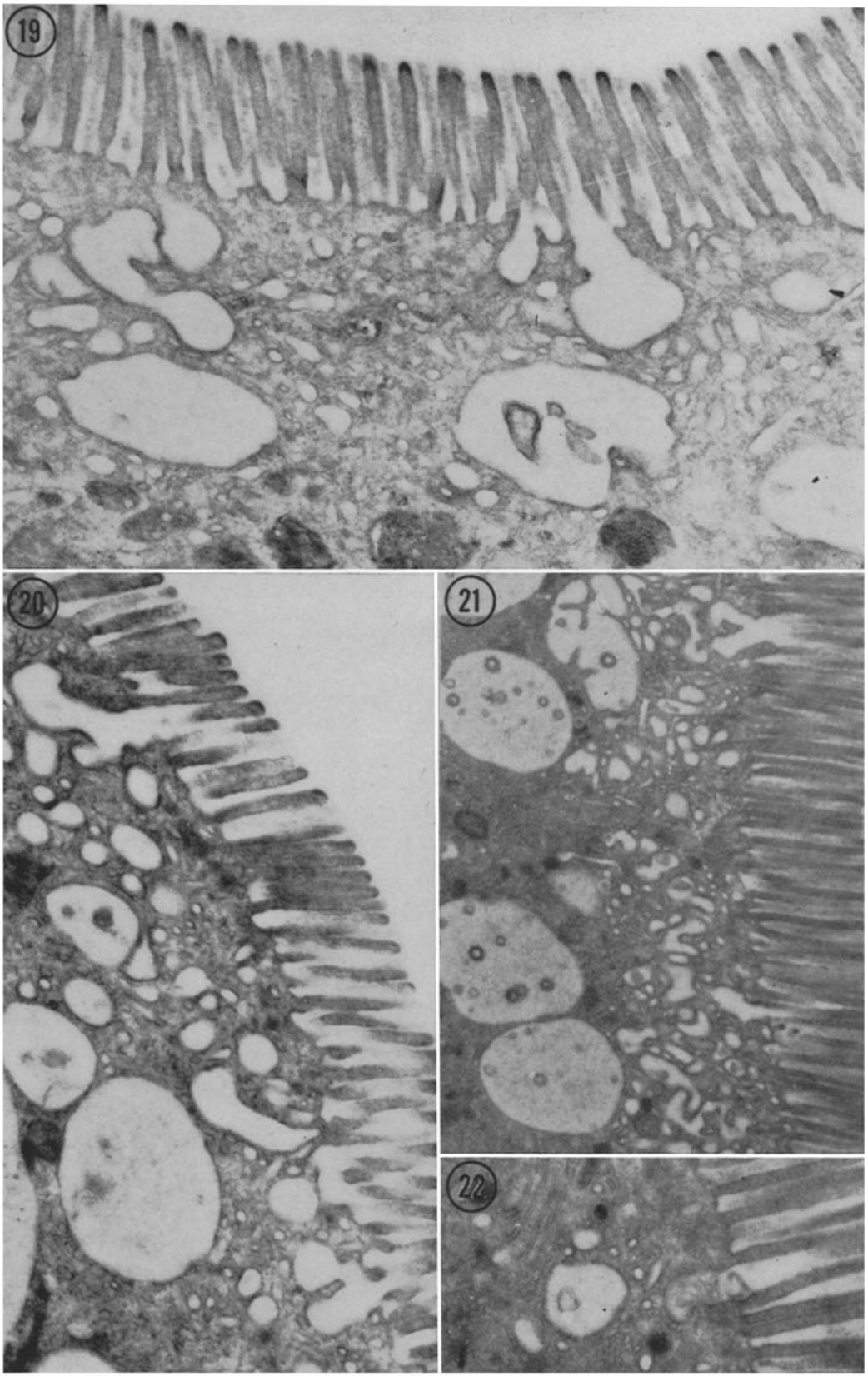
PLATE 19

FIG. 19. The apical part of a columnar absorptive cell of the jejunum taken from a 3 day old mouse 2 hours after the oral administration of colloidal gold. Although no gold can be observed with certainty in this electron micrograph, the subcuticular cytoplasm, in addition to numerous tubules, vacuoles, and dense inclusions, contains at least two large indentations of the apical cell membrane forming vacuoles in the subcuticular region which are continuous with the lumen of the intestine. × 18,000.

FIG. 20. Part of a columnar absorptive cell from the jejunum of an 8 day old rat which had received bovine serum albumin by mouth 4 hours prior to death. At least two large indentations of the apical cell membrane may be observed extending into the subcuticular region. × 23,000.

FIG. 21. A columnar absorptive cell taken from the jejunum of a 3 day old mouse 28 hours after the oral administration of bovine gamma globulin. The subcuticular zone is filled with tortuous tubules and vacuoles and there are two large indentations of the apical cell membrane. × 13,000.

FIG. 22. Part of the striated cuticular border and subcuticular region of a columnar absorptive cell in the jejunum of a 2 day old rat which had received no previous treatment. An indentation of the apical cell membrane containing some membranous material extends into the subcuticular zone. × 23,000.



(Clark: Ingestion by intestinal cells)

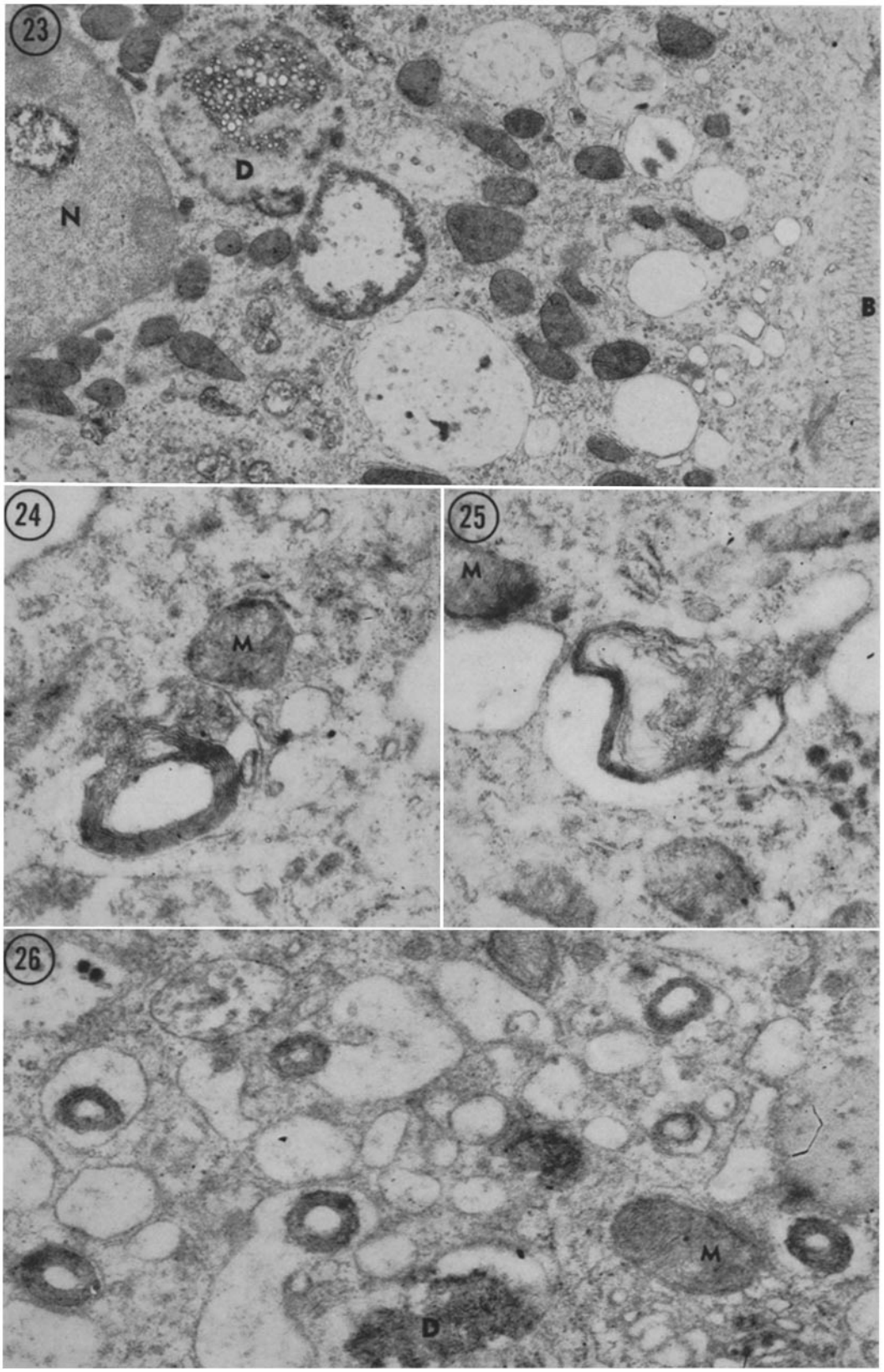
PLATE 20

FIG. 23. A columnar absorptive cell from the jejunum of a 6 day old mouse given distilled water orally 1½ hours before death. There are small tubular or vesicular structures in several of the vacuoles and in the dense body (*D*). This is one of the characteristic types of membranous structures observed in vacuoles and inclusions. The nuclear inclusion appears to contain lipide. *B*: striated cuticular border. *N*: nucleus. × 10,000.

FIG. 24. Part of the apical cytoplasm of a columnar absorptive cell from the jejunum of a 3 day old mouse given colloidal gold orally 2 hours prior to death. There is a vacuole surrounded by a single membrane which encloses a complex arrangement of concentric membranes. In this and the following two figures, the distance between the regularly arrayed concentric membranes is approximately 100 A. *M*: mitochondrion. × 30,000.

FIG. 25. Part of another columnar absorptive cell from the jejunum of the same animal as that of Fig. 24. A similar arrangement of concentric membranes enclosed in a vacuole is seen. *M*: mitochondrion. × 30,000.

FIG. 26. Part of the apical cytoplasm of a columnar absorptive cell from the jejunum of a 3 day old mouse which had received India ink orally 6 hours prior to death. Several of the numerous vacuoles contain concentric arrays of membranes. The dense inclusion (*D*) contains small dense particles presumed to be carbon. *M*: mitochondrion. × 38,000.

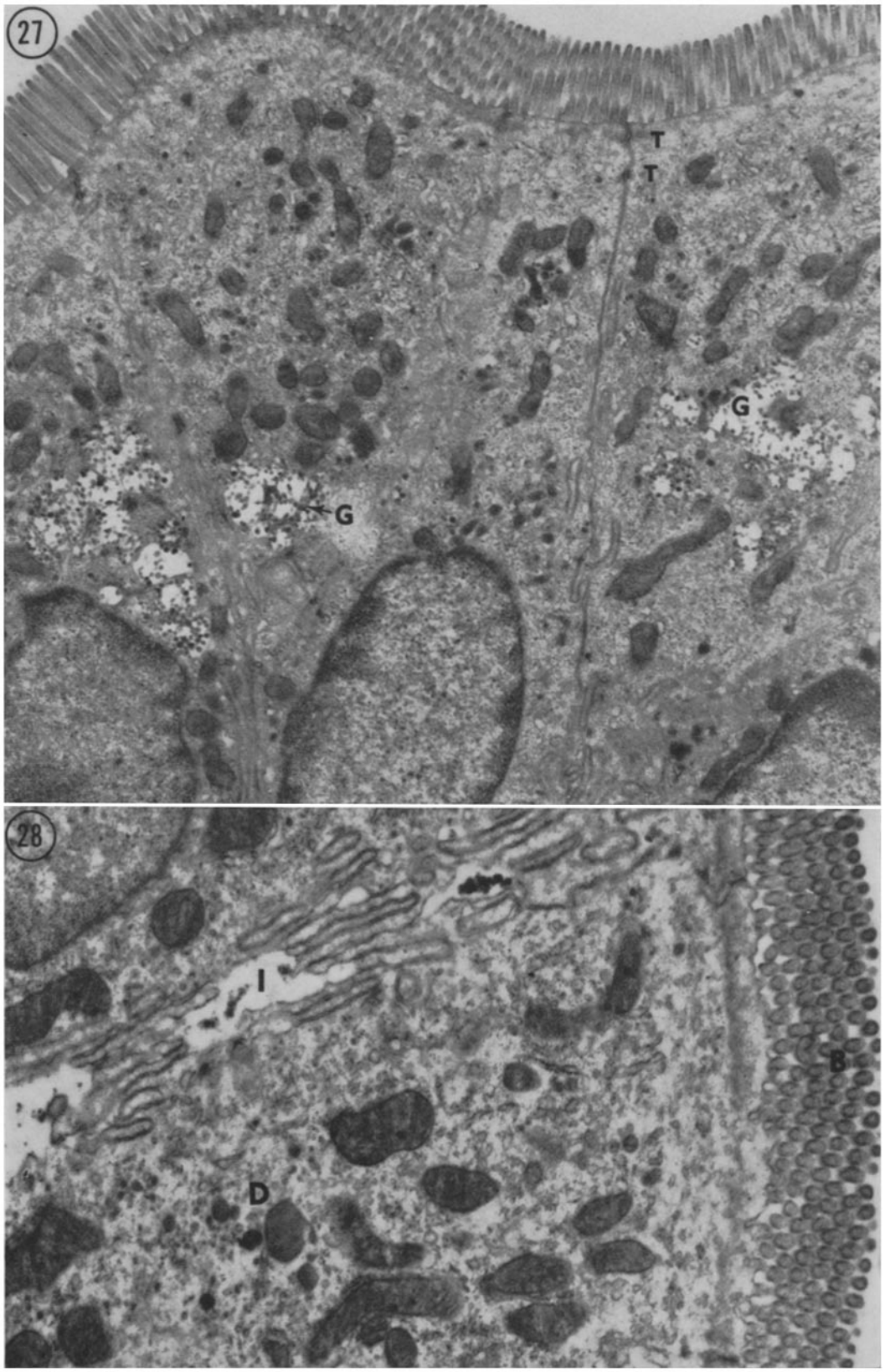


(Clark: Ingestion by intestinal cells)

PLATE 21

FIG. 27. Columnar absorptive cells from the jejunum of a 14 day old rat which had received bovine gamma globulin orally 3½ hours prior to death, but had also received a subcutaneous injection of cortisone acetate in a dose of 0.03 mg. per gm. body weight 3 days earlier. The apical cytoplasm does not contain large vacuoles or inclusions but does contain numerous fat droplets, both scattered through the apical cytoplasm and collected in the Golgi complex (*G*). In contrast with cells from animals of the same age which had not received cortisone (Figs. 11, 13, and 18), these columnar absorptive cells resemble those of adult animals (Figs. 1 to 4). *T*: terminal bars. × 7,000.

FIG. 28. Columnar absorptive cells from the jejunum of a 15 day old rat which had received bovine gamma globulin orally 3½ hours before death and 0.06 mg. of cortisone acetate per gm. body weight 4 days before death. In resemblance to the cells of adult animals and in contrast with those of suckling animals which had not received cortisone, there are only a few small indentations of the apical cell membrane, tubules are scarce in the subcuticular region, and there are few vacuoles or dense inclusions (*D*). The intercellular space (*I*) contains numerous lipide droplets. *B*: striated cuticular border. × 18,000.



(Clark: Ingestion by intestinal cells)