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THE ORIGIN, DEVELOPMENT, FUNCTION AND FATE OF THE GLOBULE LEUCOCYTE IN THE MOUSE INTESTINAL EPITHELIUM

by

ROY K.A. WESLEY

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, the City University of New York.

1973

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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ABSTRACT

THE ORIGIN, DEVELOPMENT, FUNCTION AND FATE OF THE GLOBULE LEUCOCYTE IN THE MOUSE INTESTINAL EPITHELIUM

by

ROY K.A. WESLEY

Advisor: Professor Thomas E. Jensen

This study is an investigation of the origin, development, function and fate of the intestinal globule leucocyte (crystal containing cell) in mice. Wesley and Jensen (1968, 1969) provided morphological evidence that the globule leucocyte is derived from the connective tissue mast cell. Further fine structural evidence and in vitro transformations of neoplastic mast cells under the influence of intestinal homogenates indicate that the intestinal globule leucocyte is derived from the mast cell.

A possible scheme of development of the globules and the internal crystals is presented and the entire sequence of development of the connective tissue mast cell into a globule leucocyte is described as a dynamic event in time which transforms the connective tissue mast cell morphologically and biochemically into a cell which finally deposits its contents into the lumen of the gastro-intestinal tract. It is suggested that the exocrine secretion of the globule leucocyte is composed of modified biogenic amines or possibly

prostaglandins as demonstrated by the phenolic coupling reaction using 2,6-dichloroquinoneimine (Gibbs' reagent) (Pearse, 1961). Indirect evidence indicates that the globule leucocyte secretion stimulates smooth muscle contraction in the gut with consequent diarrhea produced in a variety of experimental and pathologic conditions in which the globule leucocyte population increases in the intestinal epithelium. Consequently an alternate mechanism is proposed for the "self-cure" phenomenon occuring during nematode infestations without invoking the hypothesis presented by Carr (1967) that antibodies are produced by the globule leucocyte. Finally, it is proposed that the intestinal globule leucocyte of mice and rats be considered a modified mast cell in order to more accurately reflect its origin and general biochemical behavior.

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Special thanks to Jane Wang for performing the tedious job of mounting micrographs and for her encouragement and support. Finally, but most importantly, I would like to express my gratitude to my parents for their constant encouragement, support and help during these graduate years. Gratitude can not be expressed on paper but through the living work of life.

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INTRODUCTION

The crystal containing cell observed in the intestinal epithelium of mice described by Silva (1967) and Wesley and Jensen (1968, 1969) has been reclassified recently as a globule leucocyte (Toner, et al., 1971). The electron microscope studies of the crystal containing cell (Silva, 1967; Wesley and Jensen, 1968, 1969) are similar morphologically to the fine structural study of the mouse intestinal globule leucocyte (Carr, 1967).

The globule leucocyte is a migratory cell characterized at the light microscopic level by the presence of acidophilic cytoplasmic globules (Weill, 1919, 1920) which may contain intragranular crystals or paracrystalline inclusions at the electron microscopic level (Carr, 1967; Silva, 1967; Carr and Whur, 1968; Wesley and Jensen, 1968; Murray et al., 1968; Takeuchi et al., 1969). The globule leucocyte invades the epithelium of a variety of organs in most vertebrate classes (Heidenhain, 1888; Weill, 1919, 1920; Kirkman, 1950; Toner et al., 1971).

The function of the globule leucocyte is unknown, but it has been implicated in antibody production (Carr, 1967; Whur and Johnston, 1967), in participating in generalized gastrointestinal stress reactions (Wesley and Jensen, 1969), in the removal of intestinal parasites (Whur, 1966; Miller, 1971), and the release of a pharmacological mediator by an allergen-reaginic antibody system (Murray et al., 1971). The origin of the globule leucocyte is not certain in mouse intestine; however, the

plasma cell (Carr, 1967), the eosinophil leucocyte (Silva, 1967) and the connective tissue mast cell (Wesley and Jensen, 1968) have been suggested as the immediate precursor cell which develops into the globule leucocyte.

The purpose of this investigation was to examine more closely the origin and development of the globules and their crystalline inclusions by electron microscopy, to determine the fate of the globule leucocyte (an event which has not been described to date), and to elucidate the chemistry of the inclusions as related to the function of the cell.

LITERATURE REVIEW

Biological Crystals

One definition of a crystal is:

> A solidified form of a substance in which the atoms or molecules are arranged in a definite repeating pattern so that the external shape of a particle or mass of the substance is made up of plane faces in a symmetrical arrangement.[1]

According to this definition, some cellular constituents may be considered "crystals" since they have a regular molecular array leading to a well defined three-dimensional structure. For example, nucleic acids are composed in part of regularly repeated nucleotide units in a precise spatial distribution which can be analyzed by X-ray diffraction (Watson and Crick, 1953). Therefore, by this definition, chromosomes, ribosomes, proteins, lipids and membranes are crystalline structures.

This is not the usual understanding of the meaning of a crystal. In cell biology, a crystalline inclusion is generally of the same order of magnitude as an organelle. However, the potential for formation of larger crystals from proteins, lipids, membrane aggregates, nucleic acids or other cellular constitutents does exist and does in fact occur under normal and pathological conditions.

Nineteenth century light microscopists identified several natural and pathologic crystalline inclusions in animals *in vivo*. Intracellular

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[1] *Webster's New World Dictionary of the American Language*. New York: The World Publishing Company, 1970.

crystals were found in living Amoeba (Auerbach, 1856 cited in Grunbaum et al., 1959), in the cells within the sputum of asthmatic patients (Charcot and Robin, 1853), in human spermatogonia (Lubarsch, 1896) and in interstitial cells (Reinke, 1896). The most extensively studied of these crystals are those found in living Amoeba.

In the 103 years between the discovery of amoebic crystals in 1856 to 1959, approximately fifteen cytochemical reports appeared on the crystals found in Amoeba proteus, Amoeba dubia and Chaos chaos (Grunbaum et al., 1959). In 1960, J.L. Griffin successfully characterized the crystas as carbonyl diurea (triuret) by comparative studies of isolated crystals and known triuret samples using X-ray diffraction, infra-red spectral analysis, and chemical, physical and optical properties. A stable monoclinic form of triuret and a less stable bipyramidal form were isolated from Amoeba (Carlström and Møller, 1961; Griffin, 1961). Since the crystals are visible by light microscopy (Grunbaum et al., 1959), they are obviously real inclusions in some living amoebae. These crystals dissolve in normal chemical fixatives used in electron microscopy so freeze-etching techniques have been used to examine the fine structure of the crystal (Gicquaud et al., 1971). Both the bipyramidal and monoclinic forms exhibited a crystalline lattice spacing of 4.5 A (±0.5 A) after freeze-fracturing (Gicquaud et al., 1971) which is in close agreement with the periodicity reported by X-ray diffraction data (Griffin, 1960). It has been suggested that the carbonyl diurea crystal is a nitrogen excretion product representing an end product of purine metabolism in amoebae (Griffin, 1960). The amoebae which contain triuret crystals are

generally carnivorous and the crystalline inclusions may represent ingested macronuclear ciliate degradation products (Griffin, 1961).

Crystals are of widespread occurrence throughout the animal kingdom in a variety of tissue types. The most extensively studied crystalline inclusions in terms of distribution is the microbody. Microbodies (peroxisomes) are membrane limited intracytoplasmic bodies containing an electron dense crystalline or amorphous core in a less dense matrix and they have been found in 166 species of 75 animal families (Hruban and Rechcigl, 1969). Some authors consider this widespread distribution in animals as indicative that the microbody is a constituent organelle of higher animal cells (Hruban et al., 1972).

In mice, microbodies have been found in proximal and distal tubules of the kidney (Rhodin, 1954 Ph.D. thesis cited by Hruban and Rechcigl, 1969; Nishimura et al., 1964) and in liver cells (Daems and Rijssel, 1961; Trump et al., 1965; Daems, 1966; Essner, 1969). The microbody core (an electron dense structure) is either anucleoid (amorphous with low electron density [Essner, 1969]) or nucleoid (a core with high electron density) containing lamellae indicative of a subcrystalloid structure (Sabatini et al., 1963; Shnitka, 1966). Catalase has been localized in the microbody matrix (deDuve and Baudhuin, 1966) while urate oxidase has been found in the inner nucleoid (deDuve and Baudhuin, 1966; Hruban and Swift, 1964; Tsukada et al., 1968; 1971). Catalase is the only enzyme common to all microbodies and is found in liver cells, kidney cells, erythrocytes and leucocytes of higher animals (Hruban and Rechcigl, 1969). D-amino oxidase is commonly found in the core of many microbodies but is absent in the mouse microbody (Endahl and Kochakian, 1956; Meister et al., 1960).

Crystalline inclusions other than microbodies have been identified throughout the animal kingdom in a variety of organs. The following examples represent only a small sampling of a vast literature which has not been reviewed to date. Fine structural studies have revealed intracellular crystals among the following invertebrate phyla: protozoa (Gicquaud et al., 1971), coelenterata (Davis, 1967), annelida (Petzold, 1959; Lindler, 1964), echinodermata (Karasaki, 1965), arthropoda (Strunk, 1959; Moran and Staehelin, 1971) and mollusca (Travis, 1968). Among the vertebrate phyla, crystals have been found in fish (Marquet and Sobel, 1969), amphibia (Karasaki, 1963; Lanzavechhia, 1965; Honjin et al., 1965; Massover, 1971) and birds (Yoshimura and Irie, 1961; Byers, 1971).

Among the mammals, crystalline inclusions have been observed in a variety of tissues in mice (see Table 1) in monkeys (Bell, 1971) and in organs of man such as the adrenal glands (Magalhaes, 1972), in the thyroid gland (Richter and McCarty, 1954; Fujita and Machino, 1964; Elfvin, 1971) in human kidneys and liver (Hruban et al., 1966; Hruban and Rechcigl, 1969; Hruban et al., 1972), in hepatocytes (Sternlieb et al., 1971; Norum et al., 1972), in teeth (Frank and Cimasoni, 1970) and bone formation (Selvig, 1970).

Nearly all of the crystals mentioned so far occur as membrane limited cytoplasmic inclusions. Occasionally crystals occur in the cytoplasm without a limiting membrane as observed in some liver cells (Minio and Gautier, 1967) and in gout and pseudogout tissues (Lack, 1969). Extracellular crystals are also found outside eosinophil leucocytes of asthmatics (Welsh, 1959) and in the gastric mucosa

TABLE 1

INTRACELLULAR CRYSTALS IN MICE

Strains	Treatment or condition	Organ or tissues with crystals	Type of crystal	Reference
Swiss	Normal or cancerous mice	Crystals in lungs, acinic cells of pancreas, lumen of endometrial glands and thigh sarcoma.	Hexagonal crystals 3 um thick X 35 um wide X 70 um long	Green, 1942
Swiss	0.5% methylcholanthrene in acetone intravaginally	Gallbladder and lung.	Needle or rod shaped crystals 10-127 um long 1-55 um wide.	Yang and Campbell, 1964
Swiss	Normal	Bone marrow eosinophil	Membrane limited granules 0.3-1.2 um with central crystal having a 30 A repeat	Miller et al., 1966
Swiss	Normal	Pancreas	Crystals 1-3 um long by 0.2-1 um wide and a 75 A periodicity	Shibata, 1967
Swiss (?)	Normal	Intestine	Globule leucocyte with 45 A periodicity	Carr, 1967
Swiss	Normal, 5-hydroxytryptophan and monoamine oxidase treated	Ileum to colon	Globule leucocyte with 60 A periodicity	Silva, 1967
Swiss	Normal, actinomycin D treated	Cecum	Globule leucocyte with 60 A periodicity	Wesley and Jensen, 1968

TABLE 1--Continued

Strains	Treatment or condition	Organ or tissue with crystals	Type of crystal	Reference
Swiss	Intraperitoneal injection of triparanol and AY9944 (cholesterol inhibitors)	Schwann cells of sciatic nerve	Intracytoplasmic membrane limited granules with a lattice	Rawlins and Uzman, 1970
Swiss	Cytochalasin B	Ova	Spherical bodies 8-10 um with filaments	Moskalewski et al., 1972
Albino	Normal 2 month old	Thyroid follicular cells	Membrane limited bodies with crystals having a 70 A periodicity	Elfvin, 1971
Albino NMRI	Normal	Pancreas	Crystalline bodies noted	Bannasch and Schultze, 1972
Albino	18 hr fasted and normal	Liver	Microbodies	Trump and Ericsson, 1964; Rhodin, 1954.
C_3H_Z	Normal	Liver	Microbodies with a 95 A periodicity	Daems, 1966
DBA	Mice with pulmonary adenomatosis and pneumonitis	Mononuclear cells of lungs	Crystalline inclusions	Horn et al., 1952
C57/BL	Normal	Coagulating gland epithelium	Lysosomal crystals with a 250 A periodicity	Rowlatt, 1968

(Helander, 1969). Crystals have been observed in the nucleus of normal dog liver cells (Thompson et al., 1959a, 1959b; Gueft and Kikkawa, 1962), dog kidney (Thompson et al., 1959a; 1959b), skin biopsies of a patient with generalized vaccinia lesions (Patrizi and Middelkamp, 1969) and frog parathyroid glands (Coleman and Phillips, 1972). Crystals have been observed in the cisternum between the membranes of the nuclear envelope (Marquet and Sobel, 1969) as well as in the cisterna of the rough endoplasmic reticulum (Bessis, 1961; Toner, 1963; Benke and Moe, 1964; Hamilton et al., 1966; Marquet and Sobel, 1969; Kobayasi and Asboe-Hansen, 1970). Intramitochondrial crystals have been reported in normal and pathologic human liver cells (Mugnaini, 1964; Minio and Gautier, 1967; Norum et al., 1972) and in the mitochondria of developing blastocysts of mice (Hesseldahl, 1971).

Gastrointestinal Crystals

Crystals in intestinal cells of invertebrates have been observed at the electron m icroscope level among the annelids (Petzold, 1959; Lindler, 1964) and arthropods (Strunk, 1959; Devauchelle, 1970; Moran and Staehelin, 1971). A crystal containing cell with extruded crystals was observed in the earthworm Lumbricus, sp. by Cerfontaine (1890) who named them "corpuscles bacilliformes" and later by Cuenot (1898) who called them "bacteroides". Both authors believed the inclusions were bacterial inclusions (Cerfontaine, 1890; Cuenot, 1898). The fine structure of this cell showed that polygonal crystals were contained in membrane limited granules within the cytoplasm (Petzold, 1959). The crystals were 0.25 to 2.87 mu long by 0.25 to 1.25 mu wide and had a

100 to 225 A periodicity (Lindner, 1964). The "bacteroid cells" are generally found among peritoneal, epithelial or muscle cells and are sometimes joined to these cells by desmosomes (Lindner, 1964). There is an extensive endoplasmic reticulum system and the Golgi apparatus is sometimes observed connected to the vacuoles containing the crystals (Petzold, 1959; Lindner, 1964). No function is known for the bacteroid cell, but Lindner (1964) believes that the cell may function in blood cell formation or in the elaboration of intercellular ground substance material. Lindner (1964) also observed the extrusion of the crystals into the coelom of the gut in a sequence of events in which crystals formed in vacuoles coalesce and are extruded by exocytosis after the vacuole merges with the plasma membrane.

Intracytoplasmic crystals up to 30 um in length containing 60 A diameter subunits which were 90 A apart were found in the midgut glands of the marine isopod Limnoria lignorum (Strunk, 1959). Intranuclear crystals with a 100 A periodicity were reported in the midgut glands of Tenebrio molitor (Devauchelle, 1970) and the crystals have been shown to be composed of 6.5 A subunits (Moran and Staehelin, 1971). Virus-like particles appear when the intranuclear crystals break down, but no direct relationship between the crystals and the virus-like particles appeared to exist (Devauchelle, 1970). No functions are known for these crystals, but a secretory protein nature has been suggested for the intracytoplasmic crystals in Limnoria (Strunk, 1969).

A summary of crystalline inclusions found in the gastrointestinal tract of vertebrate mammals is presented in Table 2. The rat small intestinal paneth cell produces a crystalline structure with a 100 A

TABLE 2

FINE STRUCTURAL STUDIES OF GASTROINTESTINAL GLOBULE LEUCOCYTE CELLS AND INTESTINAL CELLS WITH CRYSTALLINE INCLUSIONS

	Animal (Species)	Granule Dimension	Crystal Dimension	Periodicity	Suggested Cell Origin	Experimental Observations	Reference
1.	Fowl	No Data	Absent	Absent	Lymphocyte	Intestinal epithelium	Toner, 1965.
2.	Chicken	No Data	Absent	Absent	Lymphocyte	Microtubules present	Holman, 1970.
3.	Rat	No Data	Absent	Absent	Mast cell or lymphocyte		Kent, 1966.
4.	Rat	No Data	No Data	No Data	Plasma cell with Russell bodies	Increases under parasitic infection	Whur and Johnston, 1967.
5.	Rat	No Data	No Data	33-45 A		Increases under parasitic infection	Carr and Whur, 1968.
6.	Rat	Up to 3um	No Data	70 A	Mast cell	Increases under parasitic infection	Murray et al., 1968.
7.	Rat	No Data	Absent	Absent	Lymphoid blast cell to mast cell.	Increases under parasitic infection	Miller, 1971a.

TABLE 2--Continued

	Animal (Species)	Granule Dimension	Crystal Dimension	Periodicity	Suggested Cell Origin	Experimental Observations	Reference
8.	Rat	230-660 mu	No Data	No Data		Suckling rat intestines	Gonzalez-Licea, 1972.
9.	Mouse	1 um	Up to 1.5 um	60 A	Eosinophil leucocyte	Increases under 5-HTP or MAO treatment	Silva, 1967.
10.	Mouse	No Data	No Data	45 A	Plasma cell	Increases under parasitic infection	Carr, 1967; Carr and Whur, 1968.
11.	Mouse	No Data	0.25-1.5 um	60 A	Mast cell	Increases under actinomycin D treatment	Wesley and Jensen, 1968.
12.	Cow	1-5 um	No Data	No Data	Mast cell	Increases under parasitic infection	Murray et al., 1968.
13.	Sheep	No Data	No Data	No Data	Mast cell	Increases under parasitic infection	Murray et al., 1968.
14.	Cat	0.5-1.5 mu	No Data	250 A	Migratory cell	Normal adults and newborn cats	Takeuchi et al., 1969.

TABLE 2--Continued

	Animal (Species)	Granule Dimension	Crystal Dimension	Periodicity	Suggested Cell Origin	Experimental Observations	Reference
15.	Chicken	No Data	0.7 X 1.5 um	130 A	Submucosal gland endoplasmic reticulum	Found in 24 hr fasted animals	Toner, 1963.
16.	Rat	No Data	No Data	75 A or 105 A	Parietal cell	Extracellular crystals of gastric fundus	Helander, 1969.
17.	Rat	No Data	No Data	100 A	Paneth cell		Behnke and Moe, 1964.
18.	Rat	No Data	No Data	50 A	Paneth cell		Yamada, 1972.
19.	Pig	up to 18 um	0.1-1.0 um wide; 1.0-40 um long	90 A		Neonatal pigs suckling colostrum.	Staley et al., 1969.
20.	Pig	No Data	No Data	No Data		No crystals in unsuckeled neonatal pigs	Hardy et al., 1971.
21.	Man	No Data	No Data	80 A	Reticulo-endothelial cell	Cystine crystals	Morecki et al., 1968; Hummeler et al., 1970.
22.	Man	No Data	No Data	No Data		Cholesterol crystals in negative images	Partin and Schubert, 1969.

periodicity in the cisternae of rough endoplasmic reticula in two to four week old rats. As the rat reaches adulthood, the paneth cell granules observed have the same regular periodicity and typical crystalline formation in the cytoplasm (Behnke and Moe, 1964). The paneth cell secretory material within the granule has been also reported to crystallize with a 50 A periodicity (Yamada, 1972).[2]

Crystals with a 75 A periodicity were found in the secretions from the gastric mucosa and within membrane limited granules in the lumen of the fundus glands and in the intracellular canaliculi of parietal cells in Sprague-Dawley rats of all ages (Helander, 1969). Large membrane bound colostral vacuoles up to 18 um in diameter containing needle-shaped crystals 0.1 to 1.0 um wide and 1.0 to 40 um long containing a 45 A periodicity are found in the absorptive intestinal epithelial cells of neonatal pigs nursing colostrum (the initial mammary milk) (Staley, et al., 1969; Hardy et al., 1971). An attempt was made to incorporate blood pigments (hematoidin) into the crystal by feeding the piglet whole blood, but no crystals were formed (Staley et al., 1969). Histochemical reactions to demonstrate the presence of hematoidin in thin sections (Gmelin reaction iodine method, ferric iron method and chloroform extraction) were all negative (Staley et al., 1969).

In none of the gastrointestinal crystals described so far is there an analysis of the chemical composition of the crystals or experimental evidence to show the function of these cellular inclusions. However, in human patients showing symptoms of the Fanconi syndrome

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[2]Personal calculations of the paneth cell crystal periodicity using the magnification data provided and the micron markers on the micrographs (Yamada, 1972) indicate an 87 A periodicity.

(cystinosis) which is characterized by excessive aminoaciduria, glycosuria, phosphaturia and abnormal storage of cystine crystals (Morecki et al., 1968), there is direct evidence that the hexagonal crystals observed by electron microscopy in the cornea, intestine and in renal tubules are cystine. The electron diffraction patterns of renal crystals are similar to diffraction patterns of pure L-cystine (Jackson et al., 1962). Although it was suggested that cystine crystals are found exclusively in the cells of the reticuloendothelial system (Morecki et al., 1968), it has been found that leucocytes and fibroblasts contain cystine in lysosomal-like organelles which stain positively with Gomori's acid phosphatase and it is postulated that the cystine crystallizes out at high concentrations (Hummeler et al., 1970).

Crystalline inclusions have been observed in some intestinal intraepithelial globule leucocytes (Table 2). Crystalline line to line center periodicities of 45 A (Carr, 1967; Carr and Whur, 1968) and 60 A (Silva, 1967; Wesley and Jensen, 1968) have been reported in mouse intestinal globule leucocytes. Intestinal globule leucocytes observed in rats showed a 33 to 45 A periodicity[3] (Carr and Whur, 1968) or a 70 A repeat (Murray et al., 1968). The cat intestinal globule leucocyte exhibited a 250 A periodicity (Takeuchi et al., 1969).

Description of Cecal Gastrointestinal Cell Types

The cecum is a division of the large intestine not generally described in detail but considered to be similar to the colon in morphology (Piliero et al., 1965). Figure 2 illustrates a typical

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[3]Personal measurements and calculations from magnification data and micrographs presented in Carr and Whur, 1968.

section through the cecum. The mucosal layer consists of the epithelium, lamina propria, and muscularis mucosae subtended by the submucosa containing the larger vascular elements, nerves in Meissner's plexus, adipose cells and connective tissue. The submucosa is underlain by the serosa composed of circular muscle, nerves in the Myenteric (Auerbach's) plexus, longitudinal muscle and bounded by the peritoneum (Piliero et al., 1965; von Henath, 1966; DiFiore, 1968; Laguens and Dumm, 1969). Extrinsic innervation to the intestines is supplied from the excitatory sympathetic ganglia. The intrinsic nerve supply is represented by the myenteric (Auerbach's) plexus and Meissner's plexus (Bockus, 1966; Texter et al., 1968; Brooks, 1970). The area of interest for this study is the epithelium and its indigenous cell population of goblet cells (mucous secreting cells generally lining the crypts of Lieberkühn), epithelial absorptive cells characterized by microvilli on the border facing the lumen of the gut, argentaffin (also called enterochromaffin, enteroserotonin or argyophil) cells which elaborate 5-hydroxytryptamine as well as other chemicals, and a large population of undifferentiated cells (Piliero et al., 1965; von Henath, 1966; DiFiore, 1968; Bloom and Fawcett, 1968; Laguens and Dumm, 1969). Paneth cells frequently observed in the small intestine are not generally found in the large intestine or cecum (Bloom and Fawcett, 1968). The mucosal layer contains lymph nodes as a source of lymphocytes at various intervals which forms an important part of the immune system of the gut acting against bacterial invasion and other pathogens (DiFiore, 1968; Mori and Lennert, 1969).

A variety of migratory cell types are described invading the

epithelial layer in both normal and pathogenic conditions. Commonly observed invasive cells are eosinophil leucocytes, mast cells and lymphocytes (Toner, 1968; TOner and Ferguson, 1971; Toner et al., 1971) The plasma cell with Russell bodies and the plasma cell are migratory cells incapable of integrating into the mucosal intestinal epithelium (Toner and Fergusson, 1971). The globule leucocyte is a common invasive cell type in the intestinal and other epithelia but it is not commonly reported or recognized because it is easily overlooked or incorrectly classified at both the light microscopic (Kent, 1952) and electron microscopic levels (Toner et al., 1971).

The Globule Leucocyte

The term "Schollenleukocyt" was first introduced by Weill (1919) to describe a cell type occurring in the intestinal epithelium of mice, rabbits and dogs containing large acidophilic globular inclusions. The German word "Schollenleukocyt" was translated into English as "globule leucocyte" by Keasbey (1923) and this somewhat awkward and misleading designation has persisted in the literature. Keasbey (1923) had her reservations about the name and she proposed either "globule" or "plaque leucocyte" as appropriate translations of "Schollenleukozyt" since she did not believe that the acidophilic inclusions were strictly globular in shape. Since then the term globule leucocyte has been used in the English language scientific literature. The cell type was originally described, but not named, by Heidenhain (1888) as an unusual inclusion in the intestine of the dog, rabbit and guinea pig. After fixation in a saturated picric acid solution and staining with Alain carmine, intraepithelial cells were observed containing round

red granules surrounded by a bright yellow halo (Heidenhain, 1888). The cell type is distinguished light microscopically by the presence of a single round nucleus in a cytoplasm containing acidophilic globules (Weill, 1919; 1920) which also stain metachromatically (Kirkman, 1950) with toluidine blue. The globules may number from 6 to several dozen in a cell (Kent, 1952) with an average globular diameter of 0.5 to 3.0 microns (Toner et al., 1971). Globules in sheep may be as large as 10 to 12 microns in diameter (Kent, 1952).

Light microscopic studies indicate that the globule leucocyte has a fairly wide distribution. The globule leucocyte has been found in the digestive tracts of various species in fish, amphibians, reptiles and mammals (Kent, 1952). Among the mammals, the globule leucocyte is found in the intestines of mice, rabbits, dogs (Weill, 1919), cats (Weill, 1920), guinea pigs, bats, sheep, pigs, horses and man. They are also present in the mesentery of turtles, lymph nodes of irradiated dogs, urinary passages of rat, the tracheal epithelia of rats and the endometrial tissues of women and sheep (Kent, 1952) and in the uterine epithelium of ruminants (Kellas, 1961).

The advent of electron microscopy has helped to clarify the general morphology and characteristics of the globule leucocyte. Fine structural studies have shown that the globule leucocyte is an intra-epithelial cell type found in the gastrointestinal epithelium of the mouse (Carr, 1967; Carr and Whur, 1968), rat (Carr and Whur, 1968), chickens (Toner, 1965; Holman, 1968; 1970), sheep (Dobson, 1966; Murray et al., 1968), cat (Takeuchi et al., 1969), and cow (Murray et al., 1968). Although the globule leucocyte is most often found in the gut, it is also found at the fine structural level in the bile duct epithelia

of cattle and sheep (Rahko, 1970) and in livers of deer (Blazek, 1971).

Ultrastructurally the globule leucocyte is mouse intestines is found among epithelial cells but without desmosomes along the membranes of neighboring cells (Carr, 1967; Silva, 1967; Wesley and Jensen, 1968; Toner et al., 1971). The most characteristic feature of the cell is the presence of spherical, membrane limited globules which may sometimes contain a crystalline array within the globule matrix located in a cytoplasm containing a large nucleus, scanty rough endoplasmic reticulum, many free ribosomes and a moderate Golgi apparatus (Carr, 1967; Silva, 1967; Wesley and Jensen, 1968; Toner et al., 1971). Fine structural characteristics of intestinal globule leucocytes in other species are summarized in Table 2.

Kirkman (1950) reviewed the light microscopic histochemistry of the globule leucocyte and presented an exhaustive cytochemical comparative study of the globule leucocyte using 69 different reagents on globule leucocytes of rat urinary tract, the cow gastric mucosa, cat intestine and gall bladder. The globules contain a periodic acid-Schiff positive mucopolysaccharide which is poor in free aldehyde groups and combines with a strongly basic protein (Kirkman, 1950). Some of the globule leucocytes, but not all, show metachromatic staining with new methylene blue, polychrome methylene blue, safranin, thionin, toluidine blue (metachromasia is noted with alkaline toluidine blue but not with acid toluidine blue) and brilliant cresyl blue (Kirkman, 1950). Some of these results were confirmed by Murray et al., (1968) on intestinal globule leucocytes of the rat and sheep. The globules

appeared to contain a sulfated acid mucopolysaccharide which stained metachromatically with aqueous toluidine blue and the globules bound Biebrich scarlet within the pH range of 8 to 10 indicating the presence of a highly basic protein (Murray et al., 1968). Murray et al. (1968) also noted toluidine blue metachromasia within the pH range of 4 to 5 which varied from deep violet metachromasia through blue to an almost colorless reaction.

In testing globule leucocytes for argentaffin cell reactions, Kirkman (1950) found that the globule leucocyte is negative when tested with the Fontana argentaffin reaction, the chromaffin reaction and diazo reaction and he found no fluorescence in the near ultraviolet range. Using Epon embedded sections, Takeuchi et al. (1969) found that the enterochromaffin cell stain diazotized safranin does not stain the globule leucocyte of cat intestines. These results would suggest that the globule leucocyte does not contain a phenolic group or 5-hydroxytryptamine (Takeuchi et al., 1969). Previously, in 1933, Vialli and Erspamer distinguished an acidophilic granulated cell in the intestinal epithelium of dogs and cats (probably the globule leucocyte [Takeuchi et al., 1969]) from the enterochromaffin cell. They concluded that the globule leucocyte did not have any coupled phenolic compounds because of its lack of staining using chromium, silver and diazo reactions (Vialli and Erspamer, 1933). In opposition to this data are the results of Murray, Miller, Jarret and Path (1968) indicating that the globule leucocytes of the rat, sheep and cow intestines do have a biogenic amine within the globules. The globule leucocyte shows a changing fluorescence during development in the intestine using acridine orange and the fluorescence technique for monoamines (Murray et al., 1968).

The globule leucocyte shows a weak green fluorescence under ultraviolet light although the granule sometimes contains a green fluorescent center (Murray et al., 1968).

The globule leucocytes have been shown to be negative for acid phosphatase, alkaline phosphatase and peroxidase (Kirkman, 1950).

The origin and the designation of the globule leucocyte as a distinct cell type have been sources of confusion in the light microscopic literature of intestinal and other epithelial cells for a long time. Heidenhain (1888) in the first descriptive report recognized the fact that the globule leucocyte was not a normal epithelial constituent. He thought at first that the inclusions were parasitic invaders, but after treating the animals with pilocarpine the cell type became more frequent so he dropped his parasitic invader supposition (Heidenhain, 1888). He then proposed the hypothesis that the unusual cell was derived from a wandering leucocyte which invaded the epithelium (Heidenhain, 1888). This hypothesis has been subsequently supported by a number of investigators' observations (Kent, 1952; Carr, 1967; Silva, 1967; Wesley and Jensen, 1968). Keasbey (1923) also believed that the globule leucocyte was an invading parasite similar to Amoeba dysenteriae which fills itself with red blood corpuscles, but a leading parasitologist at Columbia stated that her slides showed no parasitic infection and she quickly dropped the argument. However, Keasbey maintained that the globules contained phagocytized erythrocytic debris on the basis of similarities of histochemical staining between red blood cells and the globule leucocyte (Keasbey, 1923).

Weill (1919, 1920) proposed that the globule leucocyte was a wandering cell type with a secretory function that invaded the intestinal

epithelium. There has been no support for this observation to date. Globule leucocytes have been labelled as eosinophilic myelocytes, plasma cells, Russell body cells, erythroblasts, erythrophages, macrophages, degenerating cells, atypical mast cells, mucous cells, secretory leucocytes and granular cells (Kirkman, 1950).

From the data compiled in Table 2, it is apparent that the intestinal globule leucocyte increases under conditions of parasitic infection (Carr and Whur, 1968; Murray et al., 1968; Blazek, 1971), under conditions inhibiting serotonin metabolism (Silva, 1967) and under actinomycin D treatment (Wesley and Jensen, 1968). The relationship of the globule leucocyte to nematode infestation was statistically examined by Whur (1966) who showed that the globule leucocyte in the intestine and abomasum (gastric mucosa) of uninfected sheep was less than one globule leucocyte per square millimeter and that after infection the number of globule leucocytes reached a high of 150 cells per square millimeter. In rats infected with Nippostrongylus brasiliensis or Hymenolepis nana, there is a sudden increase on the 12th day after infection to more than 100 globule leucocytes per square millimeter peaking to 600 globule leucocytes on day 18 and dropping to 150 globule leucocytes on day 20. On day 12 when there is a spectacular increase in the number of globule leucocytes, there is a corresponding decrease in the worm count from a high of 1225 worms on day 8 to a low of 15 worms on day 12 (Whur, 1966). This is known as the "self-cure" phenomenon and it occurs in a number of host-parasite relationships (Sinclair, 1970). Because of this relationship of globule leucocytes in freeing the infected animals from intestinal parasites, Carr (1967) and Carr and Whur (1968) suggested that the

globule leucocyte in mice and rats is related to the plasma cell with Russell bodies since this cell type contains antibodies and is important in the immune defense system. Dobson (1966) used fluorescein isothiocyanate conjugated rabbit-anti-sheep globulin on intestinal globule leucocytes from sheep and found that the globules fluoresced and concluded that the globules contain globulin. He therefore postulated that a relationship between the globule leucocyte and the plasma cell was indicated (Dobson, 1966). However, this experiment has been repeated and the results have not been confirmed (Toner et al., 1971).

The connective tissue mast cell has also been proposed as a possible originator of the globule leucocyte in the rat, cow and sheep (Murray et al., 1968), in Nippostrongylus brasiliensis infected rats (Miller,1971a) in mice (Wesley and Jensen, 1968), and in cattle (Rahko, 1970). In rats infected with the nematode Nippostrongylus brasiliensis, there is an increase in the number of intestinal mast cells as well as in the number of globule leucocytes (Miller and Jarrett, 1971). The mast cell and the globule leucocyte appeared to be cytochemically related in that both show fluorescence under ultraviolet light using the method of Falck et al. (1962), the presence of sulfated mucopolysaccharides, metachromasia using acid toluidine blue, and fluorescence using acridine orange under ultraviolet light (Murray et al., 1968). Transitional cells were observed of similar size to the mast cell, but also containing larger granules characteristic of the globule leucocyte. With acridine orange, the transitional cell small granules showed an orange fluorescence similar to mast cells, but the larger granules showed a green fluorescence similar to that of the globule

leucocyte (Murray et al., 1968). Morphologically the granules of the rat mast cell and the globule leucocyte are sometimes indistinguishable and the cellular fine structure of the cow, sheep and rat globule leucocyte is similar to the tissue mast cell in that they both have membrane limited granules, similarly situated Golgi complexes, microvilli (pseudopodial extensions of the plasma membrane) and small amounts of rough endoplasmic reticulum (Murray et al., 1968).

Three independent workers observed the globule leucocyte by electron microscopy in mouse intestine at approximately the same time. Katherine Carr in Scotland published a descriptive report of the crystalline inclusions and made the assumption that the cell was a globule leucocyte (Carr, 1967) while D.G. Silva in Australia described a proliferation of a crystal containing cell under 5-hydroxytryptophan treatment (Silva, 1967). The first observation of the crystalline inclusions in mouse intestine in Dr. Jensen's laboratory occurred in August, 1967, in Detroit, Michigan and the results of preliminary observations of actinomycin D treatment were published in 1968 (Wesley and Jensen, 1968). The crystal containing cell of mouse intestine has been placed into the globule leucocyte category based on the fine structural morphology of the cell type (Toner et al., 1971). Ultrastructurally, the globule leucocyte of the mouse intestine from mixed laboratory strains is characterized as a mononuclear round cell at the base of the epithelium with a pale cytoplasm, scanty endoplasmic reticulum, free ribosomes, a moderate amount of Golgi, and globules containing crystals with a small amount of granular substance, a homogeneous matrix of granular substance with some periodicity visible or an "empty" granule with small amounts of amorphous material (Carr,

1967).

In albino mice, Silva (1967) reported a 60 A periodicity in the crystals in the crystal containing cell which did not form tight junctions between neighboring cells. Wesley and Jensen (1968) also found a 60 A periodicity in the crystals of Swiss strain albino mouse cecal epithelium and a 60 A periodicity within the matrix of the granules. Carr (1967) reported a 46 A periodicity in the crystals of globule leucocytes of mouse intestines from mixed laboratory strains.

Carr (1967) suggested that the 20 A diameter subunit of the mouse crystal is Y shaped and may be a crystalline immunoglobulin. It was also postulated that the globule leucocyte is a modified plasma cell with antibodies against parasitic infection and that the globule leucocyte bears similarities to the Russell body in plasma cells since it could act as an antibody reservoir (Carr, 1967). However, it was not possible for Carr (1967) to verify the presence of parasitic infection in all mice examined, since only a few showed an infection with Aspiculuris tetraptera.

Silva (1967) indicated that the crystals and granules of the globule leucocyte of albino mice may be reservoirs of 5-hydroxytyrptamine (serotonin) since the administration of the immediate biochemical prevursor of serotonin, 5-hydroxytryptophan, results in an increase in the numbers of crystal containing cells. If monoamine oxidase, the metabolic inhibitor of serotnin metabolism is used, a similar increase is noted when used alone or in conjunction with 5-hydroxy-tryptophan. From the morphological and experimental evidence presented, Silva (1967) concluded that the eosinophilic leucocyte may be the immediate precursor of the globule leucocyte.

Wesley and Jensen (1968) observed an increase in the number of crystal containing cells after actinomycin D treatment in albino Swiss strain mice. An intermediate cell type was observed in the lamina propria of high dosage actinomycin D treated mice which had characteristics of both the globule leucocyte and the mast cell and they postulated that the intraepithelial intestinal crystal containing cell (globule leucocyte) was derived from a mast cell precursor which invades the epithelium (Wesley and Jense, 1969).

The Mast Cell

The original description of the mast cell was made by P. Ehrlich in 1877, but it was 110 years ago that F. von Recklingshausen in 1863 observed these granular cells in connective tissue (Fernex, 1968). Ehrlich coined the term "Mastzellen" meaning over-fed cells because he believed the granules contained within them were a source of nutrient for other cells during degranulation of the mast cell (Ehrlich, 1879, cited in Selye, 1965). Ehrlich also noted that mast cells have a strong basophilic metachromasia when stained with aniline dyes (mast cell granules stain purple with basic dyes and often change the shade of the dye) (Ehrlich, 1879).

The mast cell is found in the connective tissue of most vertebrates (Bloom and Fawcett, 1968) and are known as tissue or histogenous mast cells and they are also found in the circulatory system. In this case they are known as mast leucocytes or basophiles (Michels, 1963). Historically, histogenous mast cells have been more extensively studied. Mast cells are present throughout the digestive tract including the cecum and they are abundant in the peritoneum. They are

also found in moderate numbers in the muscularis and in the serosal layers and may be abundant in the submucosa and mucosa (Michels, 1963).

The origin and development of mast cells are unclear but it is believed that they may be derived from undifferentiated mesenchymal cells (Diggs et al., 1954). Mast cells may arise through homoplastic regeneration (through mitosis of existing mast cells) or through heteroplastic regeneration (development from precursors such as lymphocytes, non-metachromatic mononuclear basophils, plasma cells, histiocytes or fibroblasts) (Absoe-Hansen, 1954). These and other mast cell relationships are illustrated schematically in Figure 73. Using alcian blue and safranin staining, Csaba and Forgacs (1971) have shown that ontogenesis of mast cells in rat embryos begins in hemopoietic tissues of thymus, spleen and liver and may later be associated with connective tissues in adult animals.

The mast cells of mice are spherical or ovoid and contains round nuclei and membrane limited granules in the cytoplasm. (Smith, 1963; Bloom and Fawcett, 1968). The plasma membrane forms microvillous extensions in thin sections under electron microscopy (Smith, 1963) and appears to be like a thorny ball under scanning electron microscopy (Fujita et al., 1971). There are few mitochondria in the cytoplasm, sparse endoplasmic reticulum and an abundant Golgi apparatus as well as membrane limited granules (Smith, 1963) which are approximately 0.6 um in diameter (Bloom and Fawcett, 1968).

Crystalline inclusions in the intracytoplasmic granules of mouse mast cells have not been identified; however, Bloom was the first to identify a crystalline substructure in the mast cells of the dog (Bloom, 1960, Ph.D. thesis, Karolinska Institut reported by Smith, 1963).

Other investigators found 1 to 2 um diameter granules containing a 90 A periodicity in guinea pig bone marrow basophils (Winqvist, 1963), human mast cell granules containing a 120 A periodicity (Fedorko and Hirsch, 1965), granules with a 70 A periodicity in mast cells of human gingiva (Weinstock and Albright, 1967) and 0.5 to 2.5 um granules in Newt mast cells containing a matrix of 40 to 50 um dense lines alternating with electron lucid spaces (Setoguti, 1969). Another intragranular inclusion observed sometimes in normal mast cells is the concentric lamellated whorl found in rats, mice and guinea pigs (Smith, 1963) and in a variety of human tissues and blood mast cells (Stoeckenius, 1956; Braunsteiner and Pakesch, 1957; Hibbs et al., 1960; Thiery, 1963; Brinkman, 1968).

The chemical constituents of mast cells have been studied in a variety of animal species but most extensively in rat tissue mast cells. The anticoagulant heparin (a sulfated acidic mucopolysaccharide) is apparently stored within the granules of mast cells as shown by fractionation studies (Julen et al., 1950), by the fact that isolated granules are capable of increasing the clotting time of blood plasma (Benditt, 1958), and by chemical analysis of the granules (Green and Day, 1960). Rat mast cells also contain the nonsulfated acid mucopolysaccharide hyaluronic acid (Asboe-Hansen, 1959), which is a disaccharide of glucuronosyl-ethyl-glucosamine and a primary constituent of connective tissue (Bloom and Fawcett, 1968). Since hyaluronic acid is the product mainly of fibroblasts, it has been suggested that mast cells do not synthesize hyaluronic acid but take it up through pinocytosis. Experiments supporting both sides of this concept are reviewed by Sagher and Even-Paz (1967). The full chemical structures

of heparin and hyaluronic acid are not known but the compounds are similar in that the glucosamine of heparin is sulfated while that of hyaluronic acid is acetylated (Roden, 1971).

The biogenic amines histamine (the decarboxylation product of the amino acid histidine) and serotonin (the decarboxylation product of the hydroxylated amino acid tryptophan) are found in mast cells (Green and Day, 1960). Riley (1953) and RIley and West (1953) demonstrated that histamine levels increase with increasing mast cell concentration and that degraulation leads to a drop in histamine levels. The enzyme histidine decarboxylase is present in mast cells of many animal species including the mouse (Sagher and Even-Paz, 1967). The mast cells of rats and mice contain small amounts of 5-hydroxytryptamine (Benditt et al., 1955; Benditt and Wong, 1957) although it appears to be absent from mast cells of other animal species (West and Parratt, 1957). The enzyme 5-hydroxytryptophan decarboxylase is present in the mast cells of mice and rats (Lagunoff et al., 1957; Lagunoff and Benditt, 1959; Hagen, 1961). The synthesis of 5-hydroxytryptamine by mast cells derived from mouse mastocytomas and maintained in vitro has been demonstrated (Sjoerdsma et al., 1957; Schindler, 1958; Schindler et al., 1959). Although mouse and rat mast cells are capable of synthesizing serotonin, the intracellular localization of serotonin is not yet established (Fernex, 1968). West and Parratt (1957) believed that serotonin is present in the cytoplasm outside the granules while Green and Day (1960) believed that serotonin and histamine are bound with heparin within the intracellular granules.

From a study of mouse gastrointestinal enteroserotonin cells, the in vivo administration intravenously of ^{3}H-5-hydroxytryptophan and

^{3}H-5-hydroxytryptamine resulted in the uptake of ^{3}H-5-hydroxytryptophan but not ^{3}H-5-hydroxytryptamine in the gastric endocrine cells, but the tissue mast cells showed incorporation of either ^{3}H-5-hydroxytryptophan or ^{3}H-5-hydroxytryptamine into the dense granules (Rubin et al., 1971) as shown by electron microscopic autoradiography. This result indicated that serotonin is bound with heparin within the intracellular tissue mast cell granules (Rubin et al., 1971).

Thiry six years ago, the function of mast cells seemed clear after the discovery that heparin seemed to be associated with the granules (Jorpes et al., 1937); however, in the intervening years, up to twenty five diverse functions have been assigned to mast cells ranging from inflammation to nutrition (Michels, 1963) and the list continues to grow as evidenced by reviews of this subject (Padawer, 1963; Sagher and Even-Paz, 1968).

The Eosinophil Leucocyte

The eosinophil leucocyte was described as a potential source for globule leucocytes in mouse intestine (Silva, 1967). Takeuchi et al. (1969) believed that the transformed rat eosinophil leucocyte observed by Casley-Smith (1968) in the intestinal epithelium could be a globule leucocyte as demonstrated by similarities in electron microscopic morphology. Some of the transformed eosinophils found during parasitic infestation by Acanthocephalae contained normal eosinophil leucocyte granules in the cytoplasm as well as abnormal membrane limited granules which had some resemblance to globule leucocyte granules (Casley-Smith, 1968).

The eosinophil leucocyte originates from myeloid tissue in

the bone marrow of adult animals. Myelocytes from this tissue may differentiate into three basic types of polymorphonuclear granulocytes: eosinophilic (acidophilic) leucocytes, basophilic leucocytes or heterophilic leucocytes (Bloom and Fawcett, 1968). Eosinophil leucocytes are spherical cells 9 to 12 um in diameter containing a bilobate nucleus and a cytoplasm filled with oval granules which stain intensly with acid dyes (Archer, 1963). The general fine structure of eosinophil granules were first observed in bone marrow cells by Pease (1955) and in human blood cells by Bernhard and Leplus (1955). A 50 A lamellar periodicity of the internal core of mouse eosinophil granules was first reported by Sheldon and Zetterquist (1955). A later study showed that the eosinophils of rats and Swiss strain mice contained a core periodicity of 30 A (Miller et al., 1966) and these authors suggested that the core may be composed of catalase and urate oxidase. The granules of eosinophils have been isolated and found to contain peroxidase, catalase, acid and alkaline phosphatases, cathepsin, ribonuclease, arylsufatase and beta-glucuronidase (Archer and Hirsch, 1963a). The core of the granule is argenine rich and is believed to account for the cell's acidophilia while the outer matrix is periodic acid-Schiff positive (Gross and Gedigk, 1959) and rich in phospholipids (Vercauteren, 1953).

The development of the granules and packaging of enzymes from the Golgi apparatus and endoplasmic reticulum have been described (Bainton and Farquhar, 1966, 1970). Acid phosphatase, aryl sufatase and peroxidase are localized in the matrix of the granules, but not the core of immature rabbit eosinophil leucocytes using electron microscopic histochemical techniques (Bainton and Farquhar, 1970).

Ultrastructural histochemistry of mouse eosinophil leucocytes in

the connective tissue of the intestine shows peroxidase and acid phosphatase are present in the matrix but not the core of the eosinophil granules (Geyer et al., 1970). This observation is similar to earlier fine structural observations of acid phosphatase localization in rabbit eosinophil leucocyte granules (Seeman and Palade, 1967; Wetzel et al., 1967) and in man (Ghidoni and Goldberg, 1966). Seeman and Palade (1967) observed that some degree of disruption or damage to the granule membrane was apparently necessary for the acid phosphatase reaction product to accumulate within the granules since well preserved granules never showed the reaction product.

Under certain pathologic conditions in man, unusual intracellular crystalline inclusions develope from eosinophil leucocytes of asthmatics (Charcot and Robin, 1853; Leyden, 1872), in patients with amebic dysentery (Todd et al., 1953) and in eosinophilic granuloma of bone (Ayres and Silliphant, 1958). Large crystalline formations were induced in eosinophil leucocytes in vitro by application of surface active wetting agents (Ayres and Starkey, 1950). Pathologically induced crystals from asthatics and diocytl sodium sulfosuccinate (a wetting agent) induced Charcot-Leyden crystals were examined electron microscopically and were found to be 20 to 400 um long (Welsh, 1959).

Most evidence indicates that the eosinophil leucocytes play an important role in the inflammatory process (Archer, 1963a + b; Parish, 1970). The presence of acid phosphatase and other hydrolytic enzymes (Archer and Hirsch, 1963) within a membrane limited cytoplasmic inclusion indicates that the eosinophil leucocyte granule belongs to the lysosomal system (de Duve and Wattiaux, 1966). The eosinophil leucocyte is believed to be a histamine and serotonin antagonist (Archer, 1963) and is therefore

important in the inflammatory response. Specific histamine binding sites on the plasma membrane of mixed human leucocytes have been found using argarose complexed beads. Histamine-rabbit serum albumin-Sepharose complexes with leucocytes but not with red blood cells while the rabbit serum albumin-Sepharose does not bind to leucocytes or red blood cells (Melmon et al., 1972).

Riddle and Barnhardt (1965) found profibrinolysin (plasminogen) in the eosinophil leucocyte granules. They suggested that the eosinophil plays a role in the formation of inflammatory exudates (Riddle and Barnhardt, 1965).

Time lapse cinematography shows the eosinophil leucocyte is phagocytic and undergoes degranulation during the process (Archer and Hirsch, 1963b). The eosinophil leucocyte is a migratory cell capable of ameboid movement (Marchesi, 1960) and is apparently dependent on microfilaments for its locomotion and phagocytic abilities since these are abolished by administration of cytochalasin (Zigmond and Hirsch, 1972). Antigen-antibody complexes are phagocytized by eosinophils as demonstrated by Litt (1964) who injected green fluorescent dye labelled bovine serum albumin intraperitoneally and recovered peritoneal eosinophils which fluoresced yellow (the complementary color). It was found that antigen alone does not stimulate phagocytosis and the eosinophil apparently plays no role in antibody formation (Litt, 1961, 1963). Ferritin labelled antigen-antibody complexes are ingested by eosinophil leucocytes and observed by electron microscopy to be localized in vacuoles and granules (Connell, 1968). Antigen-antibody complexes have also been demonstrated to be eosinophilotactic in vivo (Parish, 1970).

The Plasma Cell

As previously mentioned, the plasma cell has been proposed as the immediate precursor cell to the globule leucocyte in mouse and rat intestinal epithelium (Carr, 1967; Carr and Whur, 1968). The plasma cells are mesenchymal cells originating from lymphoid elements and they are abundant in the lamina propria of the gastrointestinal tract (Bloom and Fawcett, 1968) but they are not observed within the epithelial layer of the gut (Toner and Fergusson, 1971).

The cells are round with a single oval shaped nucleus which often contains heterochromatin in a "cartwheel" arrangement (Toner, 1965; Bloom and Fawcett, 1968) and the cytoplasm exhibits an intense basophilia probably caused by a large ribonucleoprotein content since ribonuclease abolishes the basophilia (Bloom and Fawcett, 1968). Electron microscopic observations demonstrated that the plasma cell contains an elaborate network of rough endoplasmic reticula with expanded cisternae and sometimes the cisternum may be filled with electron dense Russell bodies or occasionally crystals (Braunsteiner et al., 1953; Thiery, 1958; Bessis, 1961; Movat and Fernando, 1962a + b).

The Russell bodies have been observed to contain a crystalline matrix with a 100 A periodicity (Wellensieck, 1957; Thiery, 1958). Pearse (1949) observed that the Russell body was composed of a muco-protein as indicated by the Russell body's positive periodic acid-Schiff staining reaction and the degree of staining correlated with the degree of development of the Russell body. The Russell bodies were surrounded by pyronin positive material which could be abolished by ribonuclease and he concluded that the mucoprotein granules were surrounded by

ribonucleic acid (Pearse, 1949). These observations were confirmed by electron microscopy in that the Russell body has been observed to develop within the cisternae of the endoplasmic reticulum studded with ribosomes (Thiery, 1958).

The plasma cell functions in the immune response by producing antibodies within the cisternae of the endoplasmic reticulum (Reiss et al., 1950; de Petris et al., 1963). Multiple intraperitoneal injections of an antigen into rabbits produced crystalline inclusions with a 120 A periodicity within the plasma cells from the mesentery (Movat and Fernando, 1962). Studies using fluorescent labelled antibody and tritiated antibody autoradiography have shown that antibodys are present in the cytoplasm of plasma cells (Coons et al., 1955; Askonas and White, 1956; Berenbaum, 1958). Direct electron microscopic localization of anti-ferritin-antibody production in immunized rabbits using ferritin as antigen showed that the cisternae of the rough endoplasmic reticulum of plasma cells were ferritin labeled while controls were not (de Petris et al., 1963). Thus, antibody production appeared to occur within the plasma cell rough endoplasmic reticulum.

Plasma cells infiltrate the gastric mucosa during inflammation (Gray and Doniach, 1970) and being the major source of antibody production (Bloom and Fawcett, 1968), the plasma cells and lymphocytes provide the intestines with a defense mechanism against infections by bacteria and other agents (Piliero et al., 1965).

The Lymphocyte

The lymphocyte has been proposed as a possible precursor cell to the globule leucocyte in the intestinal epithelium of the white leghorn hen (Toner, 1965) and the epithelium of the young white leghorn

chickens (Holman, 1968; 1970) on the basis of cell fine structural morphology alone. Weill (1919) proposed that the lymphocyte was a possible candidate for the globule leucocyte precursor and Kent (1952, 1966) suggested that the lymphocyte was a possible candidate in sheep intestin during lymphocytic migration from the tunica propria (i.e., the submucosal layer) into the epithelium and consequent development into globule leucocytes. Takeuchi et al., (1969) observed that in the cat intestine, the ultrastructure of granules of intestinal lymphocytes showed a heterogeneity similar to the globule leucocytes, but the general cytoplasmic characteristics all the two cell types to be destinguished.

Lymphocytes are abundant in the gastrointestinal tract because of the presence of Peyer's patches, lymphoid nodules on the surface of the mucosa. Most of the migratory cell types which invade the intestinal epithelium from the lamina propria are lymphocytes (Toner and Ferguson, 1971) which originate from the subepithelial lymphatic nodules (Bloom and Fawcett, 1968). Long lived lymphocytes in circulation generally arise from lymph nodes and the shorter lived lymphocytes originate from the thymus; however, lymphocytes may also arise from the spleen and tonsils (Bloom and Fawcett, 1968). In general, lymphocytes are characterized morphologically as small round cells 9 to 12 um in diameter containing an eccentrically placed indented nucleus in a small amount of cytoplasm with a small number of mitochondria and having a high nucleus to cytoplasm ratio (Yoffey and Courtice, 1970). The cells have an active surface membrane and are capable of ameboid movement clocked at a maximum speed of 30 um/ minute (McCutcheon 1924; 1955).

Lymphocytes are classified as non-granular basophilic

leucocytes, but occasionally azurophilic cytoplasmic granules are observed after Romanowsky staining (Bloom and Fawcett, 1968). The basophilia is apparently caused by the presence of ribonucleic acid since ribonuclease destroys the pyronin-positive basophilia (Pearse, 1949). Even though lymphocytes are considered non-granular in structure, it has been estimated that 34% of blood lymphocytes contain refractile granules and neutral red staining of lymphocytes in man and rat showed granules and rosette granular formations (Yoffey and Courtice, 1970). Osmiophilic lipid granules (Gall bodies) are observed and glycogen is present in some normal lympocytes (Gibb and Stowell, 1949; Ackerman, 1967) and glycoprotein granules are occasionally found (Ackerman, 1967).

The lymphocytes and monocytes of guinea pigs contain large granular inclusions termed Kurloff bodies and were first reported by Kurloff in 1889 and independently by Foa and Carone in the same year (Pearse, 1949). The Kurloff body is eosinophilic, surrounded by a basophilic cytoplasm, reacts positively to periodic acid-Schiff and remains positive to periodic acid-Schiff after hyaluronidase treatment indicating that the Kurloff body is composed of mucoprotein (Pearse, 1949).

MATERIALS AND METHODS

Living Materials

A total of 183 mice were used during the experimental studies of the crystal containing cell in the intestine. The 160 Swiss strain mice used were descendents bred from parents derived from Swiss strain stocks at Plymouth Farms, Michigan obtained in 1966, and from the animal rooms of the University of Illinois School of Medicine obtained in 1969 through the courtesy of Dr. W. Frank Hughes III. A total of 23 AKD mice were used which were the descendents of parents derived from the Jackson Laboratory, Bar Harbor, Maine in 1971. The parental generation resulted from crossing AKR/J X DBA/2J mice which yielded the filial generation AKD2/F1 female mice (Jackson Lab designation 0301, #33809) and male mice (Jackson Lab designation 0301, #33740). Both male and female mice were used from the Swiss and AKD strains for experiments and the mice were maintained separately at room temperature with food and water available ad libidum. Complete nutrient Wayne Lab-Blox food pellets with added vitamins and minerals were obtained from Allied Mills, Inc. (Chicago, Illinois). The food pellets contained no added antibiotics. A complete list of food pellet ingredients is listed in Appendix I.

The P815Y neoplastic mast cell line was a generous gift from Dr. Glenn Fischer (Roger Williams General Hospital, Providence, Rhode Island). The mast cell line arose from a neoplasm derived from a 0.2% methylcholanthrene treated DBA/F2 mouse (Dunn and Potter, 1957) and

the P815Y neoplasm was carried as an ascitic tumor in (AKR X DBA/2)F1 mice (Schindler et al., 1959). The mast cell culture was maintained according to the procedures previously outlined by Richard Schindler et al., (1959) and procedures related by personal communication with Doctors Fischer and Chu (Roger Williams General Hospital, Providence, Rhode, Island) in 1971. The in vitro culture procedures are listed in Appendix II. Fischer's medium for leukemic cells of mice, horse serum and penicillin-streptomycin solutions were obtained from Grand Island Biological Company, Grand Island, New York.

Electron Microscopy

The sacrifice of mice was accomplished in one of three ways: the most commonly used method was that of cervical dislocation. In cases which experimental procedures precluded the dislocation technique, a saturated atmosphere of chloroform was used; or, in the case of the perfusion technique for electron microscopy, a 50 mg sodium barbital (Nembutol) per kilogram weight of the mouse was used as the anesthetic dose administered intramuscularly in the gluteus maximus followed by a lethal dose of Nembutol intramuscularly or by inhalation of chloroform after the perfusion was completed. The cecum was dissected and the distil tip of the cecum placed in cold 3% glutaraldehyde 0.4M cacodylate-0.2M sucrose buffered solution pH 7.2 to 7.3. Preparations of solutions used for electron microscopy are described in Appendix III.

The cecum was cut into cubes approximately 1 mm^3 or smaller while in solution and immediately transferred to vials containing cold 3% glutaraldehyde in 0.4M cacodylate-0.2M sucrose buffer, pH 7.2 and placed at 4°C for one hour. Immediately after the glut-

araldehyde fixation, the specimens were washed in the cacodylate-sucrose buffer, rapidly dehydrated in a graded alcohol series, dehydrated in propylene oxide and allowed to rotate overnight in a 1:1 mixture of Epon 812 to propylene oxide followed by 6 to 12 hours rotation in pure Epon and embedment in flat aluminum boats. Polymerization of Epon occurred for 24 hours under a 35°C lamp followed by a 45°C oven for 24 hours and finally a 65°C oven for a period of at least three days. Complete procedures for electron microscopic preparations are listed in Appendix IV.

After the hardened Epon blocks were trimmed, ultrathin sections were cut using an LKB ultramicrotome on a DuPont diamond knife to yield silver interference colored sections indicating that the sections were 60 to 80 mu thick. The sections were picked up with uncoated copper grids dipped in an adhesive solution (Pease, 1965).

The sections were post stained with uranyl-magnesium-acetate (Stampak and Ward, 1964) alone or in combination with lead citrate (Reynolds, 1963). The sections were examined with an RCA-3H or an Hitachi-HU 11E electron microscope operating at 60 kV and 75 kV respectively. Photographic procedures are given in Appendix V.

Light Microscopy

Thick sections approximately one micron thick were cut on the LKB ultramicrotome on a DuPont diamond knife in order to make a correlation between the electron microscopic sections and the adjacent thick sections for light micrsocopy. The sections were placed on a drop of water on a glass slide and heated to dryness on a hot plate and then stained with 0.5% toluidine blue (Trump, et al., 1961) at pH 9 containing

1% Borax (sodium borate) (Trump et al., 1961; Hingson and Ito, 1971). The stain was differentiated in distilled water and 95% ethanol.

Portions of the cecum of mice were also prepared for light microscopy by fixation in 4% (v/v) formaldehyde in 0.7% NaCl solution for at least 24 hours and then embedded in paraffin. Sections were cut on a Spencer microtome to a 15 or 20 micron thickness and placed on glass slides coated with Haupt's adhesive (Pearse, 1961).

Localization of biogenic amines

Ten to fifteen ultrathin sections 60 to 80 mu in thickness were cut and placed on formvar coated copper grids with a 2 mm by 1 mm slot. The adjacent thick sections were approximately one micron thick and were placed on glass slides. The one micron section was either cleared of Epon for differential staining (Lane and Europa, 1965) or stained directly without further preparation. The Epon clearing technique is described in Appendix VI. The histochemical stains used were hemotoxylin and eosin (Gridly, 1968), Gibbs' method for argentaffin cell granules (Pearse, 1961) using 2,6-dichloroquinonechloroimide (Bordon Company Chemical DIvision, Philadelphia, Pennsylvania), the diazo method using Fast Red Salt B (George T. Gurr Ltd., London, England) (Pearse, 1961), and toluidine blue with 1% Borax (Hingson and Ito, 1971). Procedures for these stains are listed in Appendix VI. One entire thin section was photographed under the electron microscope by taking overlapping exposures of the section. The electron microscope negatives were printed and then spliced together to form a large composite of the section. The montage was then correlated to the stained one micron section which was photographed on Kodak HC-135 film using a Zeiss Standard RA microscope under bright field or

phase contrast.

The fate of biosynthetic precursors to serotonin (5-hydroxytryptamine) was followed by light and electron microscopic autoradiography using tritiated compounds. Five mCi of tritiated DL-5-hydroxytryptophan (S.A.=3.3 Ci/mMole) were injected intravenously through the tail vein of Swiss strain mice. The mice were sacrificed 4 and 18 hours after the administration of radioactive compounds. In order to retain the labeled 5-hydroxytryptamine and remove unincorporated 5-hydroxytryptophan, the excised ceca, colon and peritoneal cells were washed in physiological saline (Gershon and Ross, 1966a; 1966b) before fixation in glutaraldehyde and subsequent electron microscopic preparation using sucrose buffers (Gershon and Ross, 1966a). Sections for light and electron microscopy were made and prepared for autoradiography following standard procedures (Caro and van Tubergen, 1962; Salpeter and Bachmann, 1964; Salpeter, 1966; Caro, 1969; Budd, 1971). The procedures used for autoradiography are briefly described in Appendix VII.

Acid Phosphatase Localization

Ceca from adult female Swiss strain mice were quickly removed and fixed in 3% glutaraldehyde cacodylate-sucrose buffered solution. Small cubes of the distal portion of the cecum were cut into portions approximately 1 mm^3, After a one hour fixation at 4°C, the tissues were rinsed in a cacodylate-sucrose buffer twice and placed into the acid phosphatase test or control incubation media. The test solution used ATP disodium salt ($Na_2H_2ATP \cdot 4H_2O$) from Nutritional Biochemicals Corporation (Cleveland, Ohio) as substrate in 0.2M tris-maleate buffer,

pH 7.3, 2% lead nitrate, 0.05 M $MgCl_2$, and distilled water (Gomori, 1950; Otero-Vilardebo et al., 1964). The control solution was the same as the test solution with the only difference being the omission of the substrate. The tissues were incubated in the solutions for one to three hours at 37°C and rinsed twice in the 0.2M tris-maleate buffer, pH 7.3 followed by a brief rinse in 0.005 M acetic acid. A brief rinse in dilute acetic acid is recommended by Gomori (1952) to remove the non-enzymatic reaction products. This procedure removes not only aberrant precipitates but may also remove some normally expected reaction product (Daems et al., 1969). The samples were post-fixed in 1% osmium tetroxide in sucrose-cacodylate buffer for one hour at 4°C, rapidly dehydrated and embedded in Epon.

All solutions used were freshly made on the day of experimentation and used immediately to avoid false localization of reaction products due to aging (Maunsbach, 1966). The age and composition of the incubation medium is of importance in the Gomori method for acid phosphatase in determining the final reaction product formed (Daems et al., 1969). Maunsbach (1966) indicates that cytoplasmic and nuclear staining obtained with aged media is less apparent in tissue fixed in glutaraldehyde for more than 2 hours when apparently more acid phosphatase is inhibited. To avoid any possible confusion from lead citrate staining, electron microscopic examinations of uranyl-acetate post stained sections only were made. In some cases, completely unstained sections were examined.

Peroxidase Localization

Normal female Swiss strain mice were sacrificed by cervical

dislocation and the distial tip of the cecum was quickly removed and fixed in 3% glutaraldehyde cacodylate-sucrose buffered solution after manually cutting the tissue into 0.5mm^3 pieces. The specimens were fixed in the glutaraldehyde solution for one hour at 4°C and washed twice in buffer. The cecal segments were then placed in one of 3 media: 1) an incubation medium containing 3,3^1-diaminobenzidine tetrahydrochloride (DAB) from Sigma Corporation in 10 ml of 0.05M sodium acetate-acetic acid buffer, pH 5.0 with 0.1 ml of a 0.1% hydrogen peroxide solution (fresh), and 1.0 ml of 0.05 M manganous chloride, 2) a control medium containing the incubating solution minus the substrate, DAB, and 3) a control medium containing the incubating solution minus the hydrogen peroxide. The tissue remained in these media for one hour at 37°C and were quickly post-fixed in 1% osmium tetroxide in sucrose-cacodylate buffer for one hour at 4°C. The samples were rapidly dehydrated and placed into Epon. The technique for peroxidase localization is similar to that described by Beard and Novikoff (1969).

Protein Digestion

Thin sections of cecal cells were obtained by normal ultramicrotomy and either stained with uranyl-acetate and lead citrate and examined under the electron microscope or left unstained and examined. THe sections were placed in a 0.5% pepsin solution in 0.1N HCl to dissolve proteins in Epon embedded smaples (Perrin, 1972). Before treatment with pepsin (2X crystallized from Worthington Biochemical Corporation), the grids were placed in 10% hydrogen peroxide for 20 minutes to remove complexed osmium tetroxide. The grids were washed five times in distilled water and placed in the 0.5% pepsin in 0.1 N HCl at 37°C for time periods varying from 15 minutes up to 24 hours.

Following pepsin incubation, the grids were washed five times in distilled water, stained with uranyl-acetate and lead citrate and examined under the electron microscope. Controls consisted of incubation of grids in 10% hydrogen peroxide alone for time periods of 10 minutes to 30 minutes, and incubation of grids in 0.1 N HCl alone for time periods corresponding to pepsin incubation times. These grids were washed five times in distilled water and stained with uranyl acetate and lead citrate and examined under the electron microscope.

Actinomycin D Experiments

The procedures for actinomycin D treatment of Swiss strain mice have been previously described (Wesley and Jensen, 1969) and are listed in the appendix. The same procedures were used on the AKDF/2 mice. In addition, two AKDF/2 mice received an intraperitoneal injection of 7.0×10^5 P815Y mast cells each on day one of actinomycin D administration. All AKDF/2 mice used were litter mates weighing 13 grams each. The total concentration of actinomycin D per injection was lower for the AKD mice than for the Swiss strain mice because of the smaller size and weights of the mice. The actinomycin D dosage received by the AKD mice was 2 ug actinomycin D per mouse per injection, but the same injection scheme was followed as described in the appendix.

In vitro P815Y Mast Cell and Peritoneal Cell Incubation

Attempts were made to induce mast cell transformation and crystalline formation in vitro in the P815Y mast cell line and in normal

Swiss and AKDF/2 peritoneal cells using homogenates and filtrates of mouse cecal tissue from Swiss and AKDF/2 intestines. The following experiments were done:

Experiment 1. Swiss strain cecal homogenate added to P815Y mast cells in vitro. A normal adult female Swiss strain mouse was sacrificed by cervical dislocation. The entire cecum was removed and opened in a sterile Petri dish containing sterile Hank's Minimal Essential Medium (Grand Island Biological Company, Grand Island, New York). The tissue was washed five times in fresh solutions of sterile, cold Hank's Minimal Essential Medium for five minutes in each wash. The tissue was then placed in 2 ml of sterile Fisher's Leukemic Cell Medium (Grand Island Biological Company) in a sterile Potter-Elvehjem homogenizer and homogenized for 5 minutes. A 0.5 ml quantity of the Swiss strain cecal homogenate was then added to a concentrated culture of P815Y mast cells containing 8.15×10^5 cells per ml. The mixture was stirred and allowed to incubate from one hour to 21 hours at 37°C. Fixation was accomplished by adding a volume of 50% glutaraldehyde for one hour at 4°C. The cells were washed five times in cold Fisher's medium, placed in a fibrin clot (Sicko and Arnold, 1971) and then post-fixed in a 1% osmium tetroxide in Fischer's medium for 1 hour at 4°C. The cells were dehydrated rapidly in a graded alcohol series and embedded in Epon. Controls consisted of P815Y mast cells cultured in 5.5 ml of Fischer's medium and a 0.5 ml quantity of the cecal homogenate cultured in 5 ml of Fischer's medium for 1 to 21 hours at 37°C.

Experiment 2. Swiss strain cecal homogenate added to normal Swiss strain peritoneal cells in vitro. Peritoneal cells were obtained from normal female adult mice by injecting 6 ml of Hank's Minimal Essential Medium intraperitoneally, gently massaging the abdominal cavity and then removing the peritoneal fluid by means of a sterile pipette. The mouse was sacrificed by cervical dislocation and the cecum removed, washed and homogenized as described for experiment 1. A cell count was made using a Spencer micro-hemocytometer and 2.0 X 10^6 peritoneal cells were found per ml. 0.5 ml of the cecal homogenate was then added to 1.0 ml of the peritoneal cells, stirred and incubated for one hour to 21 hours at 37°C. As controls, 1.0 ml of the peritoneal cells was incubated in 4 ml of Fischer's medium and 1.0 ml of the homogenate was incubated in a separate tube of 4 ml Fischer's medium. All cultures were fixed by the addition of a 50% glutaraldehyde solution to a final concentration of 3% for one hour at 4°C followed by post-fixation in 1% osmium tetroxide after five rinses in Fischer's medium, rapid dehydration and embedment in Epon.

Experiment 3. AKD2F/2 cecal homogenates added to P815Y mast cell cultures. Normal AKD2F/2 mice were sacrificed by cervical dislocation. A small portion of the distal tip of the cecum was removed and fixed in 3% glutaraldehyde in cacodylate-sucrose buffer for electron microscopy and another portion in 10% formalin in physiological saline for light microscopy. The remainder of the cecum was removed and washed five times in Hank's Miminal Essential Medium for five minutes

each using a Vortex mixer. The tissue was then homogenized in a Potter-Elvehjem homogenizer for 5 minutes. 1.0 ml of the homogenate was added to each of four culture tubes of P815Y mast cells concentrated to a volume of 3.4 to 8.8 X 10^6 cells per ml. The cells were allowed to incubate with the homogenate at 37°C and samples were removed and fixed for electron microscopy after 1, 2, 4, 6, and 8 hours. Fixation was accomplished by centrifugation of the cultured cells and resuspension in a cold 2:1 mixture of 1% osmium tetroxide in 0.1 M cacodylate pH 7.4 to 2.5% glutaraldehyde in 0.1 M cacodylate pH 7.4 (Hirsch and Fedorko, 1968). The cells were fixed for one hour, washed twice in ice cold physiological saline and aggregated using the fibrin clot technique (Sicko and Arnold, 1971) followed by completion of fixation using a post-fixation in 0.25% uranyl acetate in 0.1 M acetate buffer, pH 6.3 (Hirsch and Fedorko, 1968). This procedure for fixing cells in suspension for electron microscopy will hereafter be called the modified Hirsch-Fedorko technique. Controls included the P815Y mast cells cultured alone and the incubated homogenate alone prepared for electron microscopy by the modified Hirsch-Fedorko technique. This is described in Appendix IX.

Experiment 4. AKD2F/2 cecal homogenates added to P815Y mast cells <u>in vitro</u> containing actinomycin D. Litter mates of mice used in experiment 3 were used and a cecal homogenate obtained as previously described. 0.2 ml of a 20 ug/ml solution of actinomycin D was added to each of two test tubes. The experimental test tube contained 5 ml of P815Y mast cells

(3.4 X 10^6 cells per ml), 1.0 ml of the AKD2F/2 cecal homogenate, and 2.0 ml of Fischer's medium. The control test tube contained 5 ml of P815 mast cells (3.4 X 10^6 cells per ml), and 3.0 ml of Fischer's medium. The final concentration of actinomycin D in each tube was 4 ug/ 8.2 ml or 0.48 ug/ml. The contents of these cultures were incubated for 1,2, 4, 6 and 8 hours and fixed using the modified Hirsch-Fedorko method.

Experiments 5 and 6. Sterile Swiss strain cecal homogenates added to P815Y mast cells in vitro. Normal adult Swiss strain mice were sacrificed by cervical dislocation. The ceca and colons were removed quickly from each mouse, opened and washed in cold physiological saline. The tissues were minced with a razor, ground with a mortar and pestle on ice in cold physiological saline. Some of this mixture was placed into a test tube and sterilized in an autoclave for 20 minutes and the remainder was filtered through cheesecloth. The filtrate was then refiltered through a 0.22 micron Millipore filter (Millipore Corporation) using a vacuum line. 3.0 ml of filtrate was recovered which was kept ice cold. The filtrate and the sterile homogenate were placed into separate sterile dialysis bags (Thomas Scientific #4465-A2) which retains proteins greater than or equal to 12,000 Daltons. The bags and their contents were dialyzed against Hank's Minimal Essential Medium (sterile) for 5 hours with constant mechanical stirring at ice cold temperature. 2.0 ml of the axenic cecal filtrate were added to a 5.0 ml culture of P815Y mast cells (3.0 X 10^5

cells per ml), 2.0 ml of axenic cecal filtrate which was dialyzed against Hank's medium were added to 5.0 ml of P815Y mast cells, and 2.0 ml of the sterile dialyzed homogenate were added to 5.0 ml of P815Y mast cells. These cultures plus the homogenate alone and the P815 mast cell culture alone were incubated for 15 or 24 hours. The samples were fixed by the modified Hirsch-Fedorko method for electron microscopy. This experiment was repeated with the following modifications: the Potter-Elvehjem homogenizer was used in place of the mortar and pestle to break the tissues and cells, Fischer's medium was used throughout the experiment instead of Hank's Minimal Essential Medium and the incubation periods were for 12, 24, 48, and 72 hours.

In vivo Uptake of P815 Mast Cells

In order to follow P815Y mast cells after injection into living mice, attempts were made to label the mast cells with a non-toxic persistent marker which would be readily identifiable at the electron microscopic level. The P815 mast cell cultures were labeled with tritiated thymidine (International Chemical and Nuclear Corporation, Irvine, California), Ruthenium Red (E. Fullam Company) or Ferritin (Polysciences, Inc). The tritiated thymidine was a generous gift from Dr. S. Wallace of the City University of New York.

Tritiated thymidine was added to cultures of P815 mast cells and incubated at 37°C for times from 15 minutes to 16 hours (one division cycle of cultured P815 mast cells [Bergeron, 1971]). After incubation in the hot label, the cells were chased with cold thymidine

by washing at least three times in Fischer's medium containing cold thymidine. The cells were then injected into Swiss strain or AKDF/2 mice according to the schedule presented in Table 3.

TABLE 3

EXPERIMENTS ON THE IN VITRO ^{3}H-THYMIDINE LABELING OF P815 MAST CELLS AND THEIR IN VIVO UPTAKE IN MICE

Specimen Number	Amount of ^{3}H-thymidine	Length of in vitro labelling	Organism and period of in vivo incorporation of ^{3}H-thymidine labelled P815 mast cells
90	14.7 mCi/ml	15 min.	Swiss strain mouse injected with 1.5 X 10^{6} cells for 1 hr.
91	14.7 mCi/ml	15 min.	Swiss strain mouse injected with 1.5 X 10^{6} cells for 1 hr.
102	14.7 mCi/ml	30 min.	Swiss strain mouse injected with 1.5 X 10^{6} cells for 12 hr.
106	14.7 mCi/ml	30 min.	Swiss strain mouse injected with 1.5 X 10^{6} cells for 24 hr.
110	14.7 mCi/ml	30 min.	Swiss strain mouse injected with 1.5 X 10^{6} cells for 48 hr.
114	14.7 mCi/ml	30 min.	Swiss strain mouse injected with 1.5 X 10^{6} cells for 72 hr.
154	7.3 mCi/ml	3 hr.	AKDF/2 mouse injected with 2.0 X 10^{6} cells for 12 hr.
158	7.3 mCi/ml	3 hr.	Swiss strain mouse injected with 2.0 X 10^{6} cells for 12 hr.
166	7.3 mCi/ml	3 hr.	AKDF/2 mouse injected with 2.0 X 10^{6} cells for 39 hr.
162	7.3 mCi/ml	3 hr.	Swiss strain mouse injected with 2.0 X 10^{6} cells for 39 hr.

In addition to the experiments presented in Table 3, a P815 mast cell culture (C7) was labelled for 16 hours with 14.4 uCi/ml of ^{3}H-thymidine (S.A. = 7.34 Ci/mM), chased with cold thymidine medium and then placed into a sterile dialysis bag holding a total volume of 1.3 X 10^{6} mast cells. The sterile dialysis bag was loosely tied around the cecum of Swiss strain mice anesthetized with Nembutol. The peritoneal cavity was closed by sutures and the mice recovered without showing any signs of infection. After 9 hours, the mice were sacrificed by cervical dislocation, the ceca excised and fixed for electron microscopy and the contents of the dialysis bags washed out in Fischer's medium and fixed by the modified Hirsch-Fedorko method. A control mouse was treated in the same manner without the use of radioactively labelled mast cells in the dialysis bag.

P815 mast cells were also labelled with various concentrations of rutheium red (200 ppm to 0.2%) for time intervals of 45 minutes to 24 hours followed by resuspension in fresh unlabelled Fischer's medium. In one case, P815 mast cells were labelled for 15 hours with 0.2% ruthenium red, resuspended for 1 day in fresh Fischer's medium, incubated at 37°C and placed into a sterile dialysis bag which was tied around the cecum of a normal female Swiss strain mouse for 12 hours. The same procedures were followed as previously described for the the dialysis bag experiment using tritiated thymidine labelled P815 mast cells.

The last label used to identify mast cells was a 0.2% ferritin solution added to the P815 mast cell cultures for 15 hours in a procedure similar to that for ruthenium red.

Experiments were done in vivo in an attempt to incorporate the serotonin precursor 5-hydroxytryptophan into globule leucocytes. Swiss strain mice were injected with 0.2 ml of 50 uCi/ml of tritiated-5-hydroxytryptophan (Amersham/Searle, DesPlaines, Illinois) with a specific activity of 3.3 Ci/mM. Mice were sacrificed 4 to 18 hours after injection of labeled 5-hydroxytryptophan. Specimens of intestinal ceca were then prepared for electron microscopic autoradiography and light microscopic autoradiography as described in Appendices IV and VII. Sucrose buffers were used during the fixation to halt the leaching out of radioactive serotonin and to prevent extraction during fixation procedures (Gershon and Ross, 1966a).

Quantitation of Electron Micrographs

Data measurements made from electron micrographs involved a minimum of 11 separate measurements (unless stated otherwise) in determining the standard error according to the formula

$$S.E. = \sqrt{\frac{(x - \bar{x})^2}{n - 1}}$$

where x = the individual measurement, $\bar{x}$ = the average of all measurements and n = the sample size.

Data collected for Figure 1 and Table 4 were made from electron micrographs taken at random from a collection of over 2500 micrographs of actinomycin D treated animals and 1500 normal Swiss strain micrographs. Only micrographs with magnifications (from the electron microscope) of 1700X to 2500X were used to insure a random sample of cells for the total population.

RESULTS

Morphological Observations

Globule leucocytes in normal Swiss strain cecal epithelia

Observations of control Swiss strain cecal epithelia indicate that the globule leucocyte is most frequently found in continuous association with undifferentiated cells at the basal aspect of the crypt of Lieberkühn. Globule leucocytes are less frequently found near mucous cells or absorptive epithelial cells and rarely found near argentaffin cells. These visual qualitative observations are supported by the distribution frequencies of total cecal cell populations given in Table 4. This table gives the distribution in terms of the total cell population and is not restricted to cells contiguous to the globule leucocyte. In an electron microscopic survey of 522 control epithelial cells, 84.5% of the cells were undifferentiated cells, 8.2% mucous cells, 4.8% epithelial globule leucocytes, 2.7% absorptive epithelial cells and 0.2% argentaffin cells.

In all globule leucocytes observed, no desmosomes were found connecting the plasma membranes of the globule leucocyte to any other cell as previously reported (Carr, 1967; Silva, 1967; Wesley and Jensen, 1969). It appears that the early invasive globule leucocyte can be distinguished in the epithelium by the presence of a relatively large intercellular gap between the plasma membrane of the globule leucocyte and the indigenous basal epithelial cells (Figures 3 and 4).

The plasma membranes of normal epithelial cellular constituents

TABLE 4

SUMMARY OF CELL COUNTS FROM SWISS STRAIN ACTINOMYCIN D EXPERIMENTS

Experiment	Absorptive Epithelial Cell		Mucous cell		Argentaffin Cell		Undifferentiated Cell		Epithelial Globule Leucocyte		Lamina propria Globule Leucocyte		Total Number of Epithelial Cells
	#	f	#	f	#	f	#	f	#	f	#	f	
Group I	16	0.152	8	0.076	2	0.019	70	0.667	9	0.086	0	0.000	105
Control I	4	0.019	17	0.079	0	0.000	186	0.869	7	0.033	0	0.000	214
Group II	9	0.093	5	0.051	0	0.000	73	0.753	10	0.103	0	0.000	97
Control II	0	0.000	7	0.064	0	0.000	95	0.872	7	0.064	0	0.000	109
Group III	34	0.209	9	0.055	2	0.012	110	0.675	8	0.049	3	0.018	163
Control III	6	0.059	7	0.069	0	0.000	82	0.812	6	0.059	0	0.000	101
Group IV	6	0.069	7	0.080	3	0.034	66	0.759	5	0.057	12	0.138	87
Control IV	4	0.041	10	0.103	0	0.000	78	0.804	5	0.052	0	0.000	97
TOTAL CONTROLS	14	0.027	41	0.078	1	0.002	441	0.845	25	0.048	0	0.000	522
TOTAL ACTINOMYCIN D	65	0.185	29	0.082	7	0.019	319	0.906	32	0.091	15	0.042	352
Total Cells													874

are generally in close apposition connected by tight junctions. In a later stage of maturation of the invasive globule leucocyte, the plasma membrane becomes closely opposed to the plasma membranes of surrounding cells and is distinguishable by a lack of desmosomes (Figure 5). The plasma membrane of the globule leucocyte becomes interdigitated with neighboring cells. Sometimes neighboring desmosomes may be easily confused as a part of the globule leucocyte plasma membrane system (Figure 5).

In this study, the intestinal globule leucocyte appears to differentiate from a free migratory mast cell-like precursor in the lamina propria (Figure 22). This precursor may invade the basal aspect of the epithelium and differentiate into the globule leucocyte which is defined by the presence of large globules (Heidenhain, 1888; Weill, 1919) or crystals (Carr, 1967; Silva, 1967; Wesley and Jensen, 1968) in the cytoplasm (Figures 5 and 23). Thus two forms of the same cell type are distinguished depending on whether it is found free in the lamina propria or embedded in the intestinal epithelium. The free migratory form is morphologically similar to a connective tissue mast cell occurring in the lamina propria (Figure 22) since similar intracytoplasmic granules are present. When the connective tissue mast cell is close to the epithelium, distinct segregation of dense particles occurs in the granules and a greater affinity for osmium tetroxide by the granules is observed (Figures 11, 12, 22). This cell under discussion is a connective tissue mast cell-like globule leucocyte precursor and will be referred to simply as the precursor globule leucocyte. The other form of the globule leucocyte found in the epithelium proper will be called the invasive globule leucocyte.

Three stages of development of the invasive globule leucocyte have been observed. The first stage is that of incorporation into the epithelium and will be called the early invasive globule leucocyte or early globule leucocyte. The second stage is characterized by an increase in cell dimensions and the development of granules and crystals with movement away from the lamina propria toward the lumen of the crypt of Lieberkühn. The second stage appears to be a middle stage of development. In the third stage or late stage of globule leucocyte development the globule leucocyte is at the lumen of the crypt of Lieberkühn and releases its crystalline material and granules by exocytosis.

Stage 1 Development. After incorporation into the epithelium of the precursor globule leucocyte (Figure 22) from the lamina propria, a basement membrane forms at the cell free border (Figure 43a) and pseudopodia-like extensions of surrounding cells begin to engulf the globule leucocyte (Figure 43a). Large intercellular spaces are characteristic of the early invasive globule leucocyte (Figures 3,4, 43a). The newly incorporated globule leucocyte has an irregular outline but is generally elliptical in shape and may be up to 10 u in length at its larger diameter. The early globule leucocytes in FIgures 3 and 4 are approximately 7 u in length and contain dense osmiophilic mast cell-like granules which are less than 0.5 u in diameter. The cytoplasm is typically sparse containing many free ribosomes and a peripheral rough endoplasmic reticulum. The plasma membrane of the early globule leucocyte may make close contact with surrounding cells (middle right side of Figure 4) but no tight junctions are formed as is typically observed tween neighboring indigenous epithelial cells (Figure 4).

In Figure 3, the relationship of the early invasive globule

leucocyte is seen with respect to the entire epithelium between the lamina propria and the lumen of the cecum. Stage 1 globule leucocytes are readily distinguished from plasma cells and eosinophils which may be in close contact in the lamina propria (Figure 9).

Stage 2 Development. During the middle stage of development of the invasive globule leucocyte, the cell migrates toward the central region of the epithelium and undergoes an increase in cell size and an increase in the number and size of globules occuring within the cytoplasm (Figure 5). It is generally during the middle stage of development that rectangular crystalline inclusions are found in some of the globular membranes. The globules and the crystals formed within them often show signs of coalescence when the globules approach a one micron diameter (Figure 5). The intracellular membrane limited crystals are the result of condensation of globular materials (Figures 5, 11, 12, 13). The development of crystals will be discussed separately.

The Golgi apparatus is generally well developed in stage 2 globule leucocytes and is almost always located in a central perinuclear region corresponding to a slight indentation in the nucleus (Figure 5). The cytoplasm is slightly more abundant than in stage 1 globule leucocytes and the rough endoplasmic reticulum is still generally peripheral. The plasma membrane is usually in close contact with the adjacent cells (Figure 5) unlike the stage 1 plasma membrane (Figure 4), but no desmosomes are found joining the globule leucocyte plasma membrane to adjacent cells at any stage of development.

Stage 3 Development. Globule leucocytes in the final stage

are rarely seen. The cells are most frequently found between epithelial or mucous cells at the border of the lumen of the gut when they are observed. There appears to be no further increase in cell size from the maximum attained during stage 2 development and there is quite often a slight decrease in size because of the loss of cytoplasmic contents into the lumen.

The characteristic feature of a stage 3 globule leucocyte is the extrusion of cytoplasmic crystals and globules from the cell into the lumen of the gut. The globule leucocyte in Figure 6 was surrounded by epithelial and mucous cells and appears to have exocytosed some crystals into the intercellular space which is continuous with the lumen of the cecum. At the lower right hand corner of FIgure 6, the plasma membrane appears to be discontinuous. This discontinuity may be a sectioning artefact since in some late stage globule leucocytes, the limiting membrane surrounding a collection of crystals is continuous with the plasma membrane. This indicates that the release of internal granules is by exocytosis. The cytoplasmic rhomboidal to rectangular crystals almost always contain a regular 55-70A periodicity depending on the angle of sectioning (Figure 8) through the crystalline matrix.

Development of globules in normal Swiss strain globule leucocytes.

The intracytoplasmic globules appear to develop in two different ways: either from alterations in pre-existing mast cell-like granules (Figure 12) or by the de novo synthesis involving intracisternal accumulations within the smooth endoplasmic reticulum (Figure 10) which may later receive Golgi products in middle stage development (Figure 5).

In either case, the end product of membrane limited globules is apparently identical. The internal matrix of the globules appears to condense in late stage globule leucocytes to form intraglobular crystalline inclusions (Figure 13) which are eventually exocytosed (Figure 6). During the process of globule and crystalline development, the membrane limited globules increase in size as the cell moves toward the lumen of the intestine (Figures 10, 11, 12, 13). Micrographs 10 to 13 are printed at the same magnification so that the visual increase in size represents a real increase in dimensions. The increase in granule size is apparently from intragranular product synthesis and accumulations from the coalescence of granules.

In the lamina propria, precursor globule leucocytes can be recognized with some cytological and granular similarities to the invasive globule leucocytes. The intragranular inclusions in the precursor globule leucocyte having similar counterparts in the intraepithelial globule leucocytes are the striated lamellae having a variable periodicity from 10 mu to 25 mu (Figures 10, 11, 15) and small dense spheres generally near the limiting membrane of the globules with diameters ranging from 20 mu to 250 mu (Figures 11, 15). The periodicity of the intraglobular lamellae generally decreases as the globule leucocyte migrates toward the lumen. For example, the precursor globule leucocyte in the lamina propria with small granules contains an intraglobular lamellae system with a 206.0 A ($\pm$4.0A) periodicity (Figure 10) and another precursor globule leucocyte in the lamina propria closer to the basement membrane of the epithelium containing larger and more developed granules has intraglobular lamellae with a 173.6A ($\pm$7.1A) repeat (Figure 11). A stage 2 globule leucocyte

(middle stage of development) located in the cecal epithelium contains a lamellar scroll with a 113.8 A (±8.2A) repeat within the globule (Figure 16). The intraglobular lamellae are often closely associated with the limiting membrane of the globule (Figures 10, 11, 15) as are the dense spherical bodies in late stage globule leucocyte granules (Figure 13).

THe intraglobular small dense spheres are variable in size and shape but appear to increase in size from the approximately 20 mu diameter in precursor globule leucocytes with small granules (Figure 10) to the larger dense spheres in precursor globule leucocytes closest to the epithelium containing dense spheres 50 to 250 mu in diameter (Figures 11, 15). In globule leucocytes without formed intraglobular crystals, the dense spheres are highly irregular (Figure 12) but become more uniform in size once the crystals are formed (Figure 13). The dense spheres contained within the globule with developed crystals are approximately 25 mu in diameter (Figure 13). Thus, there is approximately a 10 fold increase in diameter size of the precursor globule leucocyte intraglobular dense sphere to the early invasive globule leucocyte followed by a 10 fold decrease in spherical diameter in the middle to late globule leucocyte stages.

Globule leucocytes in normal AKD cecal epithelia.

The development of a globule leucocyte in AKD mice (Figures 17 to 21) from a precursor globule leucocyte cell in the lamina propria to its invasion and subsequent development in three distinct stages in the cecal epithelium is similar to that observed in Swiss strain mice (Figures 10 to 13). There are, however, some significant differences in AKD globule leucocyte development. Most precursor globule

leucocytes in AKD mice appear to have granules developing within the cisternae of rough endoplasmic reticula (figure 17) rather than the smooth endoplasmic reticula observed in Swiss strain mice (Figure 10). The cisternae of the rough endoplasmic reticula in AKD mice range in diameter from 55 to 110 mu and the developing granules apparently are derived by coalescence or aggregation of the rough endoplasmic reticula cisternae. The early developing granules (Figure 17) range in size from 250 mu to 1 u. The precursor globule leucocyte observed in Figure 17 is typical of many others in AKD mice in the lamina propria very close to the basement membrane of the epithelium and occasionally subtended by an incorporated globule leucocyte or one which was close by. After the precursor globule leucocyte is taken into the epithelium at the basal aspect (Figure 18), the globules develope dense spherical bodies and some intragranular peripheral lamellae (Figures 18, 19, 20, 21). The newly incorporated stage 1 globule leucocyte contains a variety of sizes of globules ranging from spherical globules approximately 100 mu in diameter to elliptically shaped globules up to 2.0 u in the long axis.

Stage 2 AKD globule leucocytes have a round globule containing short bar-like crystals in cross section (Figures 20b, 21) which contain an average line to line center periodicity of 59.1 A ($\pm$5.5A). At low magnification, the stage 2 globule leucocytes generally have a striking resemblance to eosinophil leucocyte granules; however, the periodicity and granule appearance at higher magnification is distinctive enough to be able to separate the two cell types. No stage 3 globule leucocytes in normal AKD mice have been observed. However, in

experimentally treated AKD mice, stage 3 globule leucocytes can be infrequently found (Figure 33).

Precursor Globule Leucocytes

Under normal conditions, the number of precursor globule leucocytes in Swiss strain and AKD mice appears to be small. After every stressful experimental situation studied, both the number of invasive globule leucocytes and precursor globule leucocytes appear to increase. The most readily recognized potential precursor globule leucocyte cell is the type illustrated in Figure 22. This 10.5 u long precursor globule leucocyte is a free migratory cell in the lamina propira but very close to the epithelial border and has a flattened tapered outline with microvilli projecting at the extremities. The cytoplasm is abundant with irregularly shaped granules 0.5 u to 1.4 u in length containing dense spherical bodies and some intragranular peripheral lamellae (not visible at low magnifications). The membrane limited granules containing dense spherical granules and periopheral lamellae are characteristic of the early invasive globule leucocyte. Also located in the cytoplasm of the potential precursor cell in Figure 22 are two spherical granules approximately 0.5 u and 0.7 u in diameter which are characteristic of connective tissue mast cell granules.

Another cell type frequently found in association with areas containing globule leucocytes is that found in Figure 23. This cell type is generally more amorphous in outline and between 1.5 to 2.5 u in length. Intracytoplasmic granules are sparse and usually small. Minute granules with electron dense matter within expanded cisternae of smooth endoplasmic retiuclum or Golgi are between 50 mu to 100 mu in diameter while larger

more distinct granules are also found nearby between 300mu to 700 mu in diameter (Fig. 23). This cell type may be a possible precursor to the one observed in Figure 22 or may be an entirely different cell line. The precursor globule leucocyte observed at higher magnification in Figure 10 from a normal Swiss strain mouse appears cytoplasmically similar to the intermediate cell in Figure 23; however, the precursor globule lecocyte in Figure 10 had a tapered and flattened shape similar to that observed in Figure 22.

Technically, by definition, the globule leucocyte does not exist outside the epithelial layer (Haidenhain, 1888; Weill, 1919; Keasby, 1923). Cell types identifiable as precursors to the globule leucocyte can be observed in the lamina propria of normal Swiss and AKD mice. Cells which must be called globule leucocytes by morphology and intracytoplasmic crystalline content have been found outside the epithelium and in the submucosa of actinomycin D treated mice (Figure 24 and Wesley and Jensen, 1969). Figure 24 illustrates a "stage 3 late development" globule leucocyte found in the submucosa near smooth muscle cells and fibrocytes. The globule leucocyte has the general morphology of connective tissue mast cells found in the area (compare Firues 25 and 26) and contains crystalline inclusions within a central vacuole as well as peripheral cytoplasmic dense bodies. The 0.2 u to 1.0 u diameter dense bodies are apparently not restricted to the globule leucocyte and are also found in the cytoplasm of nearby fibrocytes and even within the smooth muscle cells (Figure 24).

Slightly abnormal connective tissue mast cells are observed in the cecal submucosa of Swiss strain mice treated by placing a dialysis bag containing P815 mast cells around the cecum (Figure 25).

The mast cell illustrated in Figure 25 is abnormal in that the cytoplasm does not contain the characteristic spherical 0.6 u diameter homogeneous granules observed in most normal mast cells in the submucosa. The granules are irregular in shape, highly variable in density and range in size from 0.3u to 1.8 u with an average granule diameter of 0.8u (±0.3u). Similarly, a mast cell found in the submucosa of an AKD mouse treated by injecting 1.2 X 10^6 mast cells per ml intraperitoneally and sacrificed 39 hours later also shows irregularities not observed in normal mast cells (Figure 26).

Results of Actinomycin D Treatment

Normal Swiss strain mice. The results of actinomycin D treatment in Swiss strain mice and its effect on the cecal globule leucocytes population have been previously publsihed (Wesley and Jensen, 1969). It was originally believed that the number of epithelial globule leucocytes (crystal containing cells) increased directly with increasing actinomycin D dosages given over a three day time period. Quantitation of the numbers of globule leucocytes by direct counting in the four experimental conditions and controls provides further information on the change in the globule leucocyte population during actinomycin D treatment (Table 4, Figure 1).

The overall result of increasing dosages of actinomycin D on the globule leucocyte cells of Swiss strain mice cecal epithelium is an initial 5.5% increase in globule leucocytes compared to control levels after two days of treatment. The initial increase is followed by a rapid decline to near normal levels of globule leucocytes in the cecal epithelium population during the third day of continued increasing dosages of actinomycin D (Figure 1). The rapid decline

of globule leucocytes in the epithelium during the third day of treatment is accompanied by a moderate increase of induced stage 2 "globule leucocytes" appearing in the lamina propria. Thus, the general trend of all crystal containing cells appearing after actinomycin D treatment is a continued increase in numbers which is consistent with the earlier report (Wesley and Jensen, 1969).

General morphology of actinomycin D treated globule leucocytes.

In general, the actinomycin D treated globule leucocytes resemble those of normal mice except that the number of crystals and globules formed in treated mice tend to be more numerous (Figure 27).

The nucleus of actinomcyin D treated globule leucocytes is spehrical to ovoid with a slight indentation in the central region and occasionally the nucelus may be bilobate. The nucleolus, if visible, is condensed into a dense circular body and is characteristic of cells treated with actinomcyin D (Schoefl, 1964; Recher et al., 1971). The cytoplasm contains large globules up to 2.5 u in diameter which are irregularly shaped. Mitochondria are sparse, irregular in outline and often have swollen cristae. Free ribosomes appear to be more numerous than in controls and the rough endoplasmic reticulum is sparse and peripheral. The Golgi complex is generally well developed in the central region of the globule leucocyte and is composed of enlarged electron lucid cisternae. Stage 1 globule leucocytes are less frequently found in actinomycin D treated animals than in normals. Stage 2 globule leucocytes are most numerous while stage 3 globule leucocytes which are rarely observed in control animals are slightly more frequent in treated mice.

The number of crystals formed as condensation products of the globular matrix may vary in number and size. SOme globules produce only one large crystal ranging in size from 0.5 u to 1.5 u while other membrane limited "globule" vacuoles may produce up to 114 crystals each approximately 0.3 u by 0.3 u (observed in a stage 2 globule leucocyte of a 20 ug actinomycin D treated Swiss strain mouse).

TABLE 5
AVERAGE CRYSTAL AND GLOBULE CONTENT OF SWISS STRAIN MICE GLS

	Sample Size (N)	Average Granule # Cell	Average Crystal # Cell	Granule:Cryst Ratio
Control	509	7.2	24.0	1:3
20ug Act. D	240	10.2	63.0	1:6
30ug Act. D	247	7.3	49.0	1:7

Actinomycin D treatments at low 20 ug dosages appear to increase both the number of granules contained within globule leucocyte cells and the number of crystals developing from the globules (Table 5). Continued treatment decreases the number of cytoplasmic globules, but the ratio of globules to crystals increases with increasing dosages of actinomycin D.

Group I Actinomycin D. All three mice treated with 20 ug actinomycin D over 24 hours survived. The mice in this group looked and behaved similarly to the control group except for the presence of a slight degree of diarrhea at the end of the 24 hours.

The frequency of the invasive epithelial globule leucocytes increased to 8.6% of the total epithelial population (a 3.8% increase over all controls [Table 4]). No globule leucocytes with cyrstalline

inclusions are observed in the lamina propria, but an increase in the precursor globule leucocytes is apparent.

Group II Actinomycin D. One mouse died before the end of the 48 hour period leaving two mice for sacrifice and examination in the 33 ug actinomycin D treated group. By 48 hours the treated mice were more active, nervous, and viciously irritable than the control mice. Diarrhea was present and there was a slight build up of ascites in the peritoneum causing abdominal distension.

The frequency of globule leucocytes increased to 10.3% of the total epithelial population (table 4). This represents a 5.5% increase over the frequency of globule leucocytes in the total control population. The numbers of crystals and globules contained within Group II globule leucocytes appeared to increase (Figure 27). There seems to be an increase in stage 2 globule leucocytes in the epithelium. Stage 2 globule leucocytes were also observed at the basal aspect of the crypts with their fully differentiated crystalline inclusions. Stage 3 globule leucocytes occur rarely. No globule leucocytes with crystalline inclusions were found in the lamina propria of precursor globule leucocytes with large dense granules filled with dense spehrical bodies and intragranular lamellae.

Group III Actinomycin D. Two of the three experimental mice died in the 40 ug actinomycin D treated group before the end of the 52 hour period. By this time the mice were less active than group II mice and the diarrhea was severe.

The globule leucocyte population decreased rapidly to a normal level while the glouble leucocytes with crystalline inclusions in the

lamina propria appeared for the first time (Figure 3).

Group IV Actinomycin D. Two of the three experimental mice died in the 63 ug actinomycin D treatment administered over 66 hours. The remaining mouse was sacrificed at 72 hours. The mouse in this group was lethargic, anorexic, dehydrated and ematiated. The abdomen was rigid and the muscles appeared to be in constant tetany.

Invasive epithelial globule leucocytes remained at a normal level compared to controls but there was a pronounced increase in extraepithelial globule leucocytes with crystalline inclusions in the lamina propria (Figures 3 and 24).

Actinomycin D Treatment of AKD Mice

Insufficient data exists to quantitate the numbers of globule leucocyte cells in AKD mouse epithelia as was done for Swiss strain mice. Preliminary electron microscopic examination indicates that actinomycin D treatment affects the AKD strain globule leucocytes in the same ways that it affects the Swiss strain. No significant differences have been observed.

The Fate of the Globule Leucocyte Crystals

Lysosomal degradation by absorptive epithelial cells. Stage 3 globule leucocyte crystals appear to be exocytosed into the lumen of the gut in Swiss strain (Figure 6) and AKD mice (Figure 33). Sometimes the exocytosed crystals are phagocitized by neighboring cells and degraded by secondary lysosomes. In actinomycin D treated animals, crystals contained within lysosomes of absorptive epithelial cells

were not uncommon (Figures 28a,b,c). The crystals show the characteristic periodicity of globule leucocyte crystals averaging 59.2A (±2.6A) (Figures 28b,c). The phagocytized crystals are generally rectangular in shape and range in size from 0.3u to 1.5u in length. Cells distal to the epithelial cells bordering the lumen never contained crystals within lysosomes.

The phagocytized globule leucocyte crystals in absorptive epithelial cell lysosomes are readily distinguished from crystalline inclusions occasionally found in some untreated absorptive epithelial cells (Figures 29a,b). The periodicity is irregular for normal lysosome crystals and is 146.0A (±19.3A) but is considerably larger than that observed for globule leucocyte crystals. Normal absorptive epithelial lysosomes range in size from 0.5u to 1.5u while the lysosomes containing globule leucocyte crystals range in size from 0.5u to 3.0u.

Incorporation of globule leucocyte crystals into mucous cell granules. Membranous whorls within lysosomal bodies of mucous cells have been reported (Toner et al., 1971) but not within the mucous granules themselves. In normal Swiss strain mice, membranous whorls are rarely found either within mucous cell granules near the lumen of the cecum or nearby the granules (figures 30a,b). Treatment of mice with actinomycin D or stress from the P815 mast cell filled dialysis bag results in an increase in these whorls found within mucous cell granules (Figure 31). The membraneous whorls have an average line to line periodicity of 132.9A (±9.1 A) and appear to be arranged in concentric circles surrounding a dense core approximately 220 A in diameter (Figure 30b).

In epithelial cells at the mouth of the crypt of Lieberkühn stage 3 globule leucocyte crystals are occasionally observed mixed within mucous cell granules in actinomycin D treated Swiss strain mice (Figure 32) and in normal AKD mouse cecal epithelia (Figure 33). In the actinomycin D treated Swiss strain mouse, the lamellar whorls have a line to line periodicity of 127.3 A (± 6.5 A) while the crystals mixed in with the lamellae have a line to line periodicity of 56.7 A (± 3.3 A). (Figure 32). There is no limiting membrane surrounding the crystals or scrolls, but fragments of unit membranes are visible in the area (Figure 32). The cell illustrated in Figure 32 was open to the lumen of the gut and apparently about to be discharged at the time of fixation. All cells illustrated in Figure 33 are at the surface of the lumen and the mucous cells are shedding their granular contents of mucopolysaccharides into the gut as evidenced by the light density of the granular content, the absence of limiting granule membranes, and the general cytoplasmic disruption. Portions of mucous cells distal from the lumen near these cells were found to be normal in granule morphology. At some point between the globule leucocyte and one of its neighboring mucous cells, there has been breakdown of the plasma membranes and the crystals have passed from the globule leucocyte into mucous cell granules (Figure 33 arrows).

Exocytosis. As previously mentioned, the usual fate of stage 3 globule leucocytes is exocytosis of the crystals into an extracellular space which is continuous with the lumen of the gut (Figure 6).

Protein Digest

Incubation of thin sections for electron microscopy in water at 37°C for time periods up to four hours does not alter the nomal structure of the cells. Treatment of thin sections for 10 minutes in 10% hydrogen peroxide at room temperature resulted in the extraction of some hydrogen peroxide soluble component within the cytoplasm of the globule leucocyte as well as in the intercellular spaces between the globule leucocyte and neighboring epithelial cells (Figure 40). The crystals and most of the larger globules of the globule leucocyte are apparently unaffected by the 10 minute 10% hydrogen peroxide treatment (Figure 40). Collagen in the lamina propria, granules in mast cells, mucous cells and granulocytic leucocytes are not affected by the peroxide treatment (Figure 40).

If the time of 10% hydrogen peroxide treatment is increased to 20 minutes, increased removal of the hydrogen peroxide soluble component is noticed around some crystals and within or around some of the globules of the globule leucocyte (not shown) and of the granules of the eosinophil leucocytes (Figure 34). Otherwise, the crystals and granules of the globule leucocytes and eosinophil leucocytes remain unaffected structurally by the increased time of hydrogen peroxide treatment and all other cellular components remain intact.

When Epon sections are incubated in 0.1 N hydrochloric acid for time periods varying from 10 minutes to 4 hours at 37°C, there is an increasing extraction of acid soluable components from a variety of cells. Figure 35 demonstrates that there is an acid soluble component in the nucleus of all cells (mostly in the heterochromatic

regions), in granular inclusions of some cells, in the mucous secreting cells and in a portion of the protein matrix of collagen in the lamina propria. Specific cell types are recognizable in spite of the absence of the acid soluble components. Most mast cell granules appear to be intact, although some appear to be partially soluble and mechanically damaged (Figure 35). Globule leucocytes show no cytoplasmic disruption of crystals although the globules may occasionally be either partially soluble or mechanically disrupted.

Incubation of thin sections pretreated with 10% hydrogen peroxide in the complete incubation medium of 0.5% pepsin in a 0.1N hydrochloric acid solution at 37°C for varying time periods of 15 minutes to 24 hours resulted in increasing removal of protein components from the cells with increasing time. After five to six hours incubation, there was such extensive protein removal that identification of cell types and components became impossible at the electron microscopic level.

A two hour pepsin treatment resulted in the removal of most of the mucopolysaccharide granules in connective tissue mast cells observed in the lamina propria. A two hour pepsin digestion is sufficient to remove nearly all mucopolysaccharide granules of normal connective tissue mast cells as well as the collagen in the lamina propria (Figure 38). Pepsin effectively removed proteins of argentaffin granules (Figure 37) but not from the control tissues (Figure 36). After the 1 hour protein digestion, mast cell-globule leucocyte intermediate cells tend to have their mast cell-like granules extracted, but larger irregularly shaped granules characteristic of globule leucocytes are not removed. Longer incubation with pepsin up to 4.5 hours results in removal of mast cell granules and decreased cellular membrane resolution as well as the total removal

of collagen in the lamina propria but does not extract the irregularly shaped granules (Figure 39).

Neither the two hour nor the 4.5 hours incubation in pepsin was sufficient to remove the granules and crystals from the globule leucocyte (Figure 41). There appears to be no significant difference between the globule leucocytes observed after 10 minutes of hydrogen peroxide extraction plus 4.5 hours incubation in 0.5% pepsin in 0.1 N hydrochloric acid at 37°C. The globules leucocyte observed in Figures 40 and 41 are portions of sections within the same cell from the same ribbon of approximately 10 sections placed on different grids and treated either in the complete pepsin incubation medium (Figure 41) or in hydrogen peroxide alone (Figure 40).

Localization of Biogenic Amines

One micron sections placed on glass slides and cleared of Epon by the Lane and Europa (1965) technique were strongly stained using Gibbs' reagent (2,6-dichloroquinonechloroimide) but reacted weakly with the diazonium salt 5-nitroanisidine at the light microscopic level. After clearing the sections of Epon, there is some collapse of cellular materials resulting in a slight loss of light microscopic resolution. Nonetheless, the staining reaction using Gibbs' reagent is strong enough to make a 1:1 correlation between the light micrographs and electron micrographs of adjacent sections (Figures 42; 43a and 44; 45a and 46). Attempts to stain one micron sections without prior clearing in saturated NaOH results in an inability of the stain to penetrate the tissue properly.

Diazo Method for argentaffin cell granules. One micron sections

cleared of Epon and stained with 5-nitrosidine (Fast Red Salt B) in 0.1 M veronal acetate, pH 9.2, and counterstained with Mayer's haemalum exhibited a very slight staining reaction of some cells. Argentaffin cell granules appeared as a faint orange-yellow color, Some granules in in the cells in the lamina propia showed the same light color, but no globule leucocytes could be identified positively in general morphology or by the staining reaction. No micrographs of this reaction product are presented because of the extreme lightness of the one micron sections after staining and the difficulty of photography the sections.

Gibbs' Reagent for Argentaffin cell granules. One micron sections cleared of Epon and stained with 2,6-dichloroquinonechloroimide (Gibbs' reagent) in veronal acetate at pH 9.2, and counterstained with 1% neutral red show intense accumulation of a black reaction product in some cells while the nuclei are stained a light red (Figures 42; 43a and 44; 45a and 46). The globules of the globule leucocyte stain intensely with the black quinoneimidephenolic reaction product at the light microscopic level (Figure 42 inset; 44; 46), but can not be recognized at the light microscopic level specifically as globule leucocytes on this basis alone. Correlation of the light microscopic micrographs with adjacent thin section electron micrographs no further than 800 mu distant from the original thick section shows that the stained granules belong to globule leucocytes (Figure 42; 43a; 45a) and not to argentaffin cells which are specific for the stain. The argentaffin cells stain differently on the Epon sections than on paraffin sections using the Gibbs' reagent. In the Epon sections, the argentaffin granules are not resolvable as individual granules under high power light

microscopy. The entire cytoplasmic content appears to stain readily with Gibbs' reagent in a diffuse manner (Figure 46). In paraffin sections which are about 5 microns thick the argentaffin granules stain darkly and are resolvable as individual or groups of granules under high power light microscopy (personal observations and Pearse, 1961). The globule leucocytes stained after Epon clearing of one micron sections strongly resemble the normal five micron paraffin sections' argentaffin cells after staining with Gibbs' reagent under high power light microscopy.

Other cells which appear to accumulate the reaction product are red blood cells observed in capillaries (Figure 42) and mucous cells which show some staining in the generally disrupted mucous granules (Figures 42 and 44). Mast cells, mast cell-globule leucocyte intermediate cells and eosinophils also show varying degrees of staining in the lamina propria (some of these cells may be identified in the composite micrograph of Figure 42).

Acid Phosphatase Localization

The minimal incubation time which showed an optimal lead phosphate reaction product was one hour at 37°C incubation with the substrate. These specimens were used exclusively for the study since the three hour incubation period resulted in the loss of fine structural detail compared to the one hour incubation. Incubations of tissue in Acid phosphatase medium for time periods less than one hour generally resulted in the absence of reaction product in most cells. Controls for the one hour incubation at 37°C without the substrate do not

localize free lead ions in the cytoplasm of epithelial cells, including globule leucocyte cells, or the cells of the lamina propria (Figure 47). Occasionally aggregates of lead were noticable intranuclearly. Control cells exhibit symptoms of necrosis after the prolonged incubation period of one hour in that the cristae of the mitochondria are swollen and filled with a dense granular material, ribosomes in the cytoplasm are disrupted and disperse, the endoplasmic reticulum of some cells are swollen and filled with a dense material, and the structure within mucous cell granules are not well preserved (Figure 47).

Mouse intestinal ceca incubated in the presence of the ATP substrate show globule leucocyte cells which incorporate the reaction product in the nuclei, and a moderate degree of product in the cytoplasm and along the plasma membrane, but no reaction product is formed within the globules themselves (Figure 48). Connective tissue mast cells in the lamina prppria and cells which may be globule leucocyte precursors do not localize the lead phosphate reaction product in their granules, although there is an abundance of acid phosphatase apparently present in the cytoplasm. The cytoplasmic acid phosphatase is usually associated with membranes and there is localization of the reaction product in the nuclei (Figure 49).

Eosinophil leucocytes do incorporate the lead phosphate reaction product in most of the cytoplasmic granules in the matrix (Figures 50 and 51). Granules which do not take up the product are occasionally found (Figure 51). Although most eosinophil crystals never appear to incorporate the reaction product, some crystals do have streaks of reaction product in straight lines along the longitudinal axis as demonstrated

in sections which were not post-stained for electron microscopy (Figure 51). The lysosomal nature of the eosinophil granular matrix is clearly demonstrated in sections post-stained with uranyl acetate (Figure 50) and in sections which were not post-stained for electron microscopy (Figure 51). The eosinophil leucocyte in Figure 50 lies just below the cecal epithelium and was approximately 15 microns distant from the globule leucocyte shown in Figure 48.

Some cells have been located in the lamina propria which have the morphological appearance of being possible precursor globule leucocytes with dark osmiophilic granules and intragranular lamellae (Figure 52). The osmiophilic granules of the possible precursor cells do not show any intense uptake of the reaction product, however, the limiting membrane may be surrounded by the reaction product (Figure 52). The difference in density of the spherical granules compared to the black reaction product in sections not post-stained indicates that the granules are osmiophilic and not accumulations of lead phosphate. The granules in this cell type are reminiscent of macrophage granules, but do not incorporate the reaction product as intensely as macrophage granules even though there is some accumulation of stain within the cytoplasm and distinctly about the plasma membrane of the cell. In all cases of cells found in the lamina propria, there is no evidence of superfluous reaction product accumulated in the lamina propria and there is a specific localization of the product in eosinophil granules.

Peroxidase Localization

The use of the Smith-Farquar tissue chopper is generally recommended for producing thin frozen sections of material in order to obtain even penetration of reagents in the peroxidase localization test (Beard and Novikoff, 1969). Such an instrument was not available for use during the mouse intestine preparations for peroxidase localizations. The result of hand slicing the pieces of cecal epithelium resulted in an uneven distribution of the diaminobenzidine throughout the tissues.

The one hour diaminobenzidine incubation at 37°C resulted in an intense osmium black staining and considerable necrosis of the cells closest to the periphery of the tissue block. As the cells in the center of the tissue block were sectioned, less intense staining was noticable and the center of some blocks were unstained. Controls showed no cellular damage compared to the experimental tissues and no osmium black was deposited.

Because of the inconsistency of the staining reaction, no firm conclusions can be reached from this experiment concerning peroxidase localization in the globule leucocytes. However, it was noticed that no osmium black occurred inside the globules of the globule leucocytes of diaminobenzidine treated tissues. The outer limiting membrane of the globules did show varying degrees of somium black deposition. Crystalline inclusions of the globule leucocytes were obliterated during the substrate incubation period, but areas which appeared to have been crystals did not show any reaction product.

Results of in vitro P815 Mast Cell and Normal Peritoneal Cell Incubation Experiments.

Observations of control cultures of P815 mast cells. The control P815 mast cells (Figure 53) have an average cell diameter of 7.2 u (± 2.7 u) with a range of 4.5 u to 13.9 u and an average nuclear diameter of 3.9 u (± 1.31 u) with a range of 1.4 u to 2.1 u. The P815 mast cells are generally spherical in shape with a spherical to bilobate nucleus which is densely heterochromatic peripherally. The cytoplasm may contain two types of membrane limited granules: one type is large and electron dense, generally spherical with an average diameter of 1.7 u (± 0.2 u), and may sometimes have a double membrane surrounding it (Figures 53 and 55); the second type of granule is less electron dense, spherical to ovoid and rarely polygonal in shape (apparently morepolygonal when the mast cell is degranulating), and has an average diameter of 0.4 u (± 0.1 u). The P815 mast cells usually have a well developed Golgi apparatus which is near the central portion of the cell adjoining the nucleus and the cytoplasm is generally filled with Golgi vesicles (Figure 54). The mitochondria are abundant in the cytoplasm and ovoid to elliptical in cross section containing compressed or expanded cristae in an intramitochondrial matrix having about the same density and granularity as the cytoplasmic ground substance (Figures 53 and 54). The endoplasmic reticular system is not generally well developed, but the cytoplasm is filldd with ribosomes; but when the rough endoplasmic reticulum is present, the cisternae are generally distended and filled with a substance of the same density as the cytoplasm (Figure 54).

Several stages of normal cultured P915 mast cells are seen in Figure 53. Relatively immature P815 mast cells (cell A in Figure 53) are characterized by the presence of the smaller spherical granules

ranging in diameters from 0.5 u to 1.5 u, a well developed Golgi apparatus and a cell diameter approximately 5 u or less. Mature mast cells (cell B in Figure 53) show the presence of both the small granules and the more electron dense larger granules which range in size from 1.1 u to 2.0 u. Occasionally mast cells are observed in the process of degranulation by exocytosis (cell C in Figure 53) and are characterized by large "empty" vacuoles, few cytoplasmic granules and an average diameter greater than 7 u.

Summary of P815 mast cell induction experiments.

It appears that normal granules of P815 mast cells develope from Golgi vesicles (Figure 54) and have products of the smooth and rough endoplasmic reticulum added to the maturing granules (Figure 55). Dense globular inclusions and intragranular lamellar inclusions are absent from all normally cultured P815 mast cells observed.

After a one hour 37°C incubation of P815 mast cells in the presence of Swiss strain cecal homogenate which was not filtered (experiment 1), dense intragranular spherical inclusions are observed often with membrane-like boundaries developing within the larger electron dense mast cell granules (Figure 56). The bodies are osmiophilic and range in size from approximately 20 mu to 100 mu. Intracytoplasmic membraneus whorls appear after one hour incubation with the cecal homogenate (Figure 57). These inclusions are most often associated with aldehyde fixation artefacts but do not appear in normal P815 mast cell cultures fixed in the same way by the modified Hirsch-Fedorko technique.

Most of the cells incubated in cecal homogenate at 37°C

degranulate within 30 minutes and show symptoms of intense mast cell granule renewal (Figure 58). The Golgi apparatus is generally extensive in the cytoplasm with many budded Golgi vesicles, multivesicular bodies are abundant and small developing granules are present in the surviving P815 mast cell population (Figure 58).

Cultures incubated in experiment 4 with 4 ug actinomycin D per ml of P815 mast cell culture showed degranulation of all cells after 1 hour with either highly abnormal granule regeneration (Figure 59) or absence of regeneration even after 8 hours of incubation. Neither membranous whorls or intragranular inclusions were observed in any of the actinomycin D treated P815 mast cell cultures. In some long term cultures of 21 hours in unfiltered cecal homogenates, the culture conditions appear to be inhibitory to new granule formation (Figure 60).

Axenic filtered cecal homogenates (experiments 5 and 6) appear to promote cellular growth and granule regeneration in P815 mast cells at levels near the normal cultures. After one hour incubation at 37°C, there is nearly total degranulation of all cultured P815 mast cells. Regeneration of granules proceeds during the first division cycle after adding the homogenate.

At 15 hours of filtered homogenate incubation, some of the P815 mast cell granules begin to form intragranular lamellar inclusions (Figure 61) and osmiophilic dense bodies appear (Figure 62) in the cytoplasm. Complex intracytoplasmic lamellar systems (Figure 63) unlike the lamellar whorls previously observed in 1 hour unfiltered cecal homogenates (Figure 57) are present after 15 hours of filtered homogenate incubation. These lamellar complexes contain membrane

systems which appear to be composed partly by membranes which have merged together forming a pentilaminar (five layered) membrane system (Figure 63). After 24 hours incubation in the filtered homogenate medium, the complex lamellar systems form electron dense areas within the matrix of these large 1 u inclusions (Figure 64). The dense granular areas are partially replaced by vesicular membranous areas after 48 hours of incubation (Figure 65) and there is generally an increase in the size of the complex lamellar inclusions from 1 u to nearly 3 u.

The complex lamellar systems are either exocytosed or the P815 mast cells containing them degenerate leaving only relatively normal appearing mast cells after 70 hours of incubation in the cecal filtrate (Figure 66). Occasionally abnormal mast cell granules (Figure 67) which are osmiophilic, irregularly polygonal, sometimes containing membranous inclusions are present in a few of the 70 hour incubation P815 mast cells. These abnormal granules (Figure 67) appear to be similar morphologically and dimensionally (0.5 u in diameter) to the granules observed in precursor globule leucocytes (Figures 11, 22, 39). Although most of the 70 hour cultured cells have normal cytoplasmic features (Figure 66) and well developed reconstituted Golgi (Figure 68), a few possess the abnormal granules (Figure 67) and a highly irregular type of cytoplasmic inclusion not previously observed (Figure 69).

AUTORADIOGRAPHY

Over 800 sections of radioactively labeled material were prepared for light and electron microscopic autoradiography. Technical difficulties resulted in background emulsion contamination in most of the sections.

Incorporation of tritiated-thymidine was successfully accomplished into the P815 mast cell cultures (Figures 70, 71) *in vitro*, but the *in vivo* uptake of injected labeled mast cells is still being investigated. Results to date indicate that the cells are incorporated into the intestinal epithelium, but the labeled cells appear to be damaged in both AKD and Swiss strain mouse ceca.

The *in vivo* incorporation of the serotonin precursor 5-hydroxytryptophan in its tritiated form was successful (Figure 72) at the light microscopic level. Incorporation was noted in mast cells and in some cells within the cecal epithelium (Figure 72), but the identification of the epithelial cells can not be accurately ascertained at the light microscopic level.

DISCUSSION

The Origin of Globule Leucocytes

The main problems of this thesis are the origin, development and fate of the glouble leucocyte in the intestine of mice. In the mouse gastrointestinal system, the globule leucocyte has been thought to be derived from eosinophil leucocytes (Silva, 1967), plasma cells with Russell bodies (Carr, 1967) or from connective tissue mast cells (Wesley and Jensen, 1968).

Eosinophil Leucocycte. Support for the eosinophil leucocyte as the immediate precursor to the globule leucocyte is based on the morphological appearance of crystalline inclusions within the granules of the eosinophil (Silva, 1967) and the acidophilia of both cell types (Kirkman, 1950). The range of diameter of the globule leucocyte granules in mice is 0.5 - 3.0 u (Toner et al., 1971) and the crystals have an average periodic repeat of approximately 60 A (Silva, 1967; Wesley and Jensen, 1969). The range of diameters of the eosinophil leucocyte granules is 0.3 - 1.2 u and the crystals exhibit a 30 A repeat (Miller et al., 1966). Therefore, the morphological similarity of the cytoplasmic inclusions is superficial. The distinction is clearly presented in Figures 7 and 8.

The globule leucocyte may be histochemically distinguished from the eosinophil leucocyte in that the eosinophil granules do not stain

for acid phosphatase at the electron microscopic level (Figure 48) or at the light microscopic level (Kirkman, 1950; Kent, 1952) and they apparently lack peroxidase. Preliminary results of this thesis using diaminobenzidine to localize peroxidase activity at the electron microscopic level indicate that no peroxidase is present within the granules of the globule leucocyte although there is often a dense ring surrounding the globule unit membrane of the reaction product. Peroxidase and other oxidases have been shown to be absent from globule leucocytes at the light microscopic level (Kirkman, 1950). In contrast to this, acid phosphatase, peroxidase, esterases and other hydrolytic enzymes have been found in association within the granules of eosinophil leucocytes of mice (Geyer, et al.,1970) and other species (Vercauteren, 1953; Archer and Hirsch, 1963a). The granules of the eosinophil leucocytes are considered part of the lysosomal system of the cell (deDuve and Wattiaux, 1966; Cohn and Fedorko, 1969). Since acid phosphatase is absent and no hydrolytic enzymes have been demonstrated in globule leucocytes, they can not be considered as lysosomes in function and this gives further evidence that the two cell types are not related.

If the eosinophil leucocyte were the immediate precursor to the globule leucocyte, it should be possible to identify an intermediate cell type in the epithelium or lamina propria with characteristics of both the globule leucocyte and the eosinophil leucocyte. There is no evidence for such an intermediate in mice. There is an electron microscopic report of a transformation of intestinal eosinophils during helminth infestation (Moniliformis dubius) in which eosinophils are

observed containing characteristic eosinophilic granules with the matrix and core as well as larger membrane bound abnormal granules within the same cells (Casley-Smith, 1968). Takeuchi, Jervis and Sprinz (1969) believed that these transformed rat granules strongly resembled the globule leucocyte granules. However, careful examination of the micrographs shows that each cell illustrated shows incomplete plasma membranes, many extracellular granules, mitochondria and debris indicative of cellular necroses. The phagocytic activity of the eosinophil leucocyte has been reported (Archer and Hirsch, 1963). It has been demonstrated that eosinophils accumulate in tissues in which mast cells shed their granules (Parish, 1970) and the phagocytosis of released mast cell granules by the eosinophil leucocyte has been clearly demonstrated by electron microscopy (Welsh and Greer, 1959). Therefore, it can be surmised that the abnormal globule leucocyte appearing granules in the eosinophil leucocyte are not products of in situ eosinophil synthesis, but ingested debris being acted upon by the eosinophil leucocyte lysosomal system.

Furthermore, a localized cecal eosinophilia occasionally occurs in some mice. This is characterized by an unusually large number of eosinophil leucocytes found in the lamina propria. Under these conditions no eosinophil transformations have been observed even though the number of glouble leucocytes found in the same area is also high.

The Plasma Cell. The plasma cell with Russell bodies has been considered as a potential precursor to the globule leucocytes on the basis of the immune reaction observed in parasitic infestations in the mouse intestinal epithelium (Carr, 1967). There is also a morphological similarity of the Russell body observed by Thiery (1958) and the globules

of the globule leucocyte (Carr, 1967; Whur and Johnston, 1967). The nuclear chromatin of the plasma cell often has a "cartwheel appearance" in light microscopic paraffin sections and this heterochromatic arrangement is also occasionally observed in the nuclei of some globule leucocytes at the light and electron microscopic levels (Toner, 1965).

A significant difference between the plasma cell and the globule leucocyte is that the plasma cell contains an elaborate system of rough endoplasmic reticulum which occupies a large portion of the extensive cytoplasmic area (Thiery, 1958) while the globule leucocyte has a sparse generally peripheral system of rough endoplasmic reticulum placed within a small amount of cytoplasm which is generally crowded with globules. The precursor globule leucocytes observed in Swiss strain mice (Figures 10 and 22) and in AKD mice (Figure 17) do not show the elaborate rough endoplasmic reticulum of immature or mature plasma cells (Figure 9). During the development of the rat intestinal globule leucocyte from its precursor cell, the endoplasmic reticulum is also very sparse (Murray et al., 1968).

The plasma cell occasionally forms crystals within the rough endoplasmic reticulum cisternae (Thiery, 1958). The mesentery plasma cells of rabbits injected with antigen respond by forming antibody mucopolysaccharide crystalline complexes with a 120 A periodicity (Movat and Fernando, 1962b). The micrographs of this antigen stimulated plasma cell with crystals show that the elaborate endoplasmic reticulum system is absent and the crystals are mostly membrane limited. This cell looks similar to the globule leucocyte except for its greater size and the larger periodicity of its crystals (nearly twice that of the globule leucocyte crystal in mice).

It is the opinion of Jarrett et al., (1967) that plasma cells are readily distinguished from globule leucocytes. This may be true only in certain stages of plasma cell and globule leucocyte cell development. The plasma cell has been observed to alter its cytoplasmic morphology when activated by injections of heterologous proteins in vivo intraperitoneally or intravenously. The first response is dense granular formation in the widened cisternae of the rough endoplasmic reticulum, condensation of this material into Russell bodies 12 days after injection and occasional crystallization (Thiery, 1958; Bessis, 1961). There is also a decreased amount of cytoplasmic rough endoplasmic reticulum with increasing granule and crystal development (personal observation of literature). Hence, during the final stages of crystal development in the plasma cell, there may be superficial structural similarities with the final stage of globule leucocyte development except for the variation in periodicity and cell size. The early plasma cells and plasma cells with Russell bodies in early to middle stages of development are clearly distinguished from globule leucocytes by the extensive rough endoplasmic reticulum system; however, late stages of plasma cells with Russell bodies which have crystallized having a sparse rough endoplasmic reticulum do resemble the middle to late stages of globule leucocyte development in general cell morphology.

Aside from the 120 A periodicity of Russell body crystals being twice as large as the globule leucocyte crystal periodicity and the larger size of plasma cells over globule leucocytes, the strongest argument against the plasma cell as an intermediate precursor to the globule leucocyte is its general lack of ameboid movement (Bloom and

Fawcett, 1968) and the fact that the plasma cell has never been observed within the epithelium of the gastrointestinal tract (Toner and Ferguson, 1971). Nonetheless the plasma cell is sometimes abundant in the intestinal lamina propria (Piliero et al., 1965).

The Mast Cell. No further reports since 1968 have implicated the plasma cell or the eosinophil leucocyte as potential precursors to the globule leucocyte. In contrast to this, the mast cell has been suggested as the possible precursor to the globule leucocyte in a variety of reports in mice (Wesley and Jensen, 1969) in rats (Murray et al., 1968; Jarrett et al., 1967), in intestinal globule leucocytes of cows and sheep (Murray et al., 1968), in the biliary epithelium of sheep and cows (Rahko, 1970) and in deer (Blazek, 1971).

The results of the graph in Figure 1 and the observations of cell types with granules intermediate in appearance between normal globule leucocytes and mast cells and the occurrence of crystalline inclusions in the intermediate cell type (Figures 13-16) provide evidence that the mast cell is the immediate precursor to the globule leucocyte in mouse intestinal epithelia. These precursor globule leucocytes are similar to the intermediate mast cell - globule leucocytes reported in the intestinal lamina propria of rats (Jarrett et al., 1967; Murray et al., 1968), cows and sheep (Murray et al., 1968). A relationship between the mast cell and the globule leucocyte is suggested in the epithelial layer of the bile duct in cattle and sheep (Rahko, 1970).

The characteristic feature of mast cells is the metachromatically staining membrane limited spherical cytoplasmic granules. Normal mouse peritoneal cell granules have an average diameter of 0.5 u (Hagihara,

1960) and the average granule diameter reported for normal connective tissue mast cells is 0.6 u (Bloom and Fawcett, 1968). The granules in normal untreated globule leucocytes of mouse intestinal epithelium are found to be ovoid with average axes of 1.8 X 1.3 u (range 0.7 to 3.0 u). Thus, the average globule leucocyte granule is larger than the average mast cell granule by a factor of approximately three. The small spherical granules observed in the intermediate mast cell - globule leucocyte cell (Figure 13) are approximately 0.5 u in diameter. (characteristic of the mast cell) while the larger granules in Figure 14 are ovoid and about 1.8 u in the long axis, or approximately three times the smaller granules. It appears that the larger granules are characteristic of the globule leucocyte and develop from smaller mast cell-like granules. A comparison of cross sectional areas and cell volumes is probably irrelevant since normal connective tissue mast cells vary in shape from spheres with minimal diameters of about 3.4 u to longitudinally stretched cells up to 30 u long (Michels, 1963). However, it does appear that there is a general increase in size during the development and transition from the connective tissue mast cell to the globule leucocyte (Figures 4 to 6).

Granules of mast cells and globule leucocytes. One interpretation of the three-dimensional structure of the globules of the globule leucocyte is that they form a system similar to that described for lysosomes (deDuve and Wattiaux, 1966; deDuve, seminar, The Rockefeller University, 1972). The globules appear to anastomose forming a continuous complex (Figures 13, 19, 45a). This suggests that the globules form a complex interconnected labyrinth within the cytoplasm

of the globule leucocyte. The globules are not, however, lysosomes since no hydrolytic enzymes have been found and this investigation shows that acid phosphatase is absent from the matrix and crystals of the globules (Figure 48). Similarly, the granules of normal mast cells also lack hydrolytic enzymes characteristic of lysosomes and acid phosphatase is absent (Pearse, 1949; Kirkman, 1950; Dobbins et al., 1969).

Fine structural intragranular inclusions have been observed in the mast cells of normal mice (Smith, 1963; Tanaka et al., 1970; (Figure 14), in the globules of the globule leucocyte (Wesley and Jensen, 1969; Figures 10, 11, 15, 16) and in the granules of the precursor globule leucocyte (Figure 22). This appears to give further evidence for the hypothesis that the globule leucocyte is derived from a free migratory mast cell in the lamina propria since the intragranular inclusions are similar.

Intragranular lamellae are observed in normal mature Swiss strain mast cells (Figure 14) containing parallel membranes which are thiner in cross section (49 A $\pm$ 1 A) than the outer limiting membrane (79 A $\pm$ 6). The line to line periodicity of the parallel intragranular lamellae is 211.0 A ($\pm$ 29.4 A). The mature granules of mouse mast cells are 0.6u to 1.5u in diameter and are usually electron dense and osmiophilic. The immature mast cell granules (usually of a diameter less than 0.6u) do not have intragranular lamellae characteristic of the mature mast cells.

The intragranular mast cell lamellae have a resemblance to the intragranular lamellae observed in the precursor globule leucocyte and

the invasive epithelial globule leucocyte. However, the precursor globule leucocyte membranes are generally dense lines in cross sectional appearance (Figure 10, 11) which can be resolved into dense lines containing three distinct bands (Figure 15). The dense line itself is 102 A (± 5.3 A) in width and composed of an inner periodicity of three lines separated by 51 A (± 5 A) (Figure 15). The inner periodicity is very similar to the periodicity found within the crystalline portions of the globule leucocytes.

Normal mast cell intragranular lamellae generally have a tripartite cross sectional appearance having lines which are 49 A (± 1 A) in width (Figure 14). In Figure 15, a granule is observed to the right of the granule containing lamellae which is characteristic of an early mast cell granule. The cell shown in Figure 22 under low magnification has mast cell - like features in its granules, cytoplasmic characteristics and general outline, but higher magnifications indicate that at least some of its granules are not characteristic of normal mast cells (for example, the intragranular inclusions observed in Figure 15). It is concluded from observations of cells such as seen in Figure 22 that these cells are precursor globule leucocytes (modified mast cells) which have the potential of being incorporated into the intestinal epithelium.

There also appear to be morphological intragranular alterations indicative of a shift in biochemical activities in the modification of mast cell granules during the transition into globule leucocytes. Aside from the appearance of intragranular lamellae, there is the appearance of the dense spherical osmiophilic bodies scattered throughout the globules during early globule leucocyte development (Figures 11 and 15)

which become peripheral in late stage globule leucocyte development (Figure 13). During the period of intragranular inclusion development, the outline of the granules becomes irregular and there is an increase in granule size (Figures 10 to 13; 17 to 21). These morphological alterations of the mast cell may also be correlated with the biochemical changes found in the rat mast cell development into the globule leucocyte indicated by decreased 5-hydroxytryptamine yellow fluorescence to the dull green fluorescence indicative of catecholamines (Murray et al., 1968). A biochemical shift is also indicated by the fact that the globule leucocyte demonstrates a strong acidophilia (Kirkman, 1950) while the mast cell is basophilic (Bloom and Fawcett, 1968).

Intraepithelial Reproduction of the Globule Leucocyte

The globule leucocyte has been thought to originate from migratory connective tissue mast cells in mice (Wesley and Jensen, 1969) and in rats (Jarrett et al., 1967; Murray et al., 1968). It is possible that the invasive globule leucocyte is capable of cell division within the epithelium on the basis of light microscopic reports of rarely occurring globule leucocyte mitoses (Weill, 1919; Miller and Jerrett, 1971). This would indicate that the globule leucocyte may form a stable epithelial population of cells similar to other epithelial components. There have been no observations of mitotic figures of globule leucocytes reported at the electron microscopic level. However, Kataoka (1970) found nuclear labelling at the electron microscopic level using tritiated-thymidine in some crystal containing cells (globule leucocytes) of mice in the duodenum two hours after injecting the labeled thymidine intraperitoneally.

This may indicate that the globule leucocytes had recently divided and may proliferate in the epithelium by mitosis (Kataoka, 1970). It should be noted that self replication of the globule leucocyte is probably not a major source for intraepithelial globule leucocytes. The relatively rapid turnover of mouse intestinal epithelial cells has been reported to be between 1 to 2.5 days (Creamer et al., 1961) and three days (Leblond and Messier, 1958). This rapid rate of cell replacement makes it improbable that the epithelial globule leucocyte originates primarily by mitosis. It has also been noted that chromosomal figures indicative of mitosis in globule leucocytes are rare events (Weill, 1919) and it can be concluded that the major source of globule leucocytes in the intestinal epithelium is from the invasion of the epithelium by mast cells with their concommitant differentiation into globule leucocytes.

Although Padawer could not find evidence of mitoses in mast cells after colchicine treatment (Padawer and Gordon, 1955) and thought it unlikely that differentiated mast cells could multiply mitotically (Padawer, 1957; 1961a; 1961b; and personal communication, 1972), there is evidence that mature mast cells do rarely undergo mitoses (reviewed by Fernex, 1968; Burton, 1960). The rare frequency of differentiated mast cell mitoses may be similar to that of the rate of mitoses for the globule leucocytes.

Tissue Culture Experiments in Relationship to the Origin of the Globule Leucocyte

Theoretically, it seems reasonable to attempt an induction of a transformation of tissue culture mast cells in the environment of the

intestinal epithelium, if it is true that globule leucocytes arise from mast cells. This was attempted in the P815 mast cell culture induction experiments using extracts of homogenized cecal epithelium added to the cell cultures. Serious contamination problems resulting from the indigenous intestinal flora occurred under most experimental conditions. Only experiments 5 and 6 described in the Materials and Methods were completely free of all signs of contamination.

The results of the attempted globule leucocyte induction from P815 mast cells were successful in producing alterations within the cells (Figures 54 to 69). The cytoplasmic changes induced are reminiscent of inclusions found in the precursor globule leucocyte cells and in invasive globule leucocytes (Figures 55, 56, 61 to 65, 67). The production of intracellular crystals characteristic of stage 3 globule leucocytes was not obtained. A continuation of these experiments is suggested. The continued addition of sterile intestinal homogenates may be advisable in order to stimulate the transforming cells into a globule leucocyte. At some future time, it may be possible to extract and identify the specific epithelial factor responsible for the transformation of the mast cell into the globule leucocyte.

Interrelationships among Migratory Cells

Figure 73 shows the possible relationship of the globule leucocyte to other cell types. Most of the experimental and observational evidence for the cell type developments and transformations is given in Bigelow (1961), Michels (1963) and Bloom and Fawcett (1968) with slight modifications. Evidence for some of the relationships are not given in the above references and morphological evidence for the connective tissue mast cell transformations into the globule leucocyte

are from Wesley and Jensen (1969) and Murray et al., (1968), Miller (1971a; 1971b), Miller and Jarret, (1971), Blazek (1971) and the results of this thesis. The histological evidence for this transformation comes from Murray et al., (1968) and from the results of this thesis.

It is possible that there is a relationship between some fibroblasts or fibrocytes and the globule leucocyte since some of the apparently de novo granule forming precursor globule leucocytes appear to have characteristics of fibroblasts: rough endoplasmic reticular formations found within tapered cells (Figure 17). The accumulated product found within the cisternae of the endoplasmic reticulum is not collagen and there is no evidence that the stored product is released into the lamina propria as in the case of fibroblasts. The cell and cytoplasmic contents observed in Figure 17 are nearly identical to some electron micrographs of fibroblasts in a report by Movat and Fernando (1962b). The only differences are that the 250 mu to 1u granules observed in cells similar to that in Figure 17 are absent in normal appearing fibroblasts and that fibroblasts are not generally observed in such close contact with the epithelial basement membrane.

Transformations of blood lymphocytes and blood plasma cells into ciculating mast cells have been reported (Bloom and Fawcett, 1968) but these phenomena would occur before reaching the epithelial tissue and the blood mast cell would be considered a connective tissue mast cell at the time of entry into the mucosal layer. There have been suggestions that the tissue lymphocyte is capable of transformation directly into the globule leucocyte (Heidenhain, 1888; Weill, 1919; Kent, 1952; Holman, 1968). There has been some morphological speculation that the tissue lymphocytes may be the source of intestinal globule leucocytes at the

light microscopic level (Heidenhain, 1888) and at the electron microscopic level based on the developmental stages of the lymphocyte which appear to be similar to that of the globule leucocyte (Kent, 1952;1966) and the appearance of microtubules in the globule leucocyte was considered sufficient evidence that the cell was a lymphocyte (Holman, 1968). Microtubules are occasionally observed in invasive globule leucocytes of mice (Figure 5), but this is not sufficient cause to call the cell type a lymphocyte. It has been postulated that small lymphocytes migrate from the tunica propria and invade the epithelium to differentiate into globule leucocytes (Kent, 1952). There is no support for this theory.

Michels (1963) presented a scheme of development in which the globule leucocyte is presented as a possible precursor to the tissue mast cell and may also develope into a plasma cell erythrophage or Russell body cell. It was also postulated that a plasma cell may become a globule leucocyte (Michels, 1963). These formulations were based on the authors investigations but no experimental evidence was provided. The scheme appears to be incorrect. Connective tissue mast cell development from globule leucocytes implies that the direction of movement of movement of the globule leucocyte is in opposition to the normal epithelial cell population from the basement membrane to the lumen. DIrect observations of this thesis and other workers (Weill, 1919; Takeuchi et al., 1969; Murray et al., 1971) indicate that globule leucocytes move in the same direction as all other normal epthelial components. The arguments against the plasma cell as a precursor to the globule leucocyte have been put forth earlier in this discussion. The development of the plasma cell from the globule leucocyte is implausible for the same reason that the connective tissue mast cell development from the globule leucocyte is unlikely.

The Function of Globule Leucocytes

Morphological implications. Direct observations by electron microscopy of normal intestinal globule leucocytes (Figures 3 to 6; 10 to 13) and actinomycin D treated animals (Figures 24, 27; Wesley and Jensen, 1968) indicate that the globule leucocytes migrate through the crypto of Lieberkühn and deposit their crystalline and granular contents by exocytosis into the lumen of the gut. Degradation of crystals and granules probably follows although there may be some phagocytosis of the excreted product by absorptive epithelial cells or general diffusion of degraded granular products back through the epithelium into the circulatory system via capillaries.

This pattern of development and exocytosis suggests similarities with other exocrine cells such as the argentaffin cell in the gut or the chromaffin cells of the adrenals, kidneys and the uterus. The intestinal argentaffin cell contains the vasoconstrictor and muscle stimulent 5-hydroxytryptamine (Erspamer and Asero, 1952; Benditt and Wong, 1957; Erspamer, 1961) in dense cytoplasmic granules as shown by tritiated 5-hydroxytryptophan uptake (Gershon and Ross, 1966a; 1966b; Ito et al., 1969; Rubin et al., 1971). The cell migrates toward the lumen of the gut and releases its granular content by exocytosis (Chang and Leblond, 1971a; 1971b; Ferreira, 1971; Ferreira and Leblond, 1971). This sequence of events in argentaffin cells has an external developmental similarity to the globule leucocyte.

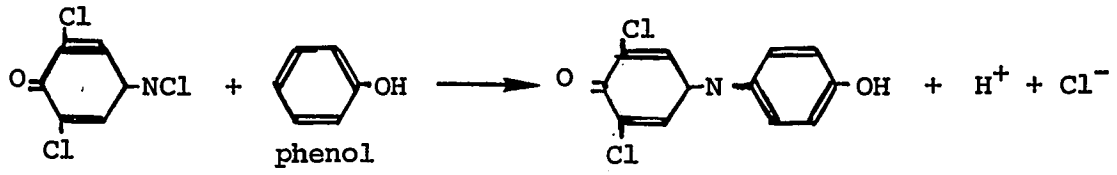
Histological Implications. In the case of the globule leucocyte, the granular constitutents are still unknown. Silva (1967) suggested that the crystals of the globule leucocyte may contain serotonin since there

appeared to be an increase in both the number of crystals as well as the number of cells after blocking degradation of serotonin using monoamine oxidase. Addition of the precursor to serotonin synthesis, 5-hydroxytryptophan, also resulted in an increase in the number of crystals and cells (Silva, 1967). This suggests that globule leucocytes should show similarities to intestinal argentaffin cells in terms of histological reactions such as ultraviolet fluorescence and staining reactions. Yellow serotonin fluorescence is routinely used to identify intestinal argentaffin cells at the light microscopic level (Falck et al., 1962) but the yellow fluorescence is apparently absent in globule leucocytes (Whur and Gracie, 1967; Murray et al., 1968). Instead of a yellow ultraviolet stimulated fluorescence, a dull green fluorescence has been noted in globule leucocytes in the rat (Murray et al., 1968) which is characteristic of the biogenic amine dopamine.

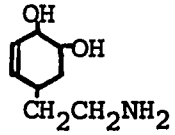
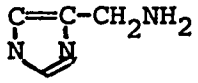
Cytological stains for argentaffin cells are Cowdry's diazo reaction which is negative for both mast cells and globule leucocytes (Kirkman, 1950), Pearse's diazo reaction (apparently negative according to the results of this thesis), diazotized safranin which is negative for cat globule leucocytes (Takeuchi et al., 1969). Gibbs' reagent was found to be positive for globule leucocytes, intestinal enterochromaffin cells and mast cells in the results of this thesis. These conflicting results may be explained in part by the inability of light microscopy to adequately distinguish the globule leucocyte from enterochromaffin cells. Because of technical difficulties, there have been no studies to date correlating the light microscopic staining reaction for enterochromaffin cells and the electron microscopic observation of the same cells (until this thesis).

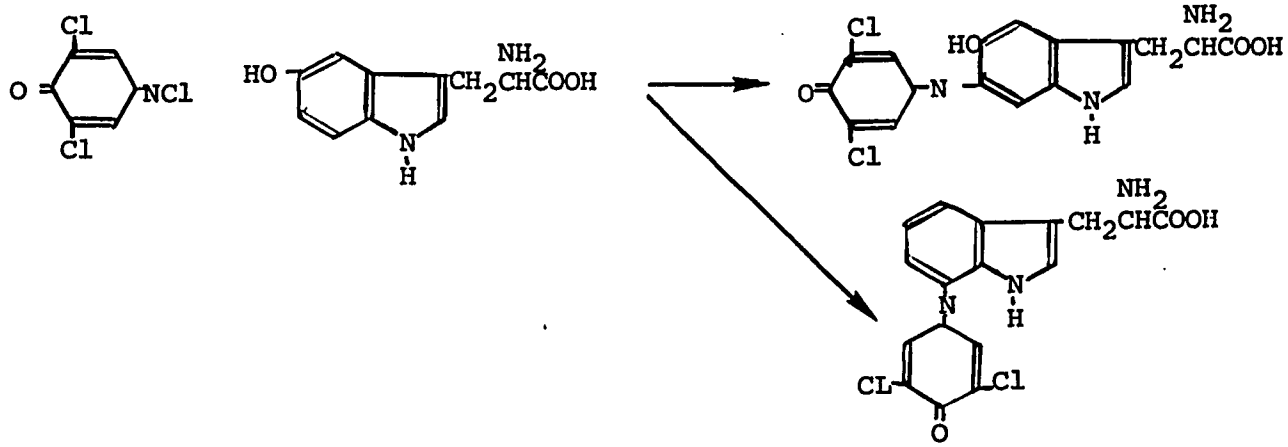
One micron thick Epon sections have been stained using diazotized safranin (Takeuchi et al., 1969) and Azur 2-methylene blue-borax (Miller, 1971), but no correlation has been made of the adjacent ultrathin sections. The positive staining of both the argentaffin cells and the globule leucocytes (Figures 42 to 46) using Gibbs' reagent indicates that it is possible to confuse the two cell types at the light microscopic level using this reagent.

The Gibbs' reagent was found by spectroscopy to have a sensitivity of detecting 1 part of phenol in 2 X 10^7 molecules, but it does not react with all phenolic compounds. The primary prerequisite for interaction is that the position para to the hydroxyl group must be unsubstituted (Gibbs, 1927). The general reaction is:



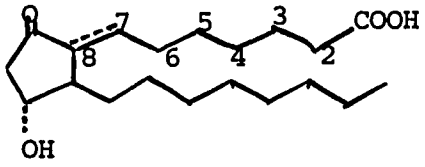
2,6-dichloroquinonimide (After Pearse, 1961)

Thus, some degree of specificity is implied in the reaction since the Gibbs' reaction does not couple with catechols or parasubstituted phenols (Pearse, 1961). In the mouse mast cell, Gibbs' reagent probably does not react with dopamine  or histamine  but may react with 5-hydroxytryptamine in one of the two following ways:

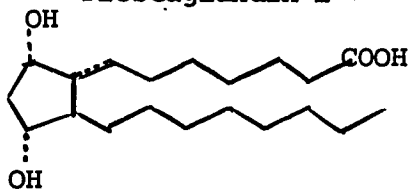


Dopamine is present in the intestinal mast cells of rats and probably globule leucocytes (Murray et al., 1968; Jarrett et al., 1967), but is not the reacting compound giving the dark black reaction product with Gibbs' reagent.

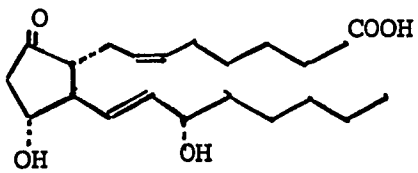
Another possibility is that Gibbs' reagent may be attacking some as yet undiscovered compound in the globule leucocyte to cause the very intense reaction exhibited (Figures 42 to 46) which was unexpected from the thin one micron Epon sections. A candidate for such an undiscovered compound could be one of the prostaglandins. The prostaglandins E and F are capable of stimulating the gastrointestinal smooth muscle, although the origin of the prostaglandins is unknown (Wilson, 1972). Most of the human stomach prostaglandins occur as prostaglandin E_2 in the gastric mucosa (Bennett and Fleshler, 1970). The general structure of some prostaglandins are:



Prostaglandin E



Prostaglandin F



Prostaglandin E_2

(After Bennett and Fleshler, 1970).

Prostaglandin E_2 appears to be a candidate for possible attack by Gibbs' reagent in that the unsubstituted and unsaturated carbon atom opposite the hydroxyl group on carbon-15 is somewhat similar to the phenolic grouping required. This hypothesis, of course, requires testing and it must first be demonstrated that prostaglandins are localized within the globule leucocyte.

There is mounting evidence in rats that the globule leucocyte plays a role in the expulsion of infecting intestinal parasites (Whur, 1966; Carr and Whur, 1968; Sinclair, 1970). The "self-cure" phenomenon is observed in sheep and cattle (Sinclair, 1970) and is often associated with the presence of globule leucocytes (Whur, 1968; Stewart, 1955; Toner et al., 1971). Wild deer infected with parasites also show a large number of globule leucocytes in the intestines and liver (Blazek, 1971). The globule leucocyte has also been observed in the endometrial uterine lining of women (Kent, 1952) and ruminants (Kellas, 1961). The occurrence of globule leucocytes in epithelial tissues with smooth muscle linings and the "self-cure" phenomenon involving expulsion of parasites by smooth muscle contractions suggests that the globule leucocyte may have a compound which stimulates this activity. Murray et al., 1971, have found that an increase in 5-hydroxytryptamine in rats infected with helminths is associated with increasing numbers of mast cells and globule leucocytes.

In Swiss strain and AKD mice used in experiments for this thesis, there is an increase in the number of globule leucocytes which appears to be correlated with abdominal contractios and diarrhea (observed in the actinomycin D treated mice and mice treated by intraperitoneal injections of isolated P815 mast cell viruses or P815 mast cells). Mice infected

with parasites have an increased number of globule leucocytes and diarrhea (Carr, 1967). From Table 4 it appears that the globule leucocytes increase after actinomycin D treatment as well as the number of argentaffin cells. Both of these cell types may play a role in causing the generalized diarrhea and abdominal contractions observed under different experimental conditions.

The mechanisms involved creating water and peristaltic dysfunction in the production of diarrhea are as yet unknown (Bockus, 1966) although histamine, serotonin, and acetylcholine are involved as humoral agents (Texter et al., 1968) as well as prostaglandins (Bennett and Fleshler, 1970; Wilson, 1972). It is likely from the observed symptoms of mice treated by experimental stress of drugs, tumors, and parasites that the globule leucocyte plays a role in the production of diarrhea.

Fate of the Globule Leucocyte

Kirkman (1950) suggested that the apparent migration of the urinary epithelial globule leucocyte to the lumen of the bladder might indicate that the granules may be discharged. Takeuchi, Jervis and Sprinz (1969) noted that globule discharge was observed in cats treated with pilocarpine, but do not present any evidence for this phenomenon.

Evidence that the ultimate fate of the globule leucocyte in mice is migration to the surface of the lumen of the epithelium and the subsequent exocytosis of both crystals and globules is presented for the first time in the results of this thesis (Figures 6, 32, 33). The fate of the globule leucocyte cytoplasmic inclusions is apparently

similar to that of the exocrine intestinal enterochromaffin cell in terms of discharge and potential resortpion of molecules into the circulatory system. The crystals, unlike argentaffin cell granules, may also mix with mucous cell granules before discharge (Figures 32 and 33) or be phagocytized by absorptive epithelial cells and degraded by lysosomes (Figure 28). This indicates at least two methods of increasing the concentration within the intestine of the compounds stored within the crystals of the globule leucocyte. Once the crystals are released into the lumen of the gut they are apprently rapidly degraded since free crystals in the gut are never found apart from the degranulating globule leucocytes.

CONCLUSION

The experimental observations produced results which may lead to the following conclusions:

1. The intestinal globule leucocyte originates from a connective tissue mast cell which invades the epithelium from the lamina propria.

2. Three stages are distinguished during the continual process of differentiation of the globule leucocyte. The early invaseive stage contains mast cell-like granules and is located near the basement membrane of the epithelium. The middle stage cell shows an increase in cell and granule size with the beginning of intra-granular crystal formation. The late stage globule leucocyte is at the lumen of the gut and deposits its crystals and globules through exocytosis.

3. The actinomycin D experiments show the formation of intermediate mast cell-globule leucocytes in the lamina propria. Early precursor globule leucocytes were identified as potential globule leucocytes in the lamina propria of normal mice.

4. The granules of early globule leucocytes and precursor globule leucocytes are probably protein and susceptible to pepsin digestion, but the late stage crystals and globules are resistant to protein digestion.

5. The globules of globule leucocytes do not stain for acid phosphatase and preliminary results indicate that they are peroxidase

negative.

6. The globule leucocyte globules stain intensely using Gibbs' reagent for argentaffin cells at the light microscopic level. An electron microscopic correlation of the same cells shows that these cells are not argentaffin cells but globule leucocytes.

7. Preliminary results of *in vitro* P815 mast cell cultures incubated with mouse intestinal homogenates indicate that it is possible to induce a degree of transformation of the mast cells into cells having similar morphological features of early globule leucocytes.

8. *In vivo* uptake of ^{3}H-thymidine labeled P815 mast cells in mice is possible, but most labeled cells are severely damaged when observed in the cecal epithelium.

9. No function can be clearly assigned to the globule leucocyte but the cell type appears to have a role aiding in the removal of some intestinal parasites during infection and in the production of diarrhea during a variety of stress conditions.

10. It is proposed that the globule leucocyte be renamed the modified mast cell to more accurately reflect the origin of the cell type.

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APPENDIX I

Ingredients in Wayne Lab-Blox Food Pellets

A. General Analysis.

Crude Protein.....	(Min.)	24.0%
Crude Fat.....	(Min.)	4.0%
Crude Fiber.....	(Max.)	4.5%

B. Ingredients.

Animal liver meal, fish meal, dried whey, corn and wheat flakes, ground yellow corn, soybean meal, wheat middlings, cane molasses, soybean oil, brewers dried yeast, vitamin A palmitate, irradiated dried yeast (source of vitamin D2), D-activated animal sterol, vitamin E supplement, menadione sodium bisulfite (source of vitamin K activity), riboflavin supplement, niacin, calcium pantothenate, choline chloride, thiamine, ground limestone, dicalcium phosphate, salt, manganous oxide, copper oxide, iron carbonate, ethylene diamine dihydriodid, cobalt carbonate and zinc oxide.

C. Reference: Allied Mills, Inc., General Offices, Chicago, Illinois.

APPENDIX II

In vitro P815 Mast Cell Culture Procedures

A. General Procedures.

All procedures were carried out under sterile conditions using a Hemco transfer hood (Hemco, Inc., Independence, Missouri) equipted with a 30 watt General Electric ultraviolet germicidal lamp. All equipment used in the transfer of cultures or preparation of media were sterilized in a Barnstead Autoclave (Barnstead Still and Sterilizer Company, Boston, Massachusetts) at 250°F and approximately 20 p.s.i. for 30 minutes.

B. Preparation of Culture Medium.

1. Solutions.
   a. Sterile 100 ml bottles of GIBCO Fischer's medium for leukemic cells of mice.
   b. 20 ml of penicillin-streptomycin solution containing 5000 units of penicillin and 5000 mcg streptomycin per ml.
   c. 100 ml of horse serum.
2. The modified Fischer-Chu medium.

   Ten ml of horse serum and 20,000 units of penicillin with 20,000 mcg streptomycin were added to 100 ml of sterile Fischer's medium. The mixture was shaken well and used immediately.

C. Culture Techniques.

1. Cell cultures from Dr. G.A. Fischer of P815Y mast cells were counted in a Levy-Hausser corpuscle counting chamber (Fisher Scientific Company, Pittsburgh Pennsylvania) and the density of the cell suspension was adjusted to approximately 3 X 10^5 mast cells per ml by dilution with fresh Fischer's medium. Portions of the original cell cultures were frozen in liquid nitrogen for future use.
2. For general maintenance of the cell cultures, fresh Fischer's medium was added every three days to the cultures and the cell count was adjusted to 3 X 10^5 cells per ml in the new cultures.
3. For experimental purposes, the cell cultures were agitated daily and the number of mast cells adjusted to 3 X 10^5 cells per ml every day for one week before use in an experiment. This procedure insured that the cultures were maintained in the logarithmic phase of growth (Schindler et al., 1959).

APPENDIX III

Solutions for Electron Microscopy

A. Fixatives and Buffers.

1. A 50% stock solution of glutaraldehyde (Fisher Scientific Comapny, Fair Lawn, New Jersey) was diluted to a 3% solution in one of the following buffers:

   a. Cacodylate-sucrose buffer, pH 7.2 (modification of buffer in Berlin *et al.*, 196).

      1' 25 ml of a 0.2 M sodium cacodylate solution.

      2' 4.2 ml of a 0.1 N hydrochloric acid solution.

      3' Solutions 1' and 2' were mixed and the pH was checked and adjusted as necessary.

      4' Equal parts of solution 3' were added to a 0.88 M sucrose solution previously made up in the 0.2 M sodium cacodylate buffer at pH 7.2.

   b. Phosphate buffer, pH 7.2 (Berlin *et al.*, 196).

      1' The following solutions were mixed:

0.1 M KH_2PO_4	13 ml
0.1 M Na_2HPO_4	37 ml
	50 ml

      2' The pH was adjusted, when necessary, to pH = 7.2.

2. 2% osmium tetroxide post-fixative.

   a. One gram of osmium tetroxide was dissolved in 50 ml of distilled water, cacodylate-sucrose buffer, or phosphate

buffer and kept as a stock solution at 4°C.

b. At the time of tissue fixation by immersion, the 2% osmium tetroxide solution was mixed in a 1:1 ratio with the appropriate buffer to make a 1% solution.

3. Perfusion fixative (personal communication with Dr. W. F. Hughes). The perfusion fixative was composed of:

a. 3% glutaraldehyde in cacodylate-sucrose buffer, pH = 7.2.

b. 1% paraformaldehyde.

c. 2% NaCl

B. Post stains.

1. Uranyl acetate in methanol (Stampak and Ward, 1964).

a. Fifteen grams of uranyl acetate were dissolved in 50 ml of absolute acetone free methanol.

b. After mechanical stirring for at least 8 hours, the solution was filtered and stored at 4°C.

c. Grids with ultrathin sections were stained in the uranyl acetate for 10 to 15 minutes.

d. The sections were washed by dipping in at least four fresh vials of absolute methanol.

2. Lead citrate (Reynolds, 1963).

a. Distilled water was either sterilized or boiled for use in preparing the lead citrate so that the solution would be carbon dioxide free.

b. Thirty ml of boiled water were added to a 50 ml volumetric flask in which 1.33 g lead nitrate and 1.76 grams of sodium citrate were placed.

c. The mixture was agitated for at least one minute and allowed to cool for 30 minutes with intermittant agitation.

d. Eight ml of 1.0 N NaOH were added to the flask and mixed.

e. The flask was filled to 50 ml with carbon dioxide free distilled water.

f. The stain was used immediately on grids. Staining time was between 5 to 10 minutes and grids were washed in cooled carbon dioxide free distilled water.

APPENDIX IV

Tissue Preparation Procedures for Electron Microscopy

A. Fixation by immersion.(Berlin et al., 196).

1. Immediately after sacrifice of the animal, the excised tissue was placed in a cold bath of cacodylate-sucrose or phosphate buffered 3% glutaraldehyde. One cubic millimeter cubes were sliced using a sharp single-edged razor blade while the tissue was immersed in the fixative.
2. The cubes were placed into vials and covered with cold buffered 3% glutaraldehyde and fixed for one hour at 4°C.
3. The sections were washed at least five times in the same buffer that was used in making the fixative. The tissue was allowed to remain for five minutes in each change of buffer with intermittant swirling.
4. The specimens were post-fixed in buffered 1% osmium tetroxide for one hour at 4°C.
5. After post-fixation, the cubes were rapidly dehydrated using the following procedure:
   a. 5 minutes each in 50%, 70%, and 95% ethanol with periodic swirling.
   b. 5 minutes in 3 changes of absolute ethanol with swirling.
   c. 5 minutes in 3 changes of propylene oxide with swirling.
   d. Overnight rotation in a 1:1 mixture of Epon and propylene oxide.

6. Preparation of Epon (Luft, 1964).

   a. Mixture A.

      62 ml Epon 812

      100 ml Dodecanyl succinic anhydride (DDSA)

   b. Mixture B.

      100 ml Epon 812

      89 ml Nadic methyl anhydride (NMA)

   c. Mixture A and Mixture B were combined in a 1:1 ratio and 0.2 ml of DMP-30 (polymerization catalyst for Epon) was added for every 10 ml of mixed Epon.

   d. The mixture was stirred very thoroughly.

7. Fresh Epon was placed into clean vials and the specimens were transferred into the new Epon and allowed to infiltrate with rotation for at least 12 hours.

8. The specimens were poured into aluminum boats and numbered.

9. Polymerization was carried out as follows:

   a. 12 to 24 hours at 35°C (under a 60 watt light bulb).

   b. 24 hours at 45°C (in a controlled temperature oven).

   c. 3 to 7 days at 65°C (in a controlled temperature oven).

B. Fixation by perfusion (W.F. Hughes, personal communication).

1. Sacrificed mice were fixed by gravity fed vascular perfusion. A small guage hypodermic needle was inserted through the left ventricle of the heart to the base of the dorsal aorta and attached to an elevated bottle containing the perfusion fixation fluid described in Appendix III.

2. A small puncture was made through the sinus venosus and

the perfusion fluid was allowed to flow. The perfusion was allowed to continue for 15 to 30 minutes after all blood was removed from the circulatory system.

3. The desired tissues were excised and treated in the same manner as described in Appendix IV steps 1 through 9.

APPENDIX V

Photography

A. Electron Microscope Negatives. Two types of negatives were used:

1. Kodak 0.050 inch thick 3.75 X 4 inch glass projector slide plates were exposed to the electron beam using the Hitachi automatic photometer.
2. Kodak Electron Microscope Film, estar base (#4489) or Electron Image Film was used (6.9 X 9 cm).
3. Development of both types of negatives was as follows:
   a. Two minute development with gentle agitation in Kodak D-19 plate developer, 68°F.
   b. A 20 to 30 second rinse in a running water bath at 68°F.
   c. Clearing in Kodak Fixer at 68°F for 5 to 10 minutes.

B. Photographic Enlargement and Printing.

1. A Durst S-45 EM enlarger was used.
2. Printing was done on Kodabromide papers graded F2 through F5.
3. Development was in Kodak Dektol developer at 68°F followed by a 20 to 30 second immersion in a midly acetic stop bath and then at least 10 minutes in Kodak Fixer.
4. Prints were washed for at least one hour at 68°F in a rotating print washer.
5. Drying was accomplished by an Arkay drum print dryer.

C. Reproductions.

1. A Polaroid MP-3 enlarging system was used for all reproductions.

2. Kodak Ektapan film (4 X 5") was used for reproduction negatives and developed in Microdol-X for 11 minutes at 68°F.

APPENDIX VI

Light Microscopic Staining of One Micron Epon Sections

A. Epon clearing and differential staining (Lane and Europa, 1965).

1. An Epon solvent was prepared by making a saturated solution of NaOH in absolute ethanol. The solution was allowed to stand for 2 to 3 days before using.
2. Glass slides with one micron Epon sections were placed in the Epon solvent for 8 to 12 hours in a covered Coplin jar. Fifty ml of the solvent were used to process 10 slides.
3. Slides were removed, drained on bibulous paper and placed into absolute ethanol for 5 minutes.
4. The slides were drained on bibulous paper again and placed in fresh absolute alcohol for 5 minutes. This was repeated until four baths of absolute alcohol were used.
5. The slides were immersed in phosphate buffer, pH = 7.0 for 5 minutes and then washed three times in distilled water.
6. The slides were then immersed in phosphate buffer, pH = 4.0 for 5 minutes and then washed in running tap water for another 5 minutes.
7. The slides were then ready for staining by one of the methods listed below.

B. Haemotoxylin and eosin stain (Gridly, 1968; Lane and Europa, 1965).

1. Slides prepared by the Epon clearing technique were stained

for 20 minutes in Bullard's, Harris', or Weigert's iron hematoxylin.

2. The slides were washed in water, quickly dipped in acid alcohol, washed well and placed in a weak solution of ammonium hydroxide.
3. The slides were washed well, placed in 95% ethanol for 1 minute and stained for 30 seconds in Triosine.
4. The Triosine stain consisted of:

Eosin Y	620 mg
Orange G	280 mg
Erythrosine	100 mg
Absolute EtOH	100 ml

5. Slides were rinsed in 95% alcohol followed by 2 quick changes of absolute ethanol and mounted in diaphane.

C. Diazo method for argentaffin cell granules (Pearse, 1961).

1. The sections were brought into distilled water after Epon clearing.
2. Slides were treated for 30 seconds with a dilute (1 mg/ml) solution of the stable diazotate of 5-nitroanisidine (Fast Red Salt B, ICI Ltd.), also known as Echtrotsalz B, in 0.1 M veronal acetate buffer, pH 9.2.
3. Slides were washed thoroughly in running water and counterstained with Mayer's hemalum for 6 to 10 minutes.
4. The slides were washed in running water for 30 minutes, dehydrated in alcohol and mounted in diaphane.

D. Gibbs' method for argentaffin cell granules (Pearse, 1961).

1. Preparation of the staining solution.

a. Twenty mg. of 2,6-dichloroquinone-chloroimide (Gibbs' reagent) were dissolved in 20 ml of veronal acetate buffer at pH 9.2 by warming to 70°C and shaking.

b. The solution was colled and filtered before using.

c. The reagent 2,6-dichloroquinone-chloroimine (molecular weight = 210) was purchased from Bordon Company, Chemical Division, 5000 Langdon Street, Philadelphia, Pennsylvania.

2. Procedure.

a. The sections were brought into distilled water after Epon clearing.

b. The sections were treated with Gibbs' reagent for 10 to 15 minutes.

c. The slides were washed well in running water and then counterstained with 1% aqueous neutral red for 3 minutes.

d. The slides were dehydrated and the nuclear stain was differentiated by rapidly passing them through 70% and absolute ethanol.

e. Slides were mounted in diaphane.

E. Toluidine blue staining (Trump et al., 1961; Hingson and Ito, 1971).

1. Epon one micron sections may be cleared or left intact.

2. The slide is heated gently over a hot plate and a few drops of a 0.5% toluidine blue solution containing 1% borax at

APPENDIX VI (CONTINUED)

pH = 9.0 were placed over the sections.

3. A few drops of distilled water to wash the slide were added quickly followed by a few drops of absolute ethanol. This process was repeated 3 to 4 times to differentiate the stain.
4. The slides were ready for examination immediately upon drying.

APPENDIX VII

AUTORADIOGRAPHIC PROCEDURES

A. Light Microscopic Autoradiography.

1. Bottles of cold Ilford L4 or Kodak NTB2 emulsions were melted in a hot water bath at 45°C until the emulsion became liquid. All procedures were carried out in a photographic darkroom equipted with a Wratten Series number 2 safelight (for Ilford L4 emulsion) or a Wratten Series number 1 safelight (for Kodak NTB2 emulsion) with a 15 watt bulb placed at least three feet away from the working area.
2. New bottles of emulsion were tested for background contamination by dipping a clean glass slide into the emulsion, allowing the slide to dry for 2 to 3 hours, developing the slide in D-19 for 2 minutes at 22°C and checking the developed slide under a light microscope. To remove small traces of background, dipped slides were placed in a saturated atmosphere of hydrogen peroxide overnight.
3. The melted emulsion was poured into a Coplin jar and slides with mounted one micron Epon sections were dipped into the emulsion slowly for about 4 or 5 seconds.
4. The covered slides were allowed to dry vertically for several hours or overnight.
5. The slides were placed in a black slide box containing a small package of dessicant. The edges of the box were sealed with

black tape and the box placed in storage at 4°C for 1 to 4 weeks.

6. Slides were developed in Kodak D-19 at 22°C for 2 minutes, washed in a 1% acetic acid bath for 20 seconds, and fixed in Kodak Fixer for 5 to 10 minutes.

B. Electron microscopic autoradiography.

1. Procedures for Kodak NTE emulsion (Salpeter and Bachmann, 1964; Salpeter, 1966).

a. Silver thin sections were cut on the ultramicrotome of radioactively labelled material.

b. The sections were transferred to a collodion covered glass slide using a transfer loop.

c. The sections were stained using aqueous uranyl acetate for 5 minutes followed by lead citrate for 10 minutes.

d. The slides were coated with a 50 to 60 A layer of carbon to prevent oxidation of the emulsion by osmium tetroxide.

e. The Kodak NTE emulsion was heated to 55°C in a hot water bath until liquid. A test slide was checked for background and cracking of the emulsion.

f. Four grams of emulsion were removed from the bottle and 40 ml of distilled water were added. This was divided equally into four clean centrifuge tubes.

g. The emulsion was centrifuged in a clinical IEC centrifuge at 14,000 G for 10 minutes.

h. The centrifuge tubes were placed in an ice bath briefly and the supernatant was checked for clarity under a Kodak Wratten series 1A safelight.

i. The concentrated emulsion was reheated and diluted with 1 to 4 ml per gram of the original emulsion (i.e., 4 to 16 ml of water when starting with 4 grams of emulsion. The actual amount used was determined empirically depending on the emulsion thickness desired.

j. The diluted emulsion was placed on the sections by dropping from sterile pipets. The slides were allowed to dry vertically for several hours and stored in light tight black plastic boxes.

k. A dessicant and sometimes nitrogen were added to the boxes before being placed at 4°C for about 2 to 8 months exposure.

l. Slides were developed in Dektol (1 Dektol to 2 parts water) for 2 minutes at 22°C, washed in water for 30 seconds, and fixed for 5 to 10 minutes.

m. For electron microscopy the collodion film was floated off on a clean distilled water surface and grids were placed directly over the sections. The grids were picked up by another glass slide, dried and examined under the electron microscope.

2. Procedures for Ilford L4 emulsion (Caro and van Tubergen, 1962).

a. A fresh bottle of Ilford L4 emulsion was heated in a hot water

bath at 49°C until liquid. During the melting and gentle stirring process, many air bubbles are created. The emulsion was filtered through several thicknesses of cheese cloth into a 250 ml beaker to remove the bubbles.

b. The emulsion was allowed to gel. 15 grams were then scooped out and several dilutions were made: a 1:1, 1:2, 1:3 and 1:4 dilutions were made using glass distilled water.

c. The mixtures were stirred gently in a 45°C water bath for about 1 minute and then placed on an ice bath for 5 to 15 minutes to gel. They were then allowed to stand at room temperature for 10 minutes.

d. The gel was then placed on the sections previously picked up on Formvar coated nickel 100 mesh grids by using a transfer loop made of copper and 4 cm in diameter. The sections were previously stained and carbon coated after being placed on the Formvar coated grids.

e. The slides were allowed to dry vertically for several hours before being stored in black plastic boxes with a dessicant at 4°C for several weeks to 10 months.

f. The sections were developed in Microdol X at 23°C for 5 minutes, washed in a 1% acetic acid bath for 10 seconds and fixed for 5 to ten minutes in Kodak Fixer.

g. The sections were then dried and examined by electron microscopy.

APPENDIX VIII

Actinomycin D Treatment Schedule

A. Procedure.

1. Sixteen mice (either Swiss strain or AKD) were used for actinomycin D treatment using 4 mice as controls.
2. Control mice were injected with 0.25 ml of physiological saline at times corresponding to the actinomycin D injections. One control mouse was sacrificed at times corresponding to the sacrifice of mice in each major group of experimental animals.

B. Experimental Groups.

1. Group I. These mice received a total of 24 ug actinomycin D injected intraperitoneally over an 18 hour time period. Each of the three mice in this group received 8.0 ug actinomycin D every 6 hours and were sacrificed 24 hours after the beginning of the experiment.
2. Group II. A total of 33 ug of actinomycin D was administered within a 42 hour time period. These three animals received the same dosages as Group I plus 2 injections of 5.0 ug actinomycin D every 9 hours beyond the first 18 hour time period. The animals were sacrificed after 48 hours.
3. Group III. These animals received a total of 40 ug of actinomycin D during a 42 hour time period. One injection of 7.0 ug actinomycin D was given at the 42nd hour of treatment

and the three animals were sacrificed after 52 hours.

4. Group IV. A total of 63 ug of actinomycin D was injected over a 60 hour time period. Two injections of 8.0 ug actinomycin D were administered every 6 hours followed by a 7.0 ug actinomycin D injection 6 hours later (at the 60th hour of treatment). The animals were sacrificed after 72 hours.

APPENDIX IX

Modified Hirsch-Fedorko Technique (1968)

A. Preparation of solutions.

1. Stock solutions:

   a. 2.5% glutaraldehyde in 0.1 M cacodylate, pH 7.4 at 4°C.

   b. 1.0% osmium tetroxide in 0.1 M cacodylate, pH 7.4 at 4°C.

2. Two parts of the osmium tetroxide solution were mixed with one part of the glutaraldehyde at 0°C in an ice bath and was used within one hour after mixing.

3. A 0.25% uranyl acetate solution in 0.1 M acetate buffer at pH 6.3 was kept at room temperature.

4. Preparation of fibrinogen and thrombin:

   a. The fibrinogen was made by dissolving 0.7% bovine fibrinogen (Nutritional Biochemicals, Cleveland, Ohio) in distilled water with salts of Furtado (0.16% sodium citrate plus 0.85% NaCl).

   b. The thrombin was prepared by placing 100 NIH units/ml of thrombin in distilled water or saline. This was generally kept frozen for several months in a ten-fold concentrated stock solution.

B. Procedure.

1. The cell culture was centrifuged into a pellet of at least 5×10^6 cells and the pellet was suspended in 2 ml of cold fixative (A2 from above) for 2 minutes.

2. The suspension was centrifuged in an IEC Clinical Centrifuge at 300G for one minute. The supernatant was decanted and the precipitate was resuspended in another 2 ml of cold fixative and aspirated with a pipet. The suspension was left on ice for 10 to 30 minutes.
3. The suspension was centrifuged for one minute and the pellet washed in cold physiological saline. This was repeated twice.
4. The suspension was centrifuged and resuspended in the uranyl acetate solution and allowed to stand at 0°C for 15 to 30 minutes.
5. The suspension was centrifuged for one minute and the pellet washed in cold physiological saline twice.
6. The supernatant was decanted after centrifugation and a volume of fibrinogen solution was added equal to the volume of the sample (approximately 0.3 ml).
7. The sample was stirred with a glass rod and approximately 0.3 ml of thrombin was added and stirred. The fibrin clot forms on the rod and is complete in less than 90 seconds. (Reference for the fibrin clot technique: Sicko and Arnold, 1971).

Figure 1. A graph of the frequency of globule leucocytes in the Swiss strain cecal epithelium during increasing dosages of actinomycin D. This graph is derived from direct counts of the epithelium using 522 normal cells and 352 actinomycin D treated cells for a total epithelial population of 874 cells.

Legend:

●—●—● The frequency of invasive globule leucocytes in the intestinal epithelium .

▲—▲—▲ The frequency of induced globule leucocytes with crystalline inclusions in the lamina propria.

FIGURE 1. GRAPH OF THE FREQUENCY OF GLOBULE LEUCOCYTES VERSUS ACTINOMYCIN D CONCENTRATION.

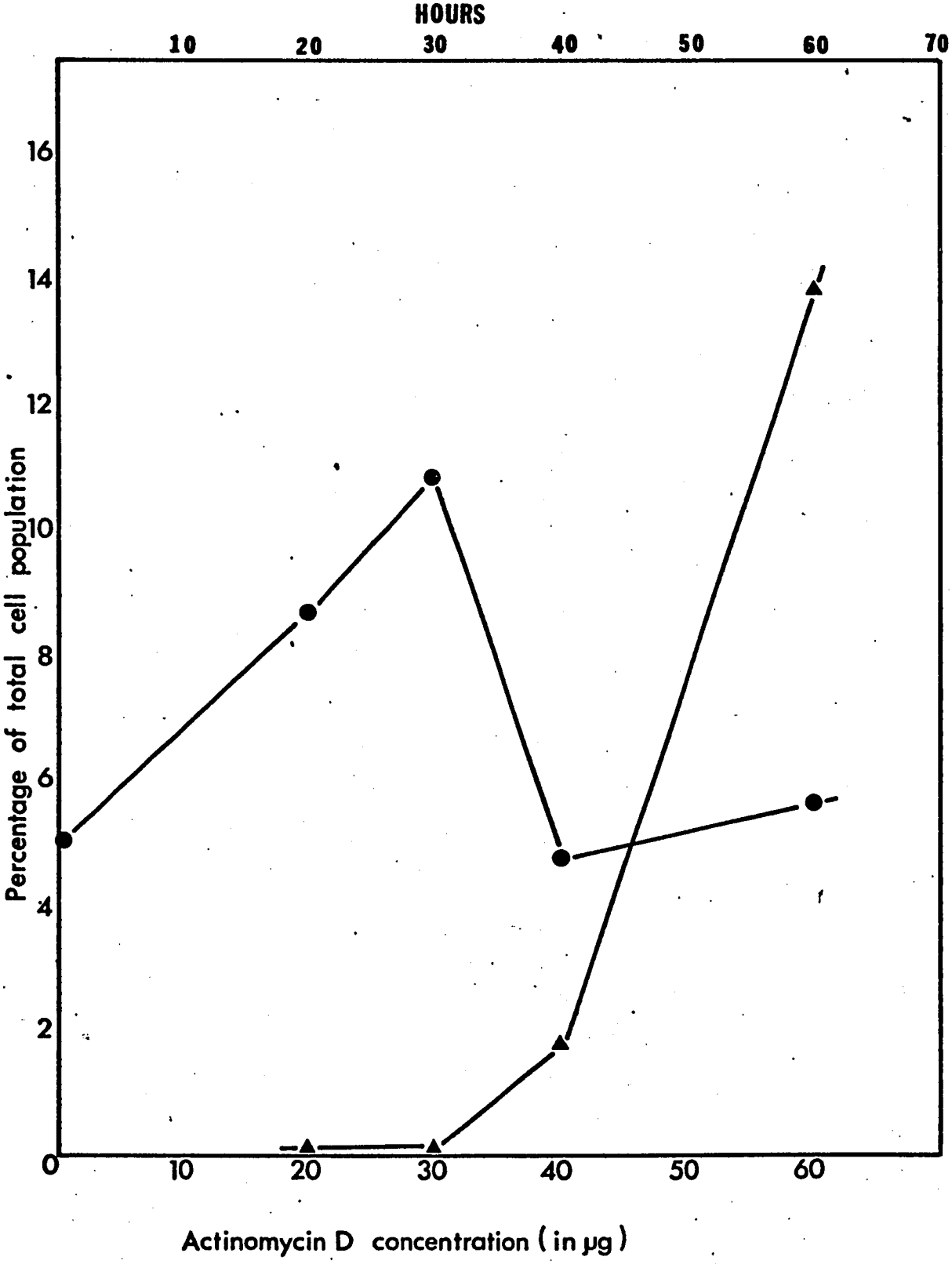
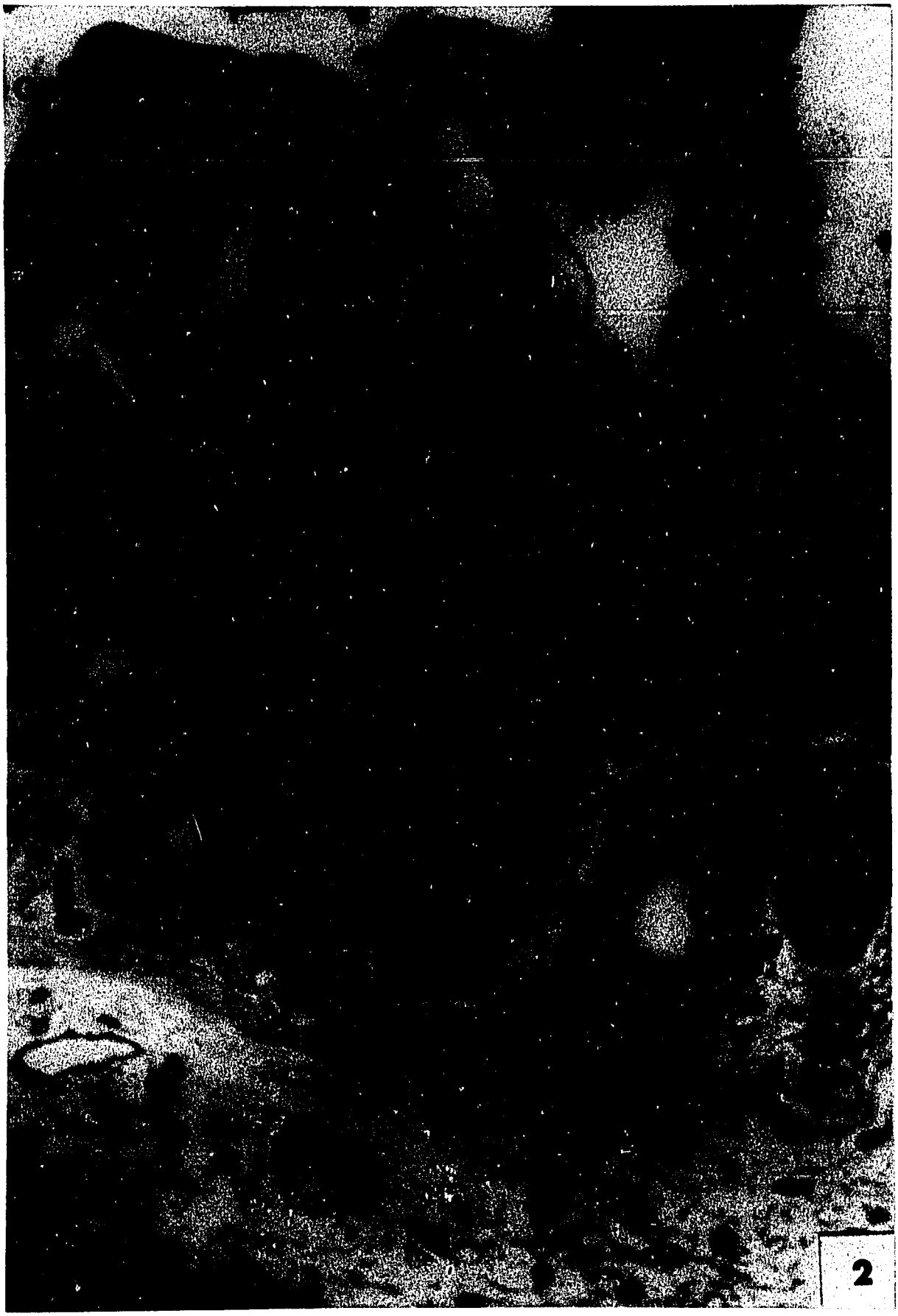


Figure 2. A light microscopic micrograph of a one micron section of a normal Swiss strain cecum stained with 0.5% toluidine blue. The cecum is generally composed of deep folds (F) which gives the appearance illustrated in cross section. A globule leucocyte (GL) is present as well as many mucous cells (McC) and absorptive epithelial cells (E) in the epithelium. There are many crypts of Lieberkühn (C) surrounding portions of the lumen (L) which are separated by the lamina propria (LP). The epithelium and the lamina propria are subtended by the muscularis mucosae (MM). Below this is the submucosa (SM) containing nerves, connective tissue cells and blood vessels. A portion of the serosa (S) is below the submucosa. Magnification 700X.



161A

Figure 3. Electron micrograph of a section of the normal Swiss strain mouse cecal epithelium near the basal aspect of the epithelium showing the relationship of a globule leucocyte (GL) recently incorporated into the epithelium to the surrounding undifferentiated cells (UC), a mucous cell (MuC), the lamina propria (LP), and the lumen of the gut (L). Note the presence of red blood cells in a large capillary near the crypt of Lieberkühn and the presence of microvilli (MV) on the absorptive epithelial cells. Magnification 8900X.

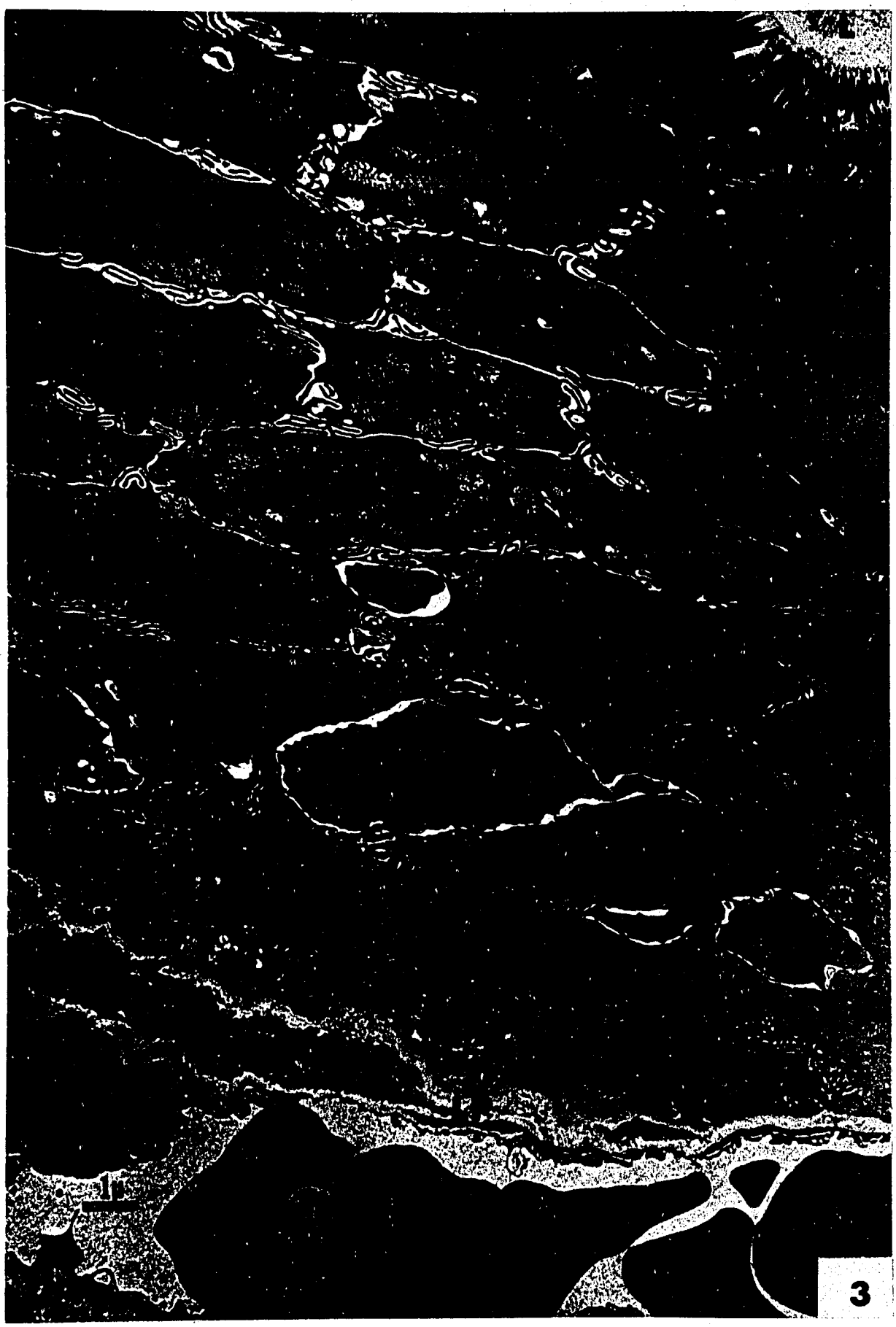

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Figure 4. Higher magnification of a normal globule leucocyte observed in the preceeding figure. Characteristic features of a globule leucocyte which has recently migrated into the cecal epithelium are demonstrated by this cell. Mast cell-like granules (G), large intercellular spaces (IC) between the globule leucocyte and adjacent cells, the absence of desmosomes (although a desmosome (D) is clearly observed in the neighboring undifferentiated cell), and a general lack of intracellular organelles are all features of the early invasive cell. Magnification 59,400X.

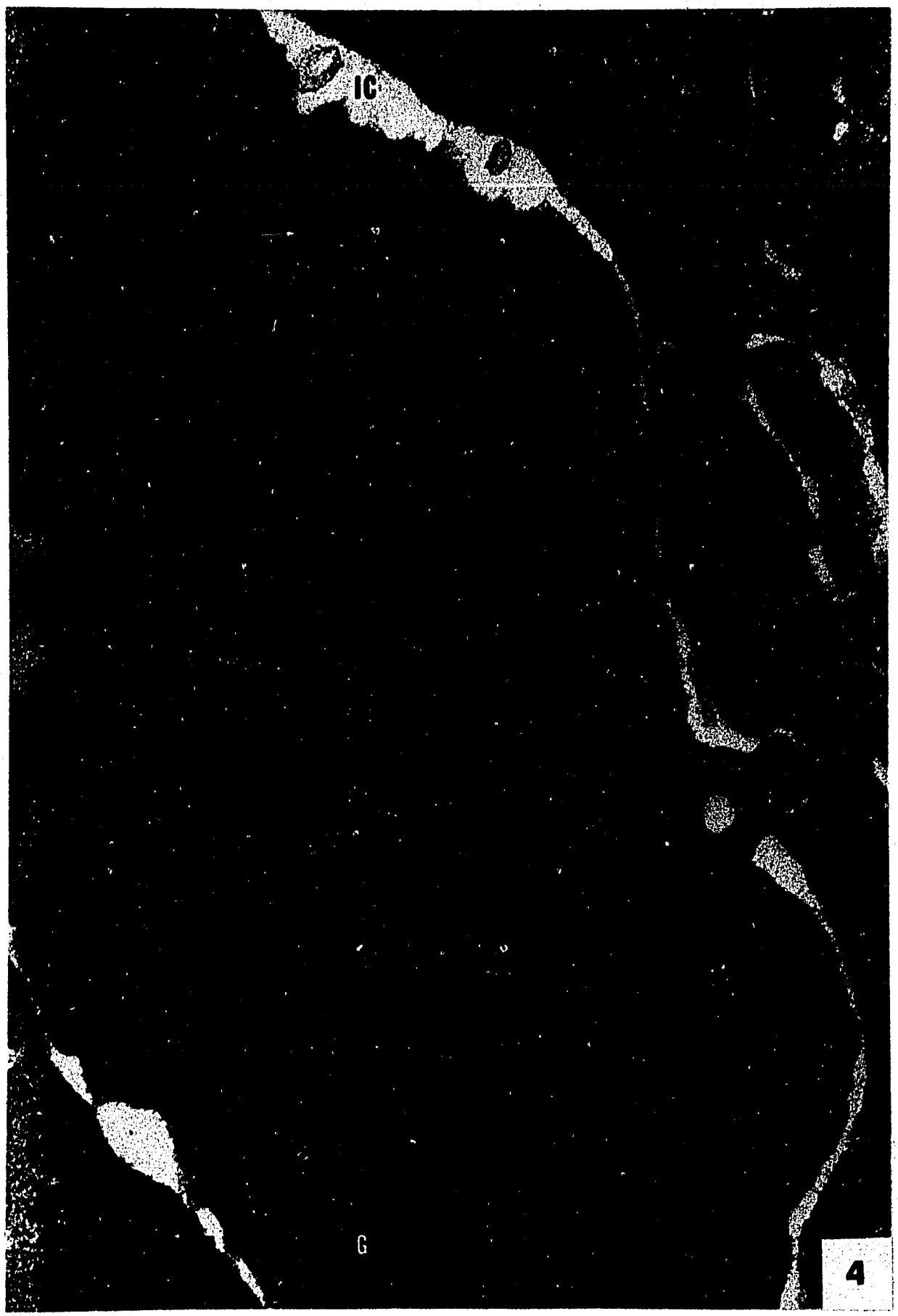
IC
G
4

Figure 5. A middle stage globule leucocyte from a normal Swiss strain mouse cecal epithelium. The globule leucocyte observed was no longer at the basal aspect of the crypt of Lieberkühn but migrating toward the lumen of the intestine. The large intercellular space of early invading cells is no longer present and the plasma membrane has made a close contact with plasma membranes of the surrounding cells. A desmosome (D) is present, but it connects the plasma membranes of two adjacent cells. The globules (G) have increased in size and have differentiated into crystals (C) in some cases. A moderate Golgi apparatus is present (G) near the central indentation of the n ucleus and a microtubule (MT) is nearby. Mitochondria are small and sparse while free ribosomes are abundant. Magnification 33,600X.

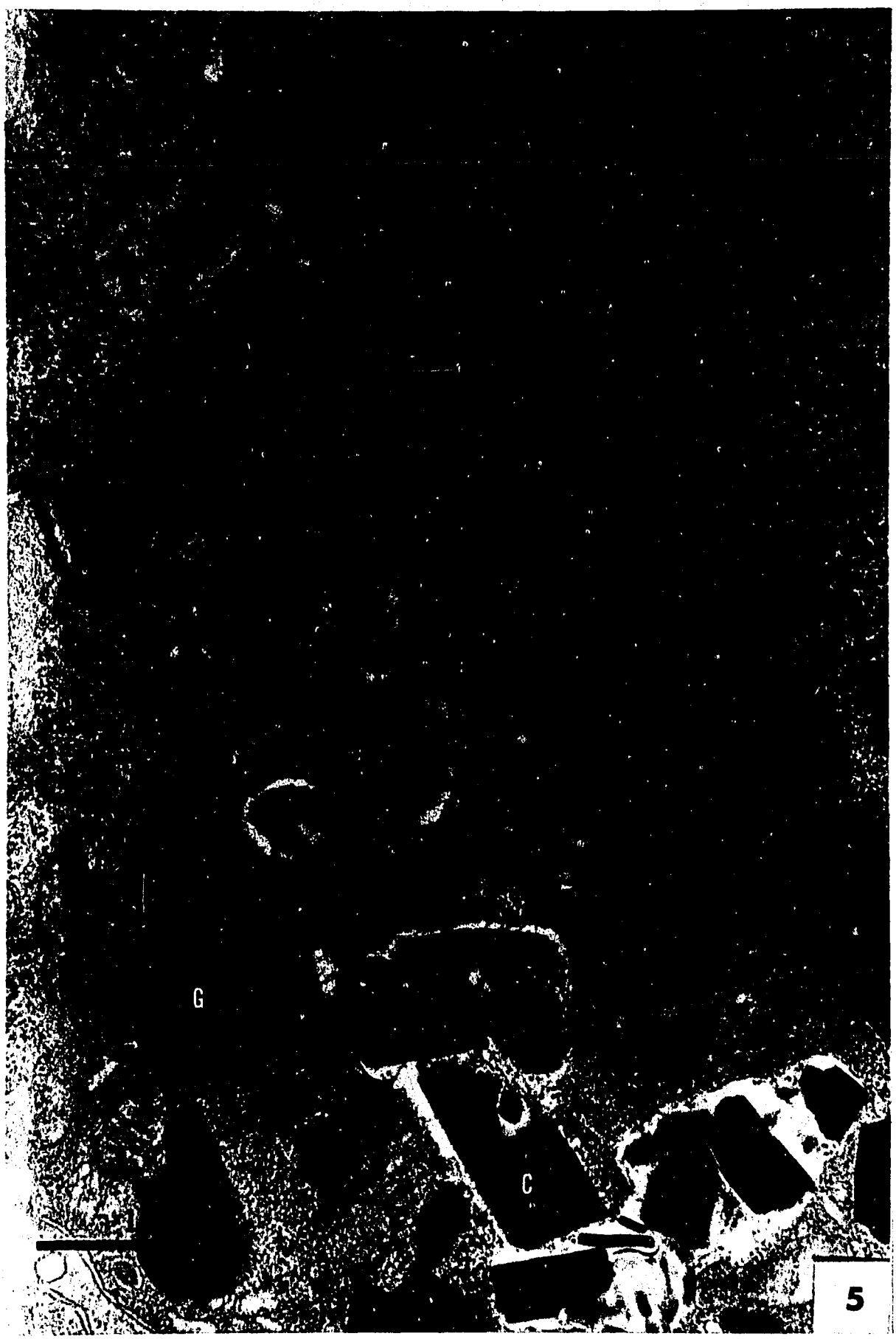
G
C
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Figure 6. A late stage globule leucocyte from a Swiss strain mouse injected with P815 mast cells intraperitoneally. This globule leucocyte was observed at the lumen of the cecum adjacent to the absorptive epithelial cells and was undergoing exocytosis of accumlated crystalline material into the intercellular spaces (IC) and into the lumen. Magnification 48,000X.

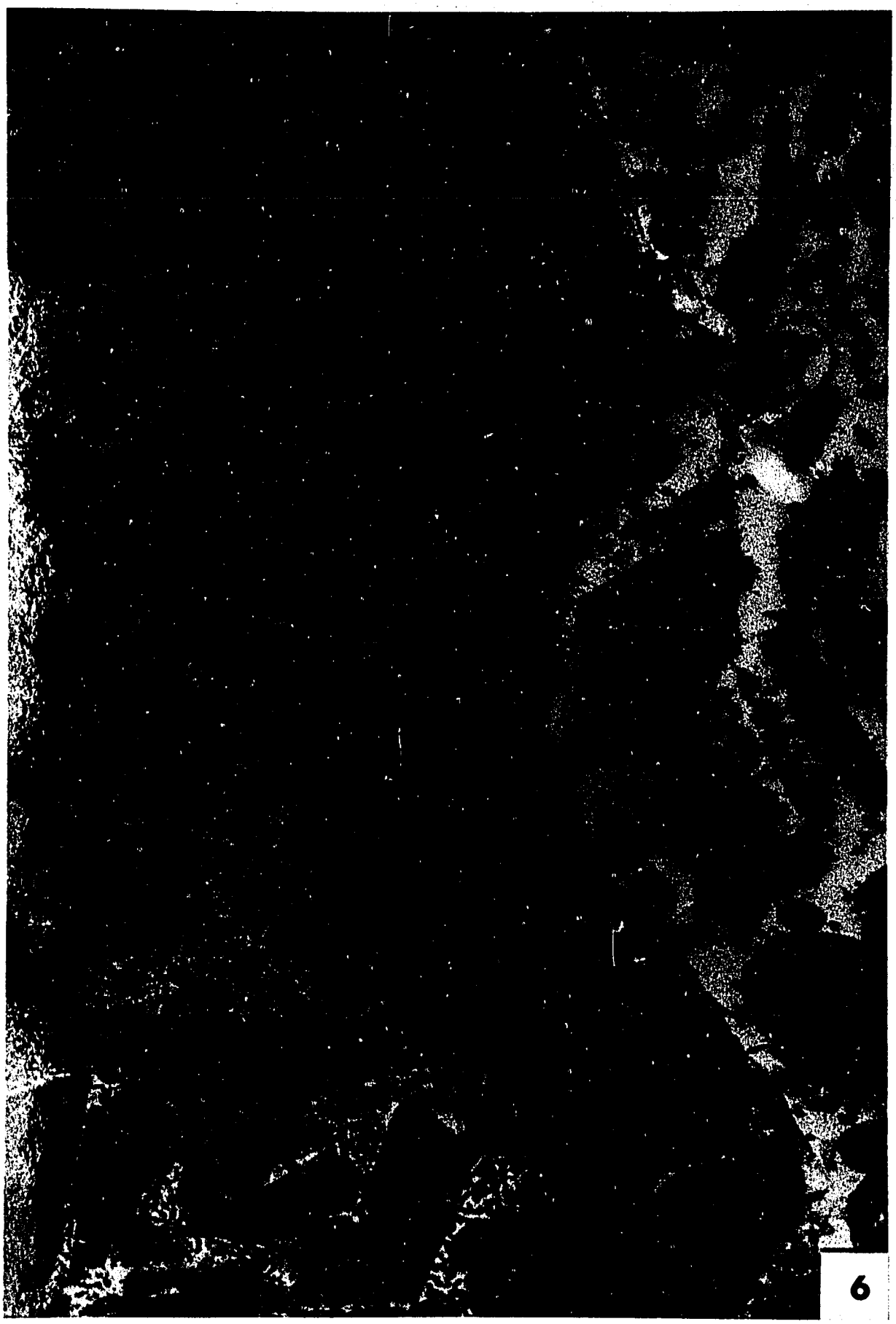
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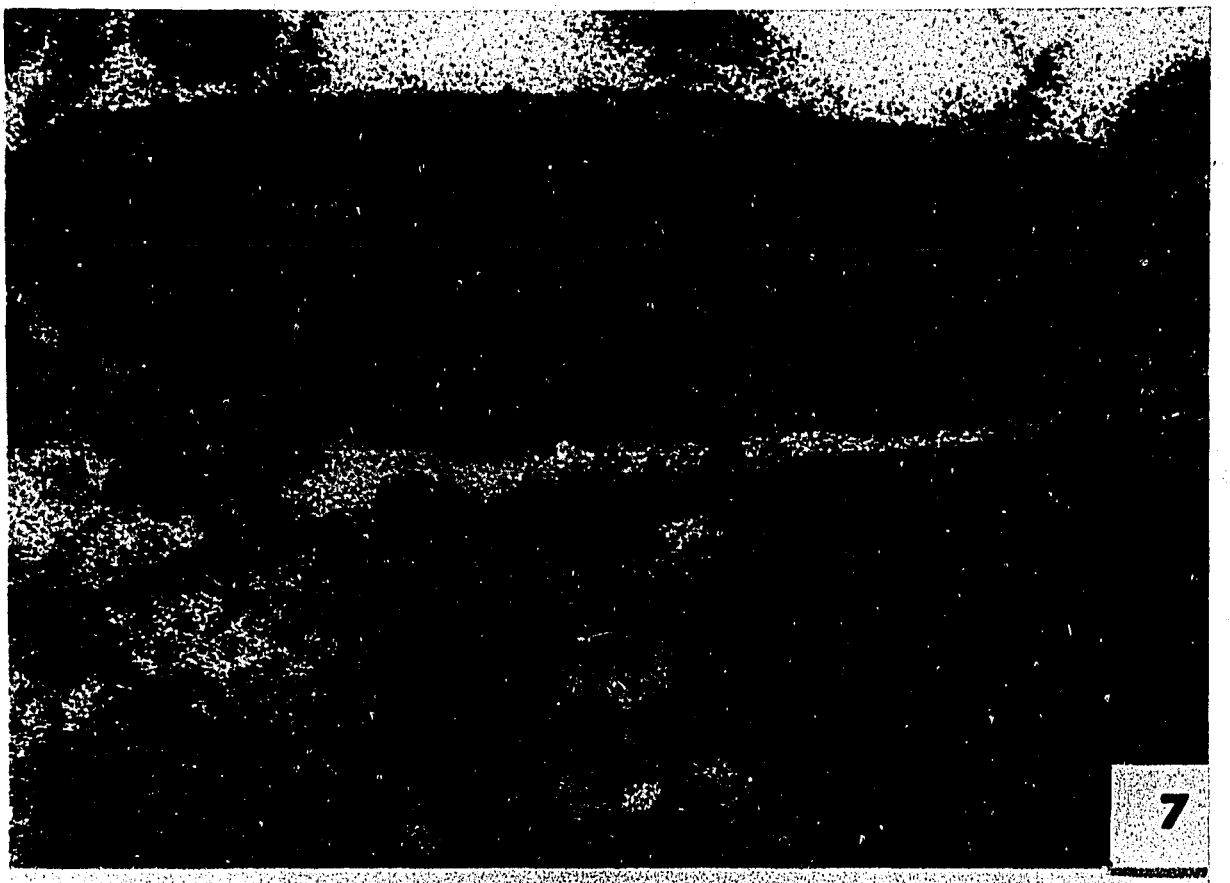
Figure 7. An eosinophil leucocyte granule from a normal Swiss strain mouse showing the characteristic 30A periodicity within the crystalline core of the granule, the surrounding amorphous matrix and the limiting unit membrane. The length of the long axis of the eosinophil granule is 0.6 micron. Magnification 234,000X.

Figure 8. A globule leucocyte crystal within a granule of a middle stage globule leucocyte in the cecal epithelium of a Swiss strain mouse treated with P815 mast cells in a surrounding dialysis bag. The crystals show varying periodicities depending on the sectioning angle. At the top of Figure 8, the internal repeat is 64A ($\pm$5A) while the bottom crystal has a 62.6A periodicity ($\pm$1.3A). A diagonal periodicity is seen in the bottom crystal. The diagonal periodic repeat is 40.0A ($\pm$2.0A). The top line of the bottom crystal is clearly divided into cubes which are 35.9A ($\pm$1.8A) in diameter. The entire crystal from which these crystals are sectioned was rectangular and 0.4 X 0.7 microns. Magnification 234,000X.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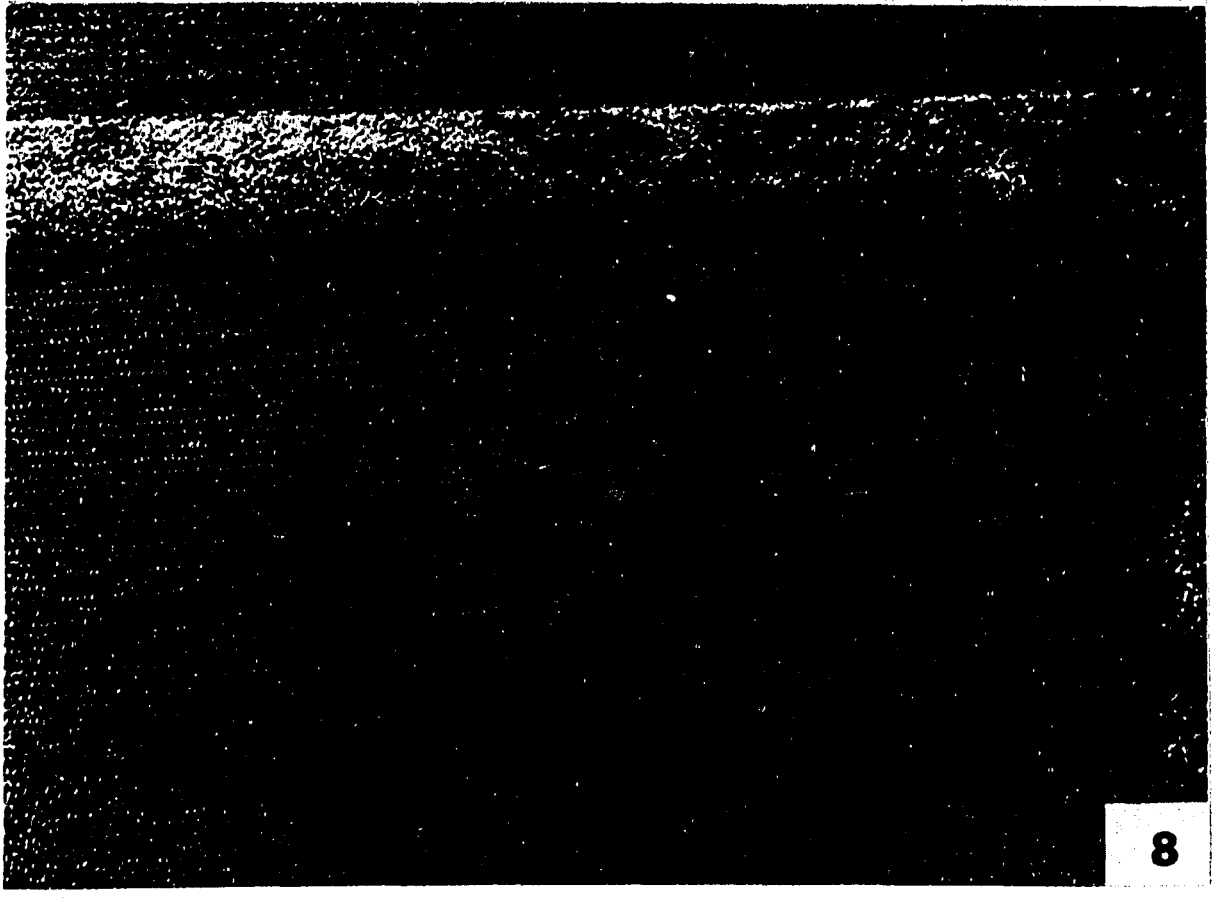
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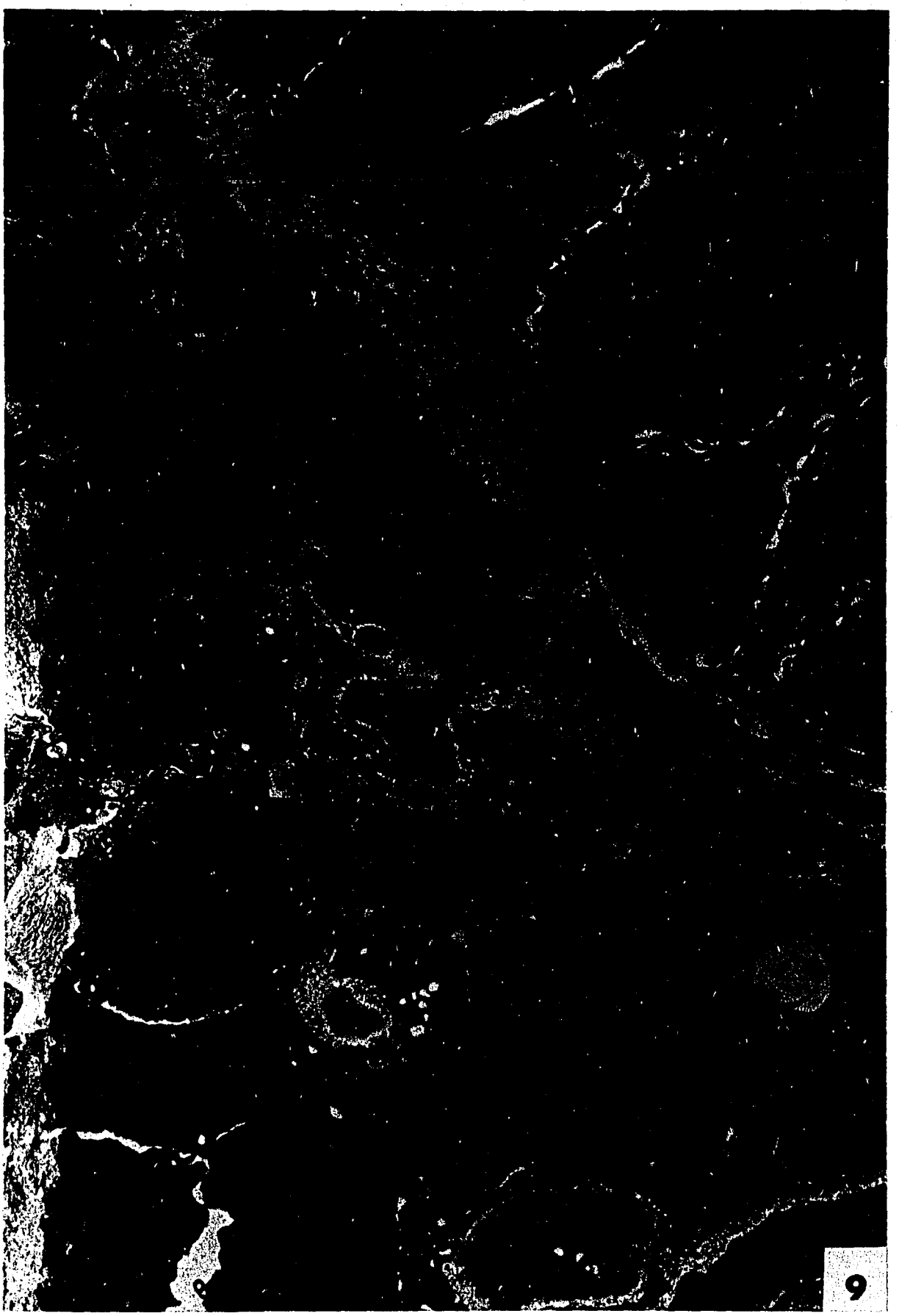
Figure 9. Section through a normal Swiss strain cecal epithelium which shows signs of infection since the lamina propria is distended with many plasma cells (PC) some of which contain Russell bodies (RB), eosinophil leucocytes (EL) and other cell types. The epithelium shows the presence of a mucous cell (MuC) and undifferentiated cells. Magnification 7,200X.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Development of crystals in Swiss strain Globule Leucocytes.

Figure 10. Section of a mast cell-like cell at the basal aspect of the epithelium in the lamina propria below the basement membrane of the epithelium. Characteristic mast cell like granules are observed but containing uncharacteristic lamellar inclusions with a 206.0 (±4.0A) periodicity. The smooth endoplasmic reticulum contains vesicles with electron dense material similar to that of the granules. Magnification 38,000X.

Figure 11. Section of another cell similar to a mast cell in the lamina propria below the basement membrane of the epithelium. The intragranular lamellae have a 173.6A (±7.1A) repeat. Magnification 38,000X.

Figure 12. A globule leucocyte in the cecal epithelium of a normal Swiss strain mouse containing globules with some dense bodies and a larger granule containing lighter areas with blocked out crystalline inclusions. Magnification 38,000X.

Figure 13. A globule leucocyte in the cecal epithelium of a Swiss strain mouse containing crystals clearly blocked out. Magnification 38,000X.

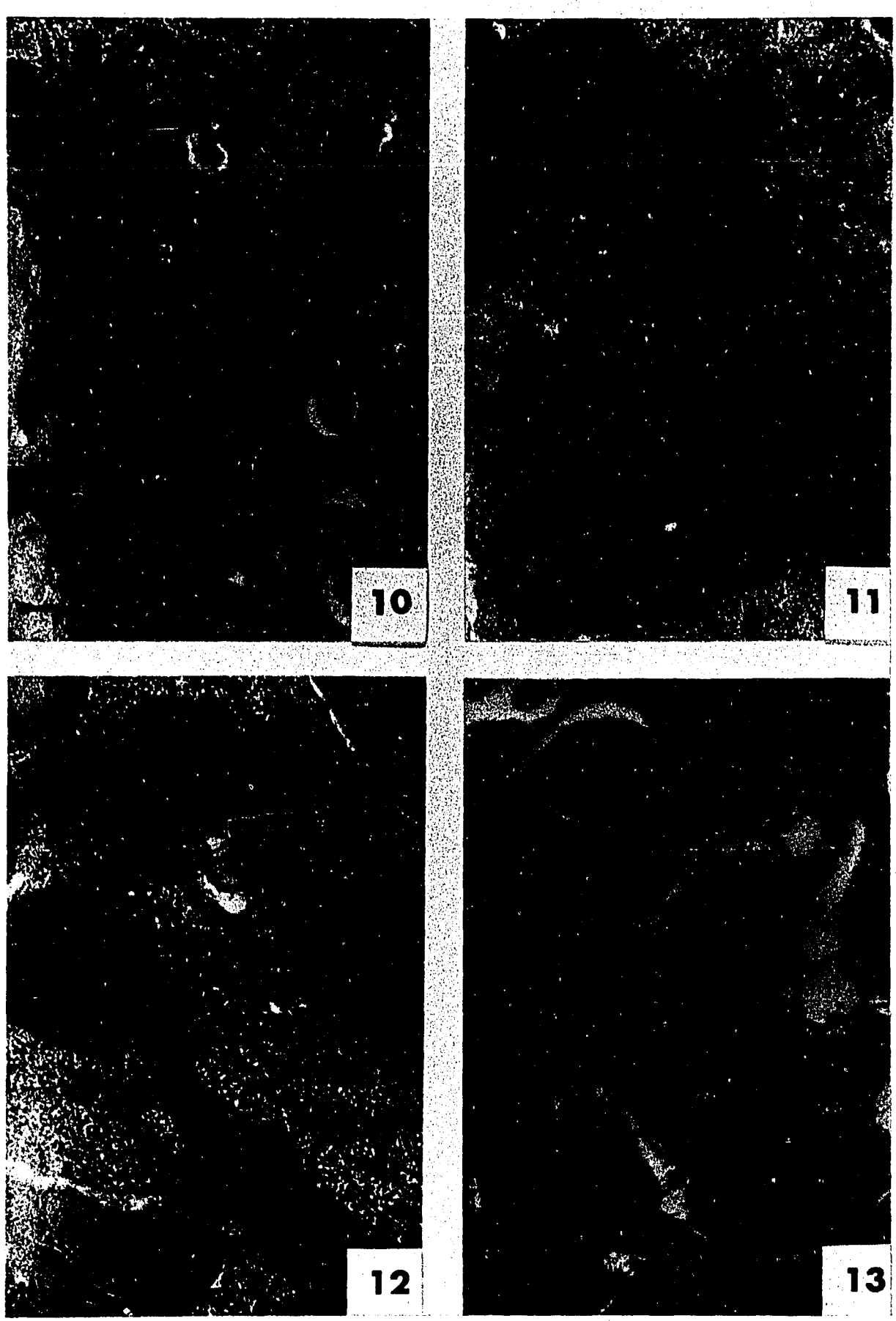
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Figure 14. Normal Swiss strain connective tissue mast cell granules showing lamellar inclusions. The line to line lamellar repeat is 211.0A (±29.4A). Magnification 93,000X.

Figure 15. A mast cell-like cell found at the basal aspect of the epithelium in the lamina propria containing developing granules with lamellar inclusions. The major periodic repeat is 181.2 (± 5.5A), but each line contains three smaller lines with a 51.0A (± 5.0) repeat. Magnification 105,000X.

Figure 16. Lamellar scroll inclusion and crystals with a 43.4A (± 2.1A) periodicity (at the arrow) within the granules of a late stage of the invasive globule leucocyte in the cecal epithelium of a Swiss strain mouse. The line to line periodicity of the scroll is 113.8A (±8.2A). Magnification 90,000X.

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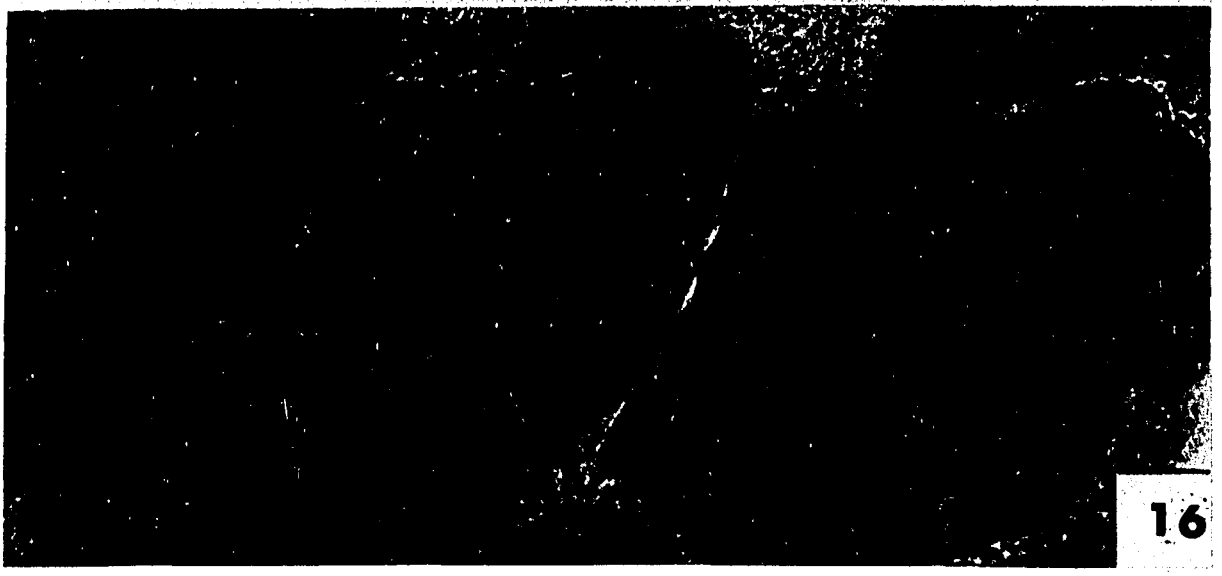

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Figure 17. A mast cell-like intermediate cell below the basement membrane of the epithelium in the lamina propria (LP) containing expanded rough endoplasmic reticulum cisternae filled with electron dense material. There are two areas of apparent rough endoplasmic reticulum cisternae coalescence forming mast cell-like granules (See stars). Magnification 36,000X.

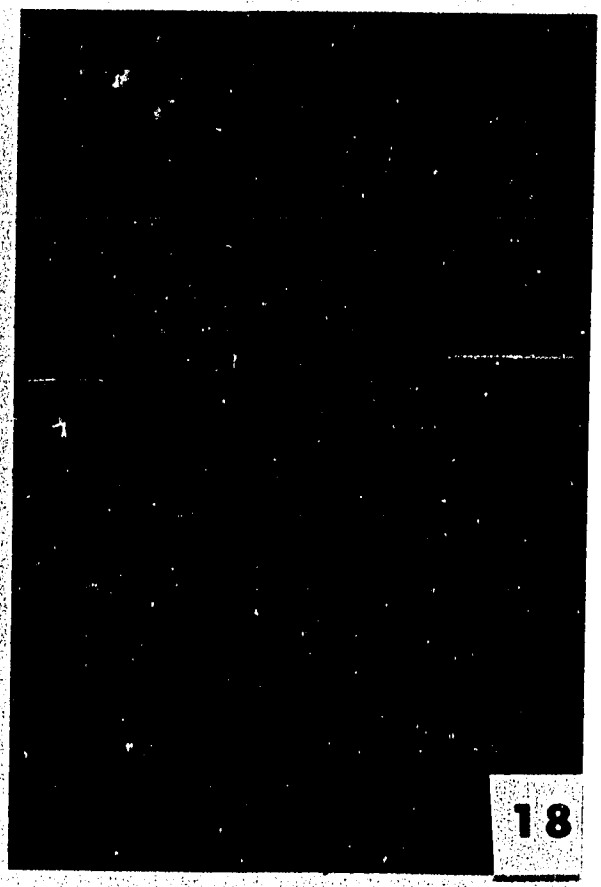
Figure 18. A globule leucocyte (GL) contained within the cecal epithelium next to the basal portion of a mucous cell (MuC) with an elaborate rough endoplasmic reticulum and an undifferentiated cell (UC). Magnification 36,000X.

Figure 19. A globule leucocyte in the middle stage of epithelial development containing lamellar striations within the globules. Magnification 48,000X.

Figure 20A. A scroll like figure in a late stage globule leucocyte found within a globule. Periodicity of the scroll is 119.4A ($\pm$7.6A). Magnification 120,000X.

Figure 20B. Crystalline development within another granule of the same cell as in FIgure 20A showing a 58.4A ($\pm$ 0.6A) periodicity. Magnification 120,000X.

17



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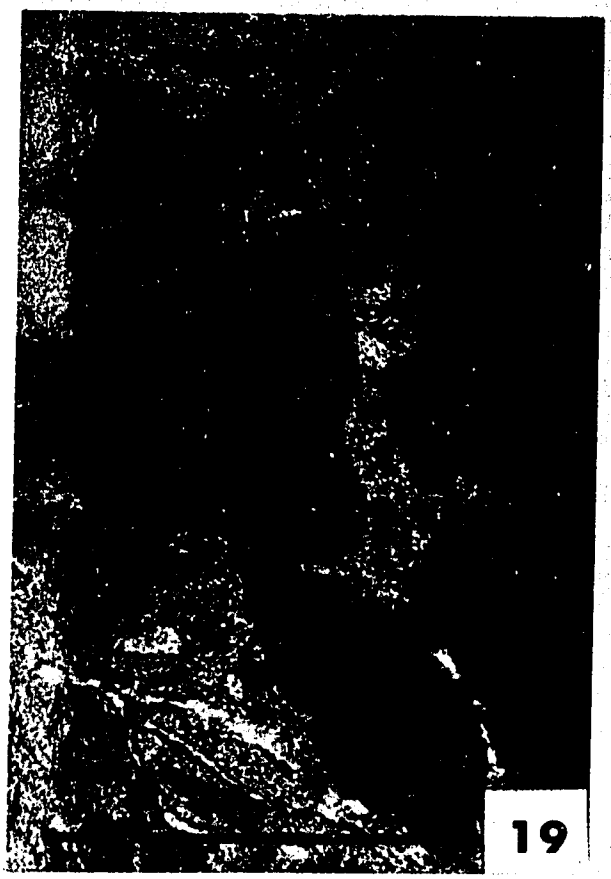

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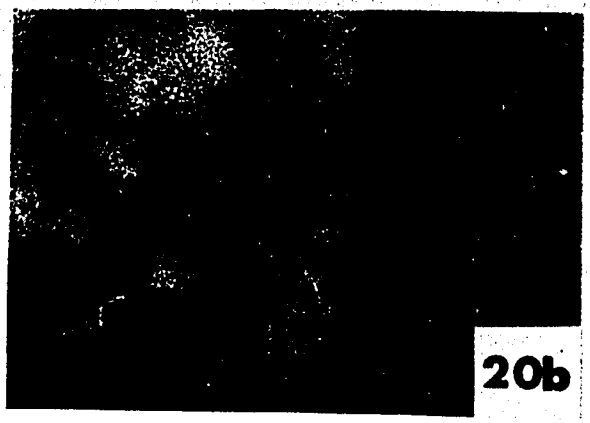
20a



20b

FIgure 21. Inclusions in the globule leucocyte of cecal epithelium in normal AKD mouse. The globule is surrounded by a unit membrane and contains crystals with a 59.8A (±0.5A) period develpping within an amorphous matrix. Another granule contains scroll-like figures near the limiting membrane. Magnification 121,400X.

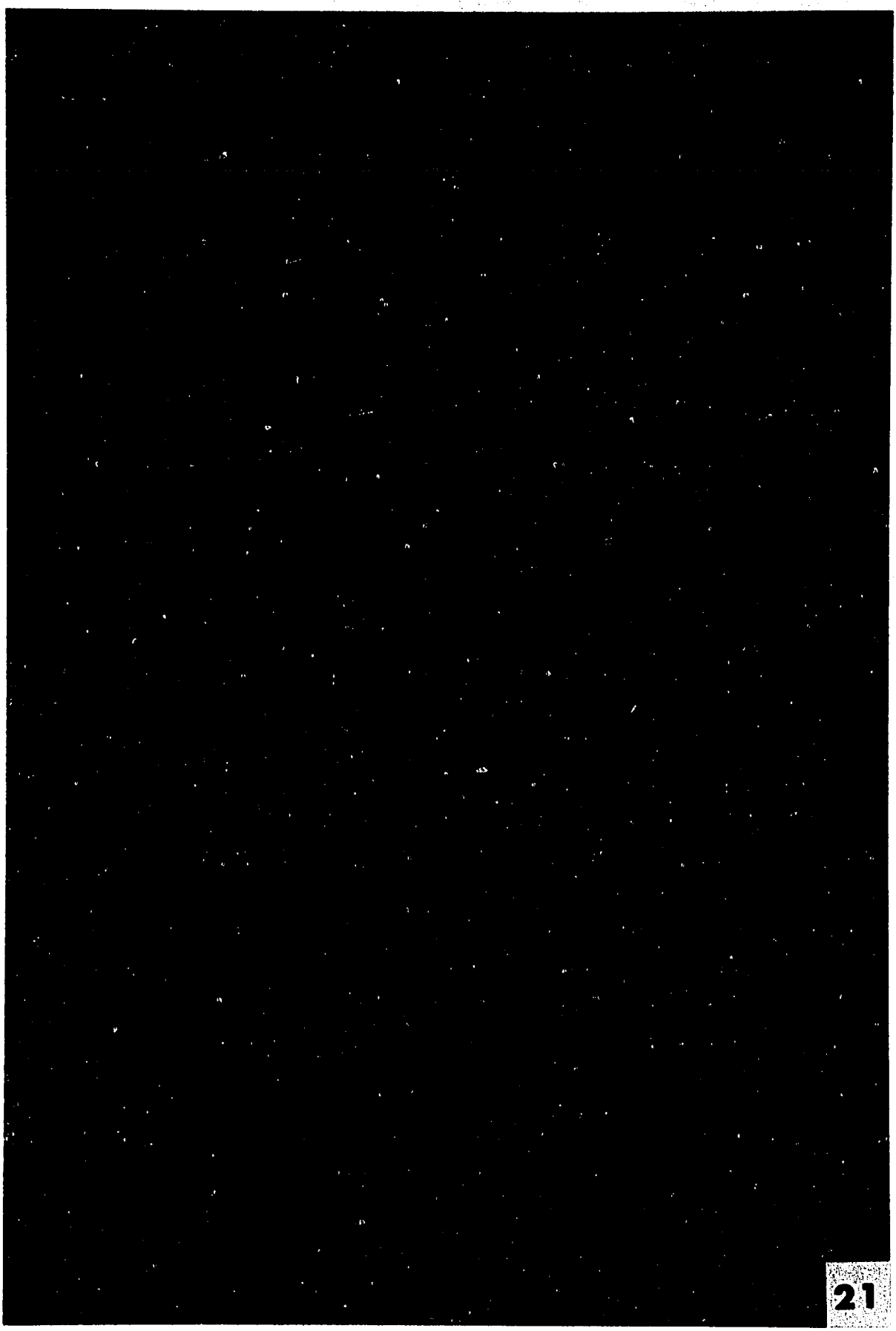

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Figure 22. A mast cell-globule leucocyte intermediate (IC) below the basement membrane (BM) of the crypt of Lieberkühn in the lamina propria of a normal Swiss strain mouse cecum. Note the capillary containing a red blood cell (RBC). Magnification 12,750X.

Figure 23. A globule leucocyte (GL) recently incorporated into the basal portion of a normal cecal Swiss strain epithelium near a mucous cell (MuC) subtended in the lmaina propria by a cell which morphologically could have the potential for becoming a mast cell-globule leucocyte intermediate (IC). Magnification 9,000X.

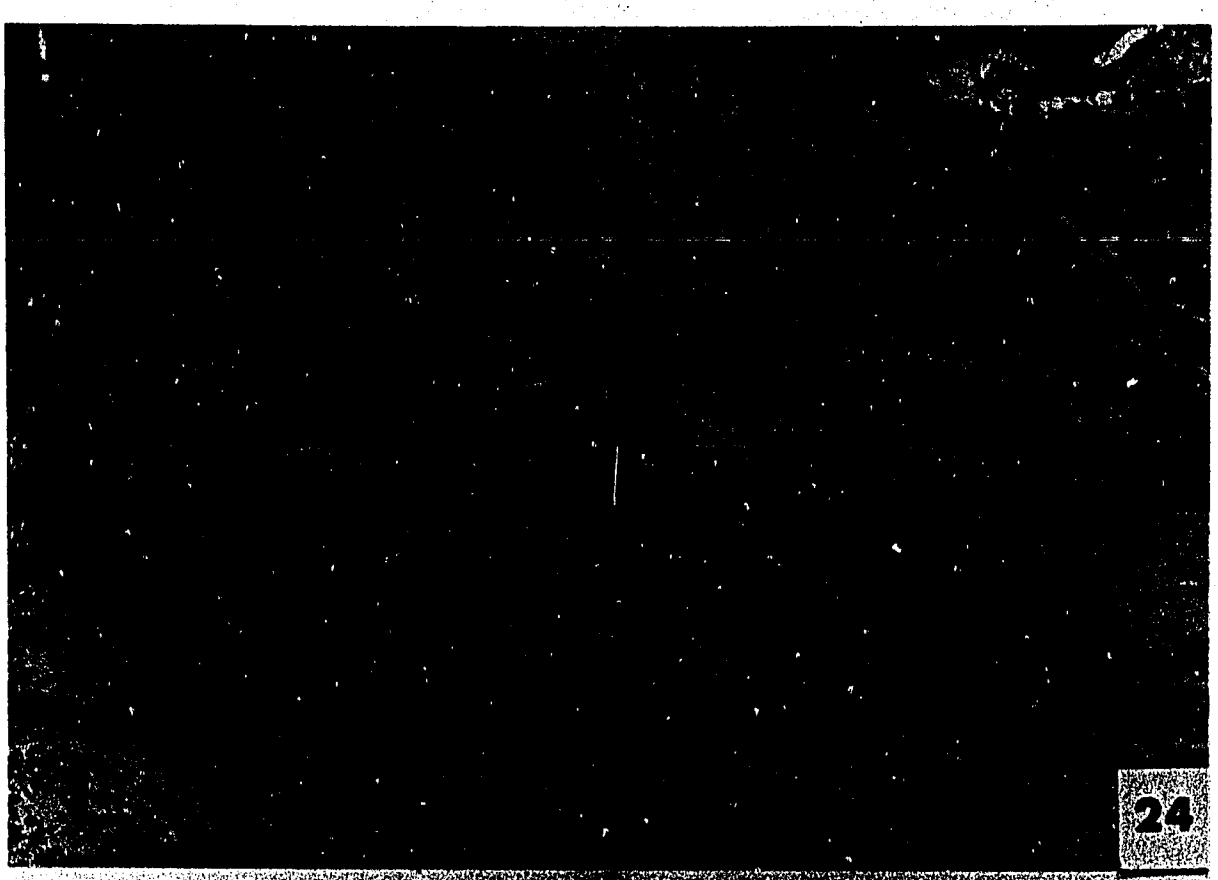
22

23

Figure 24. An intermediate cell containing crystallin inclusions in a large vacuole induced by actinomycin D treatment (high dosage). The cell was near the muscularis mucosae characterized by smooth muscle cells (SM). Magnification 7,000X.

Figure 25. A mast cell in the submucosa of a Swiss strain mouse cecum surrounded by a dialysis bag containing P815 mast cells in vivo for 24 hours. Magnification 10,200X.

Figure 26. Mast cell in the submucosa of an AKD mouse treated by injecting 1.2 X 10^6 mast cells/ml intraperitoneally for 39 hours. Note the presence of a nerve (Ne) in Meissner's plexus and the smooth muscle cells (SM). Magnification 10,200X.



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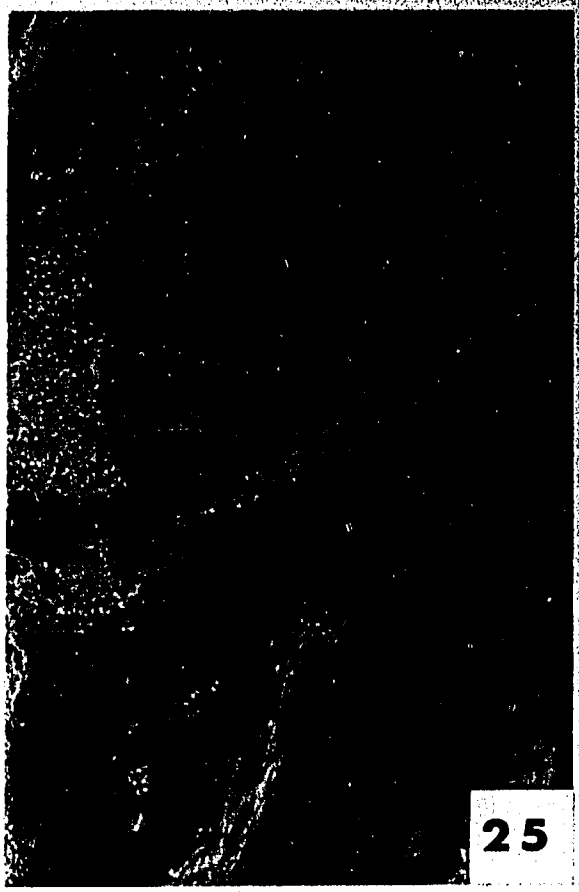

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Figure 27. An intermediate globule leucocyte with nucleus (N) found in the lamina propria of a Swiss strain mouse injected with 30 ug actinomycin D over 48 hours. A variety of granules (CG) and crystals is observed. Magnification 19,000X.

a. Magnification 95,200X.

b. Magnification 54,500X.

c. Magnification 95,200X.

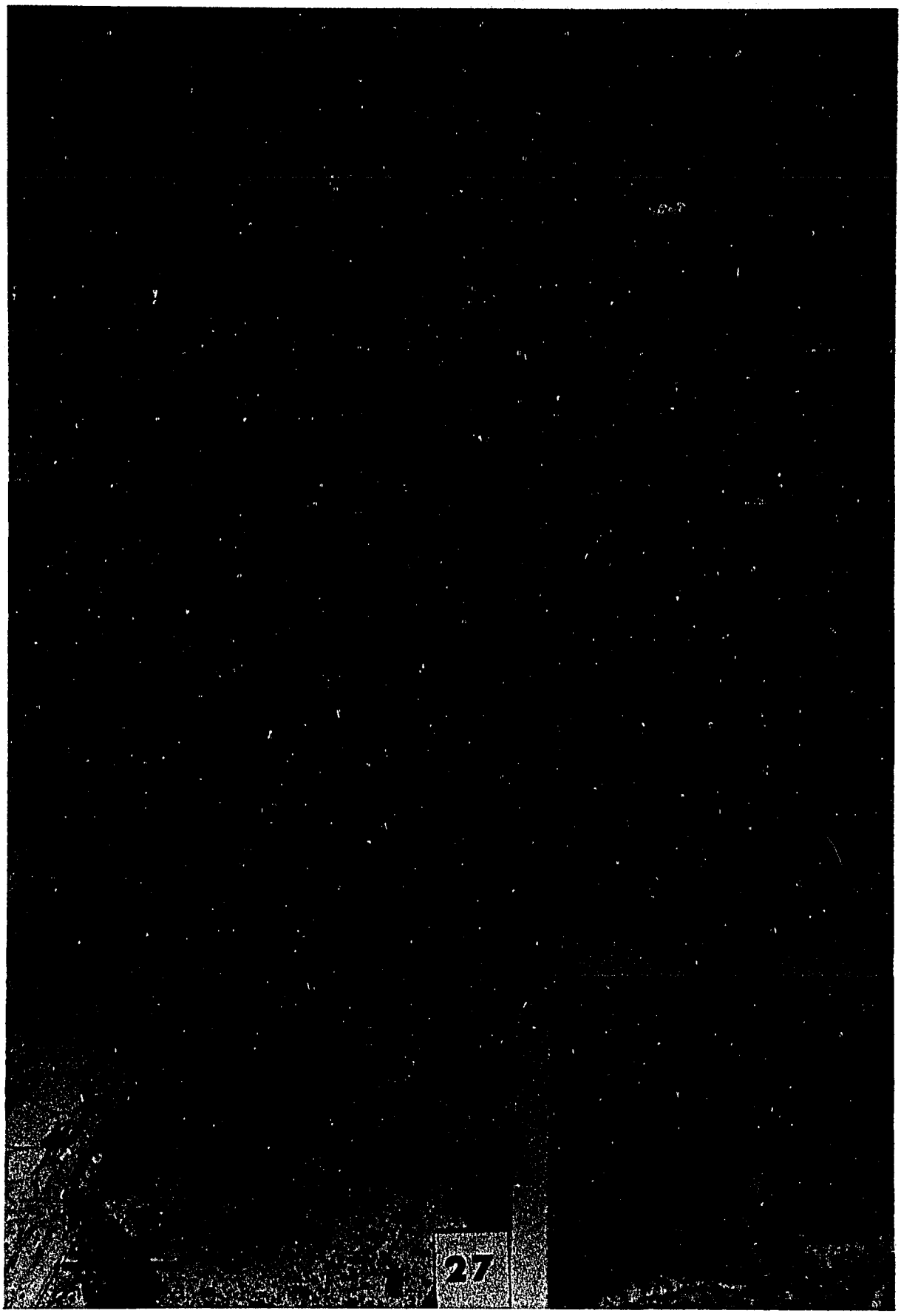
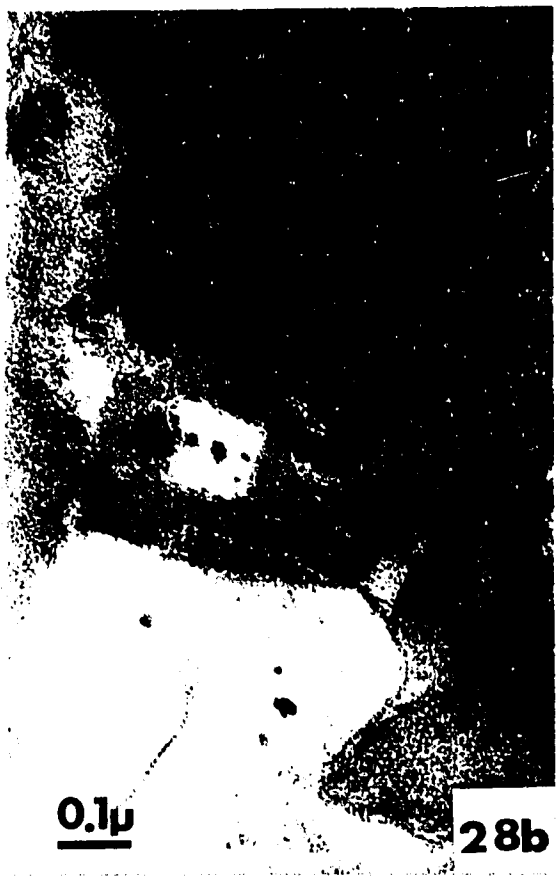

27

Figure 28a. The fate of globule leucocyte crystals after exocytosis in this 63 ug actinomycin D treated Swiss strain mouse seems to be incorporated into a secondary lysosome of an epithelial absortive cell at the border of the cecal lumen. At the upper left of the micrograph microvilli are present on the border of the cell. Enlargements of lysosome crystals (b) and (c) are shown in Figures 28b and 28c. Magnification 32,000X.

Figure 28b. Portion of the globule leucocyte crystal in the secondary lysosome of Figure 28a. Magnification 92,000X.

Figure 28c. Portion of the globule leucocyte crystal in the secondary lysosome of Figure 28a. Magnification 92,000X.

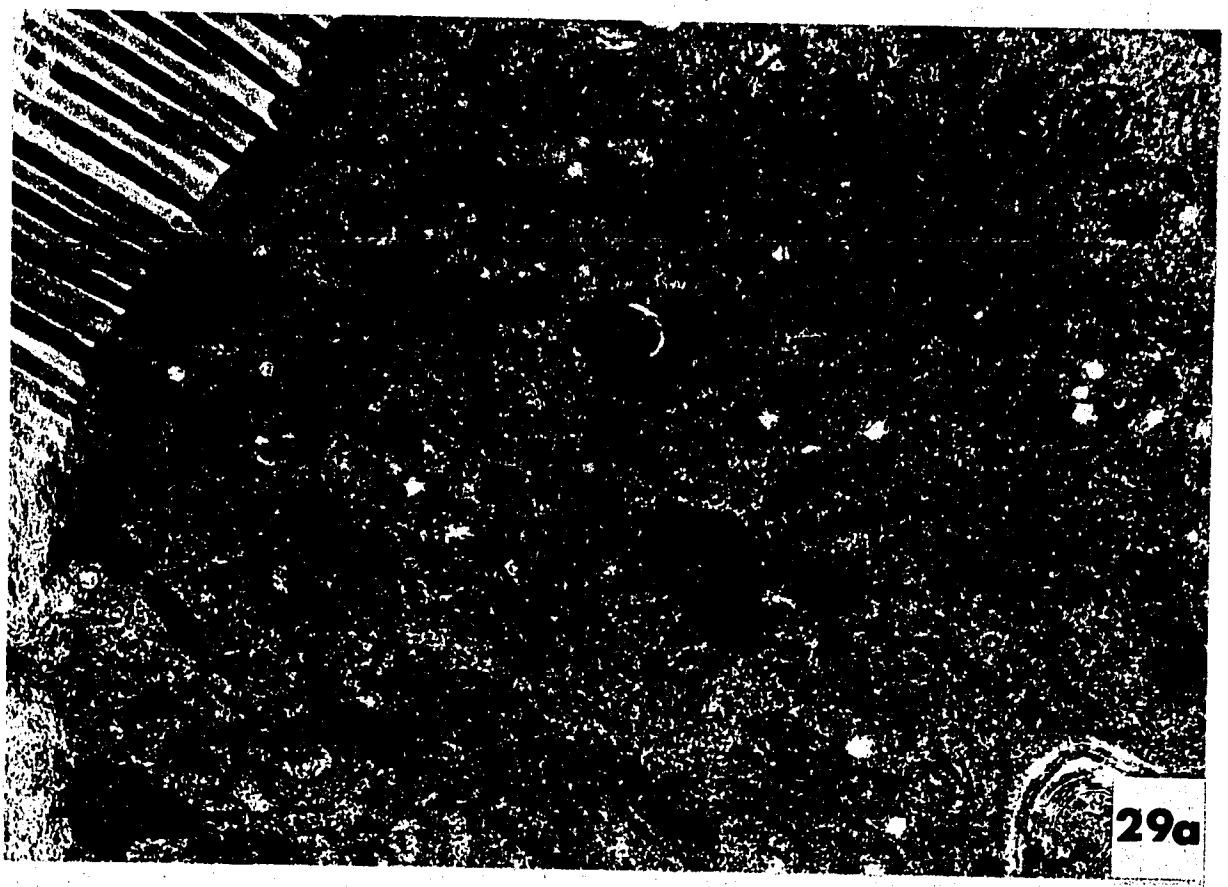

28a


0.1μ
28b

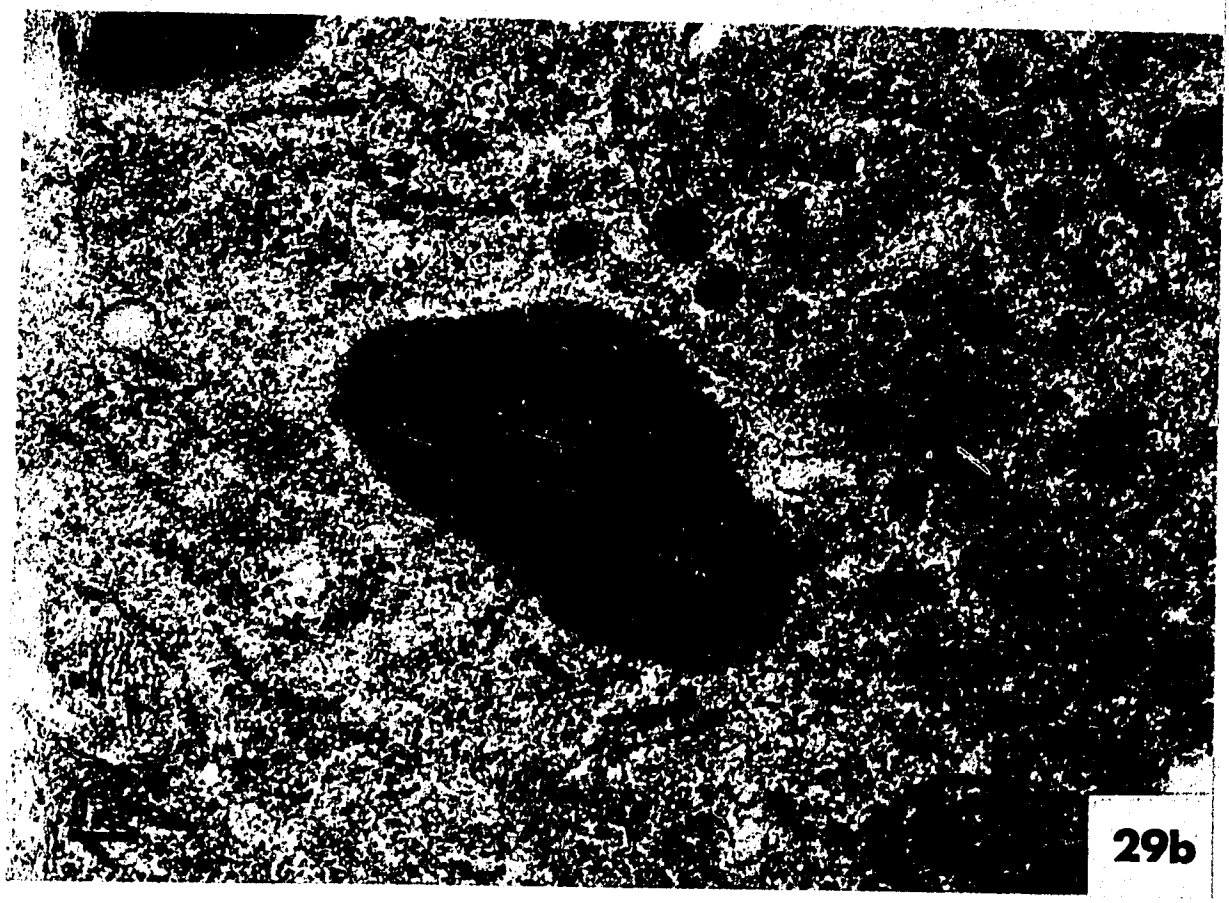

0.1 μ
28c

Figure 29a. An absorptive epithelial cell with microvilli (MV) from a normal Swiss strain mouse cecum and a lysosome (L) which has a crystalline inclusion not related to the globule leuoccyte crystal. Magnification 24,000X.

Figure 29b. Higher magnification of lysosome in 29a. Magnification 72,000X.



29a

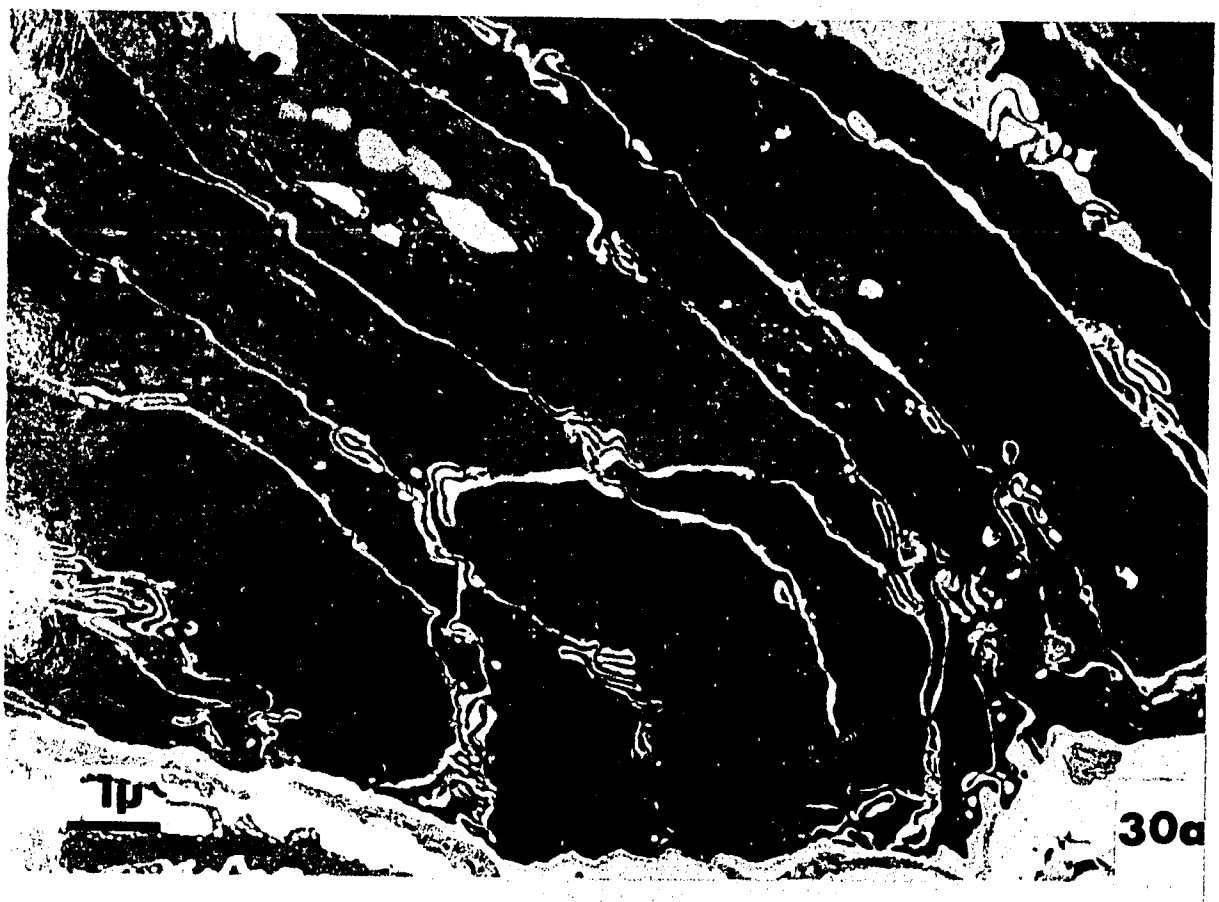


29b

Figure 30a. Cecal epithelium of a normal Swiss strain mouse which had a dialysis bag surrounding the cecum containing P815 mast cells for 8.5 hours in vivo. A mucous cell (MuC) is present at an early stage of mucous granule development. Magnification 11,700X.

Figure 30b. Higher magnification of mucous granules (starred) in Figure 30a showing lamellar concentric inclusions with a 135.9A (±6.5A) periodicity. Magnification 90,000X.

Figure 31. Another Swiss strain mouse stressed by placing P815 mast cells in a dialysis bag around the cecum for 48 hours. Mucous cell granules show concentric whorls with a line to line center periodicity of 141.3 A (± 11.6A). Magnification 90,000X.


1μ
30a


0.1μ
30b


31

Figure 32. Globule leucocyte crystals (Cr) and globules (cg) mixed with concentric whorls in a cell close to the lumen of the cecum. A mitochondrion (M) is present. Magnification 54,400X.

Inset: A crystal (Cr) shown in rectangle of Figure 32 with a 56.7A (±1.8A) periodicity mixed with concentric whorls of mucous cells. The mucous cell whorls have a 127.2A (±6.6A0 repeat. Magnification 95,200X.

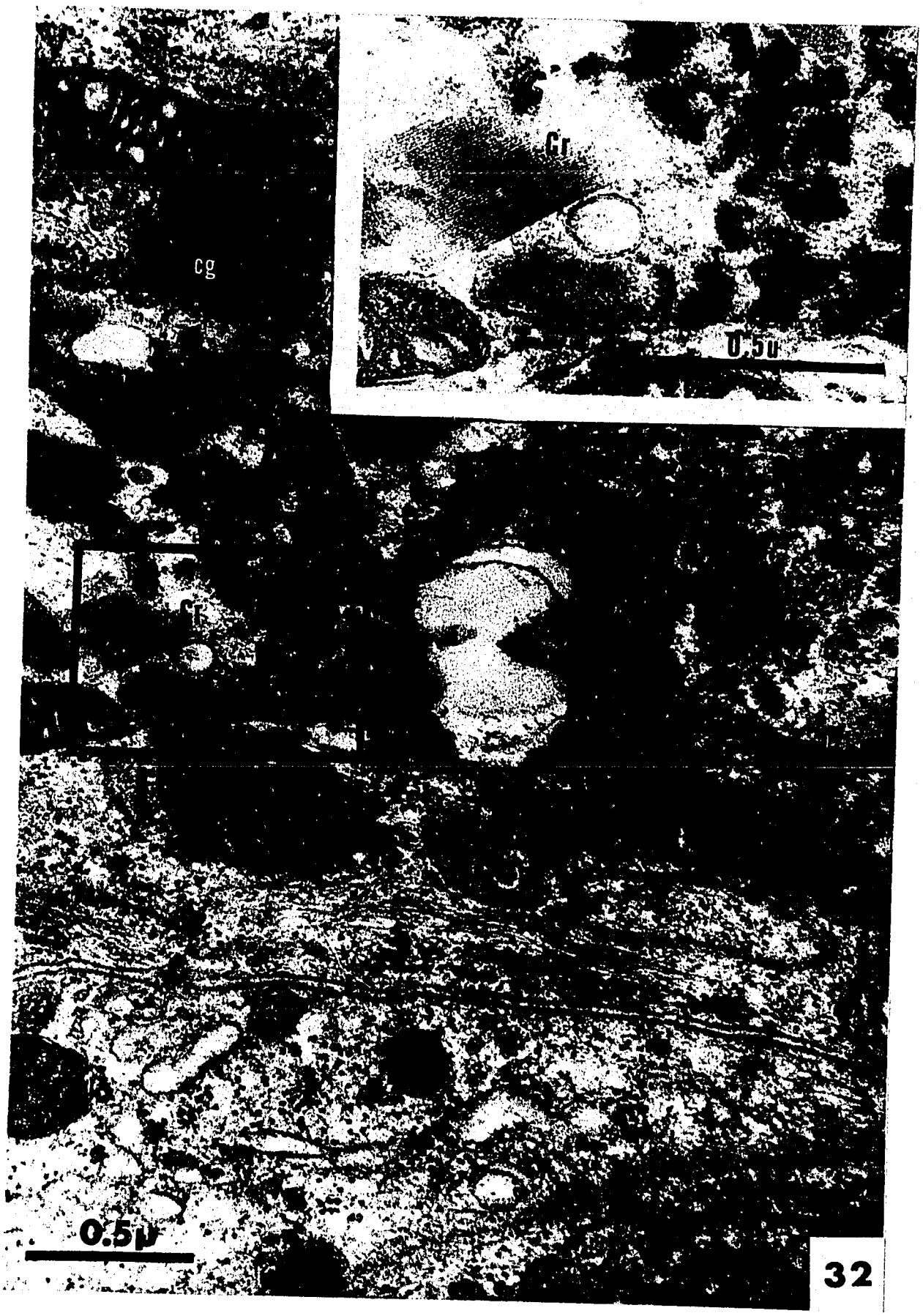

cg
Cr
0.5μ
Cr
0.5μ
32

Figure 33. Cecal epithelium of a late stage globule leucocyte (GL) undergoing exocytosis of crystals at the lumen of the crypt of Lieberkühn in an AKD mouse injected with purified P815 mast cell virus intraperitoneally. Large collections of degranulating mucous granules are observed (Mu) and neighboring mucous cells (MuC) show incorporated crystals (see arrows) from the globule leucocyte (GL). Magnification 15,300X.


Mμ
Mμ
1μ
33

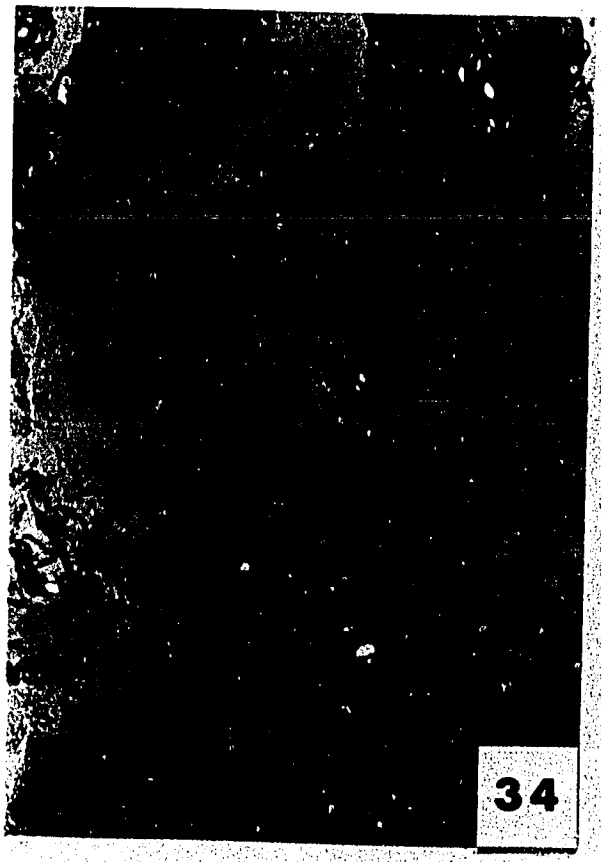
Protein Digest

Figure 34. Control section treated with 10% H_2O_2 for 20 minutes at room temperature. An eosinophil leucocyte (EL) is present with a few portions of the amorphous matrix surrounding the crystalline core unmoved. Collagen (COL) and cells remain intact although there is loss of contrast because of the partial removal of osmium tetroxide from the specimen. Magnification 5400X.

Figure 35. Control section treated with 10% H_2O_2 for 10 minutes at room temperature followed by a four hour incubation in 0.1N HCl at 37°C. The nuclei (N) of cells apparently contain large areas of acid soluble material but most of the granules of a mast cell (MC) appear to be acid resistant (arrows) except for one large granule which is partially removed. The collagen (col) remains intact. Magnification 10,200X.

Figure 36. Control section showing an argentaffin cell and granules incubated 10 minutes in 10% H_2O_2 at room temperature. Magnification 48,000X.

Figure 37. Section of an argentaffin cell and granules incubated 10 minutes in 10% H_2O_2 followed by 4.5 hour incubation in 0.5% pepsin in 0.1N HCl at 37°C. The granules of the argentaffin cell are digested and heterochomatic nuclear staining is reversed. Magnification 37,400X.


34


35


36

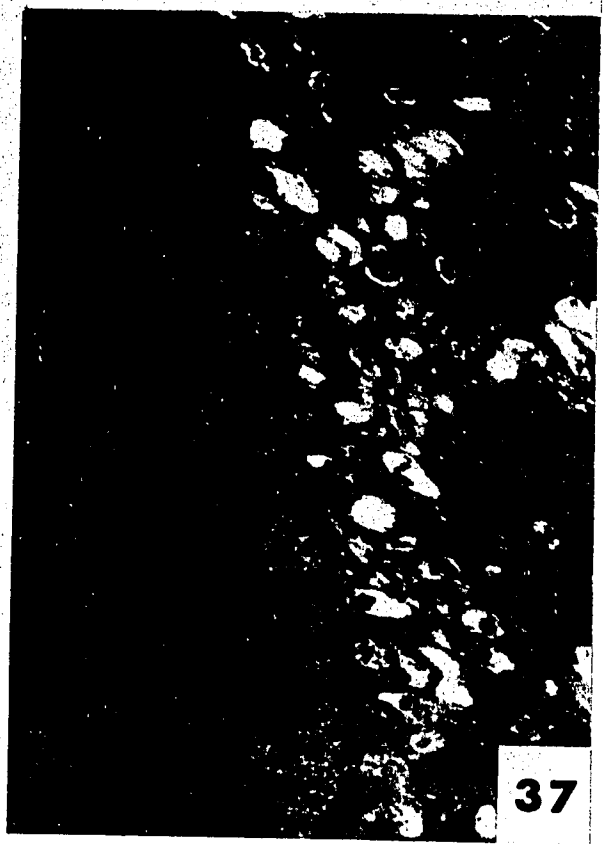
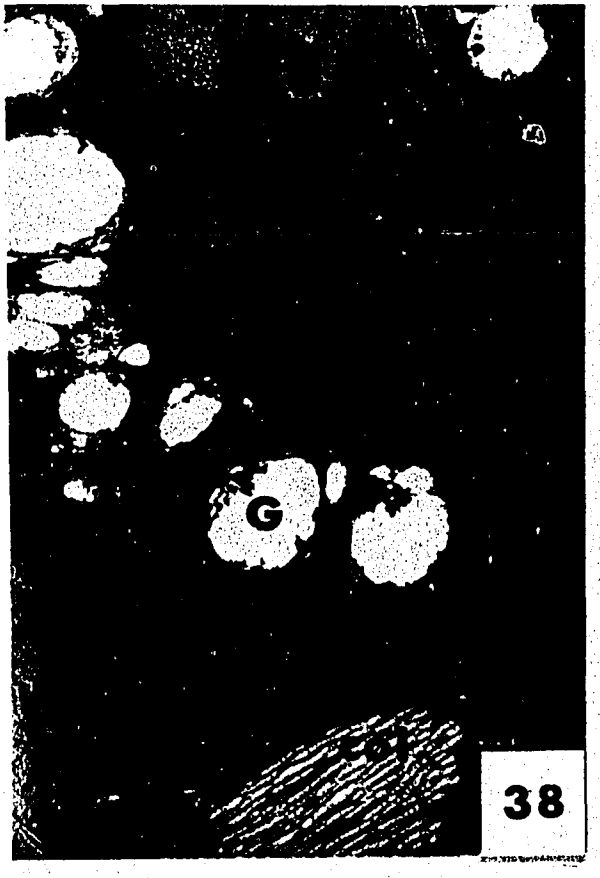

37

Figure 38. A connective tissue mast cell of a Swiss strain mouse treated for 20 minutes with 10% H_2O_2 followed by 4.5 hour 0.5% pepsin in 0.1N HCl at 37°C. Most of the nucleus (N) is indistinct, but the sites of normal mast cell granules (G) are clearly removed as is the collagen (COL). Magnification 12,600X.

Figure 39. An intermediate MC-GL cell in the lumina propria of a Swiss strain mouse treated with 10% H_2O_2 for 10 minutes at room temperature followed by a two hour 0.5% pepsin incubation in 0.1N HCl at 37°C. Characteristic mast cell granules are removed but an irregularly shaped intermediate type granule (IG) is resistant to digestion. Magnification 25,500X.

Figure 40. A globule leucocyte in the ceral epithelium of a Swiss strain mouse after 10 minutes incubation in 10% H_2O_2 at room temperature. Intercellular spaces of Epon appear susceptible to breakdown during the procedure although nearly all ganules can be distinguished as undigested. Magnification 14,700X.

Figure 41. This section is an adjacent section from the same specimen block as Figure 40. This H_2O_2-pepsin treatment leaves the crystals undigested byt smaller mast cell-like granules are digested. Magnification 22,500X.


G
38


IG
39


40


41

Gibbs' Reagent

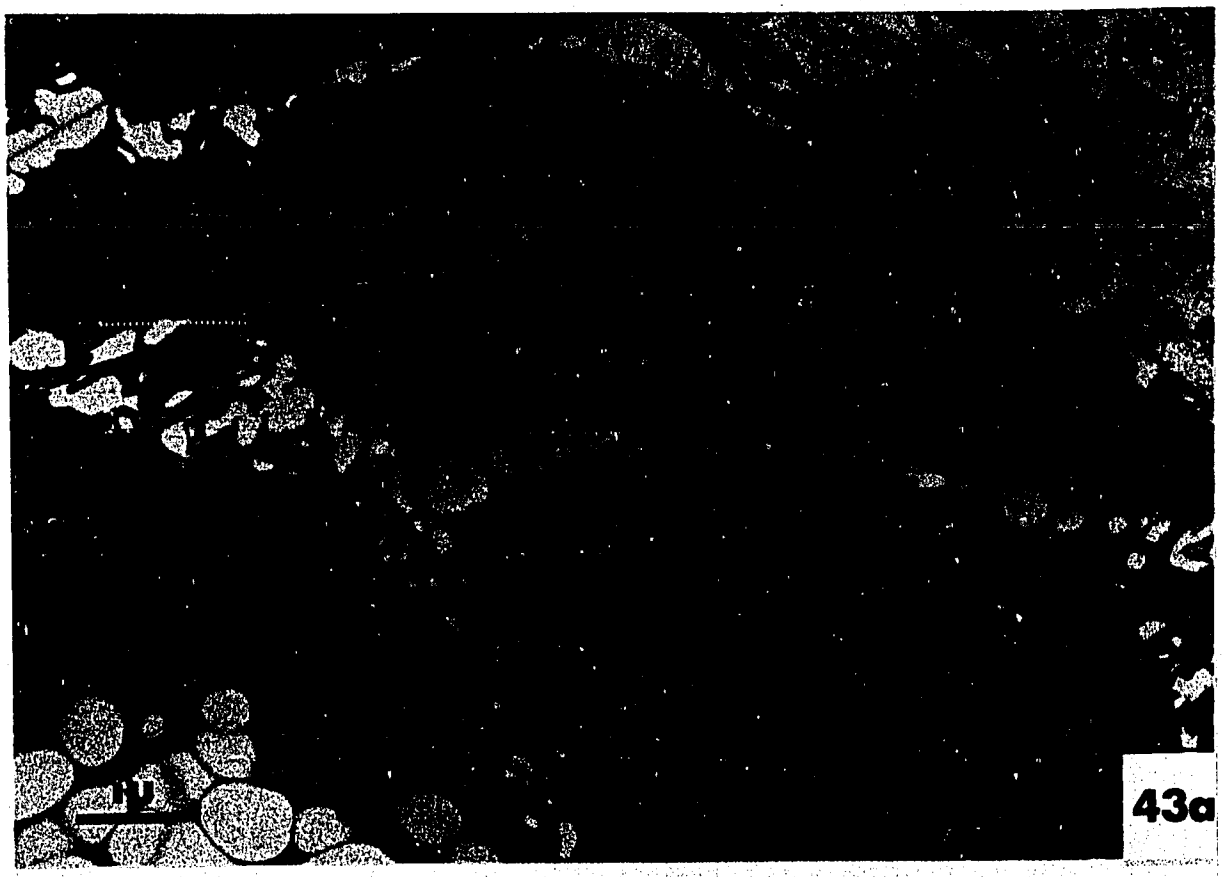
Figure 42. Cross section through a crypt of Lieberkühn with a portion of the lumen of the gut (L) in a Swiss strain mouse cecal epithelium. The electron micrograph is a montage of 10 electron micrographs photographed as overlapping sections at 3600X magnification. The light microscopic inset is a portion of an adjacent one micron section showing the same cells as found in the electron microscopic montage but stained with the argentaffin stain 2,6-dichloroquinonimine. The three red blood cells (rbc) in the bottom of the light micrograph designated a and b correspond to the electron micrograph cells designated A and B. These cells are clearly shown to be invasive globule leucocytes at the basal aspect of two different crypts and not argentaffin cells. EM magnification 3,500X. LM magnification 672X.

42

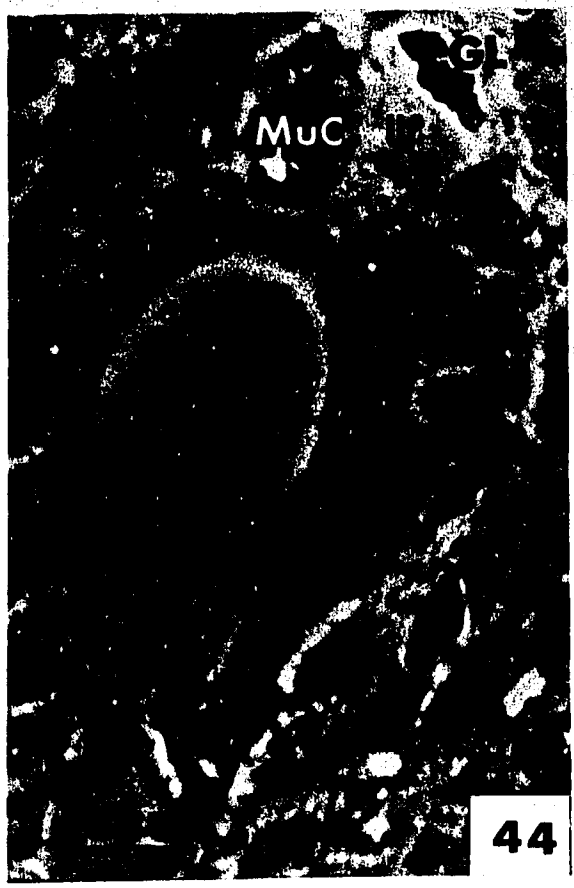
Figure 43A. A globule lecocyte (GL) corresponding to the cell (C) in Figure 42 light micrograph of the adjacent thick section stained with Gibb's reagent. Below the globule lecocyte is a mucous cell (McC) with cohorls within the developing mucous granules and an undifferentiated cell (UC). A newly formed basement membrane (BM) already surrounds the newly invading globule lecocyte. Magnification 12,200X.

Figure 44. Adjacent lu section corresponding to the same cells in Figure 43 showing the globule leucocyte (GL) stained darkly with 2,6-dichloroquinonimine above a mucous cell (MuC) which also shows staining of mucous granules and an undifferentiated cell (UC). Magnification 1500X.

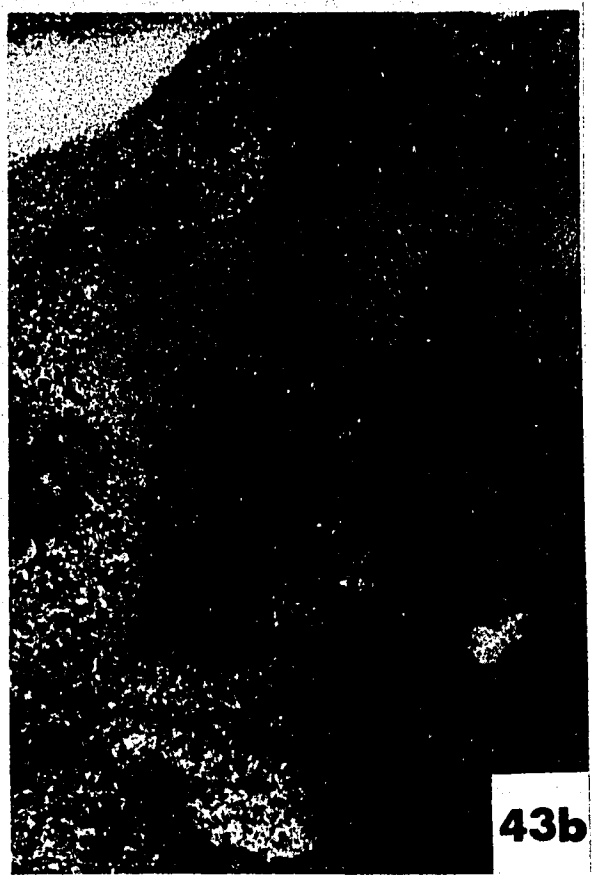
Figure 43B. A portion of a granule is Figure 43 indicated by the rectangle showing the development of a 65.2A ($\pm$3.9A) periodicity (arrow) within the granule. Magnification 75,000X.



43a



44

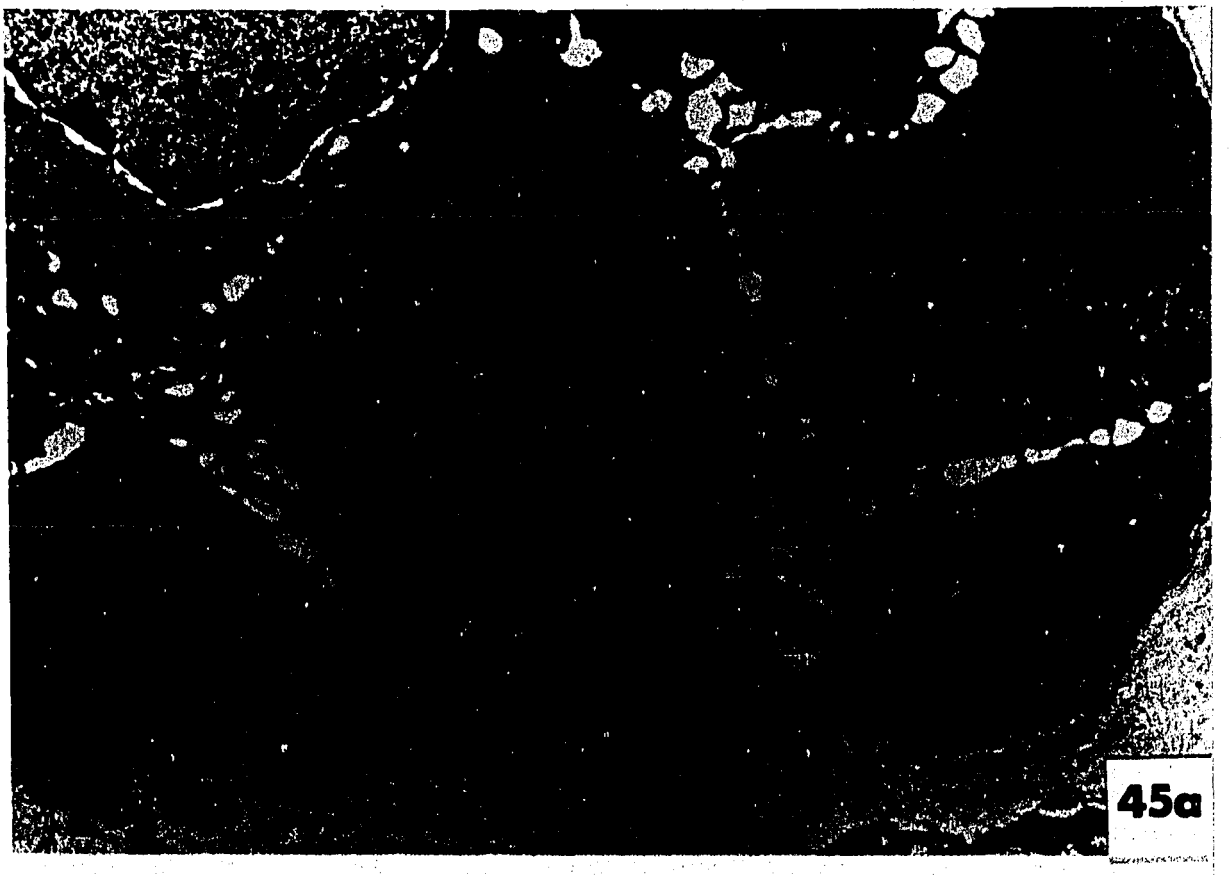


43b

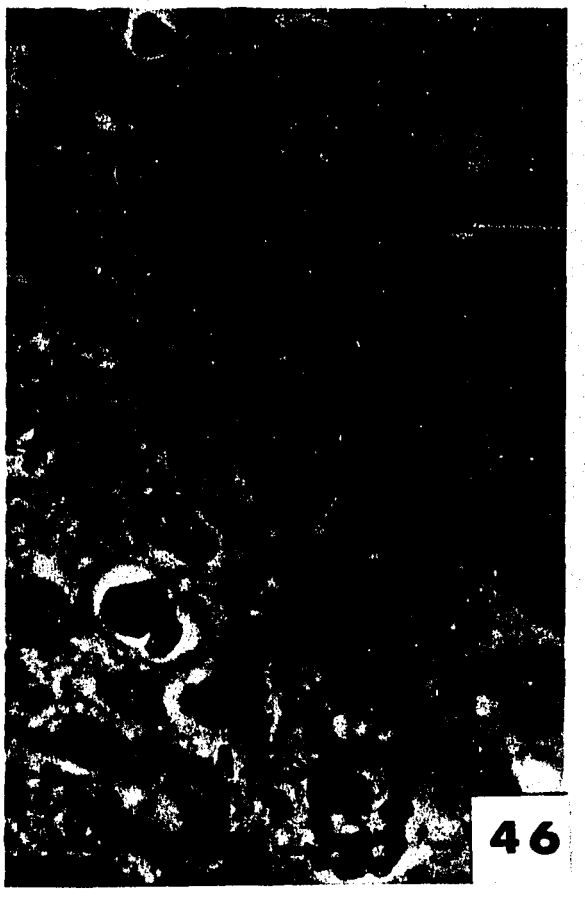
Figure 45A. A portion of the electron microscope composite showing two types of argentaffin cells (AI) and (AII) and a globule lecocyte (GL). Magnification 8,640X.

Figure 46. Light microscope an adjacent lu section stained with 2.6-dichloroquinonimine showing the staining of argentaffin cell type I (AI), argentaffin cell type II (AII) and the globule lecocyte (GL). Magnification 1660X.

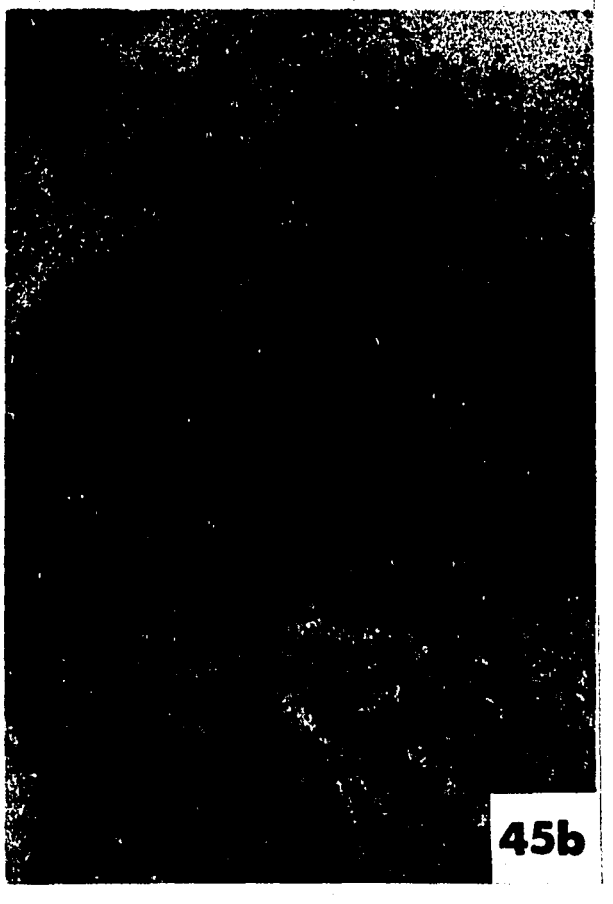
Figure 45B. A portion of the granule indicated the star in the globule lecocyte in Figure 45A showing a 54.7Å (±4.1A) periodicity. Magnification 136,000X.



45a



46

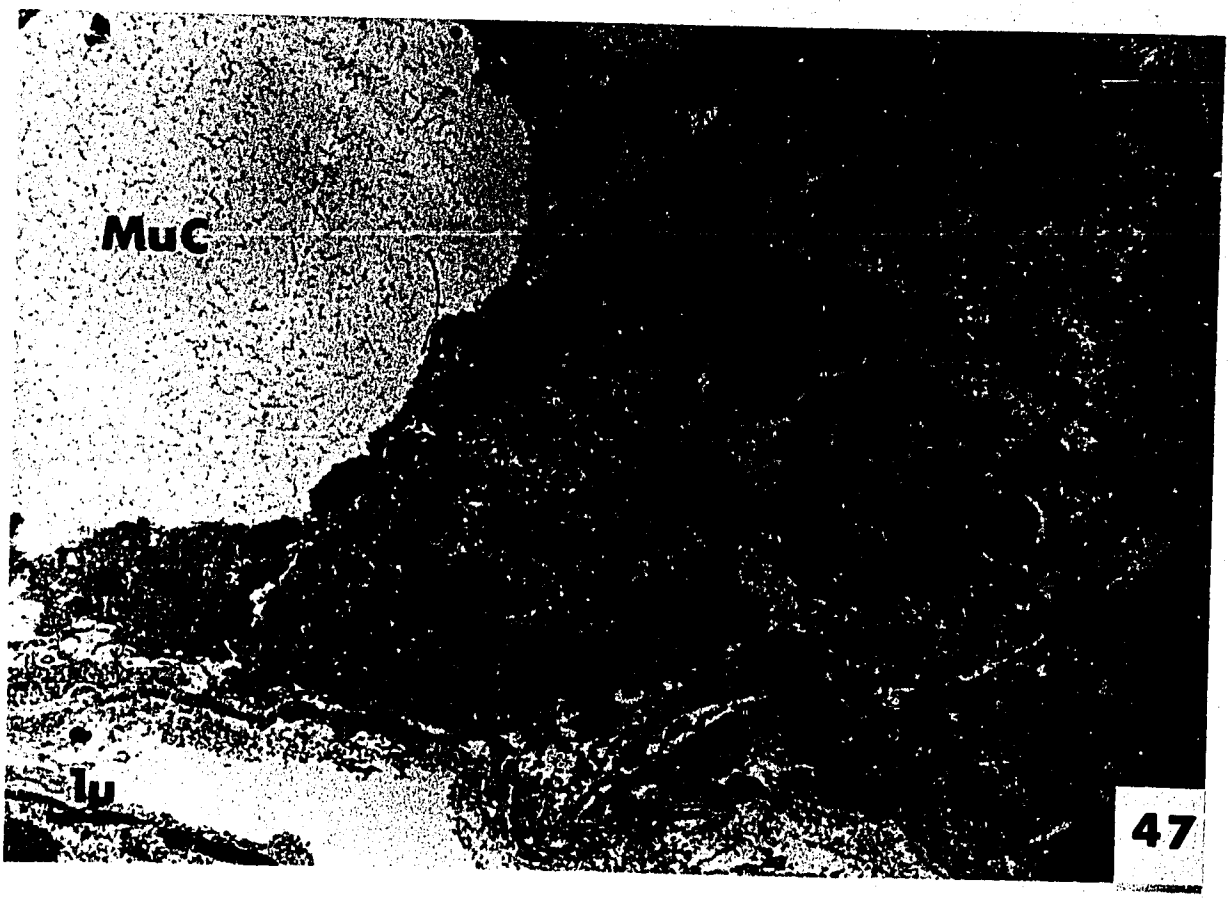


45b

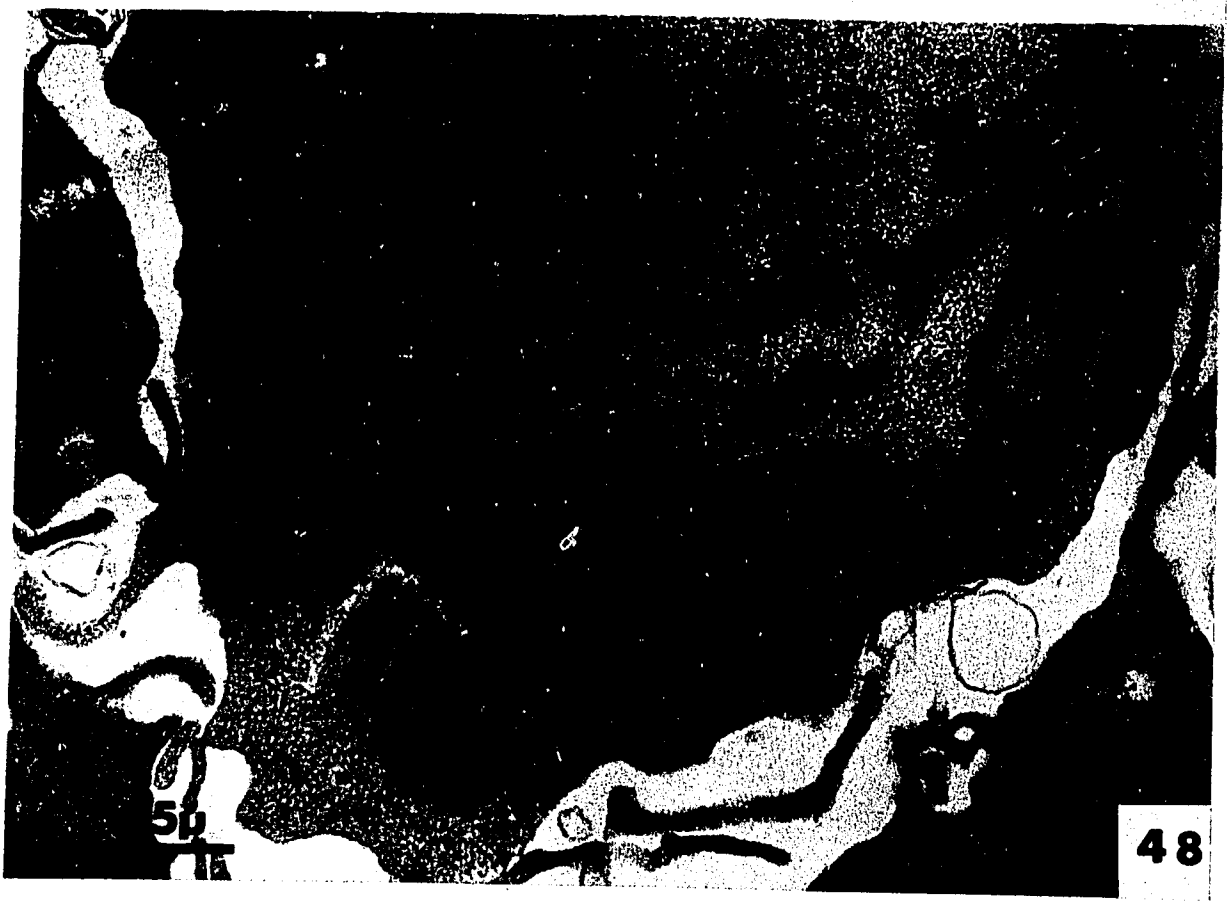
Acid Phosphatase

Figure 47. Control tissue of Swiss strain cecal epithelium incubated for one hour at 37°C. in the acid phosphatase incubation medium minus substrate before fixation and embedment in Epon. No extraneous uptake of lead ions is evident. The mucous cell granules (MuC) have been destroyed during the incubation period but the globule leucocyte (GL) is relatively intact. Uranyl acetate post-stain only. Magnification 10,700X.

Figure 48. A globule leucocyte (GL) in the cecal epithelium of a Swiss strain mouse incubated one hour in the acid phosphatase test solution at 37°C. Acid phosphatase label localized in the nucleus but not in the cytoplasm or within granules of the globule leucocyte (GL). Uranyl acetate post stain only. Magnification 44,500X.



47



48

Figure 49. One hour test incubation of cecum for acid phosphatase subepithelial mast cells in the lamina propria showing acid phosphatase reaction product in the nuclei and in the cytoplasm but not in the granules. Uranyl acetate post-stain only. Magnification 30,200X.

Figure 50. One hour test incubation for acid phosphatase of cecum showing uptake of acid phosphatase reaction product in some granules of an eosinophil leucocyte (EL) in the lamina propria. Uranyl acetate post-stain only. Magnification 16,900X.


49

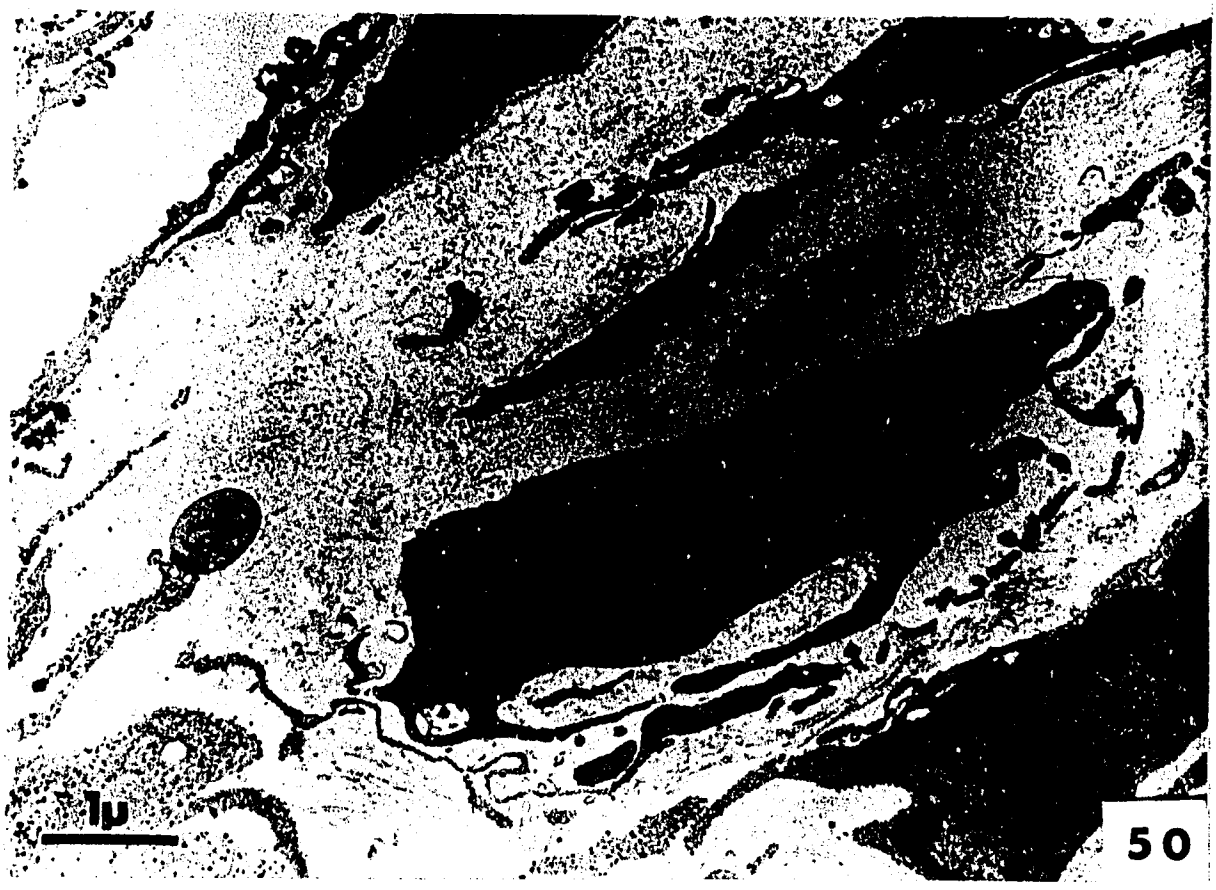
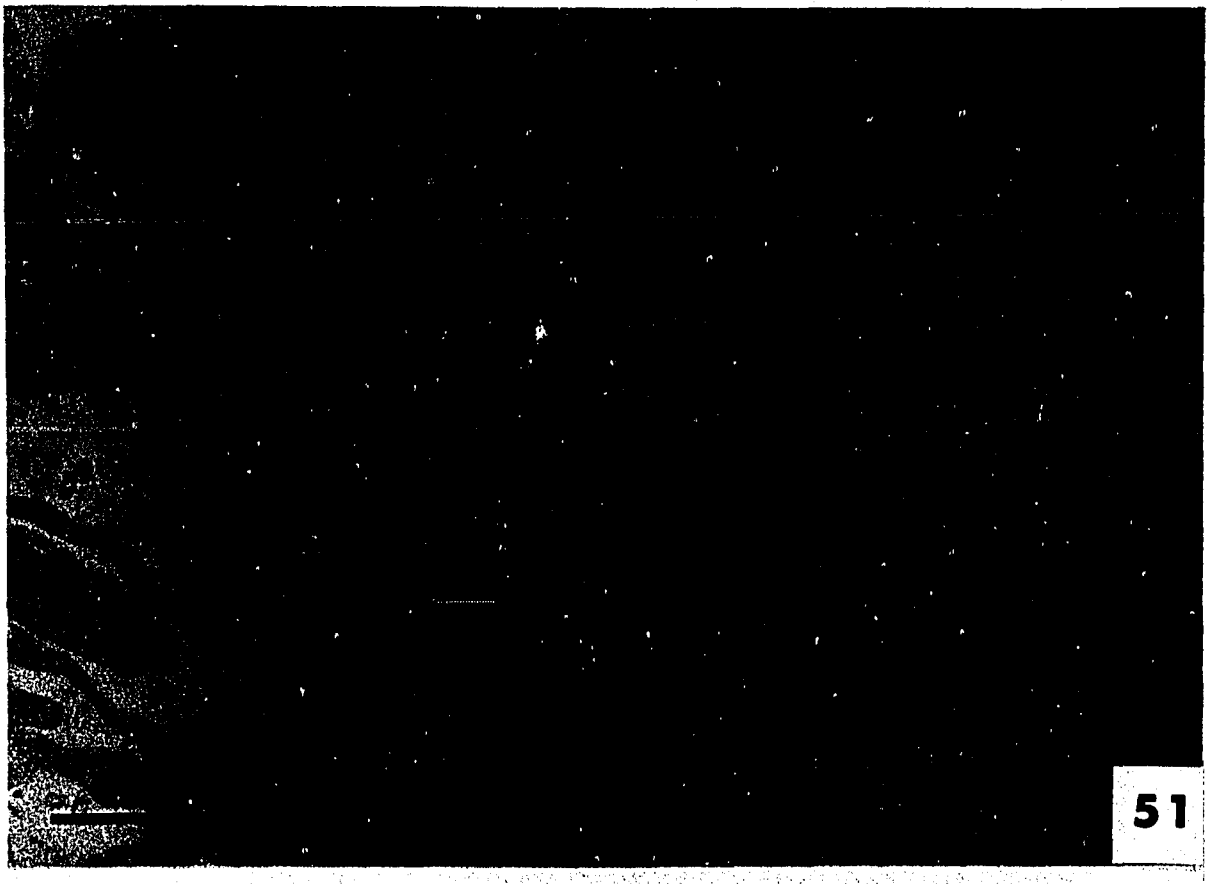

1μ
50

Figure 51. A one hour test incubation cecum for acid phosphatase showing an eosinophil leucocyte with most granules showing incorporation of reaction product in the matrix but not the crystal core of the granules. One intact granule (arrow) shows no uptake of reaction product. A nearby nerve (Ne) also shows acid phosphatase positive sites. No post-stain. Magnification 44,500X.

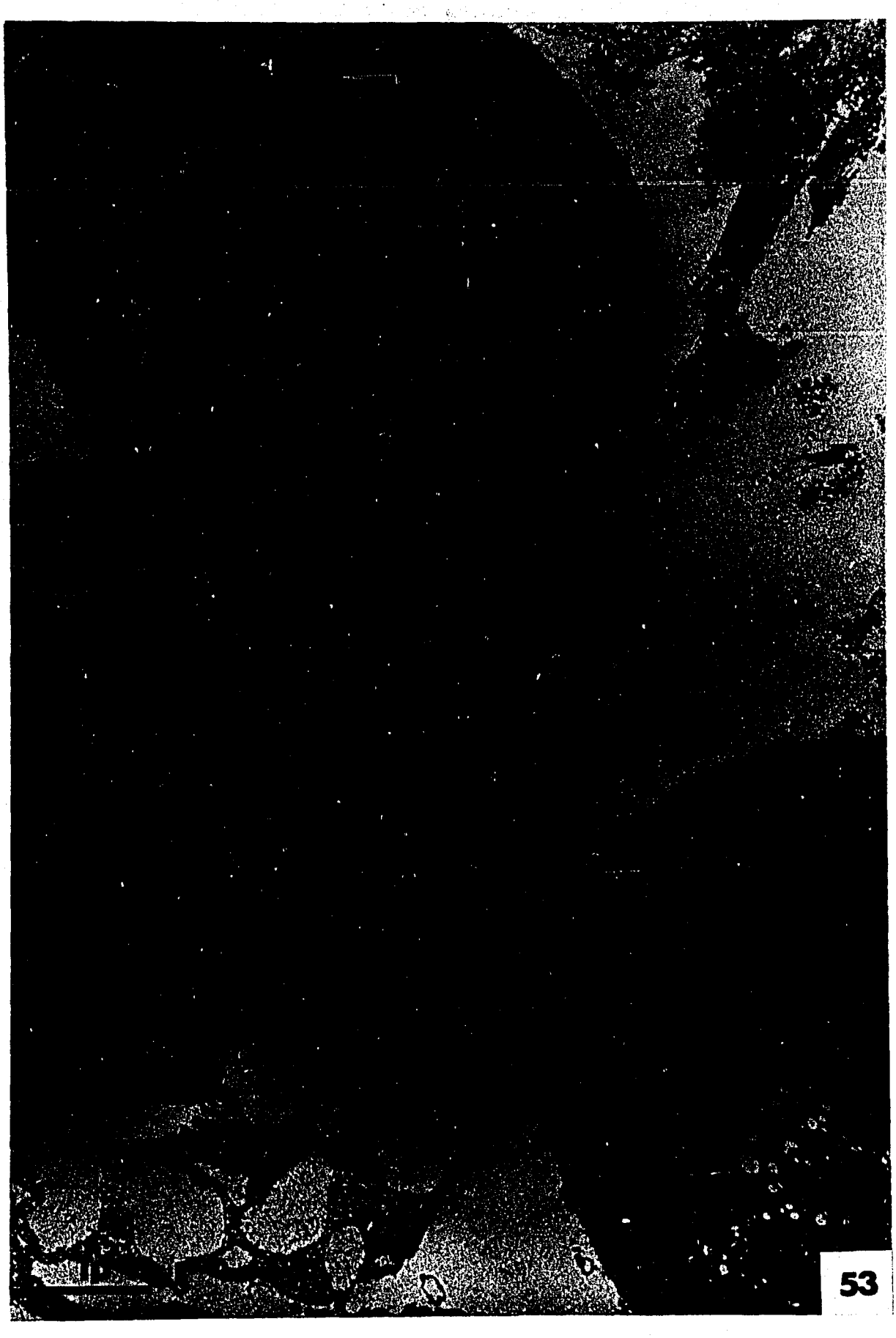
Figure 52. A one hour test incubation cecum for acid phosphatase showing a possible mast cell-globule lecocyte intermediate cell with accumulation of reaction product in the cytoplasm but not in the developing granules (G) which contain osmium tetroxide bound dark inclusions and some membrane-like lamellae. No post-stain. Magnification 44,500X.



51

52

Figure 53. Untreated culture of P815Y mast cells showing three separate stages. Cell A is a young developing mast cell with an active Golgi apparatus (Go) and small developing granules ranging in size from 53mu to 150mu. Cell B is a portion of a mature cell with dense osmiophilic granules having a range of 1.1u-2.0u. There is also a small cluster of developing granules less electron dense than the large granules. Cell C is a degranulated mast cell which has exocytosed most of its granules. Magnification 18,900.



188A

Figure 54. An untreated P815 mast cell showing early granule formation from expanded Golgi cisternae (GO). Magnification 90,000X.

Figure 55. A normal P815 mast cell granule in a later stage of development. This granule has a mitochondrion (M), smooth endoplasmic reticulum vesicle (SER) and the nucleus (N) nearby. Magnification 90,000X.

Figure 56. A peritoneal mast cell from a normal Swiss strain mouse which was isolated and cultured in vitro in Swiss cecal homogenate for one hour. A membrane whorl-like body is seen in a developing mast cell granule. Magnification 90,000X.

Figure 57. A P815 mast cell cultured in Swiss strain cecal homogenate for one hour at 37°C. Uncharacteristic membrane whorls begin to develop near normal mast cell granules. Magnification 90,000X.

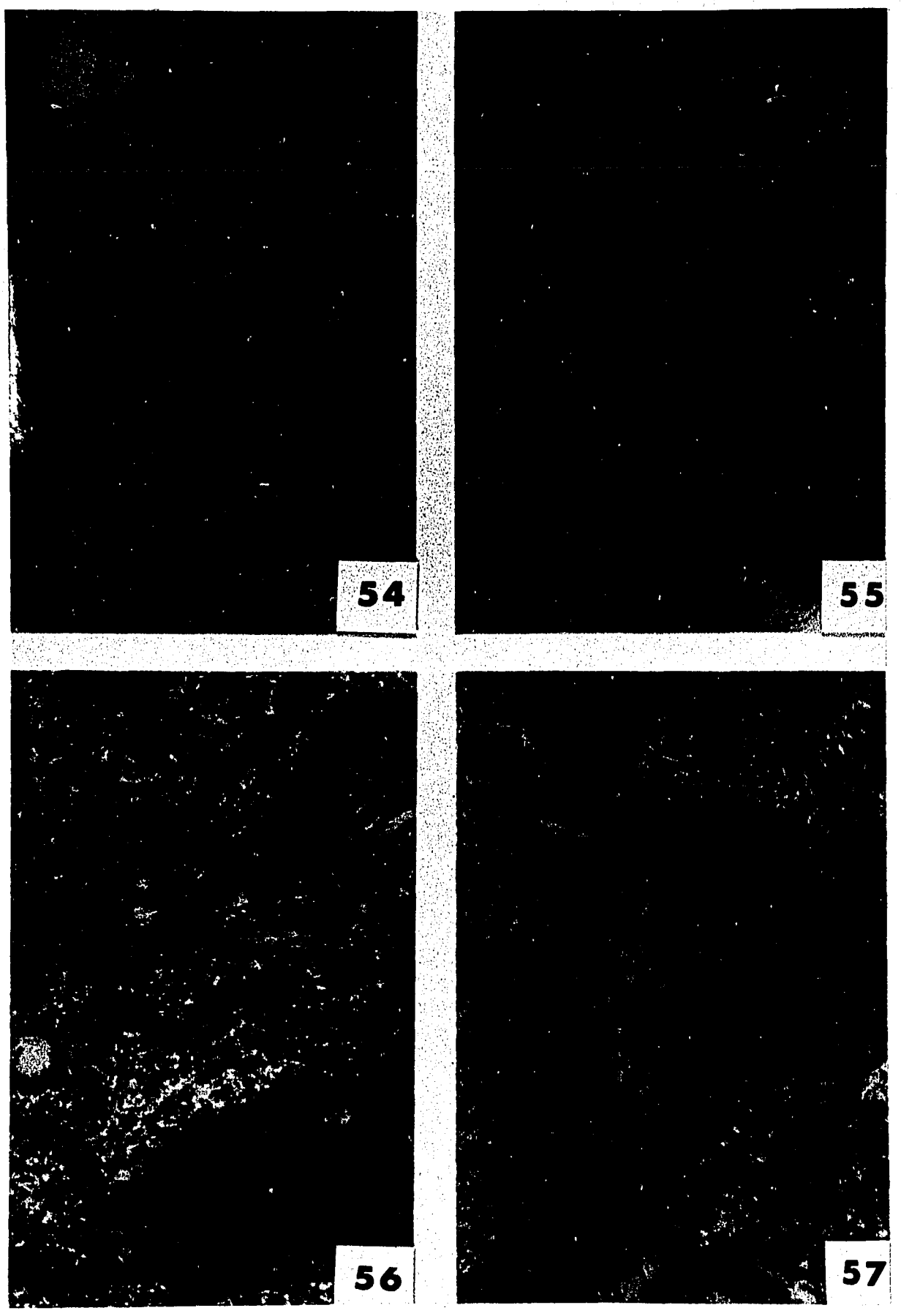
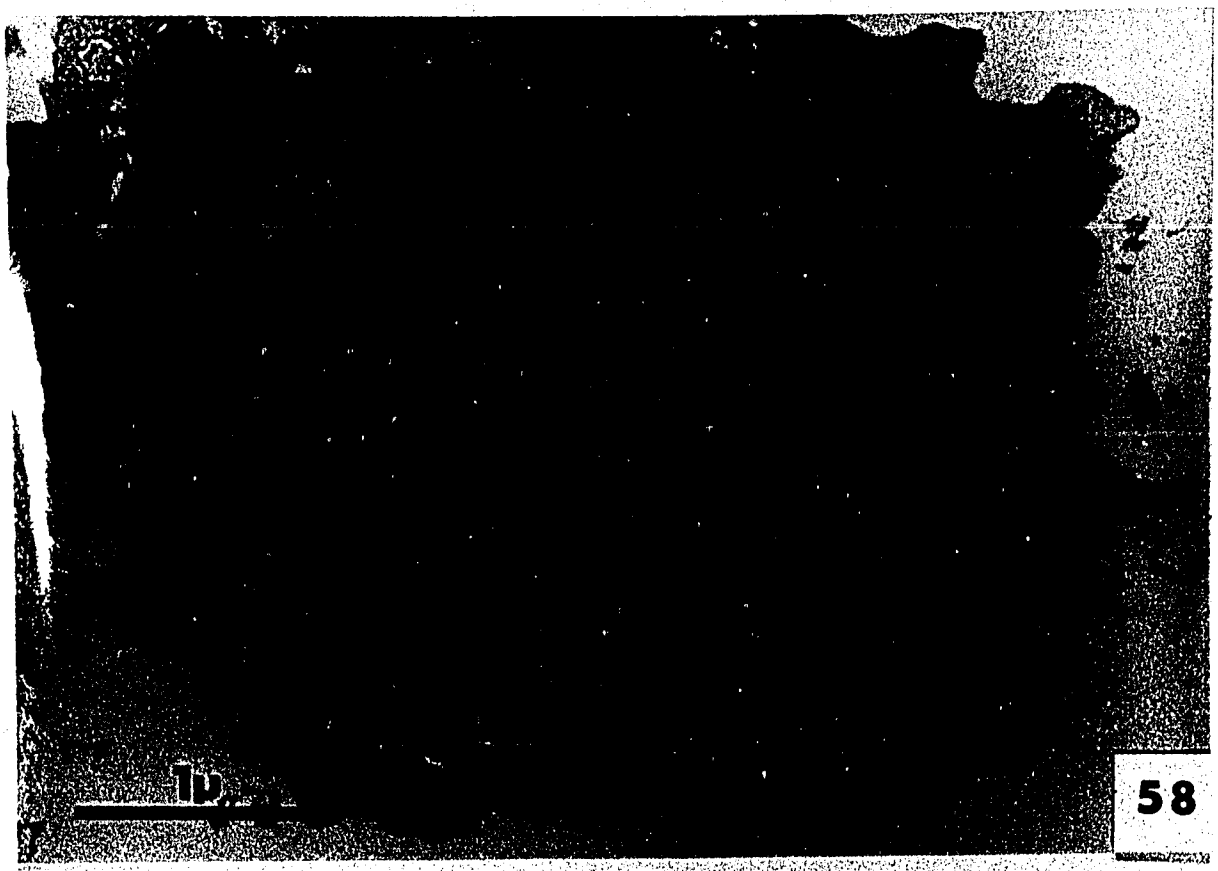

54
55
56
57

Figure 58. A one hour incubation of P815 mast cell and cecal homogenate results generally in total degranulation of most P815 mast cells but new granule synthesis is generally evident as indicated by the active Golgi areas and multivesicular body formation (MVB). Magnification 30,000X.

Figure 59. A one hour incubation of P815 mast cell and cecal homogenate and 0.48 mg/ml actinomycin D results in total cellular degranulation and abnormal de novo granule synthesis. Magnification 30,000X.


1μ
58


1μ
59

Figure 60. After 21 hours incubation of P815 mast cells in cecal homogenate there is little development of granule formation apparent. Modified Hirsch-Fedorko fixation. Magnification 18,000X.

191A

Figure 61. P815 mast cell and cecal filtrate incubated 15 hours at 37°C. results in formation of membrane limited bodies often containing membrane systems. Hirsch-Fedorko fix. Magnification 90,000X.

Figure 62. P815 mast cell and cecal filtrate incubated 15 hours at 37°C. shows another typically electron-dense abnormal mast cell granule.

Figure 63. In some P815 mast cells incubated in cecal filtrate 15 hours, large membrane filled bodies develop bounded by a single unit membrane. Each internal "membrane" is composed of three lines having a total width of approximately 100Å. Hirsch-Fedorko fix. Magnification 90,000X.

Figure 64. After 24 hours of P815 mast cell incubation with cecal filtrate, large bodies nearly one micron in diameter form in the cytoplasm. Magnification 90,000X.

Figure 65. The "granules" become larger (1.1-2.0u in diameter) after 48 hour incubation. Magnification 90,000X.

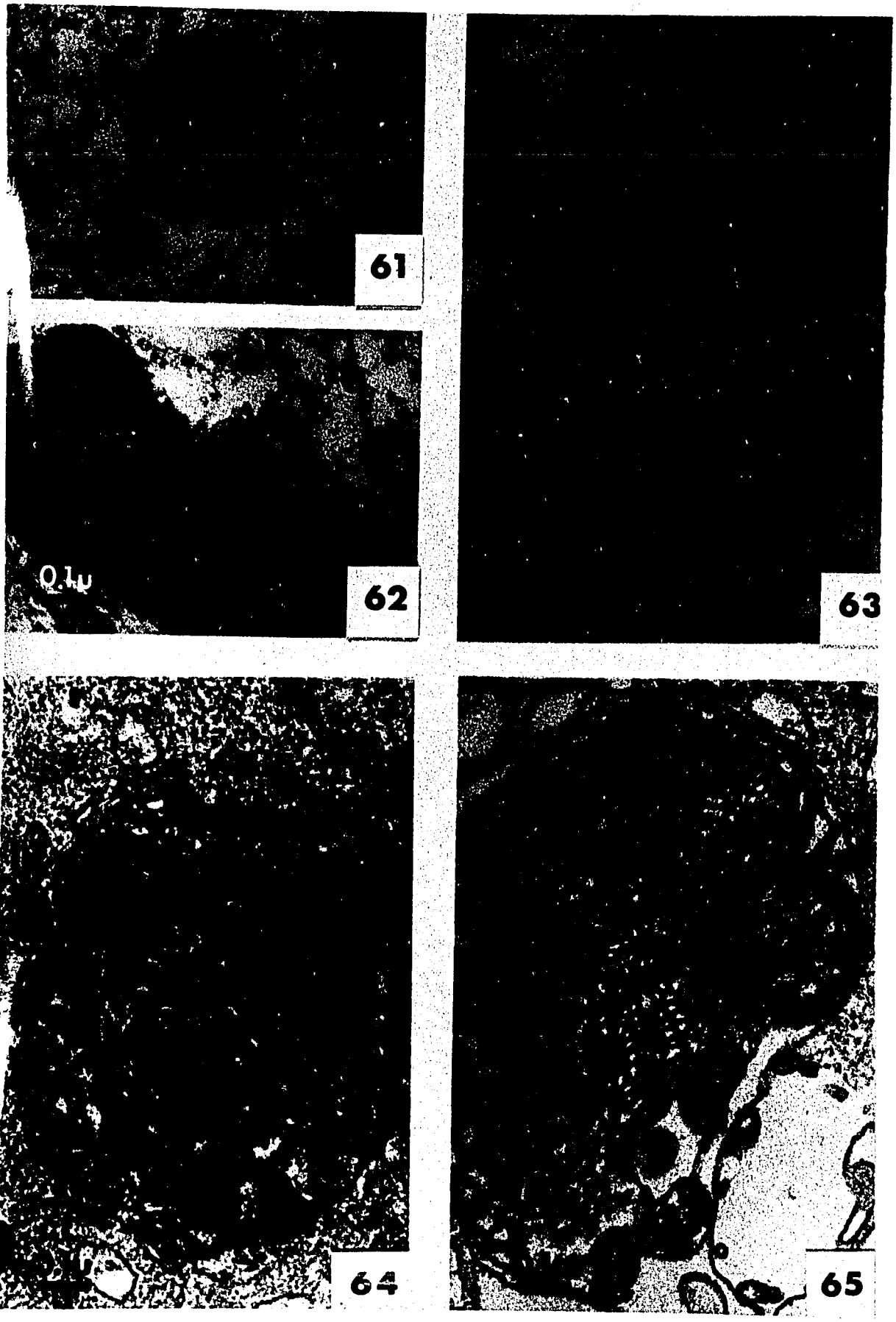

61
0.1μ
62
63
64
65

Figure 66. P815 mast cells incubated 70 hours at 37°C. with mouse cecal filtrates results mainly in a return to a stable population of normal appearing P815 mast cells. Hirsch-Fedorko fix. Magnification 8,300X.

Figure 67. Occasionally abnormal-appearing granules reminiscent of a mast cell-globule leucocyte intermediate appera in the cytoplasm of some P815 mast cells incubated 70 hours in the cecal filtrate. Hirsch-Fedorko fix. Magnification 69,000X.

Figure 68. The Golgi region of most P815 mast cells has a normal appearance after 70 hours incubation in cecal filtrate. Hirsch-Fedorko fix. Magnification 69,000X.

Figure 69. A rare cell showing signs of abnormal cytoplasmic granules after 70 hours P815 mast cell incubation in cecal filtrate at 37°C. Hirsch-Fedorko fix. Magnification 64,400X.

66

67

68

69

Figure 70. A culture of P815 mast cells labelled with 14.7 mCI ^{3}H-thymidine for two hours *in vitro*. This light microscopic autoradiogram was from a slide covered with Ilford L_4 emulsion and exposed for 25 days. The nuclei of the mast cells are labelled with dark spots representing exposed silver grains indicating the presence of incorporated radioactive tritium (arrows). Magnification 350X.

Figure 71. A portion of an electron microscopic autoradiogram of the same mast cell culture observed in Figure 70.showing nuclear labelling with ^{3}H-thymidine. Magnification 18,500X.

Figure 72. A light microscopic autoradiogram of a Swiss strain cecum showing three areas of ^{3}H-5-hydroxytryptamine localization. The mouse was injected intravenously with a 0.2 ml of 50 uCi/ml of ^{3}H-5-hydroxytryptophan (a total of 10 mCi). Exposure time was 45 days using Ilford L_4 emulsion. The three areas of silver grain exposure (arrows) includes one over a connective tissue mast cell in the lamina propria. Magnification 400X.

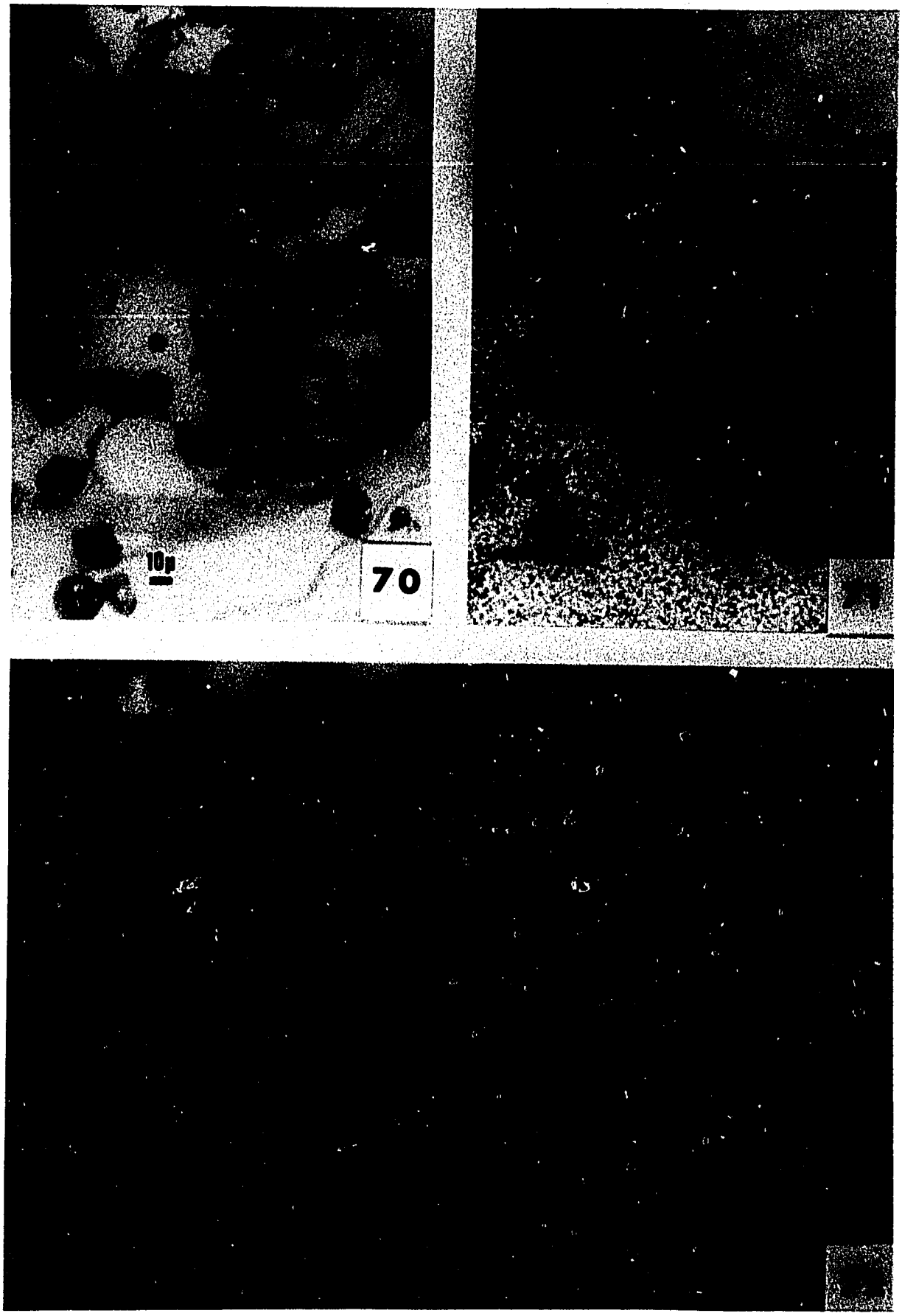

10μ
70

Figure 73. Some of the potential relationships which may exist among some of the migratory cells. See text pages 94 through 96 for explanation.

FIGURE 73 - RELATIONSHIPS AMONG MIGRATORY CELLS