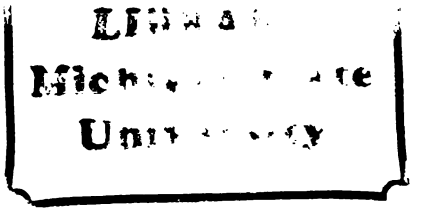

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PATHOGENESIS OF GASTROINTESTINAL HYPERPLASIA IN RATS INFECTED WITH TAENIA TAENIAEFORMIS

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David Michael Blaies

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PATHOGENESIS OF GASTROINTESTINAL HYPERPLASIA IN RATS INFECTED WITH *TAENIA TAENIAEFORMIS*

by

David Michael Blaies

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ABSTRACT

PATHOGENESIS OF GASTROINTESTINAL HYPERPLASIA IN RATS INFECTED WITH TAENIA TAENIAEFORMIS

BY

David Michael Blaies

Greatly enlarged stomachs and intestines are characteristic of chronic infections with the immature liver-dwelling parasitic tapeworm Taenia taeniaeformis. These severe hyperplastic changes were measured postmortem in rats subjected to long-term infections with metacestodes. The resulting stomach weights in infected rats (> 16g) were markedly greater than the controls (ca. 2 g) after 63 days postinfection (DPI) and most reached their greatest masses after 80 DPI. The small bowels enlarged primarily due to growth of the villi and crypts, and serum gastrin levels rose dramtically in many infected rats but without a clear trend. Soon after infection, mucosal mast cells permeated the rat's intestinal lamina propria until 40-50 DPI when maximum levels were reached. Parasitized rats generated nonuniformly great elevations in histamine concentration throughout the small bowel, however, the histamine was nearly always distributed in a gradient increasing from the duodenum toward the ileum. Remarkably, no measureable changes in any of these parameters were produced if 10-40 large strobilocerci were surgically implanted in the peritoneal cavity.

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I must credit Dr. Forbes at Oakland University (Michigan) for first stimulating my interest in parasitology and to Dr. Jeffrey Williams who then had the patience to see me through three years of graduate study. Thanks to them both!

HISTORICAL PERSPECTIVE

"THE LACTEALS commence upon the inner surface of the intestines, and absorb, or such up the chyle, the milky-like fluid, formed from the digestive process, and from which the blood is renewed, and the general system built up, pouring the chyle, as before remarked, into the thoracic duct. And Dr. Gunn, in his "Domestic Physician" says that he thinks that it is a reverse action of the LACTEALS, in cholera, by which they pour back their contents in the intestines, or rather I should say, WANT OF ACTION, in not taking up the chyle, leaving it to be passed off in the milky and watery stools."

From: Dr. Chase's Family Physician, Farrier, Bee-Keeper and Second Receipt Book, 1887, Chase Publishing Company; Toledo, Ohio.

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I. The Tapeworm Model

The larval stages of Cyclophylidean tapeworms of the genera Taenia and Echinococcus develop in muscular and visceral tissues of man and domesticated animals causing the diseases known as cysticercosis and hydatidosis, respectively. The parasites may lodge in vital organs causing serious health problems, or they may develop in the muscle tissues creating economic loss due to condemnation of domesticated animal carcases at the slaughter house or embargoes on the international sale of meats from endemic countries. There are few satisfactory approaches to therapy, and the increasing prevalence in recent years has given rise to renewed interest in the immunology and pathology of cestode infections.

Taenia taeniaeformis in the rat shows many similarities in its life cycle and immunological characteristics to the medically and economically important tapeworms (Gemmell and Johnstone, 1977; Williams, 1979). In addition, T. taeniaeformis induces pathological and hormonal changes which appear similar to those of several important naturally occurring non-infectious human diseases (Cook and Williams, 1981; Cook et al., 1981a, b). Examples include the cystic mucosal growth of the stomach as seen in patients with Menetrier's disease (Scully et al., 1978), the thickened intestinal wall with mastocytosis that appears in Crohn's disease (Robbins, 1974), and hypergastrinemia comparable to that seen in the Zollinger-Ellison syndrome (Grossman et al., 1961). The relationship

between these pathological changes and parasite survival and growth are not clear, but the suitability of this model for laboratory study provides a good opportunity for research on the underlying mechanisms.

Adults and larval stages are easy to maintain and none of the developing forms are infective to human beings. Cats are hosts of the adult tapeworms, and eggs of the parasite are collected from the feces of the cat, washed, then fed to rats via a gastric tube. In the stomach and intestine the keratinized blocks which form the outer surface of the eggs swell and fall off, leaving a series of oncospheral membranes surrounding the hexacanth (6-hooked) embryo. After entering the small intestine, the embryo undergoes a poorly understood process of activation, in which the membranes are broken and the newly liberated oncosphere migrates into an intestinal villus. Penetration is achieved with the aid of enzymes secreted by the parasite and the oncospheral hooks (Heath, 1971). The oncosphere enters a venule in the mucosa and is then transported passively to its site of predilection in the liver. There, development into a larval tapeworm takes place within a connective tissue capsule.

The work reported in this thesis concerns some aspects of the relationships between the gross pathologic changes in rodent cysticercosis and the mast cells which proliferate in affected tissues. The characteristics and the sequence of the pathological events after infection, the nature and functions of mast cells and their constituents, and the physiological regulation of gastrointestinal secretion and growth are reviewed in the following sections.

II. Pathology of Taenia taeniaeformis Infection in Rats

Pathological changes in rats with heavy infestations of T. taeniaeformis were first described by Bullock and Curtis (1930) and then Coleman and De Salva (1963). An account of the sequence of events has been developed by Cook et al. (1981a) with special reference to the liver, thymus, and lymph nodes. In the liver, fibroblasts rapidly proliferate in the capsule around the parasite at about 14 days post-infection (DPI). At this time, mast cells and plasma cells begin to permeate the capsule region. Hepatic mast cells increase in numbers reaching a peak at about 28 DPI and then decline. An acute thymic atrophy becomes grossly evident by 44 DPI.

Cook and Williams (1981) described the dramatic changes in the stomach and small intestine of infected rats due to mucosal hyperplasia; the weight of the stomach increased up to 20 times normal and the intestines weighed up to 3 times normal. Their experiments with antrectomized rats demonstrated that the hyperplastic changes persisted in spite of reduced gastrin levels, indicating that hypergastrinemia was secondary to stomach growth. The growth is potentiated by an as yet unidentified factor or mechanism.

Eosinophils in the peripheral blood and in the intestinal lamina propria were elevated during the course of infection, reaching a peak at 40 DPI and declining thereafter. The mast cell population in the small intestine increased significantly, with individual rats having over 10 times the normal mast cell level. The reason for the development of high numbers of intestinal mast cells in rats with hepatic cysticercosis is unknown. Local mast cell increases have been reported in the small intestine of rats infected with many different intestinal

parasites (e.g., Nippostrongylus brasiliensis, Befus, 1979), and levels are known to rise in the skin of human patients infected with the filarial worms Loa loa, Wuchereria bancrofti, and Onchocerca volvulus (Fernex and Beyes, 1962; Fernex and Sarasin, 1962). However, their roles in the host-parasite relationship have not been clarified experimentally.

III. Mast Cell Characteristics

Mast cells, which were originally described by Paul Erlich (1879), are loosely defined as mononuclear tissue cells containing granules that stain metachromatically with Toluidine Blue O. Because of the intense background staining with this dye, modern investigators use a variety of specific techniques to stain mast cells, but the variability of the results, particularly in mucosal tissues, is still troublesome. The cells are especially associated with skin, mesentery, lung, spleen, stomach, and intestine. They have been reported in all mammals, and even in amphibians such as frogs (Kapa and Csaba, 1972), although the quantity of mast cells in any tissue varies with the species.

Mast cells, in general, are warehouses of inflammatory mediators such as histamine, serotonin (5-HT), slow reacting substance of anaphylaxis (SRS-A), eosinophil chemotactic factor of anaphylaxis (ECF-A), and enzymes such as arylsulfatase. These mediators can be secreted from mast cells in specific response to immunological stimuli and non-specifically by complement anaphylatoxins, drugs, physical changes in the micro-environment, and chemical disruption.

The membrane-bound granules themselves consist of sulfated glycosaminoglycans, such as heparin, probably combined with histamine.

Keller (1966) showed that only slight release of histamine occurred from rat mesenteric mast cells in the absence of granule extrusion in vitro, but that both are secreted simultaneously in vivo. In carefully controlled experiments, Uvnas (1972) used compound 48/80 to release histamine from free peritoneal mast cells. He determined that release was related to granule extrusion, but that some histamine from granules deep in the interior of the cell could also penetrate through the cell membrane by an independent mechanism.

Enerback (1966a, b) first demonstrated that mast cells, formerly thought to be of one type, could be separated into two groups on the basis of their staining characteristics (granule-associated sulfated glycosaminoglycan type) and their reactivity towards compound 48/80. Connective tissue mast cells (CTMC) and peritoneal mast cells are degranulated by compound 48/80, but those of the intestinal or mucosal (MMC) type are not. Lindsay (1981) compared the reaction of mast cells in the liver and intestine of rats infected with T. taeniaeformis to CTMC from skin and tongue, by treatments with 48/80 compound and dexamethasome. She was able to confirm that the two groups could be differentiated on the basis of drug reactivity and histochemical characteristics and that rat hepatic mast cells are largely of the MMC type. It should be borne in mind that until recently no such distinction was made; therefore, much of the older literature and many of the functional studies only apply to the readily accessible rat peritoneal and mesentery mast cells, not to MMC.

IV. Heparin

Heparin, an important constituent of mast cells, was first found to be released from isolated dog liver after peptone shock (Rocha e Silva et al., 1947); therefore, the name "heparin" has been derived from the Greek term "hepar" meaning liver.

The heparin backbone (Jaques, 1979) is a polymer of glucuronic and uronic acid sugars, with an average of 3 sulfurous acid groups for every 2 sugar residues. This makes the molecule strongly negatively charged. The chondroitin sulfates have almost the same sugar backbone as heparin, but average only 1 sulfurous acid group per 2 sugar residues. Chondroitin sulfates are therefore less charged and, as a result, less active biologically. Chondroitin sulfates are generally present in cartilage and connective tissue.

Although basophils and mast cells in all species have metachromatically staining granules (Jaques, 1975), some investigators point out that these may not always contain heparin. For example, recent studies by Orenstein et al. (1978) show that guinea pig basophil granules, previously thought to contain heparin, actually are composed of 55% chondroitin sulfate A and C, 30-35% chondroitin sulfate B, and 15% heparan sulfate. No heparin was found. Tas (1977), by using microspectrophotometric analysis of the metachromatic specturm of rat MMC granules after exposure to Toluidine Blue O dye, has determined that these granules do not contain heparin, as do the other mast cells in the rat (Yurt et al., 1977; Robinson et al., 1978). He concluded that they contain lower sulfated glycosaminoglycans of unknown chemical structure and function.

V. Histamine

A. Biochemistry

In 1936, Werle discovered the DOPA decarboxylase enzymes in the kidney tissue of rabbits and guinea pigs. This was the only known system for endogenous formation of histamine, but in many animals such as cats, dogs, and man no DOPA decarboxylase could be detected (Waton, 1956). Since these animals with no obvious mechanism for histamine manufacture showed evidence of endogenous stores, the amine was believed to be absorbed from gut contents, or in the case of other animals which has a recognizable amount of DOPA decarboxylase, body histamine stores were believed to be maintained by both endogenous production and absorption (Kahlson and Rosengen, 1971). This led to the speculation that histamine was a vitamin.

A change in concept followed the discovery of histidine decarboxylase (L-Histidine carboxy-Lyase) in the late 1950's. This enzyme, contained in mast cells, removes the number 1 carboxyl group from L-histidine, in the presence of the coenzyme pyridoxal phosphate, to form L-histamine and carbon dioxide (Rothschild and Schayer, 1959). Most endogenous histamine in mammals is now considered to be produced by this mechanism, with minor contributions from the DOPA decarboxylase enzymes (Beaven, 1978).

The relationship between mast cells and histamine was first postulated by Riley (1953) and Riley and West (1953). This was based on the observed histamine release, along with heparin release, during peptone shock in the dog (Rocha e Silva et al., 1947) and a known tendency of the skin mast cell lesions in human urticaria pigmentosa to form an itchy

wheal and flare reaction upon mild trauma. This proposal was confirmed by Schayer (1956b) when he incubated ^{14}C-labeled histidine with isolated rat peritoneal mast cells *in vitro*. ^{14}C-histamine was produced.

After histamine has performed its function, there appear to be two methods of enzymatic degradation and one method of direct disposal. The first catabolic enzyme to be recognized was histaminase, now called diamine oxidase. A second enzyme system, discovered by Schayer (1966), deactivates histamine by the action of histamine-N-methyl transferase, which accepts a methyl group from S-adenosylmethionine (SAM), then attaches it to histamine, forming N-methyl histamine. This molecule may further be degraded to methyl imidazole acetic acid by the enzyme monoamine oxidase; however, its fate thereafter is unknown. Third, histamine may be disposed of by excretion in the urine of many mammals. Varying quantities of the total urinary histamine appear as free histamine in the rat (Gustafsson et al., 1957) which, although present in males, is much more prominent in females. The remaining histamine in urine appears in a conjugated form, tentatively identified as acetyl histamine (Kahlson and Rosengren, 1971), although it is now thought to be largely of bacterial origin (Beaven, 1978).

Extensive reviews of histamine metabolism or catabolism may be found in Kahlson and Rosengren (1971), Beaven (1978) and Maslinski (1973).

B. Concentration in tissue and mast cells

There are three methods for the determination of histamine. The oldest is the biological assay which measures the contraction of guinea pig ileum or rat uterus in the presence of histamine *in vitro*. An

improved method for histamine determination (Beaven et al., 1972) utilized N-methyltransferase to attach a ^{14}C-methyl group from SAM. The radioactively labeled N-methyl histamine is then quantitated in a liquid scintillation spectrometer. This procedure will detect as little as 0.1 ng histamine.

The analysis method developed by Shore et al. (1959) has been well accepted among investigators. In this procedure, the histamine must be extracted with butanol and heptane, then condensed with o-phtalaldehyde (OPT). May et al. (1970) have adapted the technique to small volumes of blood cells for clinical allergy testing. An extensive review of the methods and data concerning OPT-induced histamine fluorescence may be found in Hankanson et al. (1972).

Attempts have been made to quantitate the histamine in mast cells and animal tissue from several sources. Paterson et al. (1976), using cells that were dispersed from tissue enzymatically, report levels of 1.0-2.8 pg/mast cell from human lung and 1.0-2.0 pg/mast cell from rat lung. Mast cells isolated from dog stomach fundus by Soll et al. (1979) contain about 2.5 pg histamine per cell. Higher amounts have been reported by Austin and Humphrey (1963) and Enerbach and Wingren (1980) for rat peritoneal mast cells. In their reports, levels ranges from 15-40 pg/mast cell. Similarly, Veilleux and Cantin (1976), using cytofluorescent techniques, have determined that there is less histamine in duodenal mast cells than in the CTMC of the trachea or urinary bladder. Since peritoneal mast cells and CTMC contain substantially more histamine than mucosal mast cells, this may be a further indication of differences between the two types.

VI. IgE, Mast Cells, and Parasitism

Investigating the question of the mechanism of histamine release in rats, Mota (1957) used horse serum sensitization to cause anaphylactic shock, and anaphylatoxin as a histamine releasing agent, to study mast cell secretion. He theorized that there were antibodies on the surface of mast cells and that the cells would react to the antigen or anaphylatoxin by releasing granule contents and histamine into the blood stream of rats. Mota and Da Silva (1960) reported further definition of the granule extrusion phenomenon *in vitro* with rat peritoneal mast cells that had been sensitized with horse serum. Highly reactive cells were washed thoroughly and the release of chemical mediators upon exposure to horse serum provided indirect evidence for the occurrence of specific antibody on the mast cell surface. Later, Mota (1964a, b) discovered a mast cell-sensitizing antibody which is now considered to be reagin or immunoglobulin E (IgE) in most species.

IgE is the major sensitizing antibody involved with allergic reactions of all types (Austen et al., 1976; Snider, 1978), and is also one of the hallmarks of antibody response to parasites in all mammalian species. It appears, for example, in response to infection with *N. brasiliensis* (Ishazaka et al., 1976), *Fasciola hepatica* (Day et al., 1971) and *Schistosoma mansoni* in rats (Ogilvie et al, 1966; Rousseaux-Prevost et al., 1977), in monkeys infected with *S. mansoni*, and in sheep infected with *Trichostrongylus colubriformis* (Ogilvie, 1964). There is a high correlation between elevated levels of serum IgE and infections with *Ascaris lumbricoides* and/or *Necator americanus* in human patients (Turner et al., 1979).

A cardinal feature of T. taeniaeformis infections in the rat (Leid and Williams, 1974b) and T. pisiformis infections in the rabbit (Leid and Williams, 1975) is the production of circulating IgE antibody. Leid (1977) demonstrated indirect evidence for mast cell-associated IgE by the antigen-induced release of histamine in vitro from peritoneal cells and lung fragments of rats infected with T. taeniaeformis. However, it now appears that not all IgE is on the mast cell surface. Mayrhofer et al. (1976) established the relationship between intracellular IgE and MMC in rats experimentally infected with N. brasiliensis, and Lindsay (1981) has confirmed this for T. taeniaeformis. By using immunofluorescence techniques, IgE can be detected both on the surface of and within MMC, these large numbers of IgE-containing mast cells develop in the duodenum by 42 days postinfection with T. taeniaeformis in rats (Lindsay, 1981).

Murray et al. (1971) speculated that stimulation of intestinal mast cells sensitized with specific IgE antibodies to N. brasiliensis, leads to increased worm expulsion due, in a large part, to hyper-permeability of the intestine. Mast cell stabilizing drugs such as cortisone, which prevent vasoactive amine release, were found to slow the worm expulsion. These authors suggest that increased permeability in the intestinal epithelium allows a faster movement of antiworm antibodies into the gut lumen. Befus et al. (1979) measured the histamine concentration of the intestine in conjunction with MMC counts in N. brasiliensis-infected rats. Levels in normal rats averaged 3.4 MMC/VCU but rose to 58 MMC/VCU at 19 DPI with a strongly corresponding ($r = 0.88$) rise in histamine concentration from 0.5 μg/g to 23.3 μg/g after only nineteen days.

Rothwell et al. (1974) showed that direct infusion of histamine into the gut of guinea pigs caused expulsion of the enteroparasite T. colubriformis. A close association has been established between observations of increased worm expulsion and increased levels of histamine and 5-HT in the guinea pig intestine (Jones et al., 1974; Jones et al., 1978). Hypothetically, if histamine is intimately involved with worm expulsion from the gut, antihistamines should slow or stop the reaction. Rothwell et al. (1978) tested this hypothesis. Live larvae worms transplanted into immune animals were not rejected if the recipient animals were first treated with the antiallergic drug, CI 47,917, or the antihistamine, mepyramine. Although Musoke et al. (1978) demonstrated that T. taeniaeformis oncospheres exposed to histamine in vivo and in vitro show reduced viability and infectivity in rats, the results were variable and the potential for mast cell and histamine influences on susceptibility to this infection remains unknown.

Many authors have speculated on the role mast cells play in resistance to parasites (Wakelin, 1978; Ogilvie, 1974; Dvorak and Dvorak, 1972). It has generally been proposed that vasoactive amines are the most important components, but even though the vasoactive effects of histamine are well known, there has been no unifying theory on the relationship between histamine release from mast cells and either microenvironmental modulation or direct parasite death/expulsion in vivo.

VII. Histamine, Hormones and Gastrointestinal Function

Many hormones affect stomach function, such as secretin, glucagon, vasoactive intestinal peptide (VIP), gastric inhibitory peptide (GIP), cholecystokinin (CCK), and gastrin; only the latter has a proposed dual

role as a secretion stimulator and as a growth hormone. This peptide hormone and its analogs, such as the synthetically produced pentagastrin, apparently regulate acid release (Walsh and Grossman, 1975a, 1975b; Sewaga et al., 1979; Tani et al., 1979). Code (1977) suggests that gastrin stimulated acid production in the stomach is controlled by a simple pH feedback system, whereby a low pH inhibits acid secretion and gastrin stimulation.

There is now strong evidence for the role of histamine as a common final mediator in acid secretion [for comprehensive reviews see Code, 1977, or Waton, 1971], because H_2 histamine receptor blocking drugs, such as metiamide and cimetidine, inhibit acid release (Tani et al., 1979; Sewaga et al., 1979). The gastric histamine-containing cells are of two types. The first is the granulated mast cell such as that isolated from dog stomach fundus by Soll et al. (1979). These cells are in the mucosal layer and are similar in both ultrastructure and compound 48/80 responsiveness to MMC of the intestinal lamina propria. The second type is the enterochromaffin cell, first identified in rat stomach by Thunberg (1966). In other species few or no mast cells appear in stomach mucosa and the precise cellular location of histamine stores is unknown (Beaven, 1978).

Gastrin and pentagastrin are also growth-stimulating agents for the stomach and intestines. Crean et al. (1969) reported excessive acid output and mucosal growth in the fundus of the stomach after massive administration of pentagastrin, but saw only a smaller acid increase and no trophic effect after the administration of histamine. Gastrin-like hormone stimulation of DNA synthesis was found to be independent of acid secretory activity <u>in vivo</u> because it occurred in the presence of the

histamine H_2-blocker metiamide. The tropic action, however, was antagonized and drastically reduced in the presence of secretin. Histamine alone did not stimulate DNA synthesis in the mucosa (Johnson and Guthrie, 1974).

The gastrin-like hormones only have a direct effect on the mucosa, not on the underlying smooth muscle or connective tissue (Johnson, 1976). For example, a direct proliferative response to pentagastrin has been reported for human and rat gastric mucosa in tissue culture, but normal fibroblastic outgrowth was inhibited (Miller et al., 1973). There are two areas of the gastrointestinal tract that do not undergo growth in the presence of gastrin. Either with injection of pentagastrin or in hypergastrinemic conditions of human Zollinger-Ellison syndrome there is a growth of the gut exclusive of the antral mucosa and esophagus (Mak and Chang, 1976), which seems to be characteristic for this hormone. From a physiological point of view, hormones do not stimulate or influence the gland from which they originate; most of the gastrin-producing G cells are located in the antral region of the stomach. Gastrin is now the only known trophic gastrointestinal hormone. Although histamine has been shown to be associated with tumor growth and wound healing (Kahlson and Rosengren, 1971), it has never been shown to exert a trophic effect, by itself, on gut mucosa or underlying tissue.

VIII. Research Objectives

The manner in which the development of T. taeniaeformis in the liver influences the rate of growth of the stomach and intestine, the circulating level of gastrin and the proliferation of MMC is not clear. However, the connection established between parasite burden and the

degree of hypergastrinemia and mast cell increases suggests a dose-dependent interaction between these elements. A clarification of their respective roles may come from further work in the directions initiated by the research reported here. Specifically, the issues addressed are as follows:

Can pathological and hormonal changes be induced in non-infected rats experimentally with parasite-derived products? The results of Cook and Williams (1981) suggest that some factor, circulating between parabiotic rats, may be responsible for the gastrointestinal enlargement and mucosal mast cell population increase. If so, the parasite may produce a hormone-like substance which acts to influence growth, similar to that produced by Spirometra mansonoides (Steelman et al., 1971).

How long do the gastrointestinal changes persist? Studies conducted by Cook et al. (1981b) indicate that gastrin levels are erratically elevated in infected rats from 60 up to 100 DPI, but, because the relationship between gastrointestinal architecture, gastrin, and continued parasitism thereafter is unknown, it is difficult to predict long term effects. Quantitative studies are required over the extended course of infection.

Are there changes in stomach histamine levels which reflect or correlate with changes in gastrin levels, gastric pH, and stomach weight? Histamine is normally involved with stomach function, and some changes in histamine levels might be expected in the pathologically affected organs, but there is no information available on this question in experimental cysticercosis.

Finally, what is the relationship between the intestinal mastocytosis and histamine levels in T. taeniaeformis-infected rats? There are

reports from other host-parasite systems, such as N. brasiliensis and T. spiralis in the rat, that histamine is implicated in expulsion of the worms. However, these worms live in the intestinal lumen. Although there is evidence of MMC proliferation in the intestinal lamina propria of rats with T. taeniaeformis, it is not known if they contain or release histamine. The difference in timing of appearance of MMC in cysticercosis and nippostrongylosis, and the unique sustained high levels of MMC in the former justify an investigation of the histamine content, as a prelude to experimental work on characterization of mast cell secretory functions and responsiveness to parasite antigens and secretions.

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CHAPTER I

GASTROINTESTINAL HYPERPLASIA IN RATS CHRONICALLY INFECTED WITH *TAENIA TAENIAEFORMIS*: QUANTITATIVE PATHOLOGICAL AND HORMONAL CHANGES AND ATTEMPTS TO INDUCE THE SYNDROME WITH PARASITE PRODUCTS

by

David M. Blaies and Jeffrey F. Williams

ABSTRACT

Hyperplastic pathological changes in the gastrointestinal tract and serum gastrin levels were measured at post-mortem in rats given long-term infections with metacestodes of Taenia taeniaeformis. Stomach weights were greater than in controls from the beginning of the experiment at 63 days postinfection (DPI) onward, and generally reached highest levels (> 16g) in the more chronically infected rats (up to 368 DPI). Gross and histological evidence of papillary hyperplasia of gastric mucosal cells increased with duration of infection. Intestinal weights also increased with time, but to a much lesser degree than those of the stomachs. These changes were sustained throughout the period of study, and were associated with significant elongation of villi and increased mucosal crypt depth, especially in the duodenum. In the most chronically infected rats multiple cystic dilatations of crypts occurred, and within them accumulated masses of mucus and necrotic cells often became calcified. Villi, sometimes over 1 mm in length, became club-like and frequently fused together. Mucosal mast cell (MMC) numbers in the small intestine were significantly higher than in controls throughout infection. Hyperplastic changes were detected inconsistently in the colonic mucosa, but there were no effects on the esophagus. Splenomegaly was a constant finding in infected rats, which also showed an earlier onset of thymic atrophy than normal age-matched controls. Serum gastrin levels were elevated in all affected rats at 5 months postinfection, but by the end of the first year some animals had normal gastrin levels, even though they showed severe hyperplastic gastroenteropathy at necropsy. Attempts to induce changes in gastric and intestinal weights, MMC numbers

or serum gastrin by the serial inoculation of extract or *in vitro* products of *T. taeniaeformis* strobilocerci were unsuccessful. Surgical implantation of strobilocerci intraperitoneally also produced no measurable changes in these parameters. The results suggest that the hyperplastic stimulus provided by *T. taeniaeformis* is sustained for many months, and that there is a gradient of responsiveness in gastrointestinal tissues, declining from the glandular stomach mucosa posteriorly. The stimulus may be augmented by endogenous gastrin, but this hormone is not necessary for maintenance of hyperplastic changes. Our failure to induce changes *in vivo* artificially suggests that more subtle criteria, perhaps involving *in vitro* influences on gastric or intestinal cell turnover, will be necessary to establish if the parasite exerts its effects on host cells directly or indirectly.

INDEX DESCRIPTORS

Taenia taeniaeformis (*Cestoda*: *Taeniidae*); gastric hyperplasia; intestinal hyperplasia; mastocytosis; gastrin; intraperitoneal implantation.

INTRODUCTION

Rats infected with the hepatic metacestodes of Taenia taeniaeformis develop severe hyperplastic changes in the stomach and intestine which become grossly evident by 45 days postinfection (DPI) (Cook and Williams, 1981). The degree of hyperplasia is extraordinary, especially in the stomach which may reach twenty times the normal weight. Accompanying microscopic changes over the first 70 DPI include cystic gastric mucosal hyperplasia, increases in intestinal villar length and intense mast cell proliferation in the intestinal lamina propria. The gastric luminal pH becomes elevated, and circulating levels of the hormone gastrin rise abruptly during the first two months of infection; however, hypergastrinemia is not a prerequisite for induction of the lesions (Cook et al., 1981).

The mechanism whereby these changes are brought about is unknown, but similarities to some of the pathological and hormonal alterations caused by gastrointestinal nematodiasis (e.g., ostertagiasis,[Anderson et al., 1976]; trichinellosis,[Castro et al., 1976]) are striking and suggestive of common underlying pathogenetic processes. The physical separation of larvae of T. taeniaeformis from the sites of hyperplasia, combined with the observation that all the lesions can be produced in noninfected rats by parabiotic union to parasitized animals (Cook et al., 1981), indicate that chemical mediators may be involved. Furthermore, concomitant onset of a complex of uncontrolled mucosal cell proliferation, aberrant gastric acid secretion and hypergastrinemia suggests that the normal regulatory and homeostatic mechanisms of gastrointestinal growth and secretion are being compromised at a fundamental level.

We have extended our characterization of this parasite-induced gastroenteropathy and report here on the nature of mucosal and hormonal abnormalities in rats from 2 to 12 months PI, and on our attempts to stimulate gastrointestinal and hormonal changes in normal rats with inoculations of parasite-derived extracts or products, and by surgical implantation of larvae. We found a progressive distortion of mucosal architecture in affected tissues, over the long term, and persistence of severe pathologic changes even when circulating gastrin eventually declined to the normal level. We were unable to induce pathological or hormonal changes either by parenteral administration of parasite products or by prolonged exposure to live parasites in the peritoneal cavity.

MATERIALS AND METHODS

Experimental infection:

The characteristics of the strain of T. taeniaeformis and procedures for the routine maintenance of the parasite in our laboratory have been described recently by Williams et al. (1981).

Pathological changes in chronically infected rats:

Pathological changes were measured in a group of 38 female Spartan (Spb: [SD]) rats (Spartan Research Animals, Haslett, Michigan) given 1000-2000 eggs orally and killed at intervals over 63-231 DPI, as specified in the results. Twenty-six age-matched normal rats served as controls. The animals were killed by exposure to CO_2 vapor and exsanguinated; wet weights were then recorded for the liver, spleen, thymus, stomach and small intestine. Tissue samples from the stomach, duodenum, jejunum and ileum were fixed in formalin and stained with haematoxylin eosin. Tissues from each small intestinal segment were also fixed in Carnoy's solution and stained with Astra blue by the method of Blaies and Williams (1981). Samples of esophagus and colon were taken from some groups, but were not routinely included because these tissues were not generally affected even in rats showing severe gastroenteropathy.

Quantitative evaluation of small intestinal changes was based on measurement of villar length, crypt depth, and thickness of the smooth muscle layer and its overlying connective tissue at 5 sites on a complete transverse section of duodenum, jejunum and ileum from each rat. In the colon the thickness of the mucosal layer and the height of the rugae were measured. Only the overall thickness of the esophageal wall was measured. Mucosal mast cell (MMC) counts per villus crypt unit (VCU) in the small

intestine were made on the Astra blue-stained sections, as described by Cook and Williams (1981). Arithmetic mean values for all variables were computed for each rat, and for the purpose of statistical comparison of trends over the course of infection, the rats were considered to form two groups: those killed 40-150 DPI, and the remainder killed 151-240 DPI. Data from each group were analyzed by Student's "t" test.

Gastrin levels in chronically infected rats:

Serum gastrin levels were examined in a second group of 46 chronically infected rats killed 155-368 DPI. Control samples were drawn from 12 normal uninfected female rats aged 166-223 days. Gastrin was measured by radio-immunoassay with a commercial kit (Beckton-Dickinson). Fresh wet weights for stomach and small intestine were recorded for all rats.

Attempts to induce hyperplasia by parenteral inoculations or surgical implants:

Stomach and small intestine weights, MMC counts and serum gastrin levels at necropsy were recorded in two series of rats in which we attempted to induce changes by either serial inoculations of parasite-derived products or surgical implantation of live parasites into the peritoneal cavity. Saline soluble extracts (SS) of strobilocerci and products from worms maintained *in vitro* (IVP) for 24 hours were prepared as described by Ayuya and Williams (1979). Protein estimations were made by the Lowry method. Subcutaneous inoculations of SS or IVP were given twice daily for 3 weeks to groups of 8 twenty-eight day old female Spartan rats. Each dose contained at least 0.5 mg (protein) SS or 0.05 mg IVP. Age-matched control groups were given twice daily inoculations of 0.15 M NaCl, or 1000 eggs of *T. taeniaeformis* orally on Day 0, or no treatment. All groups were killed after 21 days.

Strobilocerci were extracted from hepatic cysts and implanted intraperitoneally into groups of 6 rats as described by Musoke and Williams (1976). Recipients received either 10 or 40 parasites and were sacrificed after 70 and 21 days, respectively. Gross organ weights, MMC counts and serum gastrin levels were compared with values obtained from age-matched uninfected and infected control groups killed in parallel. Gastrin levels in these rats were measured in the laboratory of Dr. Lenard Lichtenberger, Department of Physiology, The University of Texas, Houston, using the RIA procedure described by Cook et al. (1981).

RESULTS

Chronic pathological changes:

Stomach wet weights from the first (38) and second (46) groups of chronically infected rats are shown in Figure 1. From 63 DPI onwards infected rats generally had larger stomachs than the controls; those which did not always had low numbers of viable parasites (liver weight 30 g or below). However, some rats which had few parasites developed enlarged stomachs. The range for stomach weights in all normal uninfected rats was 0.8-2.25 g. There was a trend toward increasing stomach weight with duration of infection but there were many exceptions. Some individuals developed very heavy stomachs relatively early in infection. Histopathologically, the gastric mucosa in all affected rats showed severe cystic hyperplastic changes, with occasional hyperemic papillary outgrowths into the lumen, up to 1.5 cm in diameter as shown in Figure 2. These were more pronounced in rats killed in the later stages of infection (151-240 DPI).

Splenic weights of infected rats in the first group exceeded those of controls at all ages (Figure 3). Thymic weights declined abruptly in infected rats in the early stages, but did not differ from controls in the 151-240 DPI period (Figure 3). Small intestinal weights exceeded those of controls at all stages of infection, but the extent of the change was markedly less than that seen in the affected stomachs. Intestinal hyperplasia persisted but did not increase significantly after 151 DPI.

Quantitative aspects of histopathological changes in the small intestines of 36 infected rats are shown in Figure 4. The hyperplastic changes were manifested in all 3 segments of the small bowel, but were

FIGURE 1

Stomach weights at necropsy of rats infected with Taenia taeniaeformis. In normal rats the average was 1.5 ± 0.08 g.

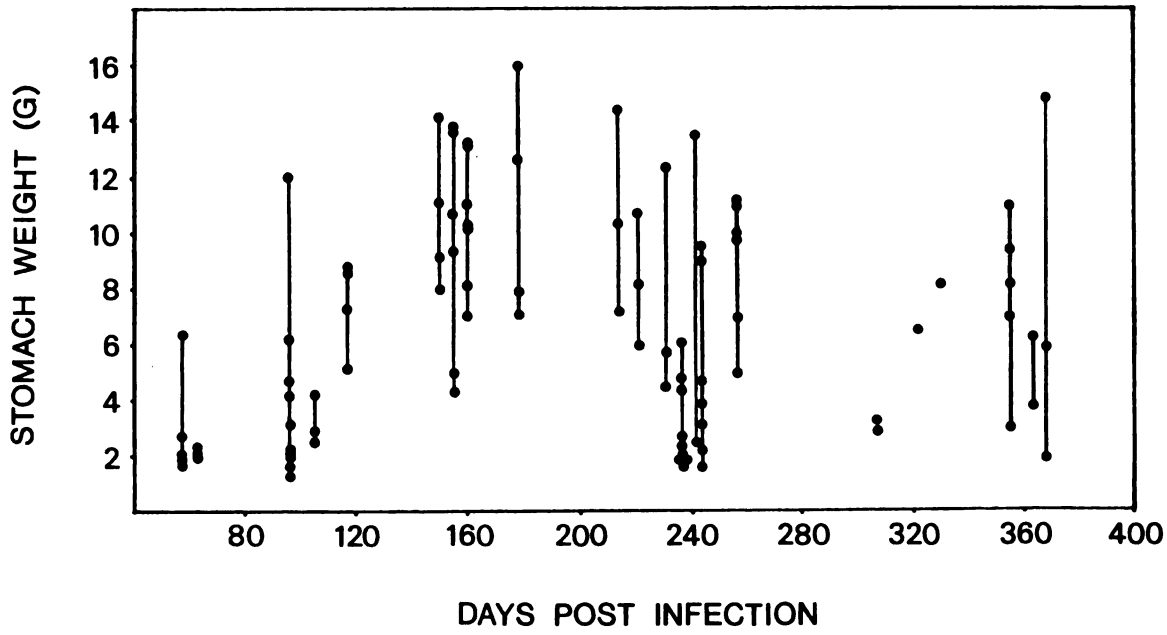
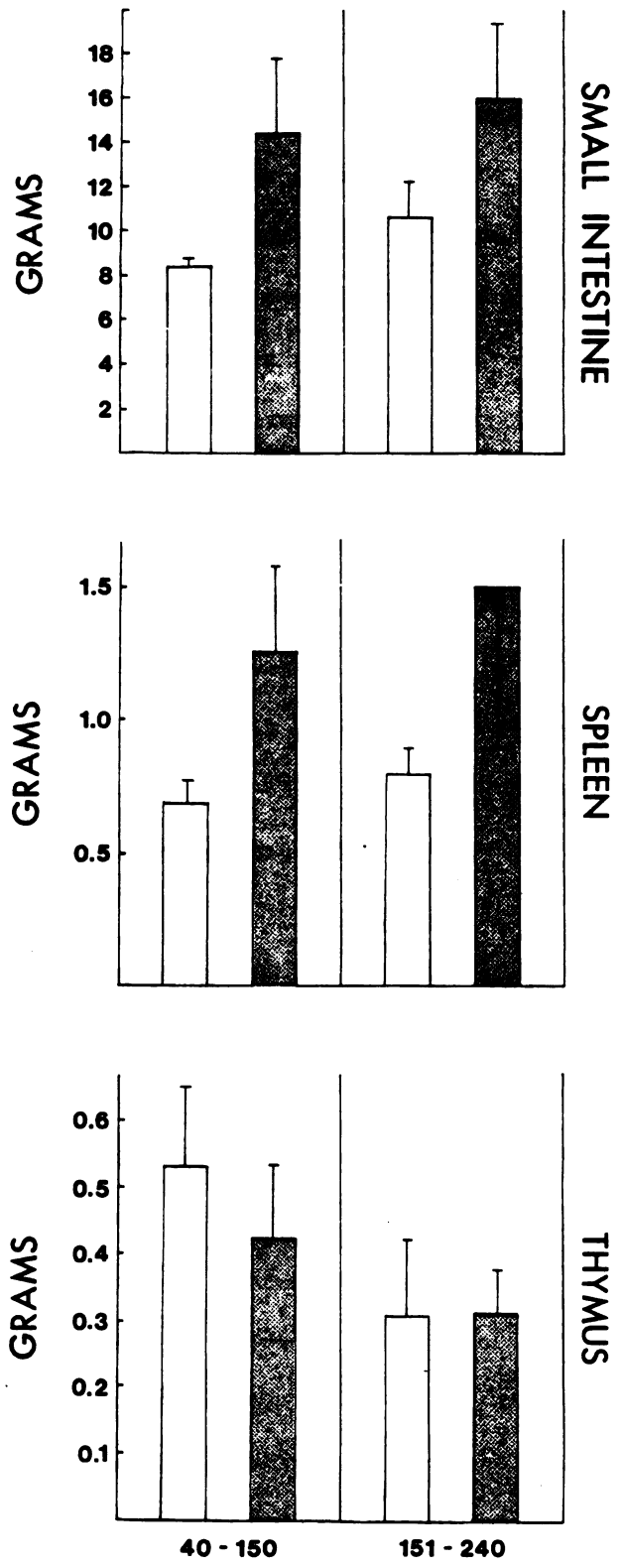

STOMACH WEIGHT (G)
16
14
12
10
8
6
4
2
80
120
160
200
240
280
320
360
400
DAYS POST INFECTION

FIGURE 2

Glandular stomach from rat infected with Taenia taeniaeformis for 160 days (above) and normal age-matched control (below). The hyperplastic stomach (16 g) shows multiple papillary outgrowths (arrowed) and the demarcation of the antrum, visible as a paler area in the normal rat, has been obliterated.

FIGURE 3

Changes in visceral organ weights in rats infected with Taenia taeniaeformis. The differences between infected (stippled bars) and normal (open bars) rats were highly significant ($P < 0.01$) in all instances, except for the thymus at 151-240 DPI. Range markers indicate ± SE.


GRAMS
18
16
14
12
10
8
6
4
2
SMALL INTESTINE
GRAMS
1.5
1.0
0.5
SPLEEN
GRAMS
0.6
0.5
0.4
0.3
0.2
0.1
THYMUS
40 - 150
151 - 240
DAYS POST INFECTION

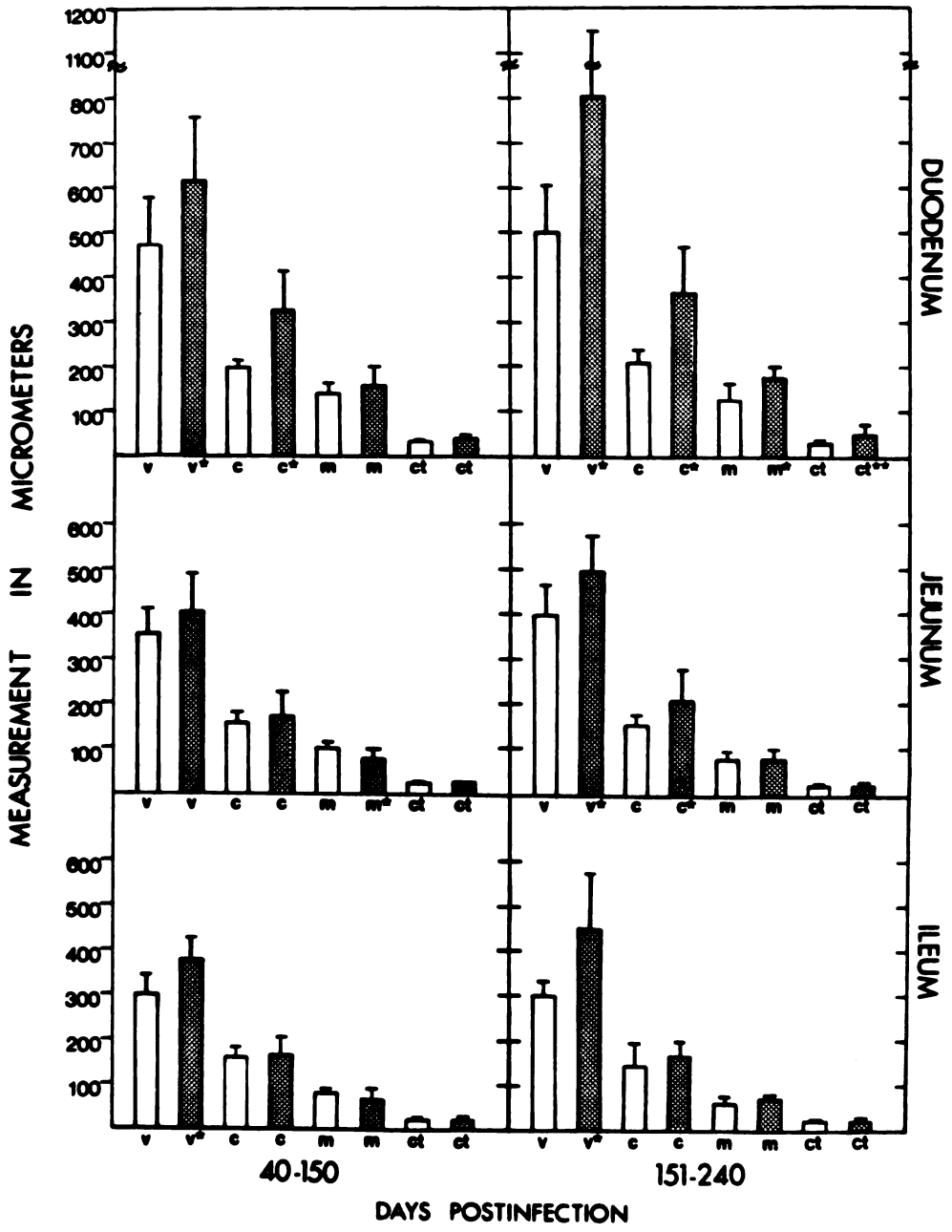
most marked in the duodenum. The dimensions of the villi and crypts were significantly affected ($P < 0.01$), with changes in the muscle and intestinal connective tissue levels being variable and less extensive. The smooth muscle layer in the duodenum of infected rats was significantly thicker ($P < 0.01$) than in control rats at all stages.

The hyperplastic mucosa of the small intestine at each level showed progressive distortion of normal architecture due to the accumulation of mucus and subsequent dilation of the crypts (Figure 5). In the most chronic cases these dilatations contained a mixture of strongly PAS-positive mucus (Figure 6), sloughed epithelial cells and pyknotic nuclear fragments, which often became calcified in rats in the second group (151-240 DPI) (Figure 7). Severely affected intestines in older animals were gritty to the touch. Villar tips eventually became club-like and there was very little epithelial integrity over their distal portions. The lamina propria of the expanded tips was heavily infiltrated with a mixture of chronic inflammatory cells, predominantly lymphocytes, macrophages and some plasma cells. In the duodenum, especially, villi were remarkably elongated, and in the oldest infections many were 1-2 mm in length. Some of these villi became fused into broad outgrowths with massive lymphocytic cellular infiltration.

Neither the esophagus nor the cardiac region of the stomach was involved in the hyperplastic changes at any stage of infection. Colonic changes were extremely variable; in most animals no gross alterations were evident but a few infected individuals had markedly thickened rugae (Figures 8 and 9). However, this did not occur with sufficient consistency to be statistically significant when data from infected and control groups were compared.

FIGURE 4

Changes in dimensions of small intestinal tissues in rats infected with Taenia taeniaeformis. Significant differences between infected rats (stippled bar) and normal rats (open bar) are denoted by * ($P < 0.01$) or ** ($P < 0.05$). Abbreviations used: V (villar length); C (crypt depth); CT (connective tissue overlying smooth muscle); and M (smooth muscle).


MEASUREMENT IN MICROMETERS
DUODENUM
JEJUNUM
ILEUM
40-150
151-240
DAYS POSTINFECTION

FIGURES 5-9

5. Histological appearance of duodenum of rat infected with Taenia taeniaeformis at 160 DPI. Cystic dilations of crypts (arrowed) contain cell debris and mucus. Tips of elongated, irregularly shaped villi lack epithelial covering and the interstitial lamina propria is heavily infiltrated with mononuclear inflammatory cells. H and E X 40. 6. Periodic acid-Schiff stained section of duodenum at 160 DPI. Dilated crypt (arrowed) contains PAS-staining mucus. X 112. 7. Calcium deposition (black staining areas) in dilated duodenal crypt by Kossa's silver deposition method. X 184. 8 and 9. Colonic mucosal thickening in rat at 80 DPI with Taenia taeniaeformis (Figure 8) compared with normal age-matched control (Figure 9). H and E X 28.

5
6
7
9

Throughout the infection mast cell numbers/V.C.U. in the small intestine remained at levels which were significantly higher than in the controls (Figure 10).

Serum gastrin levels in chronically infected rats:

Circulating gastrin levels in terminal blood samples from 46 chronically infected rats are shown in Figure 11. At 155-221 DPI gastrin levels were elevated in almost all rats showing hyperplastic gastropathy. Thereafter the relationship was much less regular, and many animals with enormously enlarged stomachs had circulating gastrin levels in the normal range (5-120). Five of the 7 rats which had been infected for 1 year or more had normal serum gastrin levels, although their stomachs were 2 to 8 times the normal size.

Attempts to induce changes by inoculation or implantation:

There were no significant differences between the wet weights of the stomach and intestines in the normal controls and any of the groups of rats inoculated with SS, IVP, or saline after 21 days of treatment. All groups showed normal increases in body weight over the course of the experiment, and terminal serum gastrin levels in all rats were within the normal range. The small intestines of rats given eggs of T. taeniaeformis were slightly but significantly heavier than those of other groups (12.38 g vs 9.48, $P < 0.01$), but the stomach weights and gastrin levels in these animals were normal. Mucosal mast cell counts in the small intestines of the infected rats were significantly higher than those in the normal controls (17.2 MMC/VCU vs 8.9 MMC/VCU, $P < 0.01$), but MMC levels in the rats which received inoculations were all in the normal range.

Rats given 40 live strobilocerci by surgical implantation 21 days previously showed no differences from normal age-matched controls in any of the parameters measured. Almost 100% of the parasites survived in recipients. When rats given implants of 10 parasites were left for 70 days the parasites survived equally well, but again there were no measurable effects on the gastrointestinal system or on circulating gastrin levels. The wet stomach weights of rats given implants 70 days previously ranged from 1.4-1.7 g, whereas those of the infected control group ranged from 6.6-18.9 g. The corresponding range for the uninfected age-matched controls was 1.4-1.6 g. Mucosal mast cell counts in implant recipients did not differ from uninfected control values, whereas in infected rats MMC numbers were approximately three times normal.

FIGURE 10

Changes in duodenal mucosal mast cell (MMC) counts in rats infected with Taenia taeniaeformis (stippled bar) and normal age-matched controls (open bar). Differences were highly significant ($P < 0.001$) for both infection periods.

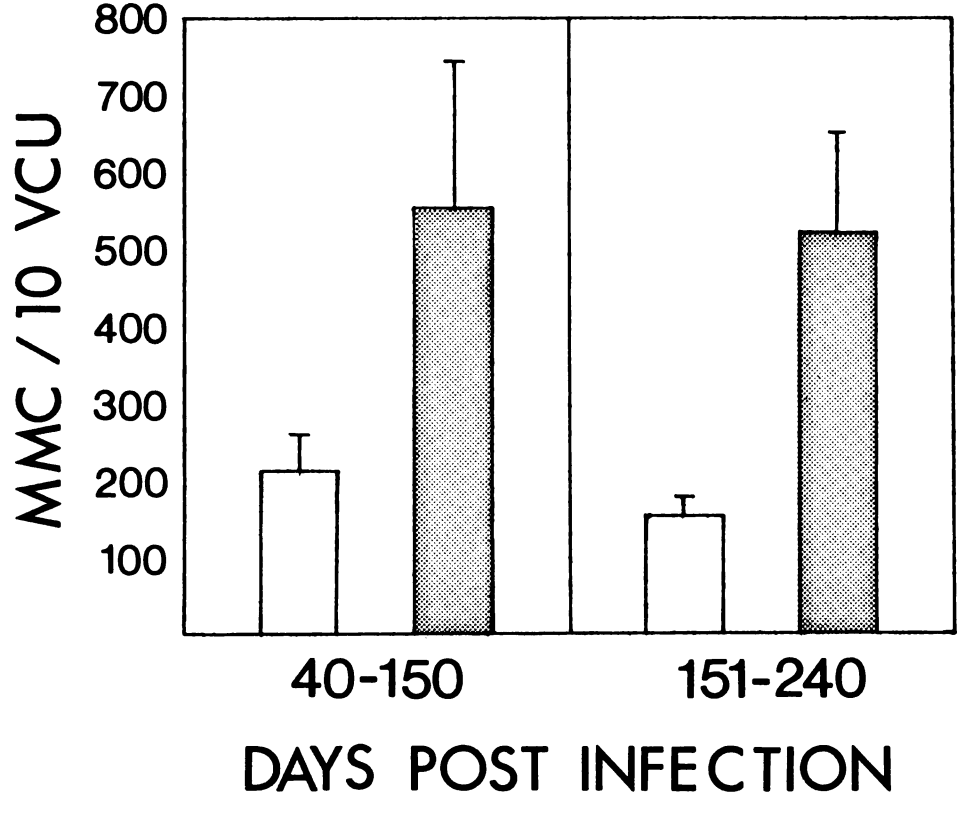
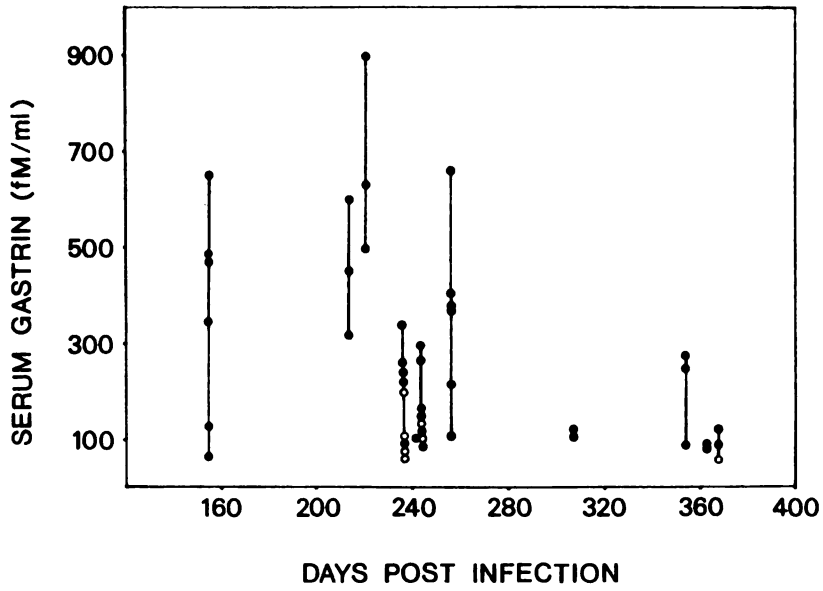

800
700
600
500
400
300
200
100
MMC / 10 VCU
40-150
151-240
DAYS POST INFECTION

FIGURE 11

Serum gastrin levels at necropsy in rats chronically infected with Taenia taeniaeformis. Closed circles denote rats in which gastric hyperplasia was grossly evident; open circles indicate infected rats in which no gross hyperplastic changes were visible and stomach weights fell within the upper limit of the normal range (2.24 g). In normal uninfected rats the mean gastrin level was 27.4 fm/ml ± 4.12.


SERUM GASTRIN (fM/ml)
900
700
500
300
100
160
200
240
280
320
360
400
DAYS POST INFECTION

DISCUSSION

Since the hyperplastic changes in the mucosa of the stomach and intestines tended to intensify than subside with duration of infection, our results suggest that the influence of hepatic T. taeniaeformis larvae is sustained for many months. However, some rats appeared to be remarkably responsive to the stimulus for gastric change, because stomach weights up to 6.2 g were recorded as early as 63 DPI. Small intestinal weight changes were always lesser in degree than those in the stomach. This observation, together with the quantitative differences we saw between the extent of the increases in villar length and crypt depth in the duodenum compared to the jejunum and ileum, and the inconsistent pattern of effects on the colon, suggest that there is a gradient of responsiveness to T. taeniaeformis in gastrointestinal tissues.

A comparable gradient has been established for the trophic effects of gastrin (Johnson, 1980). However, in gastrin-induced hyperplasia, both the esophagus and the antrum are spared, whereas in our rats only the esophagus was unaffected; the antrum became hyperplastic in all affected stomachs. This finding, and the evidence that serum gastrin levels in severely affected rats eventually declinced to normal levels, indicate that hypergastrinemia is a secondary phenomenon rather than a primary causal factor in the onset and maintenance of hyperplasia. Nevertheless, we cannot discount the possibility that the high levels of gastrin which we detected could have augmented the mucosal hyperplasia. Prolonged high levels of this hormone, induced experimentally or occurring as a feature of a natural disease state, consistently result in wet weight increases in the stomach (Crean et al., 1969).

Immunohistochemical identification of gastrin-producing cells in affected stomachs at different stages of infection may shed some light on why serum gastrin levels follow the pattern we detected here.

The spectrum of pathological changes we saw suggests that mucosal elements are almost wholly responsible for the organ wet weight increases. It seems more reasonable to attribute slight increases in the thickness of smooth muscle layers; e.g., duodenum at 151-240 DPI, to hypertrophic adaptation, rather than to a peculiar hyperplastic receptiveness of this component of the tract. We have no explanation for the slight decline in jejunal muscle thickness (Figure 4). However, recent radiographic studies of intestinal motility have demonstrated the onset of profound aberrations in peristaltic patterns in the first several months of infection with T. taeniaeformis, and these may reflect muscle layer thickness changes at different levels of the gastrointestinal tract. (Dr. Ruby Perry, Michigan State University, personal communication.)

The histological appearance of the cystic dilatation of the crypts in the small intestinal mucosa suggests that the hyperplastic villar epithelium had blocked crypts and resulted in the accumulation of mucus and sloughed epithelial cells. The ensuing calcification was most likely a response to the presence of multiple chronic necrotic foci, rather than representative of any parasite-induced abnormality in calcium deposition, although the latter cannot be ruled out as a contributing factor without further study. Calcium mobilization and deposition are affected in some animals by chronic hypergastrinemia (Krishnamara and Limlomwongse, 1978). The intense inflammatory cell infiltration which characterized villi in

chronically infected rats is probably also attributable to the uncontrolled growth of the epithelium with resulting disorganization of normal vascularization and cell necrosis.

The induction and maintenance of hyperplastic changes in the stomach and gut by parasites confined to the liver suggested to us that some chemical mediator might be responsible (Cook et al., 1981b). The demonstration that the pathological syndrome could be reproduced in non-infected rats by parabiosis supported this notion, and prompted our attempts to induce hyperplasia by inoculation of parasite products. There are important precedents for this in the discovery that metacestodes of Spirometra mansonoides manufacture a growth hormone-like chemical in mice (Steelman et al., 1979), and the recent observation that Fasciola hepatica causes bile duct hyperplasia by release of large amounts of proline (Isseroff et al., 1977). The results reported here offer no support for the proposal, although, since our approach was necessarily empirical, we cannot rule out the possibility that inoculations of larger amounts of T. taeniaeformis material given more frequently, or perhaps over a more prolonged period, would have induced some measurable changes. Likewise, the failure of implanted larvae to produce any changes might be attributable to the shortness of exposure or the low numbers of parasites involved. However, in the instance of both inoculation and implantation experiments, the total mass of parasite material to which the rats were exposed probably exceeded that experienced during infection; during the induction phase of hyperplasia the organisms are still relatively small (up to 5 mg dry weight each by 63 DPI) compared with the fully developed metacestodes (50 mg) we used here (Williams et al., 1981). Nevertheless, it is possible that the

putative trophic factor is produced in much greater amounts by young stages than by strobilocerci. A highly labile carcinogenic factor has been demonstrated in T. taeniaeformis (Dunning and Curtis, 1953), and a trophic factor with comparable lability would not have been detectable in our inoculation experiments; on the other hand, the persistence of live healthy parasites in the peritoneal cavity could have been expected to overcome this potential shortcoming.

In parallel with the proposal that some parasite product induces hyperplasia directly, is the equally tenable hypothesis that the parasites exert a primary influence on an intrinsic host regulatory mechanism, external to the gastrointestinal tract, which is eventually manifested through trophic effects on stomach and intestinal mucosal surfaces. The induction of hyperplasia by parabiosis is consistent with this possibility, and experimental approaches aimed at characterization of the agent in infected rat serum may enable us to resolve the issue. It would be especially advantageous if short-term criteria of hyperplasia, such as the proliferation of gastric or duodenal mucosal cells in vitro, could be used to detect trophic activity.

At this time the splenic, thymic and MMC changes which we saw probably should be viewed as part of the complex of immune responses to the protracted development of the foreign tissue mass in the liver. Rodents rapidly become immune to homologous challenge after primary exposure to T. taeniaeformis, and in the later stages of infection a significant hypergammaglobulinemia develops (Chapman et al., 1979). Splenomegaly may be indicative of sustained specific antibody production at a high level, although the recent demonstration that extracts of cysticerci of Taenia solium contain a potent B-lymphocyte activator (Sulivan-Lopez et

et al., 1980), raises the possibility that a comparable product of T. taeniaeformis might be responsible for non-specific stimulation of splenic cells. The acute onset of thymic involution also has immunological implications, but in the absence of any evidence of alterations in T cell-dependent reactivity in T. taeniaeformis-infected rats, little significance can be attached to the changes.

Increases in MMC numbers of much shorter duration than that reported here have been described in nippostrongylosis and trichinellosis in rats, and incorporated into hypotheses concerning acquired resistance to intestinal nematodes (Murray et al., 1971). Despite this there is little direct evidence of immunological reactivity of MMC (Befus et al., 1980), and the occurrence of the very prolonged mastocytosis of the lamina propria which we saw can only speculatively be linked to acquired resistance mechanisms. However, there is some evidence of a role for gastrointestinal hormones in the regulation of mast cell development (Upenski et al., 1977), and this observation emphasizes still further the need to characterize the biological activities of helminth products and their interactions with host endocrinological systems in the pathogenesis of parasitic diseases. The rat-T. taeniaeformis model appears to offer an especially appropriate opportunity for further research in these directions.

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CHAPTER 2

HYPERPLASTIC GASTROPATHY IN RATS INFECTED WITH *TAENIA TAENIAEFORMIS*: HISTAMINE LEVELS IN GASTRIC TISSUES

by

David M. Blaies and Jeffrey F. Williams

INTRODUCTION

In rats infected with Taenia taeniaeformis, the stomach and small intestine become hyperplastic, the gastric luminal pH is elevated, and there is prolonged hypergastrinemia (Cook and Williams, 1981; Cook et al., 1981). The underlying mechanisms which bring about these changes, and the advantages, if any, which accrue to either host or parasite as a result, are unknown. However, there is strong evidence that histamine is the final common mediator of normal gastric acid secretion (Code, 1977), and it is possible that lowered tissue levels of histamine may be involved in aberrant regulation of secretion in infected rats; altered histamine levels would, in turn, influence circulating gastrin.

We have measured the histamine concentrations in pathologically affected gastric tissues over the course of infection in rats, and report here on their relationships to the mass of the stomach. The results show that histamine levels in infected rats usually fall within the normal range, but that in some animals there are very significant elevations. Further work will be required to determine if elevated levels are associated with a specific stage of development of the lesion, or occur intermittently in most affected animals.

MATERIALS AND METHODS

Experimental Infections

Female Sprague-Dawley derived Spartan rats (Spb: [SD]) were purchased from Spartan Research Animals, Haslett, Michigan at 28 days of age and infected with T. taeniaeformis as described previously (Leid and Williams, 1975).

Twenty-four rats were given 2000 eggs each and 32 age-matched non-infected animals were used as controls. Six normal rats were killed at the beginning of the experiment to establish baseline histamine levels, and thereafter groups of 6 infected and control rats were killed at intervals of 20 days postinfection (DPI). Twenty-three other rats, infected with 1000 T. taeniaeformis eggs, were allowed to develop palpable gastric hyperplastic changes and then euthanized at 277-428 DPI in order to measure the histamine content of their enlarged stomachs.

Sampling and Processing

The rats, killed by exposure to CO_2 vapor, were quickly exsanguinated before removal of the stomach. The wet weight of the organ was recorded and approximately one gram samples were excised from the fundus for histamine extraction.

Tissue samples were placed immediately in 1.0 ml aliquots of 0.012 N HCl and homogenized with a Brinkmann Polytron (Brinkmann Instruments, Westbury, NY). Homogenates were then centrifuged for 10 minutes at 600 x G and the supernatants were collected and diluted 10-100 fold for histamine assay by the fluorometric method of May et al. (1970). The procedure was modified from the original in that 0.012 N HCl, not 0.12 N HCl, was used for extraction, and the o-phthalaldehyde condensation

step was performed without internal standards. Instead, histamine solutions of known concentration were processed in parallel with tissue samples and the results were compared with unprocessed standards. Histamine hydrochloride and o-phthalaldehyde were purchased from Sigma Chemical Co., St. Louis, MO.

RESULTS

The stomach weights of infected rats with viable parasites increased noticeably by 40 DPI and remained elevated thereafter. The largest stomach weighed 28.8 grams at 60 DPI.

Gastric histamine levels varied widely in normal rats, with an arithmetic mean of 25.8 μg/g ± 10.6 (SD). The distribution of the values obtained for both infected and normal rats is shown in Figure 1. In most of the hyperplastic stomachs the histamine levels were within ± 2 SD from the mean for normal rats. Seven of the 47 infected rats had levels above this range (Figure 2).

FIGURE 1

Distribution of gastric histamine levels in normal rats and rats infected with Taenia taeniaeformis. The solid line represents normal animals.

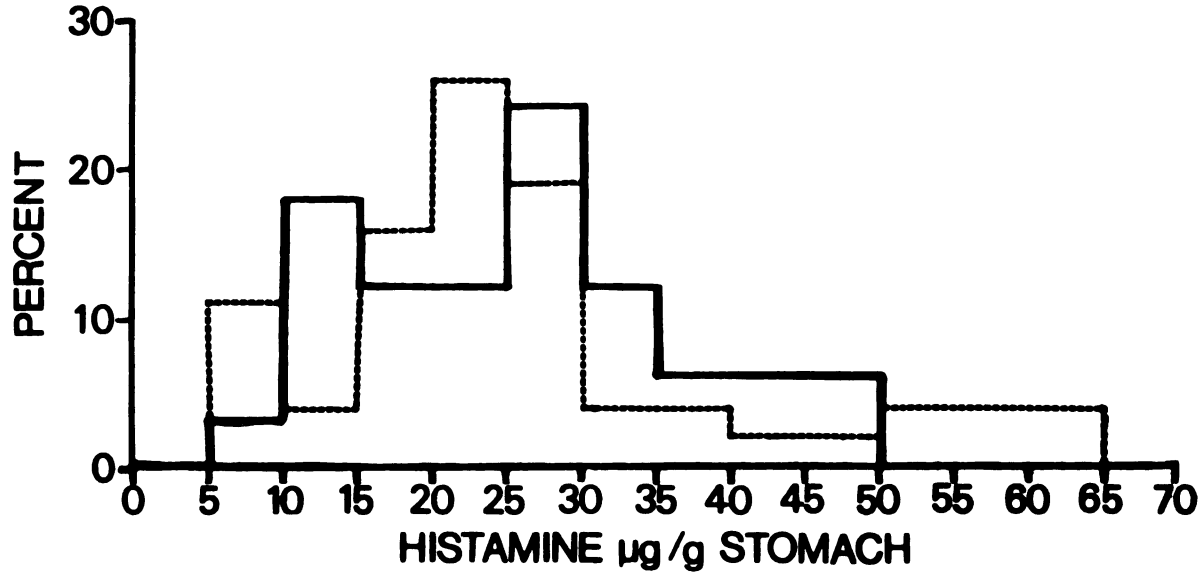
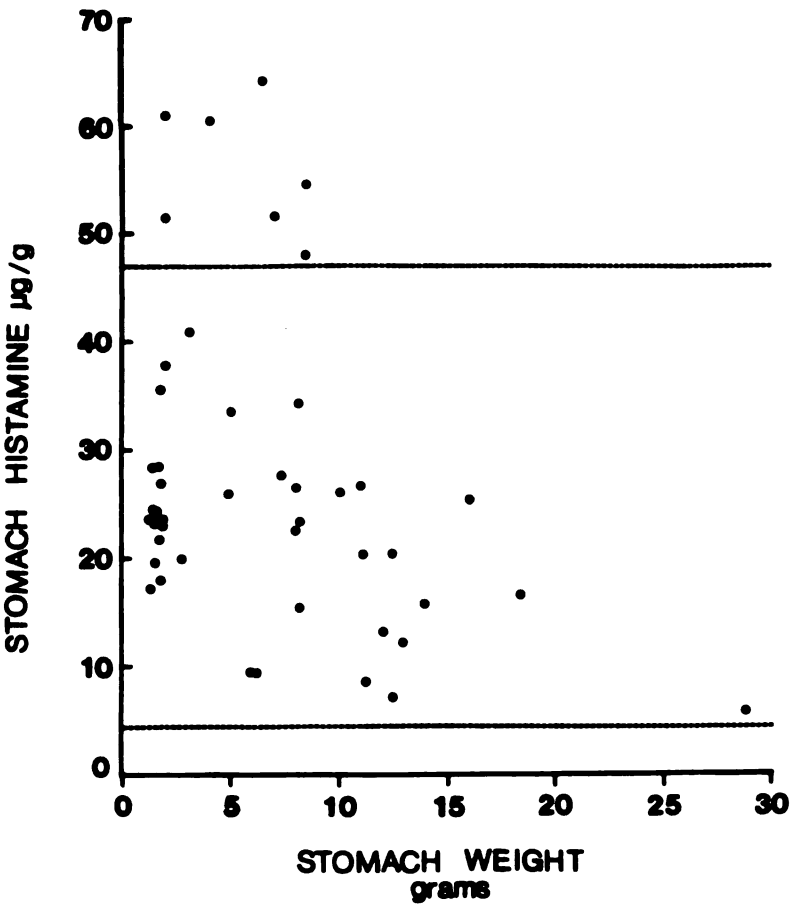

30
20
10
0
PERCENT
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
HISTAMINE µg/g STOMACH

FIGURE 2

The relationship between gastric histamine concentration and stomach weight in infected rats. The dotted lines delineate ± 2 SD from the average normal histamine concentration of 25.8 μg/g.


70
60
50
40
30
20
10
0
STOMACH HISTAMINE µg/g
0
5
10
15
20
25
30
STOMACH WEIGHT
grams

DISCUSSION

Our original hypothesis was that the histamine-containing cell population of enlarged stomachs was probably diminished, because a deficit in the final-common mediator to acid secretion could account for the hypoacidity of gastric contents in infected rats. The levels of stomach histamine in normal rats reported here are within the ranges reported by Gustafsson et al. (1957) and Kowalewski et al. (1969). Histamine levels in hyperplastic stomachs were generally normal, although in some cases they were significantly elevated. Therefore, it appears unlikely that a lack of endogenous gastric histamine could be responsible for the failure of acidification in parasitized rats.

The elevated levels of histamine, which occurred in 18% of the infected rats, might derive from supplemental histamine supplied by mucosal mast cells (Soll et al., 1979), in addition to the normal histamine-containing cells of the rat stomach (Thunberg, 1967). Mast cells have been seen in previous experiments in the mucosa of the enlarged stomachs, but there is no histological data to support the possibility that they increase at any stage of the infection in numbers sufficient to affect overall histamine levels.

The quantity of mucus in the stomach of infected rats greatly exceeds the normal amount, probably due to the disproportionate growth of mucus-producing cells (Cook and Williams, 1981). This layer of mucus may coat the surface of hyperplastic stomachs so thickly that perietal cells and other acid-secretion-modulating cells do not receive the correct stimulus. The histamine-containing cell population may then

respond by proliferating or accumulating more histamine per cell; either mechanism would lead to elevated tissue concentrations.

Gastric histamine concentration elevations could also result from the mucosa having become so hyperplastic that the gastric glands themselves are occluded and parietal cells then are deprived of access to the stomach lumen; this would prevent them from releasing acid. Circulating gastrin may increase in response to a demand for acid production, and endogenous histamine levels may rise in a viscious cycle of stimulation without feedback inhibition.

Clearly, in order to draw meaningful conclusions about the physiological responsiveness of the stomach in infected rats, more experimental analysis is needed. Other histamine-containing cells, such as gastric mucosal mast cells, must be examined to determine the extent of their impact on the total endogeneous histamine pool. Parietal cell numbers and morphology must be established and the secretory ability of these cells examined. These kinds of experiments should enable us to characterize hormonal and digestive abnormalities in rats infected with T. taeniaeformis and understand their pathogenesis in relation to the normal processes of gastric function and growth.

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CHAPTER 3

CHANGES IN HISTAMINE CONTENT AND MAST CELL DENSITY IN THE HYPERPLASTIC SMALL INTESTINES OF RATS INFECTED WITH *TAENIA TAENIAEFORMIS*

by

David M. Blaies and Jeffrey F. Williams

INTRODUCTION

Rats infected with the metacestode Taenia taeniaeformis develop prominent extrohepatic abnormalities including intestinal mastocytosis, elevated stomach lumen pH, hyperplasia of the stomach and small intestine, and hypergastrinemia (Cook and Williams, 1981; Cook, Williams and Lichtenberger, 1981). The advantages, if any, which these changes might provide for the parasite are obscure, although there is now evidence that mucosal mast cells (MMC) generated in response to intestinal helminthiasis may release amines in connection with worm expulsion (Nippostrongylus brasiliensis in rats, Befus et al., 1979 and Trichostrongylus colubriformis in guinea pigs, [Jones et al., 1978]); intestinal mastocytosis may therefore enhance resistance to further challenge in T. taeniaeformis infected rats.

Early attempts to determine if vasoactive amines would directly affect the flux of T. taeniaeformis oncospheres penetrating the gut wall yielded inconsistent results (Musoke et al., 1978). However, rather than having a direct effect on the oncospheres, endogenous amines may influence the parasite indirectly via local tissue defense factors, as has been suggested for gut by Murray et al. (1971), for mesentery by Norrby (1980), and for skin by Kahlson and Rosengren (1971). This study focusses on endogenous histamine concentrations and MMC numbers in the small intestine of rats during chronic infections with T. taeniaeformis; the results are compared to those derived from appropriate age-matched control rats.

Infected and normal rats both showed a gradient of histamine distribution which increased from duodenum towards ileum. Histamine

concentrations and MMC counts rose above normal levels in infected rats by 40 days postinfection (DPI) and remained elevated to the end of the study. Increases in histamine concentration were seen in the duodenum, jejunum, and ileum, but the pattern of responses was not the same in each segment. We were unable to correlate histamine concentrations with mast cell counts in either the enlarged intestines of the parasitized rats or those of the normal rats.

MATERIALS AND METHODS

Experimental Infections

Female Sprague-Dawley derived Spartan rats (Spb: SD), purchased from Spartan Research Animals, Haslett, Michigan, were used in the manner detailed by Williams et al. (1981). Twenty-four randomly selected rats were dosed with 2000 T. taeniaeformis eggs each, and thirty-two rats were maintained as non-infected age and sex matched controls.

Experimental Design

Rats were killed in paired groups of six parasitized animals and six control animals at 20, 40, 60, and 80 DPI, along with six normal rats killed at the beginning of the study to establish baseline values (0 DPI).

After euthanasia with carbon dioxide gas, the small intestine was removed from each rat and flushed with warm tap water to remove the chyle. The gut was then divided into equal thirds with the proximal end of each segment representative of duodenum, jejunum, and ileum. From each segment a sample piece of approximately one gram was removed for histamine analysis, along with an adjacent two centimeter length that was everted, then fixed in Carnoy's fluid for histological examination.

Tissues to be processed for histamine analysis were immediately added to 1.0 ml of 0.12N HCl and homogenized with a Brinkmann Polytron. The homogenates were centrifuged for ten minutes at 600 X g to remove solid particulate matter. This procedure was followed until all the samples to be assayed were assembeld. Preliminary calculations indicated that the supernatant should be diluted in double distilled water 10-100 fold to fall within range of the histamine standards we used.

Histamine content was determined by the fluorometric method of May et al. (1970), modified in this manner: the extraction of histamine from heptane was done with 0.012N HCl rather than 0.12N HCl as described in the original method, and the condensation step was performed without internal standards. Instead, external standard histamine solutions were used along with separate standard solutions carried through all the extraction procedures as extraction controls. The o-phthaldehyde and histamine dihydrochloride were purchased from Sigma Chemical Co. (St. Louis, Missouri).

Counting and Statistics

Assessments of MMC populations were made on histological sections stained with astra blue FM as described by Blaies and Williams (1981a). The MMC were then enumerated in 5 groups of 10 villus-crypt units as has been done by Jarrett et al. (1967) and Cook and Williams (1981). The MMC counts were pooled to give the arithmetic mean of the number of MMC per VCU per rat. These numbers were adjusted for tissue weight because of observations (Blaies and Williams, 1981b) that the predominant intestinal weight increase is due to villar hyperplasia; since MMC are exclusively located in the villi of T. taeniaeformis infected rats, this adjustment rectifies the VCU counts between normal and infected rats on a weight-proportion basis.

The statistical significance of all results was determined using a two-tailed Student's "t" test.

RESULTS

The tissue histamine assay results are illustrated in Figures 1-3 and Tables 1 and 2. A predominant finding was that the largest concentration of histamine occurred in the ileum in both normal and infected rats. As shown in Figure 2, the ileum was also the site of the primary histamine increase at 40 DPI, and the level remained high thereafter.

Total intestinal histamine, calculated as the sum of the quantity of histamine in each gut section, was markedly increased in parasitized rats over normal rats. One individual had more than 1 mg at 60 DPI (Figure 3); this is greater than 10 times the average normal level.

The changes in intestinal histamine were compared to changes in the MMC population in the ileum, the results of which are shown in Table 2. MMC were predominantly located in the lamina propria, but, rarely, astra blue staining cells were seen in the epithelium. During the course of infection, gross MMC counts rose to as high as 6 times normal, but when the numbers were corrected for tissue weight, the net MMC density approximately doubled. There was no linear correlation between MMC counts and histamine levels; the [histamine]/cell ratio was not stable during the experiment.

Two rats killed at 60 DPI and one killed at 80 DPI had overcome the parasites and both had approximately normal organ weights, MMC counts, and histamine levels. These animals were not included in data on histamine level trends since they were not representative of normal infections.

FIGURE 1

An overall view of the changes in intestinal histamine concentration following oral administration of Taenia taeniaeformis eggs. The speckled bars represent normal rats and the solid bars represent infected rats. Each of the three sections of gut we examined is represented in the order of duodenum, jejunum, and ileum as shown in the key at the upper right of the graph. Plateaus are the mean of all values in that group with +1 SE marked.

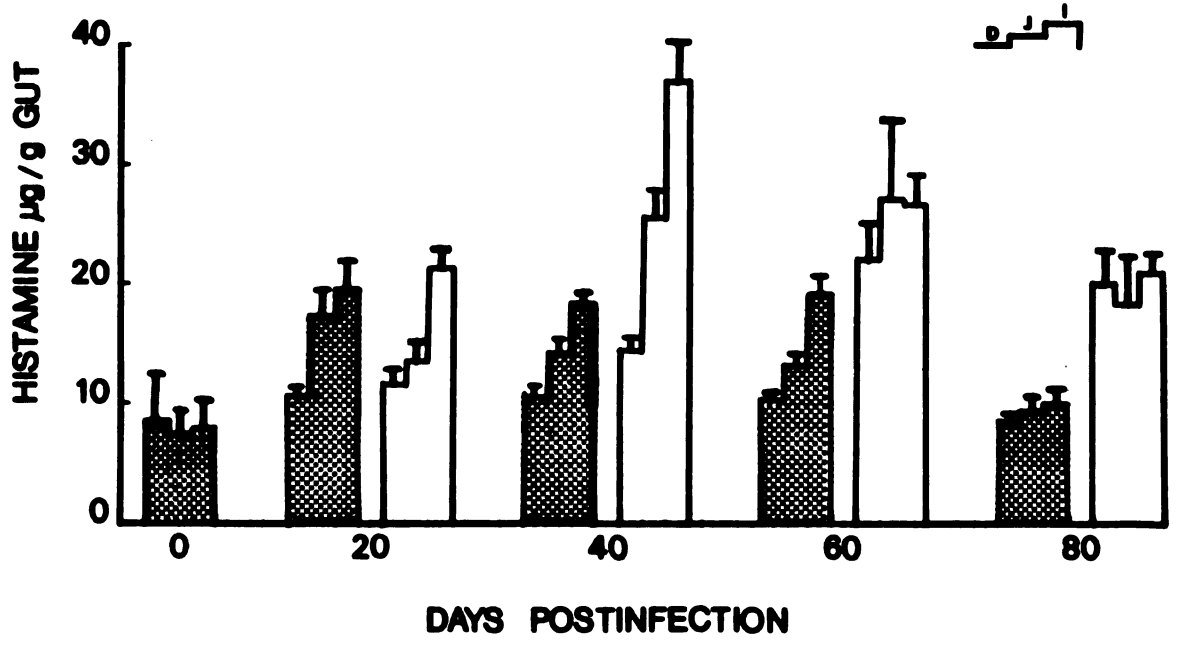
HISTAMINE µg/g GUT
40
30
20
10
0
0
20
40
60
80
DAYS POSTINFECTION

FIGURE 2

A comparison of the histamine concentration elevations in the duodenum and ileum of rats infected with Taenia taeniaeformis.

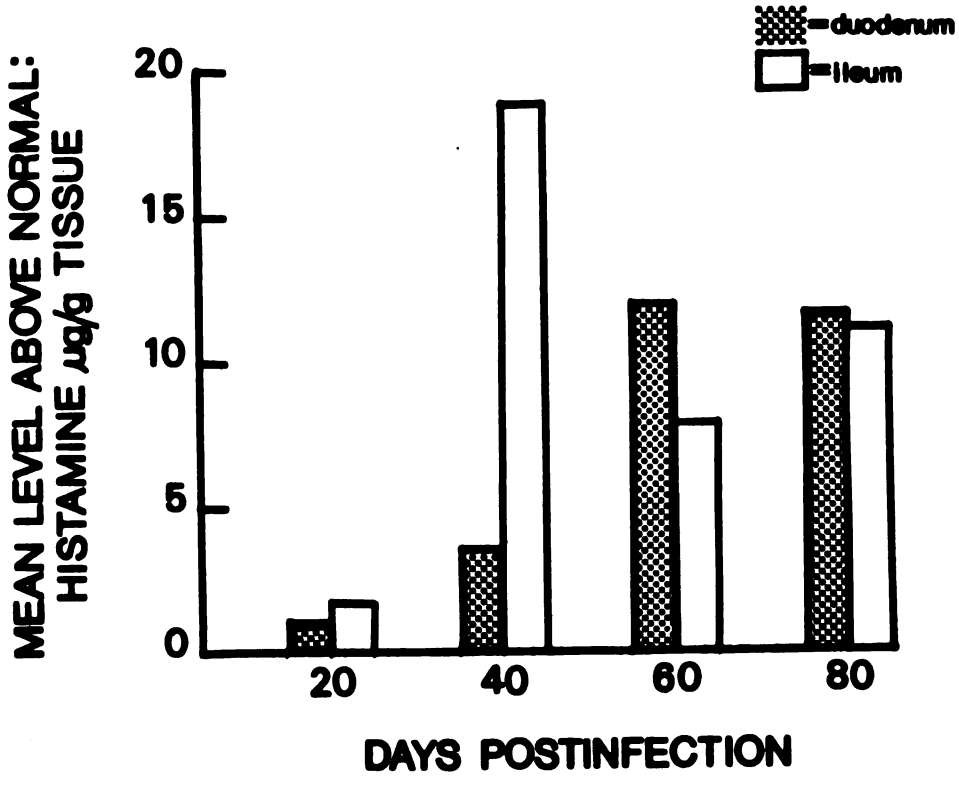
MEAN LEVEL ABOVE NORMAL:
HISTAMINE µg/g TISSUE
20
15
10
5
0
20
40
60
80
DAYS POSTINFECTION
=duodenum
=ileum

TABLE 1

Comparison of Histamine Levels in

Taenia taeniaeformis Infected and Control Rats

DPI (Days Post-Infection)	Gut[1] Section	Infected Rat Mean µg/g	Control Rat Mean µg/g	P Value
0	D		8.66	
0	J		7.31	
0	I		7.63	
20	D	11.73	10.63	NS[2]
20	J	13.73	17.35	NS
20	I	21.56	19.76	NS
40	D	14.40	10.51	< .05
40	J	25.80	14.12	< .001
40	I	37.10	18.18	< .001
60	D	22.16	10.23	< .01
60	J	27.14	13.03	< .05
60	I	26.84	19.04	< .05
80	D	20.27	8.50	< .01
80	J	18.69	9.51	NS
80	I	21.11	10.00	< .001

[1] D = duodenum, J = jejunum, I = ileum

[2] NS = not significant

FIGURE 3

The total small bowel histamine values for infected rats (large dots) and normal rats (small dots) from the start of the investigation to 80 DPI.

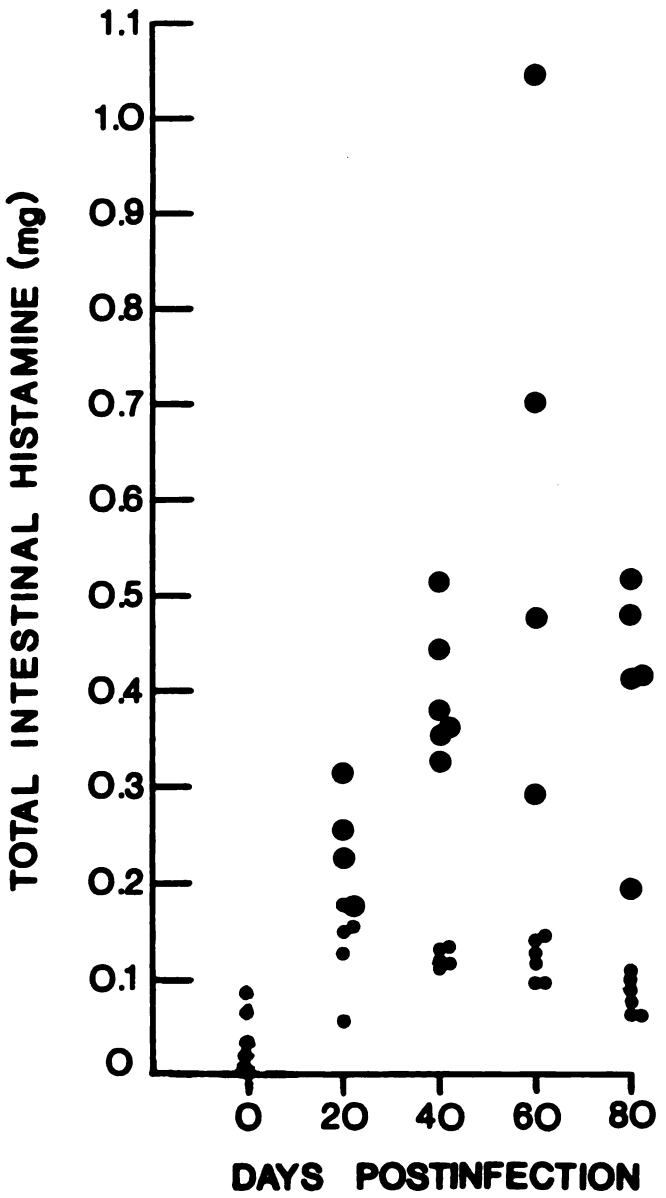
TOTAL INTESTINAL HISTAMINE (mg)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
DAYS POSTINFECTION

TABLE 2

Histamine Levels and Mucosal Mast Cells/10 Villus-Crypt Units for Normal and Infected Animals

	Normal Animals		Infected Animals	
	MMC/10 VCU	Hist µg/g	MMC/10 VCU*	Hist µg/g
0 DPI	77	11.4		
	63	6.3		
	88	3.4		
	90	13.7		
	98	13.8		
	81	15.3		
20 DPI	134	11.1	397	17.9
	192	29.1	396	16.9
	177	21.6	378	22.3
	189	18.3	358	21.7
	207	16.8	381	25.5
	209	21.6	400	25.1
40 DPI	144	17.4	517	35.9
	121	19.9	482	50.4
	159	14.4	490	25.6
	138	18.4	533	41.2
	127	20.8	587	35.9
60 DPI	138	21.3	662	21.8
	132	22.6	—	—
	168	24.8	1461	27.9
	180	15.4	—	—
	175	12.7	711	24.5
	185	17.5	882	33.0
80 DPI	203	13.6	1412	27.3
	89	10.0	825	23.1
	145	7.8	—	—
	171	6.9	442	16.3
	161	13.4	1207	23.3
	155	8.3	908	19.3

*MMC counts are corrected for weight of the tissues.

DISCUSSION

The cell responsible for endogenous stores of histamine in the small intestine has been considered to be the MMC, as a result of the work of Enerback (1969). He was able to detect histamine via visualization of fluorescent o-phthalaldehyde-histamine complexes in the granular cells of the lamina propria. The concentrations of MMC-associated histamine in normal rat intestine have been reported by Gustafsson et al. (1957), Kowalewski et al. (1969) and Enerback and Wingren (1980), and are in the range of the values reported here. However, the gradient of increasing histamine levels towards the ileum which are found has not been described previously.

There are several possible causes for our failure to detect a significant correlation between MMC numbers and the increases in histamine levels during infection. There is evidence that the histamine content of mast cells is a function of their maturity, and during the prolonged mastocytosis which characterizes taeniiasis the constitution of the mast cell population may vary considerably. Furthermore, there appears to be some histochemical heterogeneity within the MMC populations in infected rats (Lindsay, 1981), and comparable variation could occur in terms of histamine content. There is also the possibility that infection with T. taeniaeformis induces the appearance of non-MMC histamine-containing cells, similar to those which occur in the rat stomach. The pathologic changes which are associated with this hepatic metacestode are unprecedented and therefore we cannot rule out any or all of the above circumstances contributing to the result we obtained.

Histamine and MMC increases have been described in a variety of other helminthiases but the relationship between these changes and the course of the parasitic infections is still controversial. Experiments on nippostrongylosis (Befus et al., 1978a) and trichostrongylosis (Jones et al., 1978) point to a role for MMC and histamine in worm expulsion, but evidence that the amines have direct effects is equivocal. Histamine in parasitized intestines could be involved in tissue growth and repair, as has been proposed by Kahlson and Rosengren (1971) for skin, and by Norrby (1980) for intestinal mesentery preparations. Histamine could also contribute to changes in vascular permeability to immunoglobulin, as suggested by Murray et al. (1971) in their work on plasma protein movements in nippostrongylosis. These mechanisms would all implicate histamine as a mediator of changes rather than as a toxic agent which acts directly on organisms, and, to this extent, the increases seen may reflect the importance of histamine in regulatory functions, similar to the stiuation visualized for it in gastric secretory modulation (Code, 1977; Sewaga et al., 1980). Exactly what functional alterations occur as a consequence of chronic cestodiasis in the rat remains to be seen, but the potential exists for increased histamine levels to influence uptake and transport of nutrients and secretion and release of host factors into the gut.

Recently, intestinal MMC increases have been associated with diseases in man and animals which are not caused by parasitism. For example, MMC proliferate in the duodenum and kidney of rats experiencing magnesium deficiency (Veilleux, 1975); and gut mastocytosis has been reported for human diseases such as hepatitis (Astaldi et al., 1966), Crohn's disease (Befus et al., 1979b), and Zollinger-Ellison syndrome (Dobbins et al.,

1969). At this time, there is not enough evidence to propose a role for the MMC in any of these pathologic states, but further work on the highly reproducible T. taeniaeformis system may well help to clarify the role of these cells and histamine increases in host defense or repair at the intestinal level.

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APPENDIX

A SIMPLIFIED METHOD FOR STAINING MAST CELLS WITH ASTRA BLUE

by

David M. Blaies and Jeffrey F. Williams

ABSTRACT

The copper phthalocyanin dye astra blue has been used to stain differentially mast cells of the intestine; however, the procedure has not been used widely because of the difficulty in preparing and using the dye solution. Described here is a simple, reliable, and consistent method for selectively staining mast cells using a dye solution that may be prepared in any laboratory without the aid of sophisticated pH metering equipment. Astra blue is mixed with an alcoholic solution containing $MgCl_2 \cdot 6H_2O$ and the pH indicator pararosaniline hydrochloride. Concentrated hydrochloric acid is added dropwise, changing the dye mixture from purple to violet and then to blue. In this low range the weakly ionizing ethanol provides a more stable hydrogen ion concentration than the corresponding aqueous solutions used previously. Alcoholic acid fuchsin is a convenient counterstain, and this simple procedure then provides good contrast between the blue staining mast cell granules and the red tissue background.

Use of the copper phthalocyanin dye astra blue for the demonstration of mast cells in tissue sections was first described by Bloom and Kelly (1960). Since that time the stain has been used by many investigators to characterize the distinct mast cell population in the rat which develops at mucosal surfaces, especially in response to intestinal infections with parasitic helminths (Enerback 1966, Miller and Walshaw 1972, Murray et al. 1968). A modified procedure has found application in the detection of basophil granules in human peripheral blood and bone marrow smears (Inagaki 1969). Under appropriate conditions the use of astra blue results in highly selective staining of sulfated mucopolysaccharides in mucosal mast cell granules, provided these have been retained through proper fixation. The corresponding absence of background color offers considerable advantage over the less selective Bismarck brown (Gottlieb et al. 1961) or thiazine metachromatic dyes such as azure A and toluidine blue (Enerback 1966).

A major disadvantage of the above procedure, which impairs markedly the reproducibility of the results, is the critical dependence of selective staining on a pH environment in the range 0.2-0.3. Solutions with this specific degree of acidity are difficult to produce with satisfactory consistency, and pH at this low level cannot be monitored accurately with most commonly used laboratory metering equipment. By contrast, the method which we describe obviates the need for sophisticated pH detection devices. The success of this technique depends on the use of alcoholic solutions of astra blue and the pH dependent color change of pararosaniline hydrochloride in acid alcohol in the presence of magnesium. It incorporates the principle of critical electrolyte concentration in mucopolysaccharide staining (Scott and Dorling 1965).

MATERIALS AND METHODS

Reagents

1. Dissolve 2 g $MgCl_2 \cdot 6H_2O$ and 0.1 g pararosaniline hydrochloride C.I. 42500 (Sigma Chemical Co., St. Louis, MO) in 80 ml 95% ethanol in an Erlenmeyer flask.
2. Dissolve 0.5 g astra blue FM (Chroma 1B163, Chroma-Gesellschaft, Schmid Co., W. Germany. U.S. distributor Roboz Surgical Instr. Co., 1000 Connecticut Avenue, Washington, D.C.) in 10 ml glass distilled water.
3. Slowly add the astra blue solution to the alcoholic mixture while stirring constantly.
4. Add 12 N HCl dropwise, dispensing from a burette, until the color of the solution changes from purple to violet, then to royal blue. The amount needed is approximately 9 ml; however, adding several drops more will not affect the stain adversely because in the weaker ionizing ethanol environment low hydrogen ion concentrations are less liable to fluctuate widely than in wholly aqueous solutions.
5. After allowing the solution to settle for 1 hour, filter through Whatman #144v paper. This stain may be stored at 22 C for months, but it should always be refiltered before use. If the color shifts toward violet on storage a few drops of 12 N HCl will restore the royal blue tint.
6. Prepare 5% (v/v) stock HCl in 70% ethanol for the rinsing solution. The final acid concentration should be 0.6-0.7 M.

If acid fuchsin C.I. 42685 (Matheson, Coleman and Bell, Norwood, OH) is

to be used as a counterstain, prepare a stock by adding 0.25 g to 100 ml of 1% (v/v) HCl in 95% ethanol. Further dilute this solution 1:100 in 1% acid ethanol to obtain a working stain containing 0.0025% (w/v) acid fuchsin.

Staining Procedure

1. Bring paraffin tissue sections to 95% ethanol.
2. Stain in astra blue solution for 30 to 60 minutes.
3. Rinse in the 5% acid ethanol solution until all the excess stain is removed.
4. Counterstain in acid fuchsin solution for 5 minutes. The solution is dilute enough so that the exact color intensity may be varied by lengthening or reducing the time.
5. Wash in 95% ethanol.
6. Dehydrate, clear, and mount.

RESULTS AND DISCUSSION

Mast cell granules stain intensely blue with no background coloration of connective tissue or cell nuclei. The specificity of the reaction for different tissues can be ensured by adjustment of the critical electrolyte concentration. For example, intestinal mucus stains faintly blue or not at all depending on the amount of $MgCl_2 \cdot 6H_2O$ added (Fig. 1). The stability of the staining reaction is such that sections may be left in the astra blue solution for several hours without detriment to the appearance of stained structures.

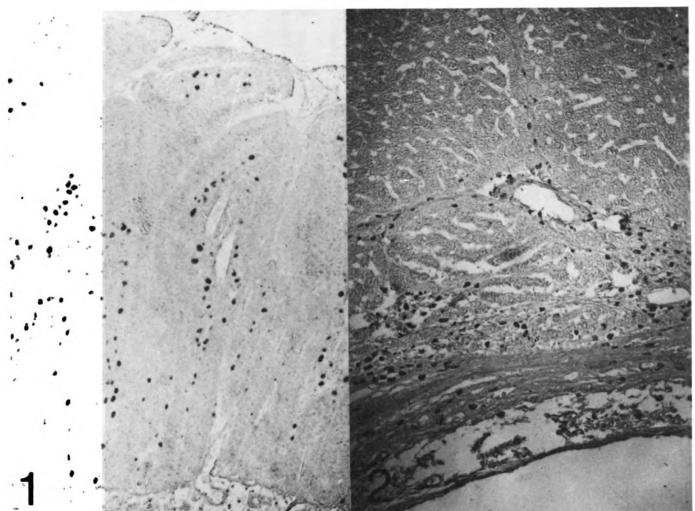
An additional advantage of the procedure is that it eliminates loss of tissue sections from slides, which frequently occurs with traditional astra blue methods. In these procedures, the low pH of the stain leads to hydrolysis of protein adhesives; when the aqueous solvent is replaced by alcohol for dehydration, surface tension changes often cause sections to float off. In our method the dye is used in an alcoholic environment and there is no abrupt solvent change to produce this effect.

The contrast between the deeply stained granules and the clear or lightly counterstained background facilitates detection and counting of mast cells, especially in circumstances where pathological increases are encountered. Mast cells in the intestinal lamina propria of both rats and humans stain well when the tissues are fixed in either Carnoy's fluid or lead subacetate. Rat liver mast cells are clearly identifiable, even when formalin is used as fixative (Fig. 2).

The simplicity and reproducibility of the procedure combined with its compatibility with a variety of tissue fixatives make it very attractive for routine use in histology laboratories.

FIGURES 1-2

1.. (left) Duodenum from rat infected with Taenia taeniaeformis. Tissue was fixed in Carnoy's fluid and stained with astra blue in the presence of sufficient Mg^{++} to prevent mucus staining. BG-38 filter. X 55. 2. (right) Liver from rat infected with Taenia taeniaeformis showing mast cells at periphery of host capsule. Formalin fixed, astra blue. X 100.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