THE ACUTE INFLAMMATORY RESPONSE DURING ENTERIC INFECTIOUS DISEASES

By

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ABSTRACT

The acute inflammatory response is an organism's immediate reaction to injury. In mammals the response is facilitated by an abundance of molecules circulating in the plasma. These molecules are responsible for clotting the blood and chemotaxis of inflammatory cells. Polymorphonuclear leukocytes and monocytes usually comprise the cell infiltrate during acute inflammation.

Within several hours of a localised injury and the start of the acute inflammatory response, systemic indications of the response are detectable. A number of plasma glycoproteins, collectively referred to as the Acute Phase Proteins, change in concentration during acute inflammation. Acute Phase Proteins are synthesized primarily by the liver parenchymal cells, the hepatocytes. The molecules which induce hepatocytes to adopt Acute Phase Protein synthesis (hepatocyte-stimulating factors) arise from the inflammatory macrophages at the site of inflammation. The phenomena of the Acute Phase Protein changes constitutes the Acute Phase Protein Response. The predictable nature of the Acute Phase Protein Response, during inflammation caused by noxious agents, has led to the speculation that the response may serve as a manifestion of infectious diseases.

Certain intestinal-dwelling nematodes elicit an inflammatory response in the small bowel of their host. Nippostrongylus brasiliensis and Trichinella spiralis are two such parasites of rodents. It was important for an understanding of the nature of intestinal inflammation, to establish whether the Acute Phase Protein Response occurred in animals harboring the parasites.

Following a subcutaneous injection, N. brasiliensis larvae pass through the rat host's lungs before reaching and maturing in the intestine. Acute Phase Protein changes were detected in infected rats at the time at which the worms were passing through the lungs and following the establishment of the adults in the intestine. Infective T. spiralis must be ingested and subsequently mature in the small intestine. Adults live, and give birth to larvae, while living in an intracellular compartment comprised of intestinal epithelial cells. No Acute Phase Protein Response was detected during infection of rats by T. spiralis.

An infection by T. spiralis did not inhibit the Acute Phase Protein Response due to a second stimuli, and macrophage secretion of factors relevant to the Acute Phase Protein Response, from sites other than the intestine, was normal. An Acute Phase Protein Response was detected in animals which harbored concurrent N. brasiliensis and T. spiralis infections. No Acute Phase Protein Response was detected in rats into which mature N. brasiliensis larvae

were transferred, in order to eliminate the lung stage of infection. It was concluded that the lung phase of an infection by N. brasiliensis was important in the genesis of the Acute Phase Protein Response during intestinal inflammation. Furthermore, it was concluded from these studies that inflammation in the rat intestine does not lead to the Acute Phase Protein Response.

When the intestinal cells of normal rats were examined for the production of factors which may induce the Acute Phase Protein Response, it was shown that constitutive secretion of the relevant molecules occurred. Secretion of these factors increased during infection of rats by N. brasiliensis but declined during infection by T. spiralis. A rat intestinal epithelial cell line was used to demonstrate that these cells, in addition to macrophages, secrete molecules important in the regulation of the Acute Phase Protein Response. It was concluded that T. spiralis likely inhibited the constitutive hepatocyte-stimulating factor activity by infecting epithelial cells of the intestine.

The pathology that the rodent infections elicit are good models of inflammation for the study of mechanisms of the acute inflammatory response of the intestine.

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α_1AGP	alpha-1-acid glycoprotein
α_1CPI	alpha-1-cysteine protease inhibitor
α_1PI	alpha-1-protease inhibitor
α_2M	alpha-2-macroglobulin
$\alpha_{2\mu}$globulin	alpha-2-microglobulin
APR	Acute Phase Reaction
BSA	bovine serum albumin
C	Complement
cDNA	complementary deoxyribonucleic acid
cm	centimeter(s)
CRP	C-reactive protein
DNA	deoxyribonucleic acid
EDTA	ethylene-diaminetetra-acetic acid
FBS	fetal bovine serum
g	gram(s)
HEPES	N-2-hydroxyethylpiperazine-N^1-2-ethanesulfonic acid
hr	hour(s)
HSF	Hepatocyte-Stimulating Factor (30kd molecule)
IBD	inflammatory bowel disease
Ig	immunoglobulin
IL-1	Interleukin-1
IL-2	Interleukin-2

IL-3	Interleukin-3
kd	kilodalton(s)
L_3	3rd stage larvae
L_4	4th stage larvae
LAF	Lymphocyte-activating factor
LPS	lipopolysaccharide
LT	Leukotriene
M	molar
min	minute(s)
ml	milliliter(s)
MLN	mesenteric lymph node
mM	millimolar
mRNA	messenger Ribonucleic Acid
NaF	sodium fluoride
NSE	non-specific esterase
OD	optical density
PBS	phosphate buffered saline
PG	Prostaglandin
PHA	phytohemaglutinin
PI	post infection
rpm	revolutions per minute
SAA	Serum amyloid A
SAP	Serum amyloid P
SDS	sodium dodecyl sulphate
sec	second(s)
TNF	Tumor Necrosis Factor (alpha)

TRIS	tris (hydroxymethyl)-aminomethane
UC	ulcerative colitis
µl	microliter(s)
µm	micrometers
xg	times gravity

INTRODUCTION

1 Introduction

Everyone has experienced inflammation- the transient reaction to trauma or infection. The reaction manifests locally as the four cardinal inflammatory signs; swelling, heat, redness and pain. This response is due to several complex physiological reactions occurring locally and systemically.

This thesis is a study of inflammation caused by intestinal parasites. The parasites serve as models of intestinal inflammatory diseases. The investigations concentrate on systemic events, and in particular the Acute Phase Protein Response, elicited by the parasites.

This review of the literature has three principle themes; 1) an overview of acute inflammation focussing on the Acute Phase Protein Response; 2) a review of the literature on human inflammatory bowel diseases and the Acute Phase Protein Response; and 3) a review of the inflammatory reaction elicited by two nematode infections of the rodent small bowel.

1.1 Acute Inflammation

Inflammation is one of the organism's major physiologic responses to injury. There are four cardinal clinical symptoms associated with the response- rubor et tumour, cum calor et dolor, or "redness and swelling with heat and pain". There may often be a consequent loss of function of the inflamed tissue or organ. These features are now known to be due to changes in vascular permeability and infiltration of cellular elements at the site of inflammation (Ryan and Majno, 1977). Changes in blood vessel permeability and coagulation involve complex pathways of interconnected enzyme cascades. The purpose of these enzyme systems is to maintain the integrity of the tissue by restricting damage to the inflamed site. The infiltrating cells eliminate foreign materials. That many plasma proteins participate in inflammatory reactions attests to the importance of the response in the survival of the organism.

The acute inflammatory response may be divided into, the local inflammatory response, entailing coagulation, kinin generation (Bennett, 1984) and cellular emigration, and secondly, the systemic Acute Phase Response, which includes changes in a number of serum constituents and physiological events distant from the site of inflammation (Kushner, 1982; Sipe and Rosenstreich, 1982). These two responses are not clearly separable; many of the systemic changes seen during local inflammation are due to the

elicitation of mediators from the site of the inflammation. One such systemic component of acute inflammation, increases in the plasma "Acute Phase Protein" concentrations, has received considerable attention as a marker for the detection of inflammation (Whicher and Dieppe, 1985; Gauldie, Lamontagne and Stadnyk, 1985; Kushner and Mackiewicz, 1987).

1.1.1 The local humoral acute inflammatory response

The plasma of healthy mammals contains a number of glycoproteins, synthesized primarily by the liver parenchymal cells (hepatocytes), that become involved in acute inflammatory reactions (Table 1). These glycoproteins are inactive serine esterases in normal blood, circulating as proenzymes. They can be divided into three cascading pathways of enzymes; the kinin, the complement, and the coagulation systems. Activated enzymes link the different pathways by triggering elements of other cascades (Figure 1). The activation events in these cascades are governed by an equally complex system of plasma proteinase inhibitors (Steinbuch and Audran, 1974). The localization of activation events to surfaces like the endothelium, may allow the enzymes to overcome the presence of inhibitors.

An important molecule in the inter-relationship of the pathways is Hageman Factor (Figure 1). Hageman Factor (Factor XII, Table 1) becomes activated upon contact with subendothelial tissue. Hageman Factor also may be activated

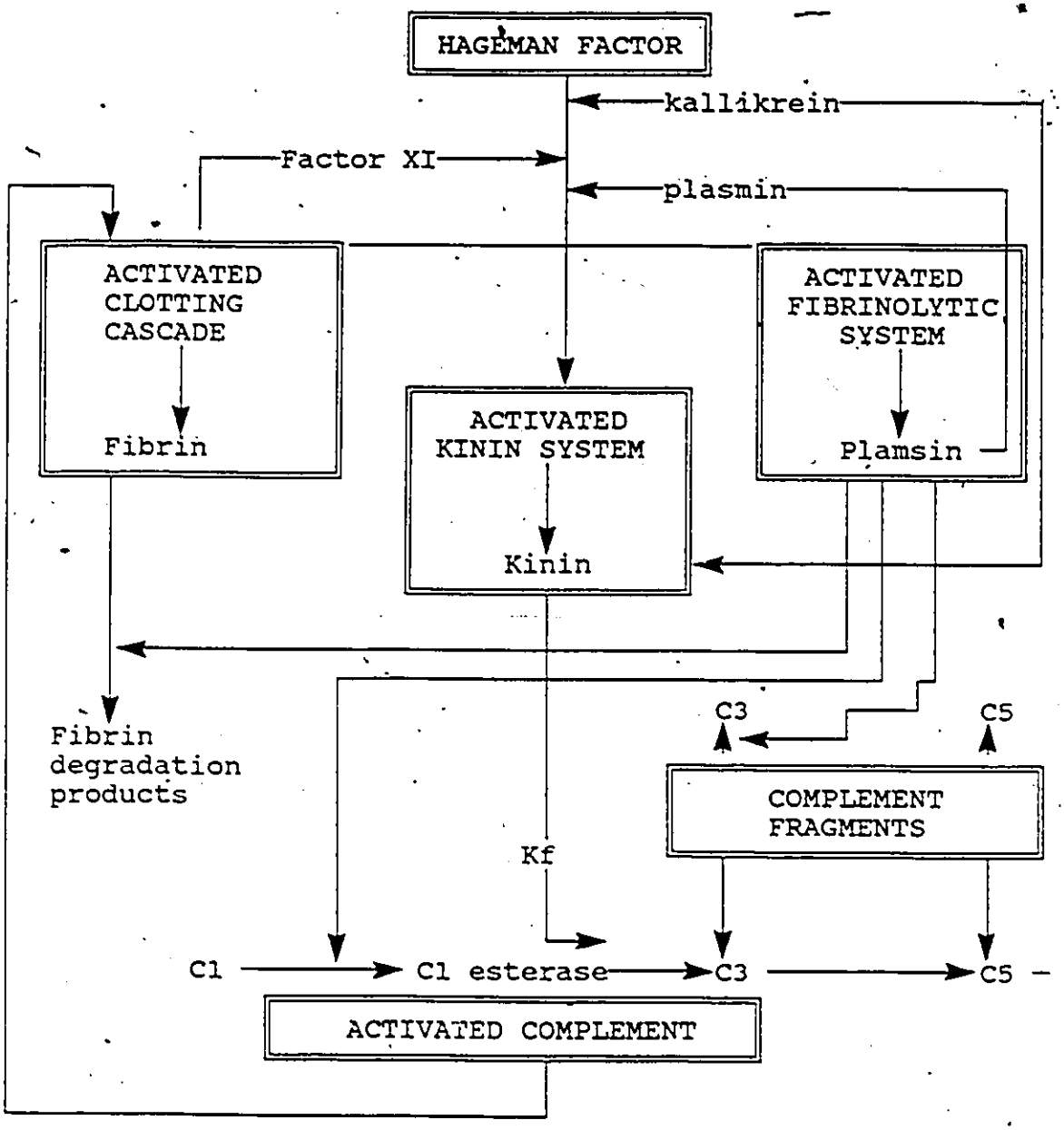
TABLE 1

Classification of Humoral Mediators of Local Inflammation

ORIGIN	MAJOR GROUPS	MAJOR MEDIATORS
Plasma	Kinin system	bradykinin
		kallikrein
		plasminogen activator
	Complement system	C3 fragments
		C5 fragments
		C567 complex
		C kinin
	Clotting system	fibrinopeptides
		fibrin degradation products
Tissues	Vasoactive amines	histamine
		5-hydroxytryptamine
	Acidic lipids	slow-reacting-substance of anaphylaxis
		prostaglandins
		leukotrienes
	Lysosomal components	cationic proteins
		acid proteases
		neutral proteases
	Lymphocyte products	migration inhibitory factor
		chemotactic factors
		lymphotoxin
		mitogenic factors
	Monocyte	interleukin 1
		hepatocyte-stimulating factors
		tumour necrosis factor
	others	substance P
		neurotensin
		cyclic AMP

Extracted from; Ryan and Majno, (1977).

Figure 1: The inter-relationships between the Kinin, clotting and complement system. Redrawn from Ryan and Majno, 1977.


HAGEMAN FACTOR
kallikrein
Factor XI
plasmin
ACTIVATED CLOTTING CASCADE
Fibrin
ACTIVATED KININ SYSTEM
Kinin
ACTIVATED FIBRINOLYTIC SYSTEM
Plamsin
Fibrin degradation products
C3
C5
COMPLEMENT FRAGMENTS
Kf
C1
C1 esterase
C3
C5
ACTIVATED COMPLEMENT

by trypsin, Kallikrein, plasmin or Factor XI. Activated Hageman factor has three effects: 1) triggering the clotting cascade (by activating Factor XI); 2) triggering the fibrinolytic cascade (by activating plasminogen proactivator) and 3) activating prekallikrein activator activity (which subsequently activates prekallikrein to form kallikrein). The end product of activation of the clotting system is deposition of fibrin, or the fibrin clot (Bennett, 1984). Activation of the kinin and complement systems leads to the release of a number of mediators of vascular permeability, smooth muscle contractility, and leukocyte chemotaxis (Table 2).

1.1.2 The local cellular acute inflammatory response

The release of certain chemoattractants at the site of inflammation dictates which cells will infiltrate. Chemoattracants may be secreted by cells; for example histamine from mast cells and serotonin from platelets (Kim, 1979; Fauve, 1981). While there are no rules for predicting the cell types in the cellular infiltrate during acute inflammation, the classical inflammatory lesion usually includes an early (4 hour) neutrophil infiltrate followed closely in time by monocytes, then lastly, lymphocytes. A prolongation of the inflammatory stimuli usually leads to continued monocytic and lymphocytic infiltrates and the development of a granuloma comprised of these cells.

TABLE 2

Mediators Potentially responsible for Chemotaxis of Leukocytes

FACTOR	LEUKOCYTE
Complement products	
C3a C5a	neutrophil
	monocyte
	eosinophil
C567 complex	neutrophil
	eosinophil
Kallikrein	neutrophil
	monocyte
	basophil
Plasminogen activator	neutrophil
	monocyte
Fibrinopeptides	neutrophil
Prostaglandins	neutrophil
Histamine	eosinophil
Eosinophil chemotactic factor	eosinophil
Collagen fragments	neutrophil
Lymphocyte factors	monocytes
	lymphocyte
	eosinophil
	neutrophil
	basophil
Transfer factor	neutrophil
Neutrophil lysosomal cationic protein	monocytes
Macrophage factors	neutrophil
	lymphocyte

Extracted from Ryan and Majno, (1977).

Macrophages, both tissue resident and circulating (monocytes) have recently been discovered to be the cell central to the control of the inflammatory response. These cells function, via a number of secreted molecules listed in Table 3, directly in the activation of blood coagulation, fibrinolysis, and the complement system. They may be directly responsible for some of the autolysis that occurs at an inflammatory site through the secretion of degradative enzymes such as collagenase and elastase (Janoff et al., 1979) and superoxide anion and free-oxygen radicals (Ward, 1986; Henson and Johnston, Jr., 1987)(Table 3). Conversely, macrophages synthesize and secrete proteinase inhibitors such as $\alpha_2$macroglobulin and $\alpha_1$protease inhibitor, which function to contain tissue damage caused by the above enzymes (Isaacson et al., 1981, White et al., 1981). Macrophages may be cytotoxic for certain tumour cells (Den Otter, Dullens and De Weger, 1983; Zoller and Matzku, 1983; Urban et al., 1986) and are able to kill parasites using antibody- or complement-dependent mechanisms (Ruitenberg et al., 1983, Mackenzie et al., 1980). Finally, macrophages provide a link between the inflammatory response and immune system by way of antigen presentation to, and activation of, lymphocytes, (Oppenheim et al., 1982; Lasser, 1983; Gemsa et al., 1982). Clearly, macrophages are ubiquitous and are able to both regulate and sustain an inflammatory response.

TABLE 3

Secretory products of mononuclear phagocytes important in inflammation

A.	Enzymes		Transcobalamin II
	Lysozyme		Fibronectin
	Neutral proteases	E.	Oxygen Metabolites
	plasminogen activator		Superoxide
	collagenase		Hydrogen peroxide
	elastase		Hydroxyl radical
	proteoglycan degrading protease		Singlet oxygen
	angiotensin convertase	F.	Bioactive Lipids
	Acid hydrolases		Prostaglandins
	proteases		Thrombaxane
	esterases		Leukotreines
	lipases		Platelet activ. factor
	sulfatases	G.	Activating Factors
	ribonucleases		Endogenous Pyrogen
	phosphatases		Colony-stim. factors
	glycosidases		Lymphocyte-activating factor
	cathepsins		of erythroid precursors
	Arginase		of fibroblasts
B.	Complement Components		of microvasculature
C.	Enzyme Inhibitors	H.	Inhibiting Factors
	Plasmin inhibitor		Interferons
	α_2-macroglobulin		of tumour cells
	$\alpha_1$protease inhibitor		of lymphocytes
D.	Binding Proteins		of Listeria monocytogenes
	Transferrin		
	Ferritin		

Extracted from Lasser (1983).

Precisely which conditions lead to a particular macrophage response and whether all macrophages are capable of performing all the functions so far detected, is not entirely clear. Much work with specific populations of cells remains to be done to answer such questions.

Macrophages are also responsible for many of the systemic changes seen during acute inflammation or the Acute Phase Response. Through the secretion of various plasma-soluble molecules (monokines; see Activating Factors, Table 3), macrophages at the site of inflammation communicate with other tissues to elicit systemic inflammatory changes (Koj, 1974; Kampschmidt, Upchurch and Worthington, 1983; Oppenheim et al., 1986; Koj, 1985; Dinarello, 1985; Beck et al., 1986; Allison, 1986; Le and Vilcek, 1987). Many of these activities have recently been ascribed to Interleukin-1 (fever induction, changes in plasma concentrations of Zn^{2+}, Cu^{2+} and some Acute Phase Proteins and neutrophils; (Kampschmidt, Upchurch and Worthington, 1983; Matsushima et al., 1985; Kampschmidt and Mesecher, 1985; Tocco-Bradley et al., 1986).

1.2 The Acute Phase Response

Episodes of local, acute inflammation are usually manifest systemically, in mammals, by a number of physiological responses. These systemic responses include a temporary fever, changes in the concentrations of plasma heavy metals (increased Cu and decreased Zn and Fe),

concentrations of some liver-derived proteins (the Acute Phase Proteins) and amino acid pools (released from skeletal muscle), and a leukocytosis, (Koj, 1974; Kampschmidt, 1978; Woloski, Kaplan and Jamieson, 1983; Gordon and Koj, 1985; Williams, Cypher and Mosesson, 1985; Milanino et al., 1986). Changes in the erythrocyte sedimentation rate, a common test for detecting inflammatory conditions in humans, is related to the changes in concentration of Acute Phase Proteins, particularly fibrinogen and $\alpha_1$protease inhibitor (Whicher and Dieppe, 1985).

The diversity of events occurring during the Acute Phase Response requires a close association between the neuroendocrine system, inflammatory cells and liver function. Macrophages and their soluble monokines are important in the initiation of many of these events. The induction of fever involves re-setting the hypothalamic temperature set-point; "endogenous pyrogen" (Table 3) has been shown to induce a potent fever, increased granulocytosis and hypoferremia when injected intracerebroventricularly or intravenously (Turchik and Bornstein, 1980). Monokines have also been shown to have a direct enhancing effect on pituitary secretion of adrenocorticostimulating hormone, combining a monocyte-pituitary with adrenal axis during the Acute Phase Response (Woloski et al., 1985).

Endocrine changes during the Acute Phase Response known to effect liver function include increased glucagon,

insulin, cortisol, catecholamines, growth hormone, thyroid stimulating hormone, thyroxin, aldosterone and vasopressin. There is a general enhancement of body metabolism; increased protein catabolism, increased gluconeogenesis and a negative nitrogen balance (Kushner, 1982).

The liver mixed-function oxidase system, including the P450 enzymes, are suppressed during the Acute Phase Response (Mahu and Feldmann, 1984, Williams, 1985). This effect would seem to leave the animal susceptible to further injury by a second agent during acute inflammation.

1.2.1 The serum Acute Phase Protein Response

There are a number of Acute Phase Proteins in mammals which increase in concentration during inflammation. Table 4 documents most of the Acute Phase Proteins and highlights some of their species specificity and biological functions. Following a subcutaneous injection of turpentine in the rat there are significant increases in plasma haptoglobin (Mahu and Feldmann, 1984), $\alpha_1$acid glycoprotein (α_1AGP; Urban, Chan and Schreiber, 1979), $\alpha_1$cysteine protease inhibitor (α_1CPI; Schreiber et al., 1982; Esnard and Gauthier, 1983), $\alpha_2$macroglobulin (α_2M; Bohannon, Kiorpes and Wolf, 1979), fibronectin (Pick-Kober, Munker and Gressner, 1986), fibrinogen (Schreiber et al., 1982; Bernuau, Rogier and Feldmann, 1983; Williams, Cypher and Mosesson, 1985), glycosaltransferases (Lombart, Sturgess and

Schachter, 1980; Fraser et al., 1984; Jamieson, Janzen and Woloski, 1987), ceruloplasmin (Aldred et al., 1987) and transferrin (Northemann et al., 1983). Alpha$_2$macroglobulin is an Acute Phase Protein only in the rat (Table 4). Significant declines occur in plasma concentrations of albumin (Aston, Jamieson and Friesen, 1970; Schreiber et al., 1982; Moshage et al., 1987), prealbumin (Dickson, Howlett and Schreiber, 1982) and transcortin and $\alpha_2$microglobulin (Faict et al., 1983). Using other inflammatory stimuli, it has been shown that angiotensinogen (Kageyama, Ohkubo and Nakanishi, 1985), prekininogen (Kageyama et al., 1985) and C-reactive protein (de Beer et al., 1982) are positive Acute Phase proteins in this species. Other negative Acute Phase Proteins include α_1-inhibitor 3 (Schweizer et al., 1987) and a poorly-defined hepatic phosphoprotein (Le Cam and Le Cam, 1985).

Some interesting observations made of Acute Phase Protein levels were related to changes in circulating hormones. There are "female-specific" Acute Phase Proteins in the rat (Schade et al., 1982) and hamster (Coe et al., 1981) which increase in concentration in response to estrogen. Pregnant and lactating women have increased plasma levels of α_1PI, ceruloplasmin, α_2M, and copper, perhaps due to increased estrogen levels (Haram, Augensen and Elsayed, 1983; DiSilvestro, 1986;). New-born rats also have elevated concentrations of α_2M and α_1CPI, yet will

TABLE 4

The Acute Phase Proteins and their biological role

PROTEIN	SPECIES	BIOLOGICAL ROLE
Major changes		
C-reactive protein	man, rabbit rat, mice	opsonin, immuno-modulating?
serum amyloid A protein	man, mice	?
$\alpha_2$macroglobulin	rat	antiprotease
$\alpha_1$cysteine protease inhibitor	rat	antiprotease
$\alpha_1$acid glycoprotein	most species	transport protein
Medium changes		
$\alpha_1$proteinase inhibitor	man, mice, rat	antiprotease
$\alpha_1$antichymotrypsin	man, mice	antiprotease
fibrinogen	most species	coagulation
haptoglobin	most species	binds hemoglobin
serum amyloid P protein	mice	immunomodulating
ceruloplasmin	most species	O_2^- scavenger
complement	mice, man, rat	complement, opsonin
angiotensinogen	rat, man	blood pressure
fibronectin	rat	cell attachment
female hamster protein	hamster	?
glycoslytransferases	rat	carbohydrate attachment
Negative changes		
albumin	most species	transport protein
transferrin	most species	transport protein
transcortin	rat	T3, T4 transport
prealbumin	rat	T3, T4 transport
$\alpha_{2\mu}$-globulin	rat	?

Compiled from Lombart, Sturgess and Schachter, 1980; Schade et al., 1982; Dickson, Howlett and Schreiber, 1982; de Beer et al., 1982; Faict et al., 1983; Koj et al., 1984 Gauldie, Lamontagne and Stadnyk, 1985; Kageyama, Ohkubo and Nakanishi, 1985; Pick-Kober, Munker and Gressner, 1986; Nielsen and Knudsen, 1987.

respond to turpentine inflammation with further synthesis and secretion (Hudig and Sell, 1978; Thomas and Schreiber, 1985).

1.2.2 The liver Acute Phase Protein Response

Increased accumulation of a molecule in the plasma may be due to either increased secretion, or a change in the half-life or tissue distribution of the molecule. Increased Acute Phase Protein concentrations are due to increased synthesis by the liver. Hepatocyte synthesis of positive Acute Phase Proteins has been confirmed using primary cell cultures (Panrucker and Lorscheider, 1982; Kaplan et al., 1983; Deshmukh et al., 1985; Nakagawa and Hirata, 1986; Sedgwick and Lees, 1986; Lonberg-Holm et al., 1987). Moreover, it has been confirmed that a single hepatocyte is capable of synthesizing multiple Acute Phase Proteins (Courtoy et al., 1981; Bernuau, Rogier and Feldmann, 1983). During acute inflammation a greater number of hepatocytes, than under normal conditions, are recruited to increase Acute Phase Protein secretion. The stimulus for this recruitment diffuses from the portal circulation, as shown by sequential immunohistochemical staining for Acute Phase Proteins in liver sections (Courtoy et al., 1981).

Increased Acute Phase Protein secretion by hepatocytes is associated with increases in specific RNA message (mRNA) for the same molecule (Schreiber et al., 1986).

Increases in mRNA may be due to either an increase in the half-life or stability of the particular mRNA (Vannice, Taylor and Ringold, 1984; Jefferson et al., 1984) or increased transcription of the DNA. Measurement of the increased Acute Phase Protein mRNA has been performed by cell-free in vitro RNA translation (Princen et al., 1981; Andus et al., 1983; Northemann et al., 1983; Faict et al., 1985), in situ hybridization (Kodelja et al., 1986; Tournier et al., 1987) and dot/blot (Kageyama et al., 1985; Yu and Gorovsky, 1986; Aldred et al., 1987) or Northern blot hybridization of cellular RNA (Aldred et al., 1987) using complementary nucleotide (cDNA) probes. The technique, "nuclear run-on", which involves isolating and radiolabelling ribosomal-bound RNA transcripts, has been used to confirm increased transcription of α_2M in the inflamed rat liver (Northemann et al., 1985; Gehring et al., 1987). Additionally, reports of increased transfer-RNA (tRNA) in liver cells isolated from inflamed animals (Cajone and Bernelli-Zazzera, 1985) and changes in amino acid pools (Woloski, Kaplan and Jamieson, 1983), are indications of increased DNA transcription.

There are few exceptions to the rule that hepatocytes are the source of Acute Phase Proteins. One exception are platelets which synthesize fibrinogen. Since the number of circulating platelets rises during acute inflammation so does the amount of fibrinogen they may contribute to the

plasma (Williams, Cypher and Mosseson, 1985). A second exception are human and rodent alveolar macrophages which have been shown to synthesize and secrete low levels of $\alpha_1$antitrypsin ($\alpha_1$protease inhibitor, α_1PI) (White et al., 1981; van Furth, Kramps and Diesselhof-Den Dulk, 1983; Lamontagne, Stadnyk and Gauldie, 1985; Takemura, Rossing and Perlmutter, 1986). During pregnancy in rats the placenta and uterus synthesize significant amounts of α_2M (Hayashida et al., 1986; Kodelja et al., 1986).

1.2.3 Mediators of the Acute Phase Protein Response

A number of hormones have been identified which are important for normal hepatocyte metabolism and which also effect Acute Phase Protein production (Miller, 1983). Primary liver-cell cultures and established hepatoma cell lines have been used extensively in these studies. The optimal culture media for these cells usually requires a source of glucocorticoid and insulin.

Glucocorticoids are directly involved in the regulation of transcription of many genes in many cell types (Rousseau, 1984). The synthetic corticosteroid, dexamethasone, has been shown to regulate aspects of normal liver metabolism (Agius, Chowdhury and Alberti, 1986). For example dexamethasone increases the number of insulin receptors (Fleig et al., 1985). Using rat hepatocytes, dexamethasone has been shown to enhance transcription of

α_1AGP (Kulkarni, Reinke and Feigelson, 1985), α_2M (Gross et al. 1984; Tsukada, Hibi and Ohdawa, 1985; Bauer et al., 1986; Kurokawa et al., 1987), fibrinogen (Grieninger, 1983), haptoglobin (Hooper et al., 1981) albumin (Nawa et al., 1986) and sialyltransferase (Dijk et al., 1986). The effect of the hormone on transcription was confirmed by the abolition of its effect by α-amanitin. It has been shown that inhibition of protein synthesis (using cycloheximide) inhibits the effect of dexamethasone enhancement of liver α_1AGP and $\alpha_{2\mu}$-globulin, implying that a second molecule is necessary for hormonal regulation of some genes (Baumann et al., 1983; Addison and Kurtz, 1986; Klein et al., 1987).

A second hormone which has a substantial impact on the in vivo function of hepatocytes is insulin. Insulin is intimately involved in glycogen metabolism by hepatocytes (Miller et al., 1986). Interestingly, this effect of insulin can be replaced by Epidermal Growth Factor (Bosch et al., 1986). Insulin, in rat hepatoma cell lines, causes the accumulation of some species of mRNA (Messina, Hamlin and Larner, 1985) and decreased mRNA for albumin (Straus and Takemoto, 1987). In some cellular functions, insulin antagonizes the effects of corticosteroids; for example, it down-regulates the number of insulin receptors on the cell membrane (Fleig et al., 1985).

Other hormones known to have effects on Acute Phase Protein production by rat liver cells include catecholamines

which have been shown to have α_2M-enhancing potential, (although the effect was lessened in adrenalectomized animals; van Gool et al., 1984). Catecholamines and triiodothyronine, in combination with dexamethasone, induced high α_2M levels (Bauer et al., 1986). Hepatocyte mRNA levels of the negative Acute Phase Protein, $\alpha_{2\mu}$-globulin, have been shown to be androgen-dependent, and levels drop considerably in hypophysectomized animals (Sarkar et al., 1986).

In addition to hormones, several monokines have been described which have enhancing effects on hepatocyte synthesis of Acute Phase Proteins. The original observation that monokines stimulated hepatocytes into increased protein synthesis was made using leukocytes collected from turpentine-induced abscesses (Homburger, 1945). More recently macrophage culture supernatants have been fractionated into several factors each of which has some hepatocyte-stimulating potential. Three notable factors are Interferon-β_2 (Hepatocyte-Stimulating Factor; Gauldie et al., 1987), Interleukin-1 (IL-1; Dinarello, 1984; Koj, 1985), and Tumour Necrosis Factor (TNF; Le and Vilcek, 1987). A fourth monokine, Platelet Activating Factor, has recently come under greater scrutiny for its role as a mediator of the Acute Phase Response, although it is not known whether it acts on hepatocytes (Issekutz and Szpejda, 1986; Martins et al., 1987).

While these molecules have been identified in culture supernatants of macrophages, their production is not exclusive to macrophages. For example, rat and human lung fibroblasts secrete Interferon-β_2, and various cells (ie. endothelium) secrete IL-1 (Oppenheim et al., 1982; Gauldie et al., 1987; Le and Vilcek, 1987).

The term "Hepatocyte-Stimulating Factor" was initially applied to the molecule(s) in human monocyte-conditioned culture supernatant that led to increased fibrinogen synthesis by fetal hepatocytes (Ritchie and Fuller, 1983). Recently, this activity was ascribed to Interferon-β_2 which was subsequently re-named Hepatocyte-Stimulating Factor (HSF; Gauldie et al., 1987). Hepatocyte-Stimulating Factor induces a great range of Acute Phase Proteins in all hepatocyte culture systems tested. In decreasing order of magnitude, human HSF stimulates, in rat primary hepatocyte cultures, α_2M, fibrinogen, α_1CPI, α_1PI, haptoglobin and α_1AGP. The molecule also induces a significant reduction in albumin and transferrin in this culture system (Koj et al., 1984).

The in vivo role of HSF has been shown experimentally in two types of studies. First, the use of actinomycin D at the site of inflammation leads to significant reductions in plasma Acute Phase Protein concentrations (Hirschelmann and Schade, 1986). Secondly, and more directly, the monokines have been administered back into animals and

increases in the plasma Acute Phase Proteins monitored (Woloski, Gospodarek and Jamieson, 1985). Hepatocyte-stimulating activities which remain to be confirmed as Interferon-β_2, have been described from a human colon carcinoma cell line (COLO-16, Baumann et al., 1986) and human epidermal keratinocytes (Baumann et al., 1984).

Interleukin-1 (IL-1) induces fewer Acute Phase Proteins in hepatocyte cultures than HSF. Murine and human recombinant IL-1 (rIL-1) stimulate production of α_2M and decreased albumin synthesis, respectively, by primary rat hepatocyte cultures (Bauer et al., 1985; Moshage et al., 1987). Human IL-1 elevates secretion of the third component of complement (C3) and $\alpha_1$antichymotrypsin and causes decreased albumin synthesis in human hepatoma cell lines. No increases in α_1AGP, fibrinogen, or C-Reactive Protein were detected (Darlington, Wilson and Lachman, 1986; Perlmutter, 1987; Goldman and Liu, 1987). Rats infused with murine rIL-1, while showing many characteristics of the Acute Phase Response, failed to manifest increases in either fibrinogen, haptoglobin or serum copper (Tocco-Bradley et al., 1986).

Tumor Necrosis Factor (TNF) shares many functions in common with IL-1 but has only recently been acknowledged as a mediator of the Acute Phase Response (Le and Vilcek, 1987). The observation that TNF was involved in the Acute Phase Response was made during investigations of the state

of cachectia during endotoxin-shock experiments (Beutler et al., 1986). Endotoxin shock can been reproduced in vivo by the infusion of recombinant TNF (rTNF; Tracey et al., 1986; Remick et al., 1987). Tumor Necrosis Factor, when applied in relatively low concentrations on human hepatoma lines, directly stimulates C3 (Darlington, Wilson and Lachman, 1986) and $\alpha_1$antichymotrypsin and decreased albumin and transferrin synthesis (Perlmutter, 1987).

1.2.4 Biological function of the Acute Phase Proteins

Table 4 lists the species-dependency and known function of the major Acute Phase Proteins. A number of these molecules are serine or cysteine proteinase inhibitors and are relatively abundant in normal plasma. This abundance (four of eight plasma protease inhibitors are Acute Phase Proteins) signifies a role for the inhibitors in hemostasis and in fact many of the enzymes of the coagulation cascade are inhibited by these molecules (Heimburger, 1974). The roles of other Acute Phase Proteins are less completely understood. For example, the biological role of C-Reactive Protein (CRP) has eluded a satisfactory explanation, despite descriptions of in vitro interactions of the molecule with cells and complement (Gewurz et al., 1982; Thompson, 1985, Baum et al., 1987). Only a few Acute Phase Proteins of the human and rat will be discussed in greater detail.

Apart from the immediate role of proteinase inhibitors in containment of damage by proteolytic enzymes (Carrell, 1986), many of the Acute Phase protease inhibitors have considerable immunomodulating potential. The function of α_2M, which has the widest spectrum of protease substrates, has received the greatest attention. Table 5 lists the known effects of α_2M on cells of the lymphoid and reticuloendothelial systems. Alpha$_2$macroglobulin has been shown to inhibit Natural Killer cell- and antibody-dependent cell-mediated-cytotoxicity at concentrations below physiological levels in humans (Gravagna et al., 1982). Furthermore, α_2M/protease complexes have been shown to have similar inhibitory activity in vitro (Hubbard, 1978; Dickinson et al., 1985). The antiproteinase has been shown to inhibit both B lymphocyte (Teodorescu et al., 1982) and T lymphocyte (Vischer, 1979, Miyanaga et al., 1982) mitogenic responses (Table 5). The mechanism by which α_2M hinders the proliferating properties of lymphocytes and cytotoxicity of cells is not fully understood. Even less clear is how antiprotease/-protease complexes work to achieve the same inhibition. Some light has been cast on this problem with the discovery of serine proteases in the membranes of killer T lymphocytes (Pasternack et al., 1986). Cytotoxic T lymphocytes are stimulated to kill specific target cells following contact with the target. It seems that proteases serve in the chain of molecular membrane events leading to signal

TABLE 5

Summary of the known effect of $\alpha_2$macroglobulin on cells of the lymphoreticular system

1.	Stimulates the development of lymphocytes and granulocytes in normal and irradiated animals.
2.	Promotes the restoration of humoral responsiveness in sublethally irradiated mice.
3.	Enhances the production of antigen-induced colony forming units *in vitro*.
4.	Inhibits the generation of kinins.
5.	Prevents the binding of chemotactic factors (C3a and C5a) to receptors on neutrophils.
6.	Inhibits the generation of macrophage slowing factor.
7.	Enhances the response to macrophage inhibitory factor.
8.	Binds macrophage activating factor.
9.	Inhibits mitogenic response to concanavalin A, phytohemagglutinin, pokeweed mitogen, lipopolysaccharide and dextran sulphate.
10.	Inhibits two-way mixed lymphocyte reaction.
11.	Binds and inhibits cationic proteases released during phagocytosis.
12.	Promotes clearance of proteolytic enzymes through phagocytosis of complexes.

Extracted from James, (1980).

transduction following this contact. Inhibition of the membrane esterases of cytolytic T lymphocytes leads to an inhibition of their killer potential (Utsunomiya and Nakanishi, 1986). Exposure of the killer cells to serine proteases triggered the membrane events in the absence of the target cells (Utsunomiya and Nakanishi, 1986). Otherwise the protease inhibitor may act by interacting with stimulating agents, for example, by binding directly to phytohemagglutinin (James, 1980).

Similar immunomodulatory effects have been reported for $\alpha_1$protease inhibitor (α_1PI). Lymphocyte surface proteases have been shown to be inhibited by α_1PI (Bata, Martin and Revillard, 1981). Lymphocyte proliferation (Bata and Revillard, 1981) and antibody-dependent cytotoxicity (Trinchieri and De Marchi, 1976) are inhibited by α_1PI. The α_1PI was shown to bind to the lymphocytes using radiolabelled glycoprotein.

A third major Acute Phase Protein/protease inhibitor of the rat, $\alpha_1$cysteine protease inhibitor (α_1CPI), is not related to the serine protease inhibitors. It is notable that the sequence for bradykinin is contained in the primary amino acid sequence of α_1CPI thus relating this molecule with the kininogens, although there is no evidence that bradykinin is release upon proteolytic cleavage of α_1CPI (Furuto-Kato et al., 1985; Anderson and Heath, 1985). To the contrary, T-kinin and bradykinin have been investigated

for anti-protease activities (Barlas, Sugio and Greenbaum, 1985). The cysteine protease inhibitors have not yet been examined for immunomodulating effects.

The biological function of $\alpha_1$acid glycoprotein (α_1AGP) is poorly understood. The molecule is heavily glycosylated (as much as 40% of the molecular mass may be carbohydrate), which allows for considerable microheterogeneity of the circulating molecule. The abundant carbohydrate indicates that α_1AGP probably serves as a carrier molecule in the plasma. The fact that α_1AGP serves a beneficial role as an Acute Phase Protein was implied by the localization of the molecule to inflammatory lesions (Shibata et al., 1978). Furthermore, concurrent injection of α_1AGP with urate crystals lead to a significant reduction in swelling due to the urate crystals (Denko and Wanek, 1984). Alpha$_1$AGP has demonstrable antiheparin activity (Andersen et al., 1980) and may inhibit platelet reactions (Costello, Fiedel and Gewurz, 1981). Recently, α_1AGP has been implicated in cell membrane-activation events, possibly interacting with the T_3 membrane antigen of T lymphocytes (Stefanini et al., 1986).

Haptoglobin has been identified as the principle transport protein for the circulating heme fraction of hemoglobin. The complex, haptoglobin/heme, has a considerably shorter half-life than the native molecule as its is removed by the liver and reticuloendothelial cells.

1.3 Models of inflammation

A number of experimental models have been established in the laboratory rat in order to study the inflammatory response. The most studied models have involved the use of noxious or toxic agents in either pleural, peritoneal or subcutaneous injections. Such agents include carrageenin, turpentine, bacterial lipopolysaccharide, and celite. Other conditions used to study the inflammatory response include sponge implants and burns or open wounds (Ammendola, Di Rosa and Sorrentino, 1975; Lundberg, Lebel and Gerdin, 1984; Johnson, DiMartino and Hanna, 1986; Sedgwick and Lees, 1986; Nakagawa and Sakata, 1986). It is clear from the use of such a variety of inflammatory stimuli that inflammatory changes are often different for the different sites and agents. Each model must be considered individually and caution must be exercised when the models are compared to naturally occurring inflammatory conditions. Few studies compare the magnitude of changes due to noxious agent with a natural inflammatory insult such as an infection. There is a paucity of reports in which the inflammatory response to natural infection has been studied.

1.4 The serum Acute Phase Protein Response in parasitic diseases

The concept of parasitism involves an intrinsic dependance of the parasite on the host whereupon the

relationship becomes obligatory for the parasite. Noble and Noble (1976) have defined parasitism as follows:

> "...an association between two specifically distinct organisms in which the dependence of the parasite on its host is a metabolic one involving mutual exchange of substances. This dependence is the result of a loss by the parasite of genetic information."

The obligatory aspect of the relationship is usually based on the nutritional requirements of the parasite (Cheng, 1986).

The host responses to parasitic infection, of relevance to the Acute Phase Response, include fever, a state of cachectia, and suppression of the hepatic mixed-function oxidase system (Cha, 1978; Srivastava, Chatterjee and Ghatak, 1985; Tekwani et al., 1987). There are no concerted research programmes investigating the role of the Acute Phase Protein Response during parasite infection.

Bout et al., (1986) reported Acute Phase Protein changes in schistosome-infected rats and showed CRP to be important in the schistosomicidal activity of rat serum. There was a significant rise in serum CRP from day 12 through to day 44 post infection, in parallel with increased IgE levels and platelet counts. Schistosoma infection of mice resulted in elevated C3 and serum amyloid P (SAP) late in the infection, between days 40 and 50, corresponding with the development of liver granulomas (Pepys et al., 1980). The C3 response was absent in T lymphocyte-deprived mice. Lamontagne et al. (1984) documented Acute Phase serum amy-

loid A (SAA), SAP, C3 and α_1PI concentrations during Nippostrongylus brasiliensis infection of CBA/J mice. Two peaks of increased SAA and SAP were seen while one peak of C3 and α_1PI was observed late in the infection. Liver production of the same molecules was documented from hepatocytes isolated from infected animals (Lamontagne et al., 1984). An infection of mice with Hymenolepis nana stimulated an Acute Phase Response at "the time of peak host responsiveness to the parasite" (Baltz et al., 1982), which did not occur in T lymphocyte-deprived mice, but was restored by transfusion with immune or naive mouse spleen cells. The T lymphocyte-deprived mice (thymectomized and treated with anti-thymocyte serum) did respond to turpentine inflammation with increased C3 and SAP.

Mice have been shown to respond with an Acute Phase Protein Response to protozoan infections. Three days following inoculation of mice with Trypanosoma brucei, serum levels of haptoglobin and haemopexin increased (Pluschke et al., 1986). The cutaneous trypanosome species, Trypanosoma cruzi, induced an early serum SAP but not C3 or C4 response in mice (Scharfstein, Barcinski and Leon, 1982). Serum C3 concentrations increased about day 9 of the infection, coincident with peak parasitemia. Serum changes due to T. cruzi infection were shown to be dependent on the number of parasites and amount of tissue infiltration by the parasite. From these few studies it can confidently be concluded that

parasitic infections do elicit the Acute Phase Protein Response.

Little has been learned about the role of Acute Phase Proteins from infectious models of inflammation. It appears that some parasites are able to incorporate host serum proteins into their membranes, possibly shielding them from the immune system. Trichomonas vaginalis adsorbs human α_2M, α_1PI, immunoglobulins, and fibronectin onto its surface (Peterson and Alderete, 1982). Fibronectin possibly serves as one of the bridges for Leishmania mexicana recognition and entry into human macrophages (Wyler, Sypek and McDonald, 1985). A host response that leads to increases in any of these molecules may hasten the infection and not the cure.

Studies have been performed in humans in which the Acute Phase Protein Response was monitored during parasite-induced illness. In one study involving human volunteer infections by various Plasmodium sp., significant increases in the α_1 fraction of plasma (likely α_1PI) was noted (Klainer et al., 1968). Similar changes were detectable in naturally acquired disease (Klainer et al., 1969). Albumin levels were not reduced in the patients. There was no relationship between the changes and the magnitude or duration of fever, severity of illness, or density or duration of parasitemia (Klainer et al., 1969). Treatment of patients with corticosteroids had no effect on serum protein concentrations. A similar finding of increased serum α_1 fraction

has been reported in humans infected with schistosomes (Mousa, Waslien and Mansour, 1976). These patients had decreased albumin and β globulin concentrations. However, unlike the murine model of schistosomiasis, the patients failed to show increased Acute Phase Protein concentrations at the time of liver granuloma formation (Manoukian and Borges, 1984).

With regards to human intestinal infection and the Acute Phase Protein Response; albumin levels were reported to be decreased in a study of Egyptian children with multiple intestinal parasite species. Lower albumin concentrations correlated with greater weight loss as the children aged through puberty (Cole et al., 1982). Treatment of an emigrating population of Vietnamese children with anthelminthics led to reductions, to normal levels, of serum α_1AGP, α_1PI and ceruloplasmin concentrations. Many of the children suffered from multiple infections. By issuing anthelminthic regimes specific for certain parasite species, and following the reductions in serum proteins, the authors thought they achieved sufficient evidence to associate a particular infection with major serum protein changes (Blom, Prag and Norredam, 1979). Using this strategy it was concluded, from among several helminth species, that Ancylostoma duodenale likely comprised the major impact to stimulate the Acute Phase Protein Response in the intestine. The difficulties in interpretation of such a study are many

but it is possible to infer that intestinal infection does induce the Acute Phase Protein Response.

1.5 The serum Acute Phase Protein Response in inflammatory bowel diseases

The Acute Phase Protein Response has been used as a prognostic tool in human inflammatory diseases, with CRP serving as the prototype. C-reactive protein levels typically rise within hours of surgery, peak in two days, and begin returning to normal by the third day (Fischer et al., 1976; Werner and Odenthal, 1967; Romette, di Costanzo-Dufetel and Charrel, 1986)). C-Reactive Protein concentrations remained elevated in those patients that suffer surgical complications involving infection and inflammation. The serum CRP response has also been used to detect systemic infections (Whicher, Bell and Southall, 1981) and as a prognostic tool for neoplasias (Rashid et al., 1982). Inflammatory conditions for which the response has not proven to be a reliable marker include connective tissue disorders, osteoarthritis, periarticular disease and spondylosis and ulcerative colitis (Whicher and Dieppe, 1985).

There is evidence from the few parasite infections discussed above, that man may undergo an Acute Phase Protein Response due to inflammation of the intestine. However, some of the helminth species reported have tissue-migrating

larval stages and an effect due to this aspect of the parasite life cycle cannot be ruled-out. The Acute Phase Protein Response has been examined in patients suffering from either of two human intestinal inflammatory conditions, Ulcerative colitis and Crohn's disease, the so-called "inflammatory bowel diseases" (IBD) (Lind et al., 1985).

Table 6 outlines and compares some of the features of the two diseases. There is no known etiology for either condition, although some viral and bacterial infections lead to similar clinical presentations (Sacher, 1985) and immunological- and dietary causes have been proposed (Strickland and Jewell, 1983; Kohler and Brown, 1982; Sacher, 1985; Persson, Ahlbom and Hellers, 1987).

Biopsies of inflamed intestine show quantitative increases in plasma cells, T and B lymphocytes and macrophages (Strickland and Jewell, 1983). Intestinal lymphocytes from patients have a diminished ability to produce Interleukin-2 (IL-2)(James, 1985). There are clearly increased populations of mast cells and increased expression of Class II histocompatibility antigens on epithelial cells, and higher levels of leukotrienes (LTB_4) in actively inflamed tissues (James, 1985; Hirata et al., 1986; McDonald and Jewell, 1987).

There are no reliable systemic markers for identifying IBD. Diagnosis invariably involves removal of a biopsy specimen, roentrograms, colonoscopy, or a combination of

these. Histological preparations allow for discrimination between certain stages of the disease (Nostrant, Kumar and Appelman, 1987). The most widely used index for measuring Crohn's disease uses primarily subjective criteria and includes the "erythrocyte sedimentation rate" as the only laboratory test (Crohn's Disease Activity Index, CDAI; Best et al., 1976). There is a need for dependable marker for following the course of the disease and, ultimately, predicting relapses (Sacher, 1987).

If macrophages play a central role in the inflammatory response, it seems reasonable to look for macrophage-derived mediators during the disease. Only recently have investigators looked for systemic macrophage inflammatory mediators in IBD patients. Variable results showing increased serum IL-1 have been reported for Crohn's disease patients (Stasangi et al., 1987) and even less consistently, in UC patients (Wandall et al., 1986). Peripheral blood mononuclear cells of Crohn's disease patients made significantly more IL-1 when stimulated in culture than non-diseased cells.

Increases in circulating Acute Phase Proteins have been observed in IBD patients since first reported in 1957, when investigators determined α_1AGP concentrations in patients with UC and recognized a relationship between increased concentrations of α_1AGP, decreased albumin, and an increased erythrocyte sedimentation rate (increased fib-

TABLE 6

Features of Crohn's disease versus
Ulcerative Colitis

FACTS ABOUT ULCERATIVE COLITIS
1. The inflammation almost always involves the rectal segment.
2. The extent of the disease demonstrated originally remains constant in most cases. In other cases it can spread proximal, coincident with clinical worsening of the disease.
3. UC can be provoked by a variety of phenomena including respiratory infections, stress and bereavement.
4. Sulfasalazine has been proven to have a useful role in preventing recurrences.
5. The risk of carcinoma increases over time and in proportion to the extent of the disease.
6. Resection of the colon results in cure of the disease.
CROHN'S DISEASE DIFFERS...
1. Crohn's disease is an extensive process involving the entire alimentary canal.
2. The pathology features transmural inflammation, granulomas, and fistulas from the diseased bowel or in the region about the rectum.
3. The most common extent of involvement is a combination of the terminal ileum with some extent of right colon.
4. The extent of the disease usually remains constant unless the bowel is transected. With clinical worsening there might be extension distally, but rarely proximal.
5. Resection or transection of the bowel will be followed by extension of disease proximal.
6. The disease has its onset and greatest virulence in young people but remains potentially virulent at all ages. UC is more likely to remain quiescent in older age.
7. There is a family history of IBD, usually Crohn's, in 20-30 % of patients.
8. No drug has been shown to permanently eradicate Crohn's disease.

Extracted from Korelitz, (1985).

rinogen concentration) (Cooke et al., 1958). The most seriously ill patients had the highest levels. This particular population of patients showed no increases in CRP levels (Cooke et al., 1958).

Increased plasma α_1AGP has subsequently been shown to be related to the staging of UC (Dearing, McGuckin and Elveback, 1969; Weeke and Jarnum, 1971) and Crohn's disease (Weeke and Jarnum, 1971; Andre et al., 1981) in adults and children (Campbell, Adinolfi and Walker-Smith, 1979). Changes in CRP levels are more variable but increases during both diseases have been reported (Shine et al., 1985; Fagan et al., 1982). When related to the staging of either disease, plasma CRP concentrations were always greater in Crohn's than UC patients for similar clinical categories (Fagan et al., 1982; Shaverymuttu et al., 1986). There was no difference in CRP increases between Crohn's patients with small or large bowel involvement (Shaverymuttu et al., 1986). Plasma antiproteases that are elevated in the two diseases include α_1PI (Weeke and Jarnum, 1971; Andre et al., 1981; Fischbach et al., 1987) and $\alpha_1$antichymotrypsin (Bohe, Genell and Ohlsson, 1986). Alpha$_2$macroglobulin, which is not an Acute Phase Protein in man, has been reported to increase in concentration (Bohe, Genell and Ohlsson, 1986) and decrease (Weeke and Jarnum, 1971), during IBD.

Changes in CRP concentrations have been emphasized in a prognostic capacity for the Acute Phase Protein

Response and IBD. Those patients with clinically quiescent disease, but elevated CRP, usually relapsed within one month (Andre, Descos and Vignal, 1979). C-reactive protein and pre-albumin showed a strong correlation with the patient's clinical response to treatment versus those patients which did not respond to treatment (oral prednisolone or surgery; Buckell et al., 1979). Plasma CRP showed a significant increase and plasma iron a decrease, during the third and second month before acute exacerbation of Crohn's disease (Wright, Young and Tigler-Wybrandi, 1987). Plasma α_1PI and α_1AGP rose significantly between the second and first month prior to the relapse, respectively. However, the positive predictive value of the Acute Phase Proteins was always less than their negative predictive value, and no better than the CDAI or other indices in predicting relapse.

1.6 Models of intestinal inflammatory diseases; intestinal nematode infections of the rat

Despite the availability of many rodent models of inflammation, few involve natural causes of inflammation and few involve the bowel. A number of parasitic infections of the rat are available which cause local intestinal inflammation. Two nematode infections of the rodent small bowel were chosen for investigation: Nippostrongylus brasiliensis (Travossos, 1914) and Trichinella spiralis (Owen, 1835). Local inflammation occurs at three sites in N. brasiliensis-

infected rats: the skin, the lungs, and the intestine. Infection of the rat by T. spiralis leads to inflammation of the small bowel followed by inflammation of skeletal muscle. These two infections were chosen for the investigation of Acute Phase Protein changes because of the temporal sequence of the periods of acute inflammation.

The two nematode species belong to different phylogenetic classes; N. brasiliensis is a member of the class Secernentea[1], order Strongylida, and is thus closely related to the human hookworms (Ancylostomatoidea, Necator) and more closely related to Ascarids (Ascaris) than to T. spiralis. T. spiralis is a member of class Adenopherea, order Trichocephalida and is related to Trichuris the human whip-worm. Similar to human hookworms, N. brasiliensis spends a period of its life cycle as free-living larvae while T. spiralis is an obligate parasite throughout its life cycle. Both infections have a period of systemic infection in addition to a period of residence in the host intestine.

N. brasiliensis eggs are passed out of the host in the feces. The larvae hatch and remain free-living until they molt into infective third stage larvae (L_3), about one week later. The L_3 do not feed, and relying entirely on stored food, remain fecund for considerable periods when stored at cool temperatures (Hindsbo, 1983). The infective

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[1] Refer to Cheng, 1986 for more details concerning the classification of nematodes and their morphology.

third stage larvae are capable of penetrating the host's skin (Lee, 1972) but can be injected subcutaneously. Following skin penetration, the larvae undergo a period of passive migration, being carried away by the blood stream to the first capillary bed they encounter- the lungs (Croll, 1977). Maximal numbers of L_3 have reached the lungs by day 2 post infection (PI). Once in the lungs they penetrate into the alveolar spaces, molt (sexes can be differentiated at this time) and continue up the bronchi to the trachea and eventually the esophagus. The fourth stage larvae (L_4) are swallowed, pass through the stomach and arrive in the duodenum to molt once more. The worms mature to adults and live in the proximal jejunum (Haley, 1962). In the intestine, adults mate and the females begin to excrete eggs by day 6 PI. Egg production reaches maximum output between days 8-9 PI, shows significant declines in production starting on day 10 and almost no egg production by day 14 PI. Worm numbers reached a plateau by day 4 PI in the intestine and depending on the rat strain, are expelled concurrent with a specific immune response by day 14 PI. The worms that are expelled are not irreversibly damaged by the expulsion process (Kassai, Takats and Reld, 1987) and a few worms, usually male, may remain in the small bowel post expulsion (Africa, 1931). Due to the passage of the worms through the host's lungs, the host has been exposed to a parental component of the infection before the adult phase.

The T. spiralis life cycle and infection of the rat is considerably different. Infective larvae must be recovered from skeletal muscle of mammals. The larvae survive in cysts (nurse cells) in skeletal muscle as L_1, but achieve the sexual differentiation of L_4 (Kozek, 1971). Ingestion of the infected muscle by a second, susceptible host, leads to digestion and release of the larvae from the cysts. The larvae undergo rapid molts (4 within 24 hours) in the proximal jejunum (Kozek, 1971). The worms mature and mate within 36 hours of infection. Female worms are ovoviviparous and begin to deliver newborn larvae by days 5-6 PI (Despommier, 1977). The newborn (L_1) migrate via the portal vein (Wang and Bell, 1986) to skeletal muscle throughout the host where they encyst and remain until the cycle starts over (Despommier, 1975). The time-course kinetics of the adults' residence in the small bowel is similar to N. brasiliensis and few worms remain beyond day 14 PI. However, the parental stage of T. spiralis follows the intestinal adults stage, in contrast to infection with N. brasiliensis.

The adults of the two species occupy a different niche within the host's intestine. N. brasiliensis remains in the intestinal lumen, usually at the base of the villi, while T. spiralis lives intraepithelially (Gardiner, 1976). In this intraepithelial compartment, the worms lie in the cells' cytoplasm and thread through rows of enterocytes, as

if in a syncytium, without gross distortion of the cells' luminal surface (Wright, 1979). T. spiralis therefore spends most of its life cycle intracellular, with a brief period of extracellular larval migrans. Some characteristics of the life cycle kinetics, at least for T. spiralis, are worm-dose dependent- for example infections with greater than 2000 larvae are not expelled by day 14 PI (Bell et al., 1983).

Some additional similarities in life cycle kinetics are seen when the animals are challenged with a second, homologous infection. Both species of worm are expelled before they mature during challenge infections and considerably earlier than in a primary: the so-called "rapid expulsion" process (Love, Kelly and Dineen, 1974; Bell and McGregor, 1979; Miller, Huntley and Wallace, 1981). Fewer N. brasiliensis reach the intestine in animals with multiple infections and those worms that do reach the intestine are stunted and do not produce eggs (Taliaferro and Sarles, 1939; Love, Kelly and Dineen, 1974). Transfer of N. brasiliensis adults into the intestine of a second rat immunizes the recipient, which may then undergo rapid expulsion on challenge with L_3 (Ogilvie, 1965). The rapid expulsion phenomena may be elicited following a single patent infection with T. spiralis (muscle larvae establishment), or large infections of adults only (Bell and McGregor, 1979). There is no immunological cross-reactivity between the two

species, determined by a failure to show rapid expulsion on challenge of mice with the heterologous nematode (Kennedy, 1980). The two species do not seem to affect each others' development when given concurrently; however, if the second species was administered at the time that the first infection was being expelled, the challenge infection was also expelled (Kennedy, 1980).

Some other variations in life cycle kinetics occur during infections of cortisone-treated, neonatally thymectomized, nude/nude, and young (less than 28 days of age) rats or mice. In all four of these models of infection the animals fail to expel their worm burden and the adult worms continue to produce eggs or newborn larvae, for the life of the host (Coker, 1955; Markell, 1958; Jacobson and Reed, 1974; Perrudet-Badoux et al., 1980).

1.6.1. Intestinal inflammatory events due to nematode infection of the rat

Despite the differences in life cycle and adult behaviour in the host intestine, both species elicit many similar changes in host pathophysiology. Table 7 lists many of the pathophysiological changes documented in the intestine of nematode-infected rats. Many of the changes are likely due to the introduction of cells foreign to intestinal tissue, and others due to triggering of resident cells.

TABLE 7

Pathological changes due to helminth-induced inflammation and possible cell mediators

INFLAMMATORY EVENT AND RELEVANT MEDIATORS	POSSIBLE CELL SOURCE
Cell infiltration and activation	
Leukotriene (LT) B_4	epithelial, neutrophils, macrophages
HETEs	mast cells, neutrophils, endothelial
Platelet Activating Factor (PAF)	mast cells, neutrophils, eosinophils, macrophages, platelets, endothelial
Eosinophil Chemotactic Factor	mast cells
Neutrophil Chemotactic Activity	mast cells (?)
Histamine	mast cells, basophil
Mucosal edema	
Histamine	mast cells, basophil
SRS-A, LTC_4, LTD_4	mast cells, eosinophils
Prostaglandins (PG)	macrophages, platelets
Bradykinin	-
PAF	as above
Mucus hypersecretion	
Histamine	mast cells, basophil
LTC_4, LTD_4	as above
HETEs	as above
Epithelial shedding	
Proteolytic enzymes (RMCP II)	mast cells
Neuropeptides	-
Products of oxidative metabolism	macrophages, neutrophils
Major basic protein	eosinophils
Increased gut motility	
Histamine	as above
LTC_4, LTB_4	as above
PGs and thromboxane A_2	mast cells
PAF	as above
5-Hydroxytryptamine (serotonin)	mast cells, platelets

Extracted from Moqbel, (1986).

Gross changes in the intestine are obvious at necropsy. The small bowel becomes distended, watery and soft and appears to be more "thin-walled" than that of uninfected animals. Histologically, changes in the lamina propria and epithelial cellularity become obvious by the end of the first week of infection. Changes in the length of the jejunal villi accompany a hastening of epithelial cell turnover (increased movement and mitosis) with increased cell loss and deep crypt hyperplasia (Symons, 1965) until a characteristic flattening and fusion of adjacent villi is evident by day 10 PI (Cheema and Scofield, 1982). Similar changes occur in the distal, uninfected jejunum and ilium, indicating that they are probably not caused by the direct interaction of the worms with the epithelium (Symons, 1978). These changes are obvious in the duodenum and jejunum of rats infected with as few as 40 larvae and become increasingly obvious in the ileum and colon with increasing worm burdens (Cheema and Scofield, 1982). Goblet cell hyperplasia occurs and there is an accompanying escalation of intestinal mucus with infection (Miller, Nawa and Parish, 1979; Miller and Huntley, 1982).

Coincident with the physical changes in the epithelium are various changes in epithelial function (Castro and Russell, 1984). Altered intestinal epithelial transport of electrolytes, glucose, and water have been documented (Sukhdeo and Mettrick, 1984) along with reductions in brush

border amylase, maltase, and alkaline β-glycerophosphatase activity (Symons and Fairbairn, 1962), and some lectin binding potentials (Castro and Harari, 1982). Some changes in epithelial transport may be detected with doses of worms as low as 100 N. brasiliensis (Scofield, 1980).

Cellular infiltrates occur in the lamina propria and epithelium of the infected intestine. During N. brasiliensis infection of the rat, mast cell numbers initially decline until few cells remain by day 8 PI. Then mast cell numbers begin to escalate during or immediately following worm expulsion and may rise ten fold over normal numbers (Miller and Jarrett, 1971; Kelly and Ogilvie, 1972; Denburg, Befus, and Bienenstock, 1979). A similar mast cell hyperplasia occurs during infection by T. spiralis, although the early, initial decline does not occur (Woodbury et al., 1984). Changes in tissue levels of histamine and rat mast cell protease II (RMCP II) correlate with the changes in mast cell numbers (Befus, Johnston and Bienenstock, 1979; Woodbury and Miller, 1982).

Eosinophil numbers in the lamina propria usually change in concordance with mast cell numbers (Kelly and Ogilvie, 1972; Nawa and Hirashima, 1984) and the changes may be followed by measuring intestinal peroxidase activity (Smith and Castro, 1978), although neutrophils may contribute to the total detectable peroxidase.

Some of the other inflammatory events shown in Table

7 are due to the activity of the cell types discussed above. For example, changes in intestinal motility may be due to generation of prostaglandins, histamine and/or serotonin (Sukhdeo and Croll, 1981). In addition to the effects these mediators have on smooth muscle, the muscle hypertrophies and shows some alterations in contractile behaviour during infection (Fox-Robichaud and Collins, 1986).

The lymphatics which drain the intestine show considerable changes over the course of infection. Peyer's patches (PP) and mesenteric lymph nodes (MLN) both swell with lymphocytes although the PP respond later than MLN (Levin et al., 1976). The MLN also responds with a mast cell hyperplasia and increased histamine content during N. brasiliensis infection of the rat (Befus, Johnston and Bienenstock, 1979).

The changes in lymphatic cellularity are related to the development of acquired immunity against the parasites. Both pathogens elicit specific immunoglobulin responses of most isotypes, and non-specific enhancement of total IgE production (Ogilvie and Jones, 1971; Lee and Best, 1983). The potentiation of IgE may be rat-strain related (Jarrett, Haig and Bazin, 1976). Both infections elicit worm-specific cell-mediated (T lymphocyte) responses as shown by passive transfer of immunity with immune MLN cells (Love, Ogilvie and McLaren, 1976). Recently, the T lymphocytes recovered from mesenteric MLN of parasitized mice have been shown to

respond to worm antigens with production of IL-2, IL-3 and IFN-gamma (Grencis, Riedlinger and Wakelin, 1987). The role of the T lymphocyte in the host response to parasite infection cannot be overstated; most of the pathological changes do not occur in T lymphocyte-deficient animals (Ferguson and Jarrett, 1975).

1.6.2 Systemic inflammatory events due to nematode infection of the rat

Some general observations of the infected animals' health indicate the presence of a systemic inflammatory reactions. Rats lose weight during the first two weeks of infection, suffering greater weight losses with increasing worm numbers (Smith and Castro, 1978; Ovington, 1985; Ovington, 1987). Part of the weight loss is attributable to two periods of reduced appetite- one at day 2 and the second between days 6 and 9 PI (Ovington, 1985). Infected animals also endure some blood loss in the intestine which is maximal between days 7 and 10 PI (Nielson, 1969; Cummins et al., 1986). Precisely how much each of these phenomena contribute to the weight loss versus other aspects of the Acute Phase Response, such as muscle protein catabolism, is not clear.

Serum concentrations of albumin are reduced during N. brasiliensis infection of the rat (Ash, Crompton and Lunn, 1985) and T. spiralis infection of the mouse (Stewart,

1978). Plasma hormone changes documented during infection by N. brasiliensis include decreased corticosterone, adrenocorticotrophic hormone, triiodothyronine, insulin, and thyroxine concentrations, all maximal by day 8 PI, but normal by day 15 PI (Ash, Crompton and Lunn, 1985b). Infection by N. brasiliensis inhibits the hepatic microsomal drug-metabolizing system in rats (Tekwani et al., 1987).

Many of the local cellular changes which occur in the infected intestine also occur systemically. Infection with N. brasiliensis leads to increases in circulating lymphocytes (days 11-17), monocytes (day 5), neutrophils (days 7-12), eosinophils (days 3-5 and days 11-19) and basophils (days 6-13)(Roth and Levy, 1980). Leukocyte numbers increase in the lungs of N. brasiliensis-infected rats in a biphasic pattern on days 2 through 4 and about day 16 PI (Egwang, Gauldie and Befus, 1984). During infection of the rat by T. spiralis, lymphocyte numbers decline below normal, neutrophils increase on days 18 and 42, and eosinophils increase from day 6 (Lee and Best, 1983b). Monocyte numbers did not change significantly. Changes in numbers of leukocytes in the peritoneal cavity are less remarkable and only eosinophils have been reported to increase in number in T. spiralis-infected rats (Lee and Best, 1983).

1.6.3 Role of the macrophage in host resistance to nematode infection

In addition to the orchestration of the inflammatory response, host macrophages are the first line of "non-immune mediated" defence against pathogenic organisms. For this reason, macrophage function during parasitic infection has come under considerable scrutiny (Skamene and Gros, 1983). A substantial amount of investigation has been reported which characterizes the helminthocidal potential of macrophages. Enriched populations of peritoneal and alveolar macrophages have been used in *in vitro*-killing assays against different life stages of various nematodes. Rat macrophages kill *N. brasiliensis* L_3 by complement-dependent (Egwang, Gauldie and Befus, 1984b) and newborn *T. spiralis* by complement- and antibody-dependent mechanisms (Ruitenberg *et al.*, 1983). Macrophage killing of infective *T. spiralis* has yielded variable results depending on the freshness of the rat serum used (Mackenzie *et al.*, 1980). In mice, the macrophage capacity to kill *T. spiralis* via antibody-dependent mechanisms was related to the mouse strain (Perrudet-Badoux *et al.*, 1985).

It is not clear from *in vivo* investigations, whether macrophages actually function in this helminthocidal capacity in the infected animal. However, there is evidence that alveolar macrophages become activated during a primary infection of rats and mice by *N. brasiliensis*. Alveolar

macrophages secrete IL-1 and HSF and secretion increased during infection (Egwang, Befus and Gauldie, 1985; Lamontagne et al., 1985). Alveolar macrophages showed increased expression of C3 receptors during infection of the rat (Egwang, Befus and Gauldie, 1985), and IgA receptors during infection of the mouse (Gauldie, Richards and Lamontagne, 1983). N. brasiliensis-infected rats were more resistant to the growth of Walker carcinosarcoma. The tumour resistance was transferable with peritoneal macrophages from infected rats, but blocked by serum factors from late-infected animals (Keller, 1973). Mice infected with N. brasiliensis cleared plasma ^{125}I-labelled polyvinyl pyrrolidone with accelerated kinetics, relative to uninfected controls, an event associated with increased lung and liver macrophage phagocytosis (Price and Turner, 1983).

Studies have been performed which show increased resistance against T. spiralis, due to infections that stimulate macrophage activity. Mice infected with either Toxoplasma gondii or Listeria monocytogenes had significantly lower peak-worm burdens in their intestine than non-parasitized controls (Wing and Remington, 1978). Despite the lower worm numbers, the infections were expelled with similar kinetics as control mice. Similar to the parasite/-tumour studies using N. brasiliensis, peritoneal macrophages recovered as early as day 6 (Merrovitch and Bomford, 1976) and as late as day 18 PI (Wing, Krahenbuhl and Remington,

1979) from T. spiralis-infected mice inhibited tumour growth in vitro. T. spiralis infection also inhibited in vivo tumour growth in mice (Pocock and Meerovitch, 1982).

The studies suggesting macrophages are activated in T. spiralis-infected animals are in contrast to the systemic anti-inflammatory effect of infection suggested by Castro, Malone and Smith, (1980). These authors showed that the infection lead to inhibition of granuloma formation about a length of string implanted in the rat's abdominal skin. The size of the infection was inversely related to the weight of the granuloma and the effect of infection could be mimicked by injections of dexamethasone. If granuloma formation is a function of inflammation and macrophages are involved in granuloma genesis, this study would imply systemic macrophage activity is suppressed during infection.

Despite the intestinal inflammatory changes characteristic of the nematode infections, there are no studies of macrophage function in this tissue.

1.7 Summary of the literature and statement of the problem

Features of acute inflammation have been reviewed, with an emphasis on the role of the macrophage. Macrophages secrete various inflammatory mediators, (monokines or cytokines), at the site of inflammation, some of which circulate in the blood and lymph to elicit systemic events. Systemic inflammation manifests as the Acute Phase Response.

Interleukin-1 and Hepatocyte-Stimulating Factor are two notable examples of monokines that are involved in the Acute Phase Response. Greater amounts of these monokines are secreted by macrophages that have become activated.

One facet of the Acute Phase Response, the Acute Phase Protein Response, was regularly used to monitor the course of inflammation. Evidence was presented that macrophages elicit the Acute Phase Protein Response through cytokines which stimulate protein synthesis by hepatocytes. Thus the Acute Phase Protein Response served as an indicator of macrophage activity during inflammation.

It was established that nematode infection of the rat provokes intestinal inflammation. Activation of rat macrophages by the nematode infections also has been reported. It is not known whether the intestinal infections elicit the systemic inflammatory changes typical of the Acute Phase Response.

This thesis was an investigation of the Acute Phase Protein Response during nematode infection of the rat intestine. The experimental strategy was to examine the Acute Phase Protein concentrations of nematode-infected rats. The role of the macrophage in the response was assessed. The function of the intestinal cells recovered from infected animals also was investigated.

The activity of intestinal macrophages has not been studied in detail by others. Likewise, the capacity of

intestinal cells to regulate the Acute Phase Response has not been reported. A better understanding of the regulation of intestinal events will allow for an assessment the Acute Phase Protein Response as a window to the events in the intestine. The corollary to this understanding is that the occurrence of the Acute Phase Protein Response during intestinal inflammation may indicate that certain cells are actively participating in the local inflammatory response in that tissue.

MATERIALS AND METHODS

2.1 Animals

Outbred male Sprague-Dawley rats weighing 250-300 g (Charles River Biobreeding Laboratories, Montreal, Canada) were used for all experiments. All animals were allowed to remain in our animal housing facilities for at least one week before they were used. An exception was made in the experiments involving young rats, which were purchased immediately after weaning and were infected before 27 days of age. Sprague-Dawley and Specific-Pathogen-Free Lewis strain rats (Charles River) were used for passing and maintenance of *N. brasiliensis*. The *T. spiralis* life cycle was maintained in CBA/J mice (Jackson Laboratories, Bar Harbour, Maine, U.S.A.). All antisera used in rocket immunoelectrophoresis were raised against rat proteins in New Zealand White rabbits and were kindly provided by Dr A. Koj (Jagiellonian University, Krakow, Poland).

Two methods were used in order to repeatedly bleed rats. The most commonly used method was retro-orbital bleeding. This technique involved gaining access to the ophthalmic venous plexus (which in fact give albino rats their red eye colour) using a long-stem glass pipette. The pipette tip was used to disrupt the plexus allowing blood to

flow freely up the pipette. When performed correctly, retro-orbital bleeding did not disturb the animal as the eye ball fell back on the capillary bed applying enough pressure to stop further bleeding. No serum Acute Phase Protein Response was detected in animals bled this way and an autopsy of one animal failed to show gross pathological changes behind the orbit. This method proved useful for most of the earlier experiments, until the manufacturer of the pipettes changed their product and the pipette tips were no longer polished.

The second technique bleeding from the animal's tail artery. Two veins run lateral in the rat tail and a single artery lies parallel but ventral to the veins. Under light ether anesthesia and with the rat on its back, the artery was hardly visible along the centre-line of the ventral aspect of the tail. Using a 23 gauge butterfly infusion needle (Venisystems, Abbott Labs., Montreal, Canada) the artery was pierced and the animal bled of 0.25 ml of whole blood. Upon removal of the needle, pressure was applied to the tail until bleeding stopped.

In the earlier experiments, whole blood was collected into heparinized tubes in order to isolate plasma. All plasma specimens were frozen at -20°C before they were assayed. Unfortunately, upon thawing rat plasma had a tendency to clot. Subsequently, serum was collected from clotted blood for use in rocket immunoelectrophoresis.

2.2 Media

The following culture media were used: HANKS Ca^{2+}, Mg^{2+} free balanced salt solution for liver perfusion and isolation of macrophages; RPMI for cultures of macrophages and lamina propria cells; WLLLIAMS E for hepatocyte cultures, and MEMF-15 for mouse thymocyte proliferation assays and cultures of mouse cells. All media were Gibco (Grand Island Biologicals Company, Grand Island, U.S.A.) products. All culture media were supplemented with 5% (v/v) fetal bovine serum (FBS; Gibco), and 1% (v/v) each of penicillin-streptomycin, N-2-hydroxyethylpiperazine-N^1-2-ethanesulfonic acid (HEPES, Boehringer Mannheim, Germany), bicarbonate, and L-glutamine (Central media resources, McMaster University). Phosphate buffered saline (PBS: 0.03M sodium phosphate, 150 mM NaCl, pH 7.3) was sometimes substituted for HANKS solution for washing macrophages. In addition to the above, the hepatocyte media was further supplemented with 1μM dexamethasone ("Hexadrol", Organon Scientific, Toronto, Canada), 1μM insulin, and 50μg/ml gentamycin.

2.3 Parasitological techniques

2.3.1 Nippostrongylus brasiliensis

Nippostrongylus brasiliensis (Travassos, 1914) was originally obtained from Dr. Joe Urban, (U.S. Department of Agriculture, Beltsville, Maryland, U.S.A.) and was maintained in the laboratory by passing the life cycle through adult

rats. Both Sprague-Dawley and Lewis strain rats were used with no obvious differences in worm infectivity. Feces from seven to ten day-infected rats was collected and softened using excess water in a beaker. An equal weight of granulated charcoal (10-18 mesh, BDH Chemicals, Poole, England) was mixed with the feces followed by enough fine vermiculite to make the mixture a moderate slurry. The vermiculite served to retain water and the cultures did not need further watering. The mixture was placed in a flat dish and lightly compressed until flat. Larger fragments of vermiculite were erected on the mixture. Larvae hatched shortly after the feces cultures were established and began to migrate up the vermiculite. They matured to infective 3rd-stage larvae (L_3) in about 8 days at room temperature. Cultures between 12 and 25 days were choosen for use which was within the time that worms remain infective (Keymer, Martin and Wainwright, 1983). Infective larvae were collected from the feces culture by applying the pieces of upright vermiculite onto a Baermann apparatus.

The Baermann apparatus was simply a funnel with a filter through which the larvae passed, leaving the culture contents behind. The funnel was sealed with a stop-cock or fastened to a volumetric flask in order to collect larvae or adults, respectively. The filter was either cheesecloth or a single sheet of lens paper. In order to collect adult worms from the rat intestine, the funnel was attached to the

flask and was placed in a 37°C water bath.

The L_3 were washed several times using PBS containing 1% penicillin/streptomycin in order to remove most of the bacteria on the worm cuticle. Worms were counted by performing a dilution count on several samples under low power on a compound microscope and their numbers corrected to 6000 worms per ml of PBS. When adults or lung stage worms were collected all worms recovered in the flasks were counted; those remaining in the tissue represent about 10% of the population and were not counted (Egwang, 1985). Three thousand L_3 were injected subcutaneously in the dorsolumbar region of rats under light ether anesthesia, using a 23- or 21-gauge needle. In the experiments where fewer numbers of worms were injected, the final injection volume was always corrected to 0.5 ml. The day of the injection was considered "day 0" in all experiments.

Nippostrongylus brasiliensis eggs were collected and counted from fresh rat feces by a sucrose floatation method (454g sucrose in 355 ml water plus 6 ml formalin). Fresh rats feces was collected using forceps, weighed, then soaked in an excess volume of water until the stools dissociated easily. The dissolved feces was passed thorough a wire sieve into 50 ml polypropylene tubes. The tubes were then centrifuged for 10 min at 200 times gravity (xg), the supernatant discarded and the pellet resuspended in water to 40 ml. A 1 ml sample was taken and mixed with the sucrose

solution in a 12 ml polypropylene tube. This second tube was filled with sucrose solution until the meniscus became slightly convex and the fluid would contact a 50 X 50 mm glass coverslip. The coverslip was applied carefully in order to avoid spilling fluid down the sides of the tube. The tube, with the coverslip attached, was stored in a refrigerator (4°C) overnight. The cool temperature inhibited normal egg germination and they did not hatch overnight. The next day the coverslip was removed with the top layer of sucrose and placed on a microscope slide. All eggs present on the slide were counted using the low power objective of a compound microscope and the number corrected to eggs per gram feces using:

$$\frac{\text{numbers of eggs counted X 40 ml dilution}}{\text{weight of feces in grams}}$$

The top few cm of the sucrose from several specimens which contained plenty of eggs was examined for residual eggs, following removal of the coverslip. No eggs were found, indicating a successful recovery with the coverslip.

2.3.2 Trichinella spiralis

Trichinella spiralis (Owen, 1835) was originally obtained from Dr. K. Wright (University of Toronto, Toronto, Canada). The parasite life cycle was regularly passed through CBA/J strain mice but infective larvae recovered from rats were used in some experiments. Larvae recovered

from rats which had been treated with the anthelminthic "Mebendazole" (Pitman-Moore, Scarborough, Canada), although healthy-looking, were not infective for a second host. Mice or rats with infections aged between 30 and 90 days were used.

Infective larvae were recovered from the skeletal muscle of animals by a method involving pepsin digestion. Whole mouse carcasses or a quarter of a rat carcass were mulched in a blender in a solution containing 7% (w/v) pepsin and 7% concentrated HCl (v/v). The tissue was then incubated in 500 ml of the same solution in a 37°C water bath with air percolating through it, for two hours. The resulting slurry was filtered through several layers of cheesecloth into graduated cylinders and the larvae allowed to settle for one hour at room temperature. The digestion media was drawn-off and the larvae pooled into 50 ml polypropylene tubes and washed similar to N. brasiliensis.

T. spiralis infective larvae were counted in the same manner as N. brasiliensis and their concentration corrected to 4000 per ml of PBS. This sample was diluted further with an equal volume of 0.4% agar (also in PBS), in order to deliver 2000 larvae in 1 ml volumes. The agar was necessary to keep the larvae in suspension, in order to deliver the prescribed dose of worms. Without the agar the worms settled very quickly and an insufficient number may have been loaded into the syringe. Without the agar many

worms collected at the hilus of the needle and were not expelled from of the syringe. The larvae were delivered using a gastric feeding tube, or for mice, a blunt 18 gauge needle attached to a 1 ml syringe. An infection of 2000 and 500 larvae was regularly used in rats and mice, respectively. Day 0 was considered the day the worms were given to the animals.

2.4 Turpentine studies

Undiluted turpentine (Harrisons & Crossfield, Toronto, Canada) was used in a subcutaneous injection in the dorsolumbar region of rats, under ether anesthesia. The routine turpentine dose was 100 µl per 100 gm of body weight. In experiments in which the effect of the turpentine dose was examined, the turpentine was not diluted but was delivered as a smaller volume. Turpentine was injected using a glass syringe and 23-gauge needle.

Animals undergoing turpentine-induced inflammation were bled as indicated in the Results; however, their liver function or macrophages were always studied 24 hours following turpentine injection.

2.5 Cells

2.5.1 Bronchoalveolar cell preparations

Bronchoalveolar cells were recovered from rats by washing-out their lungs using warmed (37°C) PBS. Rats were first given a lethal dose of Sodium Pentobarbital ("Sonmotol", M.T.C. Pharmaceuticals, Mississauga, Canada) and exsanguinated by severing the dorsal aorta. The rat's pleural cavity was exposed by piercing the diaphragm without damage to the lungs. This step allowed the lungs to deflate. The animal's trachea was then exposed and the plastic stem from a 23-gauge infusion butterfly inserted and tied-into it. Phosphate buffered saline was infused in 5ml volumes, the thoracic cavity lightly massaged and the fluid withdrawn into a second syringe through a three-way stopcock system. The PBS infusion/recovery was repeated 10 times until a total of 50 ml of fresh, sterile PBS was used.

The cells recovered from several animals were usually pooled following the first wash by centrifugation. From the time of the washout the cells were kept on ice, until they were cultured. Pooled alveolar cells were washed in either PBS or RPMI by repeated centrifugation through fresh medium at 200 xg, 4° C. The final wash was always in RPMI supplemented with 10% FBS (complete medium) in preparation for culturing.

Bronchoalveolar cells were counted and the viability determined using a hemacytometer and trypan blue as a vital

dye (Gibco, 5% final volume). The number of viable cells was corrected to 1×10^6 per ml and the cells applied, in complete RPMI onto plastic petri dishes. The cell yield in any single experiment determined the size of the dishes used for culturing, but the cells were always cultured at the same density. There was no evidence to suggest that any single size of dish gave results different from other dish sizes.

Following a two hour incubation, during which time cells adhered to the plastic, all dishes were washed repeatedly using warmed PBS. The culture media was then replenished to its original volume with incomplete RPMI. One half of the cultures had 5 μg per ml lipopolysaccharide (LPS, E. coli, 055:B5, Difco Laboratories, Detroit, U.S.A.) added.

2.5.2 Peritoneal cell preparations

Peritoneal cells were recovered following the infusion of cold (4°C) PBS into the intact peritoneal cavity of rats. The rats were killed by cervical dislocation and their abdomen skinned. A volume of 30 ml PBS was infused using an 18 gauge needle without opening the cavity. The abdomen was massaged briefly, and (usually) at least 25 ml was recovered by using the same needle in the side of the abdomen. If the media was discoloured the cells were not used. Such discoloration may be due to excessive red blood cells or the initial injection having pierced the bowel.

2.5.3 Intestinal cell preparations

The isolation of intestinal cells involved serial exposures of the tissue to digestive media. The entire small intestine of a rat was removed, washed out using a glass Pasture pipette and PBS, and then slit open along the (longitudinal) mesenteric axis in a bath of cold PBS. Sections of intestine about 5 cm long were cut and laid onto Whatman (#1) filter paper. At this time the Peyer's Patches were discarded. The mesenteric border was trimed-off the sections and the remaining tissue was then returned to a dish of HANKS media. These sections were trimmed into smaller pieces by cutting multiple sections off the ends, perpendicular to the direction of the gut lumen. These pieces were incubated in pre-warmed, Ca^{2+} and Mg^{2+} free HANKS (37°C) containing 1.3 mM ethylene-diaminetetra-acetic acid (EDTA). One rat intestine was typically incubated in 100 ml of media, stirring at 37°C for 10 min. The EDTA digestion was repeated for a total of three times; the tissue filtered through surgical gauze and temporarily bathed in HANKS between digestion. The EDTA incubations were intended to "burn-off" the intestinal epithelium and expose the lamina propria for the next stage of the digestion.

The lamina propria was digested using collagenase. Two brands of collagenase were used over the duration of these experiments. While there was no obvious difference in

the performance of each brand, a direct comparison of their respective "units" cannot be made because each supplier had a different assay for collagenase activity. Gibco collagenase was used at 25 units/ml in 200 ml of HANKS media while the Sigma (St. Louis, U.S.A.) product was used at 70 mg in the same volume. In both cases the HANKS medium was supplemented with 20% FBS. The collagenase medium was prewarmed to 37°C and the tissues suspended in 100 ml, following the final EDTA wash. This digestion was left stirring at 37°C for one hour. The medium from this stage was saved after the tissues were filtered out and returned to fresh collagenase, for a second hour. The cells collected over the first hour of collagenase digestion are referred to as "coll I" in the results. Following the second hour of digestion the tissues were filtered and the media saved. The tissues were resuspended in a small volume of HANKS and mulched by repeatedly drawing them up and down a 10 ml syringe barrel, resulting in a slurry. The product of the second collagenase digestion was two populations of cells- the second hour incubate, coll II, and the "syringed" population.

All three cell populations were collected by centrifugation at 200 xg at 4°C for 10 min. Each fraction was resuspended in 30 ml of RPMI in preparation for nylon wool separation. The nylon wool was cleaned by boiling it in an EDTA solution followed by distilled water. Three gram pieces were packed into a 10 ml syringe which was then

moistened with RPMI to ensure there were no cavities in the column. The cells were passed through this column followed by enough RPMI to bring the final volume of the collecting tube to 50 ml. The nylon wool was intended to remove mucus and large lumps of cells and was not expected to separate cell types.

Following centrifugation, the syringed population and coll II were pooled into a single 50 ml tube, the total number of cells and viability determined in coll I and II, and the cells washed in complete RPMI. The cells were finally resuspended in 35 ml of media in preparation for centrifugation through a 30% Percoll solution (Pharmacia, Uppala, Sweden)(Ulmer and Flad, 1979; Pertoft and Laurent, 1982). This initial Percoll separation was intended to enrich for viable cells.

The Percoll gradient was prepared by mixing 26 ml of stock Percoll with 2.5 ml of 10X concentrated HANKS, 0.25 ml of HEPES and 2 drops of 10N HCl, the final volume being approximately 30 ml. The Percoll was then added to the 35 ml of coll I and coll II, to a final volume of 50 ml. Both tubes were thoroughly mixed; the final homogeneous solution becomes 30% Percoll (v/v). Centrifugation at 200xg for 10 min at 4°C separated dead (less dense) cells from viable (more dense), the viable cells spinning down into the pellet in the tube. The pellet was recovered and the cells washed repeatedly in RPMI to remove contaminating Percoll. Coll I

and coll II were always applied to the Percoll step in separate tubes in order to avoid saturating the solution. The two collagenase products were usually pooled after the single-step Percoll enrichment and represent the entire lamina propria cell population. Cytocentrifuge slides of representative samples of the intestinal preparations were always prepared using a Shandon (Johns Scientific, Toronto, Canada) table top cytocentrifuge operating at 350 rpm. For intestinal cell cultures the cell density was corrected to 5 X 10^6 viable cells per ml and to 2 X 10^6 viable cells per ml for cytocentrifuge preparations.

2.5.4 Hepatocyte preparations

Rat liver cells were isolated by *in situ* perfusion followed by *in vitro* collagenase digestion. Rats were first anesthetized using Somnotol, then given an intraperitoneal injection of 10000 units of heparin ("Hepalean", Organanon). The rat's abdominal cavity was opened and the portal vein exposed. A peristaltic pump was outfitted to perfuse at 30 ml/min. A "bubble trap" was furnished, in series, on the perfusion line to ensure uniform perfusion of the tissue.

The perfusate consisted of Ca^{2+}, Mg^{2+} free HANKS buffered with 20 mM HEPES and was maintained at approximately 35°C. Air was percolated through the perfusate using a conventional fish tank air pump and autoclaved hoses and filters. All solutions used in the isolation of liver cells

were filter- sterilized through 0.22 μm filters. The portal vein was pierced and cannulated with a plastic catheter. The catheter was tied down to the vessel using No. 000 black silk (Davis-Geck Cyanamid, Montreal, Canada). The perfusion was begun as soon as the catheter was secure. It was necessary to severe a major blood vessel to allow the perfusate to escape the animal's circulation. The animal was exsanguinated in the process. This initial perfusion usually took about 7 min during which time the entire liver was dissected out of the animal and removed to a petri dish.

The liver was then bathed in a collagenase solution maintained at 35-37°C in a water bath. The digestion medium consisted of collagenase (75 mg, Sigma) in HEPES buffered HANKS reconstituted with Ca^{2+} (0.12mM $CaCl_2$) and 2% bovine serum albumin (BSA), to a final volume of 100 ml. The collagenase digestion was allowed to incubate for 10-15 min with the solution continually perfusing through the liver. Following the digestion the hepatocytes were combed away from the lobules onto a dry and sterile petri dish. The cells were pipetted-off the dish into 100 ml of 0.2% DNAase (Sigma) and left stirring at 37°C for 15 min. The purpose of the DNAase was to disrupt cell aggregates which were thought to be due to DNA adhering between cells. The incubation in DNAase was optional and was not always performed since some batches of collagenase proved to be more effective than others in digesting the liver into

single cell suspensions. In either case, the cells were finally recovered into 50 ml polypropylene tubes and washed repeatedly using complete WILLIAMS E medium. Hepatocytes were enriched by washing the product of the liver digestion at unit gravity- leaving the cells on the bench for 15 min. Hepatocytes are considerably heavier than macrophages (Kupffer cells in the liver) and settled into the pellet in the tubes within minutes. Kupffer cell contamination was determined to be less than 2% based on morphological criteria.

Hepatocyte preparations were routinely greater than 70% viable, determined by trypan blue exclusion. The cell concentration was corrected to 8 X 10^5 viable cells per ml and 0.25 ml was applied into each well of a 24-well plastic dish ("Nunclon", N.U.N.C., Denmark) which had been previously coated with rat collagen ("Vitrogen 100", Collagen Corporation, Palo Alto, U.S.A). Following a 2 hr incubation at 37°C the adherent cells were washed using pre-warmed (37°C) WILLIAMS E and the cells cultured in a final volume of 0.25 ml.

2.6 Hepatocyte-stimulating assay

Primary hepatocyte cultures were used to assess hepatocyte-stimulating activities from two sources: the *in vivo* consequences of turpentine-induced or parasite-induced inflammation and secondly, the products of macrophages iso-

lated from animals but maintained in vitro. The first activity is a direct measure of liver stimulation in the animal and the hepatocytes were simply isolated and cultured for 3 days at 37°C, 5% CO_2. The collagen-adherent cells were washed every 24 hr and replenished with fresh WILLIAMS E (final volume 0.25 ml). The culture supernatant was saved following each 24 hr wash and applied to the rocket immunoelectrophoresis assay.

The second (in vitro) cell assay was used to measure the state of activation of hepatocyte-stimulating factor (HSf)-producing cells. In the case of macrophages, the 24 hr culture supernatant was recovered and dialyzed against PBS ("Spectropore", Spectrum Medical Industries, Los Angeles, U.S.A., molecular weight-cutoff of 15,000) for 48 hr. Following dialysis, the supernatants were filtered through a 0.22 μm filter and frozen at -20°C until assayed on hepatocytes. Serial dilutions of the macrophage supernatants were prepared in PBS and applied onto the hepatocytes in a 0.25 ml volume. The supernatant was re-applied with each 24 hr change of hepatocyte medium. Unless otherwise indicated, the 72 hr hepatocyte supernatant was analyzed by rocket immunoelectrophoresis. The positive control in this assay was the 24 hr culture supernatant from human peripheral blood mononuclear cells stimulated with LPS, while media alone served as the negative control (Koj et al., 1985). Media containing LPS alone did not stimulate

hepatocyte acute phase protein synthesis.

This approach to measuring hepatocyte-stimulating activity production was also used to indicate whether cells recovered from inflamed/parasitized animals were activated and secreting greater amounts of factor(s) than those recovered from normal rats. In these experiments, one half of the cultures of both populations, infected and non-infected rat macrophages, had LPS added to the culture. This additional *in vitro* stimulation served to identify whether the cells were maximally stimulated by the *in vivo* inflammation, or whether they were inhibited. The negative control for *in vivo* activation was normal and unstimulated rat macrophage supernatant. The presentation of hepatocyte-stimulating factor assay results is described in the next section.

2.7 Rocket immunoelectrophoresis

Rocket immunoelectrophoresis was performed according to Weeke (1973) in 2% agarose ("Seakem ME", Mandel Scientific, Rockwood, Canada) dissolved by boiling in barbital buffer (30 mM barbital, 5 mM Na barbitone, 0.25 M glycine, 0.25 M TRIS; Fisher Scientific, FairLawn, U.S.A.). Monospecific rabbit anti-rat Acute Phase Protein was mixed with the agarose at 60°C and poured onto glass slides (10 cm X 8 cm, final volume 12.5 ml). The optimal dilution of the different anti-sera was determined by trial and error and are

recorded in Table 8. The hepatocyte supernatants (7 µl) were used undiluted in the antigen wells. Rat serum specimens were diluted as recorded in Table 8 and 7 µl volumes were used in the antigen wells. Electrophoresis was run at 25V, 60mA per glass plate for at least 10 hr at 4°C for both hepatocyte and serum samples. Following electrophoresis the plates were soaked in dishes of 0.89% saline in order to leach-out free proteins, then the agarose was pressed flat onto the glass slide and dried in an oven at 60°C for one hour. The protein precipitate on the slide was stained using Coomassie brilliant blue (Merck, Darmstadt, Denmark). Excessive background stain was removed by repeated washings in a mixture of water/ethanol/acetic acid (55/30/15, v/v/v).

The precipitin bands on the stained electrophoretic plates constituted the rockets. The distance between the peak of the precipitin arc and the leading edge of the antigen well was considered the height of the rockets. The coefficient of variation for rocket peak heights, measured between replicate rockets of one sample on the same plate was less than 4% and and on different plates, using the same batch of antisera, was less than 10%. The rocket peak height was directly related to the logarithm of the antigen concentration hence dilution curves for each antigen/-antibody reaction were determined. The antigen dilution was plotted against peak height in order to compare concentra-

TABLE 8

Volumes and concentrations of antisera and antigens used in rocket immunoelectrophoresis

Acute Phase Protein	% ANTIBODY CONCENTRATION	ANTIGEN CONCENTRATION
Serum		
Albumin	1.2	1/500
$\alpha_2$macroglobulin	0.64	1/50
Haptoglobin	0.16	1/250
$\alpha_1$acid glycoprotein	2.0	1/40
$\alpha_1$cysteine protease inhibitor	0.40	1/160
Plasma		
Prothrombin	0.80	1/250
Fibrinogen	0.80	1/250

tions between samples.

Serum Acute Phase Protein concentrations are reported in one of two manners. For some experiments the rocket peak height of "unknown" samples was compared to the dilution of a standard pool of inflamed rat serum necessary to achieve a rocket of equal height. The estimated dilution of inflamed (infected) samples was divided by the estimated dilution of day 0 (normal) serum from the same rat. This calculation yielded a number representing a "percent change" compared to normal, over time for a single rat. The numbers from 4 or 5 animals bled on the same day were pooled to give a mean change ± standard error of the mean. This method gave a relative indication of changes in concentration over time and neglected variability between animals on the same day. The second measure was to determine the absolute concentration of a protein by comparing the rocket peak heights to a standard dilution curve determined from immunoelectrophoresis of purified proteins of known concentration. The second measure shows the "between animal" variability in protein concentration, including day 0, and statistical tests were used to compare day 0 with other days of infections.

Hepatocyte-stimulating assay results are expressed in two manners. One method was to use the ratio of the rocket peak height for any single Acute Phase Protein (usually α_2M) from different samples divided by the rocket

peak height of albumin for the same samples. Increases in this ratio were potentially due to a number of factors in the hepatocyte assay including a rise in Acute Phase Protein concentration, a decline in albumin concentration or both. The utility of the ratio lies in the fact that it standardized changes within and between assays. If there was a net difference between the total protein-synthetic activity of two normal batches of hepatocytes, the relative synthesis of albumin to other proteins remained constant and the ratio was similar for the two batches of cells. The normal response of cultured rat hepatocytes to the positive control was increased synthesis of Acute Phase Protein and decreased synthesis of albumin, and the ratio rose directly with increasing concentrations of the monocyte supernatant. Therefore the ratio provided a sensitive, albeit non-specific, measure of hepatocyte activity.

The second method for presentation of hepatocyte-stimulating activities is the calculation of a stimulation index based on α_2M synthesis by the liver cells. A dilution series of the positive control for hepatocyte stimulation (LPS-stimulated human peripheral blood mononuclear cells) was included in every assay and was used to determine the rate of change of rocket peak height due to factor concentration. The $\log_{10}$ transformation of the volume of monokine in each dilution was plotted against rocket peak height and the "one-half maximum hepatocyte stimulation", in

μl of cytokine, was determined (Figure 2). Serial dilutions of macrophage culture supernatants containing unknown amounts of stimulating factors were analyzed concurrently. The supernatant dilution of each of these samples was plotted against the α_2M peak height determined by electrophoresis. The "best fit" straight line was drawn between these points and was extrapolated until it intersected with the one half maximal value determined for the positive control. This point of intersection was read as a volume on the abscises and was estimated to be the volume of supernatant necessary to acheive one-half maximum assay stimulation. Hepatocyte-stimulating units were calculated as follows:

$$\frac{1000}{\text{Y-intercept, } \mu l} = \text{units}$$

The numerator 1000 corrects the units to cytokine in 1 ml. This measure of specific hepatocyte-stimulating units was used only for stimulation of α_2M. The coefficient of variation for calculating units of activity in the same assay was less than 10%. The concept of empirical units was functional for comparing "within assay" results because the α_2M rocket peak heights vary with the batch of antisera and concentration used. The advantage of this measure of hepatocyte-stimulating activity was that it is independent of changes in albumin levels.

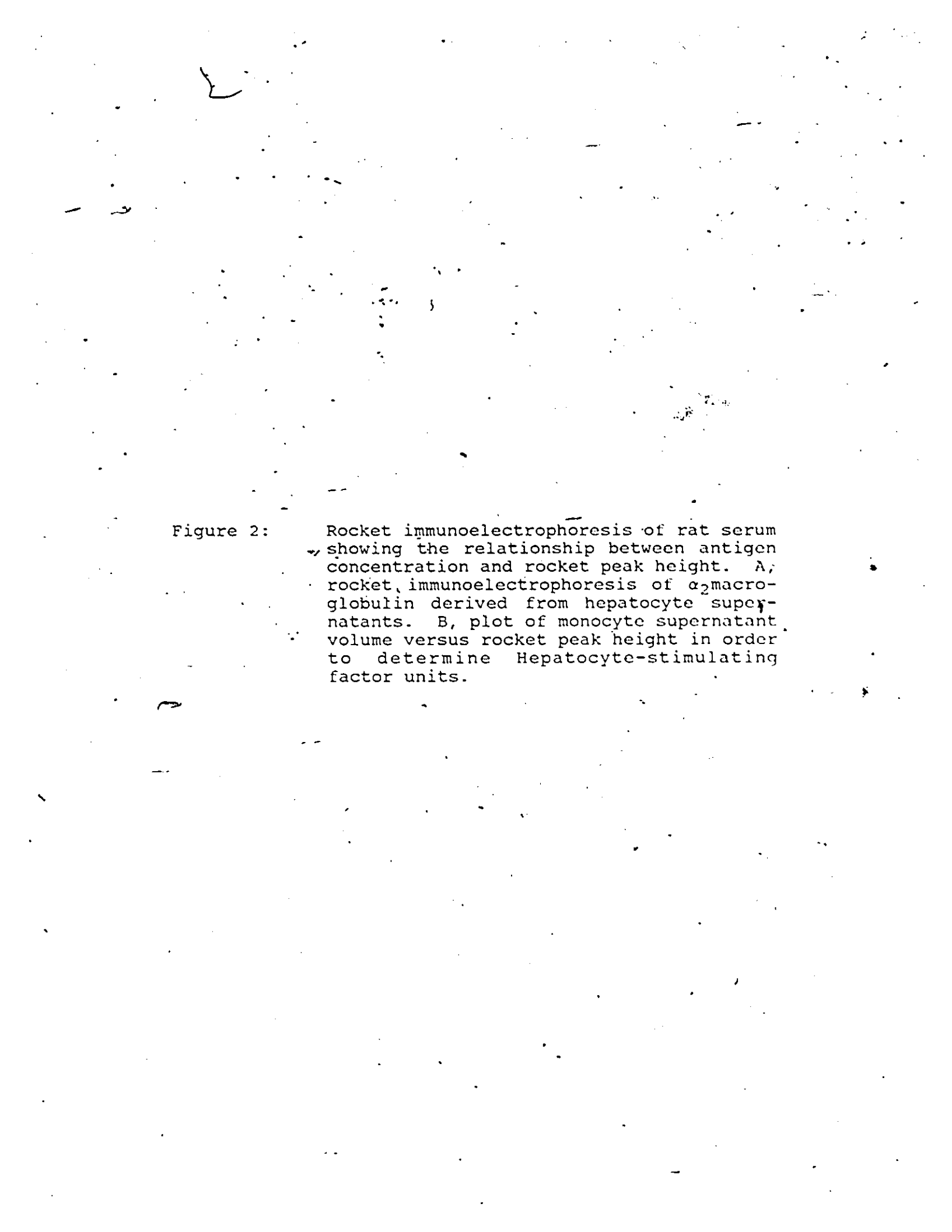
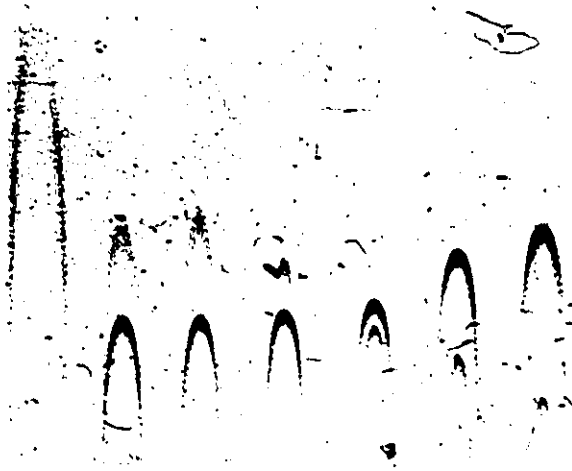
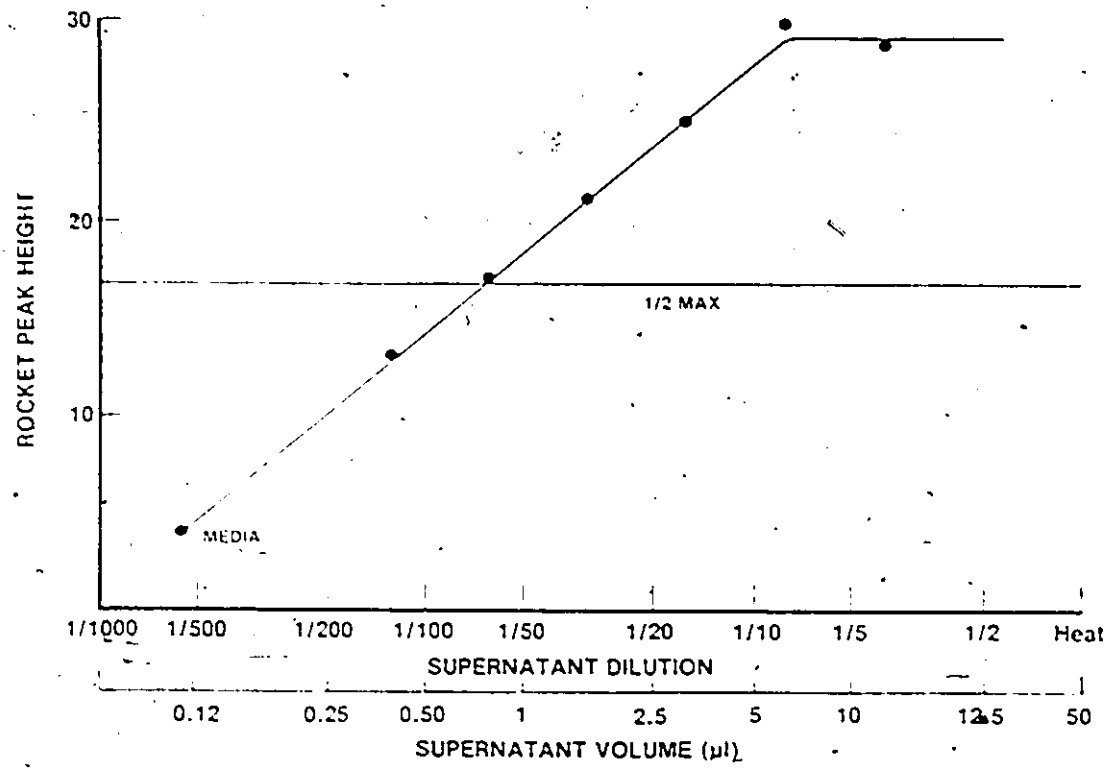


Figure 2: Rocket immunoelectrophoresis of rat serum showing the relationship between antigen concentration and rocket peak height. A, rocket immunoelectrophoresis of $\alpha_2$macroglobulin derived from hepatocyte supernatants. B, plot of monocyte supernatant volume versus rocket peak height in order to determine Hepatocyte-stimulating factor units.



PBS 1/4 1/8 1/16 1/32 1/64 1/128

Supernatant dilution



2.8 Lymphocyte-Activating Factor assay

The method chosen for measuring IL-1 activity was the murine co-mitogenic assay (Lymphocyte-Activating Factor or LAF, Farrar et al., 1978). The assay involved culturing C3H/HeJ-strain thymocytes (pooled from several animals) in MEMF-15 supplemented with FBS, penicillin/streptomycin, L-glutamine, HEPES, Na bicarbonate, Na pyruvate (1% of 100 mM stock) and β-mercaptoethanol (10^{-5} M) for three days at 37°C, 5% CO_2. The cells were cultured at 1.5 X 10^7 viable cells per ml, in 100 μl volumes in 96 well microtiter dishes. Sub-mitogenic concentrations of phytohemagglutinin (PHA, 1%, Difco) and serial dilutions of crude IL-1 were added (100 μl volumes) to the thymocytes on the first day of culture. On the third day a radioactive label was added (50 μl of 10 μCi per ml methyl-^{3}H thymidine) to the cultures and the cell DNA harvested on a cell harvester ("Minimash", M.A. Bioproducts, Waldersville, U.S.A.) 24 hr later. The proliferating cells incorporate the radiolabell into their DNA; the "hot" label on the harvester filters was counted in 5ml of liquid scintillant (22 mM 2,5-diphenyloxazole, 0.27 mM di-1,4(phenyl-5-oxazolyl)-2-benzene in toluene) in a β counter.

LAF assay results are presented as the mean ± standard error of the mean of the total radioactivity (counts per min) for three determinations at each dilution.

2.9 Measurement of Acute Phase Protein mRNA

2.9.1 Isolation of liver RNA

Total liver RNA was isolated from rats using the guanidine HCl method (Cox, 1968). The procedure first involved homogenizing 1 g pieces of fresh rat liver in 8 M guanidine HCl (BRL, Gaithersburg, U.S.A.; 10 ml, buffered with 50 mM Tris, 10mM EDTA, 0.1 M potassium acetate, pH 7.5). The product of this step was centrifuged (10,000 xg, 4°C) and the pellet discarded. Absolute ethanol (-20°C) was added (5 ml), the mixture vortexed and left to incubate at -20°C for 1 hr. The preferential isolation of RNA over DNA depended on the ratio of ethanol/guanidine. Following the first incubation the solution is centrifuged and the pellet resuspended in 5 ml of 6 M guanidine HCl (buffered with 0.1 M potassium acetate). Ethanol was added, in a 1/2 X volume of the guanidine, the solution vortexed, and left incubating for one half hour at -20°C. The 6 M guanidine extraction was repeated for a total of three times.

Following the final 6 M extraction the pellet was resuspended in (distilled and autoclaved) water. The sample was vortexed and centrifuged immediately. The water was saved and the pellet resuspended in fresh water. From this point in the isolation the RNA was kept on ice.

The optical density (OD, 260 nm) of the RNA was determined for a 1/100 dilution of the water-extracted product. Repeats of the water extraction of the pellet

continued until the OD_{260} declined; the RNA was usually recovered over three or four extractions. The RNA was precipitated from the water by adding sodium acetate (final concentration 0.3 M) and 2.5 volumes of absolute ethanol and incubating the mixture at -20°C. Following an overnight incubation the RNA was centrifuged out of the water, the pellets pooled, and reconstituted in 1ml of water. The final concentration of the RNA was determined by the fact that 1 OD_{260} equals approximately 40 μg of RNA (Maniatis, Fritsch and Sambrook, 1982).

2.9.2 Transfer of liver RNA to nitrocellulose

Liver RNA was fixed to nitrocellulose filters in preparation for molecular hybridization. Dot/blot analysis was performed to quantify specific mRNA for each protein. For the dot/blot analysis (Kafatos, Jones and Efstratiadis, 1979), a known amount of RNA was diluted in denaturant (2.2 M formaldehyde in 6X SSC: 20X SSC stock made from 175.3 g NaCl and 88.2 g sodium citrate in 800 ml water, pH 7.0), heated for 15 min at 55°C and further diluted by a factor of 2 or 3 to give a range of concentrations between 10 and 0.33 μg. A sheet of nitrocellulose was pre-soaked in 6X SSC and with an absorbent filter paper backing, laid on the stage of a dot/blot apparatus. The RNA was applied to the chambers on the apparatus in a final volume of 200 μl under light vacuum. The vacuum was progressively increased until all

the fluid had passed through the nitrocellulose. The nitrocellulose filter was then removed and oven-baked (80°C) for 2 hr and stored at room temperature between sheets of dry filter paper.

2.9.3 Molecular probes and hybridization of liver RNA

The complementary DNA (cDNA) probes for rat Acute Phase Proteins were kindly provided by Dr. H. Baumann, Roswell Park Memorial Institute, Buffalo, New York. Six cDNA probes were used, haptoglobin (pIRL-9), α_1CPI (pIRL-3), α_1AGP (pIRL-10)(Glibetic and Baumann, 1986), 18 S (ribosomal RNA), $\alpha_{2\mu}$globulin and β-actin (kindly provided by Dr. C. Harley, McMaster University, Hamilton, Ontario). The cDNA for α_1AGP was radiolabelled by the random priming procedure (Feinberg and Vogelstein, 1983) and α_1CPI, haptoglobin, $\alpha_{2\mu}$globin and 18 S were radiolabelled using the nick translation procedure (Rigby et al., 1977).

Nitrocellulose filters blotted with RNA were "pre-hybridized" in an excess volume of buffer (4X SSC, 0.1 M $NaPO_4$, 10X Denhardt's solution: 20X stock made from 0.4% each of BSA, Ficoll and polyvinyl pyrolodone; 0.2% sodium dodecyl sulphate (SDS), 1 mg/ml Salmon sperm DNA) overnight at 60°C. The next day the radioactive cDNA plasmid (10^6 counts per minute) was added to the hybridization buffer (similar to pre-hybridization buffer except with 1X Denhardt's) and used to replace the pre-hybridization buffer.

The hybridization mixture was left incubating in a water bath, shaking slowly, for at least 18 hr at 60°C. Following the hybridization the nitrocellulose was washed repeatedly in an excess of 2X SSC with 0.02% SDS. The filters were dried after washing and exposed onto photographic film overnight.

In order to quantify the radiolabel incorporated into the nitrocellulose filters, each dot was cut-out and the radioactivity counted in liquid scintillant, in a β counter. The absolute amount of RNA on each dot was determined by comparing counts from parallel filters hybridized with either actin or 18S ribosomal probes. Results of the dot blots are presented as the fold difference in radioactivity of infected liver RNA samples versus normal liver RNA dots.

2.10 Non-specific phagocytosis of neutral red

Phagocytosis of neutral red particles (Gibco) was used as an indicator of antibody- and complement-independent phagocytosis (Cohn and Weiner, 1963). Intestinal or peritoneal cells were prepared in a concentration of 2 X 10^6 viable cells per ml in complete RPMI. To 0.5 ml of cells, 50 μl of 0.1% neutral red dye (made in 0.25 M sucrose) was added. The cells were then incubated at 37°C for 20 min with intermittent shaking. Following the incubation, the cells were washed through two changes of fresh RPMI and

cytocentrifuge preparations made. Cells having phagocytosed neutral red particles were visible using a compound microscope with no further staining, and appeared slightly red. The results of the phagocytosis assay are expressed as the percent of total cells phagocytosing the dye.

2.11 Histology

Rat lung, diaphragm and intestine were prepared for histology by fixing fresh specimens in 10% neutral-buffered formalin. All tissues were allowed to fix for at least 36 hours, but one week was more common. Tissues in formalin were kept at 4°C.

Following this period of fixation all tissues were dehydrated by serial (one hour) incubations in ascending concentrations of ethanol and two final baths of absolute ethanol. Folowing the ethanols was a bath of a 50:50 mix of ethanol and xylene, then three changes of xylene and finally three changes of molten paraffin ("Paraplast", Canlab, St. Louis, U.S.A.). Both lung and intestinal tissues were infiltrated in molten paraffin under vacuum. The paraffin was allowed to harden with the tissue in a plastic cast and the blocks were stored in a freezer until sectioning.

Sections were cut using a steel knife on a microtome set for 3 μm advances. Cut sections were wafted onto a bath of warm distilled water with dilute egg albumin powder. Microscope slides were then dipped into the bath and the

sections directed onto them. The dried slides were stained using a routine procedure for hematoxylin and eosin.

Intestinal tissues that were processed for non-specific esterase staining were immediately snap-frozen in 2-methyl butane, upon dissection from the animal. Shortly after the snap-freezing they were transferred to a bath of liquid nitrogen until paraffin infiltration. Wax infiltration was performed under the vacuum of a lyophilizer. After two hours the tissue were infiltrated with paraffin and were transferred to a bath of molten paraffin and blocked similar to formalin fixed tissues. The tissues embedded in this manner were fixed in the first stages of the esterase staining protocol.

2.12 Cytochemistry

2.12.1 Non-specific esterase

The identification of cellular esterases is based on the enzyme activity splitting an exogenous α-naphthyl substrate with the production of α-naphthyl which couples with hexazotized pararosanilin to form a colored, insoluble, amorphous deposit at the site of the enzyme. The procedure for staining of cellular non-specific esterases was a modification of the protocol of Yam, Li and Crosby, (1971). The single-most important modification was a substition of their fixative with acetone/0.038M Na citrate (6:4, v/v), for both cytocentrifuge preparations and intestinal tissue sections.

The staining procedure required that a number of solutions be made in advance: Sorensen's buffer or "solution A" (0.066M solutions of KH_2PO_4 and Na_2HPO_4 mixed until the pH is 7.6); pararosanilin, "solution B" (1g pararosanilin hydrochloride (Sigma) dissolved in 30 ml of distilled water and 5 ml of 14 N HCl); "solution C" (0.4g Na nitrite in 10 ml of water); and α-naphthyl acetate (Sigma), "solution D" (0.09g dissolved in 4.5 ml ethylene glycol monomethylether). When α-naphthyl butyrate was substituted for α-naphthyl acetate it was used in similar relative concentrations. Harris' haematoxylin was used as a counterstain.

Slides were incubated in the above fixative, on ice, for 30 seconds, then run through 3 changes of distilled water. The washed slide were left to air dry at room temperature. Hexazotized pararosanilin was prepared by mixing 2.7 ml each of solution B and C and leaving it for 1 minute. Following this incubation, 80.1 ml of solution A was added, mixed, then the whole mixture added to solution D. This final mixture was adjusted to pH 6.0 and filtered through Whatman number 1 filter paper into a coplin jar. Slides were incubated in the filtered solution for as long as 45 minutes. In the experiments where NaF inhibition of non-specific esterase was examined, the NaF powder was added directly to the incubation mixture.

Positive staining was evident with the unaided eye providing the cells were applied onto the slide at a density

of at least 1 X 10^6 per ml. Positively stained cells were usually evident before the end of the incubation period but in order to be consistent all slides were left for the entire 45 min. Following the staining period slides were removed, rinsed in 3 changes of distilled water and counterstained for 1 to 2 minutes. Excess counterstain was washed out using distilled water, the slides left to dry at room temperature, and finally coverslipped with Permount media (Fisher Scientific).

A second, more rapid, procedure for staining cell in suspension, was also used and gave similar results to the above technique (Tucker, Pierre and Jordan, 1977).

2.12.2 Myloperoxidase

Cellular peroxidase was identified in cytocentrifuge preparations using the method of Kaplow (1965). The substrate solution of benzidine dihydrochloride was made by mixing 0.6 g benzidine dihydrochloride, 1 ml 132 mM $ZnSO_4$. $7H_20$, 1 g sodium acetate, 1.5 ml 1N NaOH and 0.2 g safranin O in 100 ml 30% ethanol (v/v). The solution was adjusted to pH 6.0 and filtered. Immediately before staining, 20 µl of 30% H_20_2 was added to 25 ml of the substrate in a coplin jar. Slides were stained in this final mixture for 30 sec at room temperature. Following the staining the slides were washed in distilled water, dried and mounted using Permount. The reaction product stained in black granules on a faint

pink background. This method was intended to identify eosinophil peroxidases.

2.13 Statistical analysis

All data are reported as the mean ± standard error of the mean. Where the "mean ± standard error" is stated, the standard error of the mean is assumed.

Data showing changes in the concentrations of serum Acute Phase Proteins were analyzed, and shown to be normally distributed, by the Lillifor's test for normality. In experiments in which a test for differences between untreated and treated animals was desired, the student's t-test was performed between sample populations of rats obtained on the same day. In those experiments in which a test for changes over the course of a single infection was desired, the student's t-test was applied to two time points from the same animals. The effect of bleeding did not cause significant changes in serum Acute Phase ~~Protein~~ concentrations.

All statistical analysis were performed using "StatPak" (Northwest Analytical Incorporated, Portland, Oregon, U.S.A.) Version 4.1, assuming unequal variances between sample populations. A stastical test value of $P \leq 0.05$, for a one-sided test, was assumed to prove significant differences between sample populations and is indicated on the figures.

RESULTS

3.1 Turpentine-induced inflammation of the rat

Preliminary studies of the Acute Phase Protein Response of rats during infection by N. brasiliensis showed that serum changes due to the parasite were substantially lower than those induced by turpentine. Considering that the assays for liver activation and hepatocyte-stimulating activity were developed from experiments involving high turpentine doses, it was imperative that the rat response to lower turpentine doses be examined and the efficacy of the assays be re-examined.

3.1.1 Serum Acute Phase Protein Response of rats due to turpentine

Inflammation of various laboratory animals by sterile noxious chemicals has proven to be a popular method for studying inflammation in general. Agents chosen to elicit inflammation include, carrageenin (Deshmukh et al., 1985; Sedgwick and Lees, 1986), Freund's adjuvant (Johnson, DiMartino and Hanna, 1986; Hirschelmann and Schade, 1986), LPS (Kageyama, Ohkubo and Nakaniski, 1985), and turpentine (Lombart, Sturgess and Schachter, 1980; Schreiber et al., 1986). Each agent may give a different response depending

on the site of adminstration but typically a single administration will give significant and reproducible serum Acute Phase Protein changes. Figure 3 shows the typical serum Acute Phase Protein Response due to a single subcutaneous injection of turpentine (100µl per 100g body weight) in rats. The positive proteins underwent increases that were maximal by 60 hours. Levels began to return to normal by 3 days. While all positive Acute Phase Proteins increased due to the turpentine injection, circulating albumin levels declined as low as 60% of normal levels. From Figure 3 it is also evident that the magnitude of change for each protein differed despite the same inflammatory stimulus; haptoglobin usually increased to a lesser extent than α_1AGP or α_1CPI. The concordant changes of multiple positive Acute Phase Proteins during turpentine-induced inflammation has been reported by others (Courtoy et al., 1981).

While the magnitude of changes for each Acute Phase Protein differed under a single stimulus, all protein concentrations changed relative to the dose of turpentine administered (Table 9). Table 9 shows that obvious serum changes were detectable at turpentine doses as low as 10µl per 100g rat- a dose considerably less than 500µl/100g used by other authors. A decline in the negative reactant, albumin, was evident at the low dose of turpentine.

Figure 3: Changes in the concentrations of serum Acute Phase Proteins during turpentine-induced inflammation of rats. Each point represents the mean ± standard error of four animals. ○, α_1AGP; □, α_1CPI; △, haptoglobin; ●, albumin.

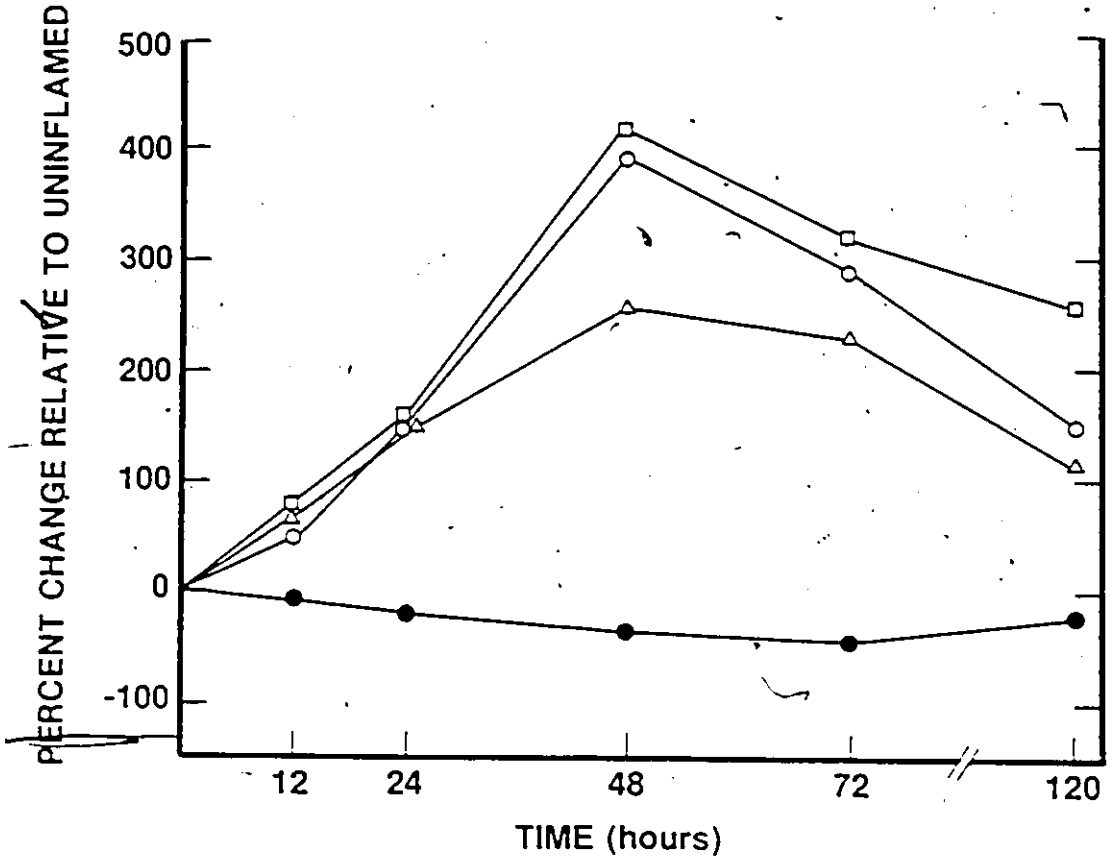

PERCENT CHANGE RELATIVE TO UNINFLAMED
500
400
300
200
100
0
-100
12
24
48
72
120
TIME (hours)

TABLE 9

Percent Changes in concentrations of serum Acute Phase Proteins in Sprague-Dawley rats given different volumes of turpentine

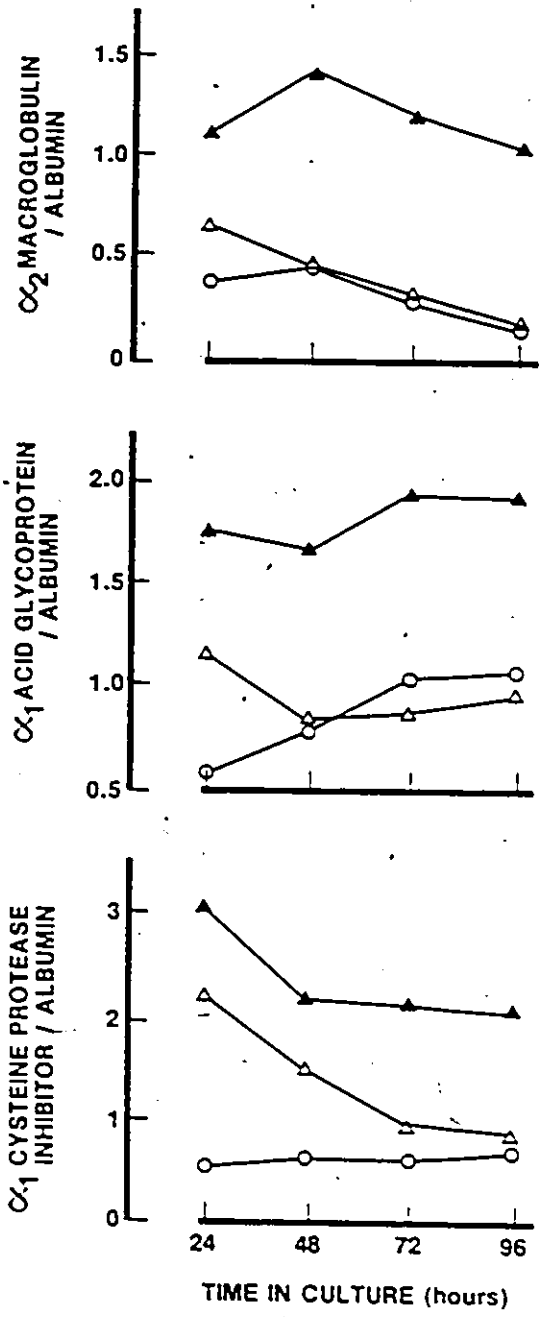
Acute Phase Protein	Turpentine Dose (µl/100 g) 10	50	100
$\alpha_2$macroglobulin	100	400	1400
$\alpha_1$acid glycoprotein	420	600	980
$\alpha_1$cysteine protease inhibitor	260	310	350
albumin	79	84	66

3.1.2 Liver Acute Phase Protein Response of rats due to turpentine

The liver is the single-most important source of serum Acute Phase Proteins. It was therefore necessary to correlate the hepatic protein-synthetic activity with the serum changes seen during inflammation. In order to measure protein synthesis by the hepatocytes of turpentine-inflamed rats, the animal's liver was removed into an *in vitro* culture system. These cells were stimulated *in vivo* by turpentine inflammation and were synthesizing and secreting greater amounts of Acute Phase Proteins in culture, than non-inflamed cells (Figure 4). Greater protein secretion by cells recovered from inflamed rats was represented as a larger ratio (Acute Phase Protein/albumin rocket peak heights) at 24 hr of culture. Over the course of the culture period the cells returned to normal synthetic rates, probably because the enhancing signals were not replenished with each (24 hour) change of media.

At the dose of turpentine used for Figure 4 it was obvious that the liver cells were not stimulated maximally since they responded with further synthesis following *in vitro* stimulation. When the stimulating agent, LPS-stimulated monocyte-conditioned supernatant, was applied at each change of media (solid triangles, Figure 4) the effect of *in vivo* stimulation by turpentine was prolonged in the *in vitro* cultures and was in fact considerably enhanced. Contaminat-

Figure 4: Rat hepatocyte production of Acute Phase Proteins in culture, following turpentine-induced inflammation. . Rat livers were perfused 24 hr following turpentine administration (100μl/100g). ○, normal non-inflamed liver; △, inflamed rat liver; ▲, inflamed rat liver with monocyte-conditioned supernatant added *in vitro*.

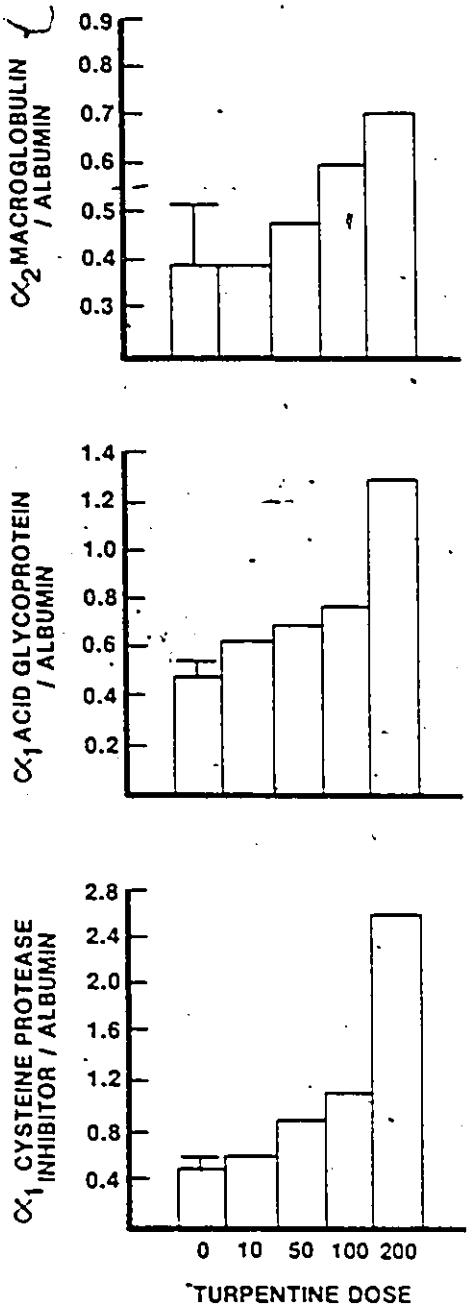

α_2 MACROGLOBULIN / ALBUMIN
1.5
1.0
0.5
0
α_1 ACID GLYCOPROTEIN / ALBUMIN
2.0
1.5
1.0
0.5
α_1 CYSTEINE PROTEASE INHIBITOR / ALBUMIN
3
2
1
0
24
48
72
96
TIME IN CULTURE (hours)

ing LPS was shown to have no effect on protein synthesis by cultured hepatocytes. Whether each hepatocyte in the culture makes more of all the proteins or whether more cells were recruited into synthesis was not investigated but work by others has indicated that all hepatocytes are capable of making all the proteins (Courtoy et al., 1981).

The rate of hepatocyte secretion of α_1AGP differed from secretion of the other proteins, as shown in Figure 4. The difference- a steady increase in α_1AGP production with time versus steady or declining synthesis of other molecules- was likely due to the fact that α_1AGP is a corticosteroid-inducible molecule. The culture media included dexamethasone, without which hepatocyte secretion was substantially reduced and viability became a concern. The α_2M synthesis by hepatocyte cultures also seemed abnormally high considering the low circulating levels in normal rats. It was expected that exciting the animal during handling, the use of somnotol as an anesthetic, and the collagenase perfusion procedure would contribute to the activation of the hepatocyte prior to establishing the cells in culture.

An enhancement of hepatocyte protein secretion by the isolation procedure had some important ramifications when the cells from animals given low doses of turpentine were examined. The "background" rate of protein synthesis due to non-specific enhancement masked the effects of lower turpentine doses. Figure 5 shows the analysis of 24 hour

Figure 5: Summary of *in vitro* Acute Phase Protein synthesis by rat hepatocytes following inflammation induced by increasing doses of turpentine. The rat livers were perfused 24 hr following turpentine administration. Each dose represents one rat except the control which is the mean ± standard error of five animals.


α_2 MACROGLOBULIN / ALBUMIN
0.9
0.8
0.7
0.6
0.5
0.4
0.3
α_1 ACID GLYCOPROTEIN / ALBUMIN
1.4
1.2
1.0
0.8
0.6
0.4
0.2
α_1 CYSTEINE PROTEASE INHIBITOR / ALBUMIN
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0 10 50 100 200
TURPENTINE DOSE

culture supernatants from hepatocytes removed from different animals given decreasing doses of turpentine. There was a threshold effect, at 200 µl turpentine, leading to a significant increase for some proteins. The lower limit for detecting in vivo activation of hepatocytes was between 10 and 50µl of turpentine, depending on the protein.

In order to circumvent any difficulties with the interpretations of the in vitro assay, another assay for liver changes was employed. Using specific cDNA probes for Acute Phase Proteins, the changes in messenger RNA (mRNA) were measured from the liver of parasitized animals. This assay was, in effect, measuring the "0" hour period of culture. Turpentine-treated rats served as positive controls for mRNA changes, and the results can be seen in a later section.

3.1.3 Macrophage activity in turpentine-inflamed rats

Local acute inflammation may affect the animals' response to a second inflammatory agent, administered at a second site. The effect is usually dampening- the so-called anti-phlogistic effect whereby the effects of two inflammatory stimuli on local swelling are less than the sum of each stimuli measured in two animals (Atkinson and Hicks, 1975). Plasma-soluble mediators serve as the communication link between local and systemic inflammatory events and this anti-inflammatory phenomena is thought to arise from and be

regulated by macrophages (Loegering, 1981).

Little is known about the cells responsible for the liver activation following subcutaneous turpentine injection. Based on previous evidence that the macrophage/-monocyte, activated in vivo, makes hepatocyte-stimulating factors (Egwang, Befus and Gauldie, 1985; Lamontagne et al., 1985) the activity of two populations of macrophages from rats undergoing turpentine inflammation were examined. Bronchoalveolar lavage and peritoneal washout cells were isolated and the plastic-adherent cells cultured from normal and 24 hr-post turpentine inflamed animals. One half the cultured cells were exposed to further in vitro stimulation using LPS. The 24 hr macrophage culture supernatants were assayed for hepatocyte-stimulating activity and lymphocyte-activating factor activity (LL-1). Table 10 shows that neither the peritoneal nor alveolar macrophages, isolated from the turpentine-inflamed rats, were activated when hepatocyte- or lymphocyte-stimulating activities were measured. However, alveolar cells ~~could be~~ stimulated by LPS to secrete greater amounts of hepatocyte-stimulating activity and both normal and inflamed cells could be induced to secrete greater amounts of IL-1. The peritoneal macrophages showed considerable variability with respect to LPS stimulation of hepatocyte-stimulating activity, and an increase in the activity of normal cells was detected in only one ex-

TABLE 10

Hepatocyte-stimulating activity and Lymphocyte-activating factor activity (IL-1) of macrophages recovered from turpentine-inflamed rats

MACROPHAGE SOURCE[1]	STIMULATING ACTIVITY Hepatocyte-[2] -LPS	+LPS	Lymphocyte-[3] -LPS	+LPS
normal alveolar	0.22	0.46	26268 ± 3481	60072 ± 4638
inflamed alveolar	0.28	0.44	31106 ± 4057	60279 ± 274
normal peritoneal	0.23	0.28	13221 ± 1782	32864 ± 5104
inflamed peritoneal	0.27	0.25	11402 ± 1137	17374 ± 1309
positive control (monocyte-conditioned media)[4]	----	1.62	-----	147220

[1] Alveolar cells were pooled from 4 animals and peritoneal from 3 animals, 24 hr following turpentine administration. Plastic adherent cells were cultured for 24 hr.
[2] Results shown as the ratio of $\alpha_2$macroglobulin rocket peak height/albumin peak height (Koj et al., 1985).
[3] Results shown are the radioactive "counts per minute" determined from a PHA co-mitogenic assay using C3H/HeJ thymocytes and are mean ± standard error.
[4] Human peripheral blood mononuclear cells cultured in the presence of LPS (Koj et al., 1984).

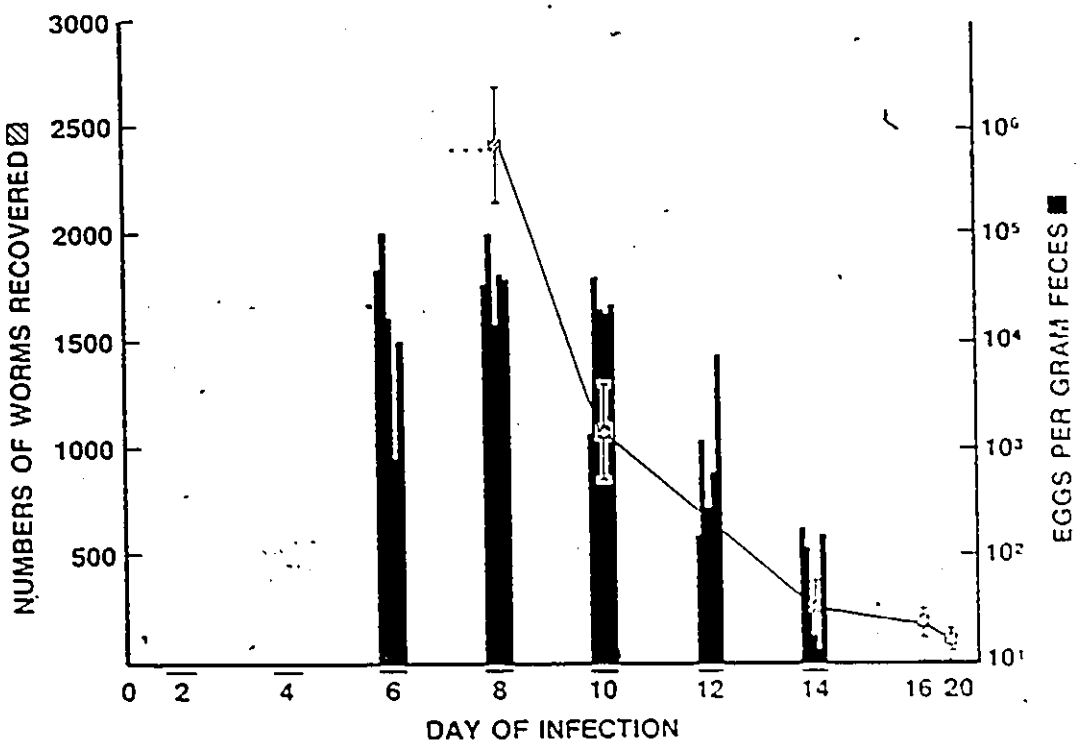
periment. The likelihood that this variability was due to the low dose of LPS used (5μg/ml), was not examined further. The failure to detect in vivo macrophage activation indicated that these cells did not participate in the Acute Phase Protein Response due to subcutaneous turpentine. Furthermore, these cells were capable of being activated to secrete inflammatory mediators important in the regulation of the response and were not affected by counter-irritation.

3.2 Nematode-induced inflammation of the rat

3.2.1 Biology of N. brasiliensis and T. spiralis infection

The host response to infection by N. brasiliensis or T. spiralis share some common general features, yet differ in other specific details. Adults of both parasites inhabit the proximal jejunum albeit in a different niche; N. brasiliensis remains intraluminal (Ogilvie and Jones, 1971) while T. spiralis mature in an intraepithelial space (Gardiner, 1976; Wright, 1979). Both infections remain in the host for similar periods of time. N. brasiliensis reach the host's intestine by 90 hours following subcutaneous injection and their numbers remain constant until day 8 PI when they begin to decline until few worms remain at day 14 (Figure 6). It can be seen from Figure 6 that egg production by the mature worms roughly reflect the changes in numbers of worms in the intestine. The few worms that remain beyond 14 days are usually male or "immune adapted" females (Africa, 1931).

Figure 6: Worm numbers and egg production by a primary *N. brasiliensis* infection in Sprague-Dawley rats. Each point on the worm recovery curve (♀) represents the mean ± standard error of five rats. The histogram shows the egg production of five rats independently, over the course of the infection.


NUMBERS OF WORMS RECOVERED ▨
EGGS PER GRAM FECES ■
DAY OF INFECTION
3000
2500
2000
1500
1000
500
0
2
4
6
8
10
12
14
16
20
10⁶
10⁵
10⁴
10³
10²
10¹

Similarly, T. spiralis larvae arrive and mature in the intestine, few worms remain beyond 14 days of infection in an immunocompetent host. Following infection by either parasite, animals are immunized against a challenge infection becoming patent.

The pathologic condition that either infection elicits is evident by following the body-weight gains of infected animals. The decline in host body weight is a function of worm dose, host age, and a loss of appetite (Smith and Castro, 1978; Ovington, 1985). Young, but mature, rats are growing more rapidly than older rats and their failure to gain weight was more evident.

The pathology that each infection elicits in the intestine was similar under light microscopic observation (Figure 7). The intestine undergoes obvious superstructural changes including villous flattening (atrophy) and partial fusion and cellular changes including a polymorphonuclear leukocyte (neutrophil) infiltration and eosinophil, mast cell, crypt epithelial and goblet cell hyperplasia (Symons, 1965; Ogilvie and Jones, 1971; Cheema and Scofield, 1982).

Systemic pathological events were also characteristic of both infections. The adult-intestinal stage of N. brasiliensis was preceded by a systemic exposure to the worm, in the lungs, as infective larvae migrate from the skin (Figure 8). There are changes in the cellular differential in the alveolar spaces during this period of lung

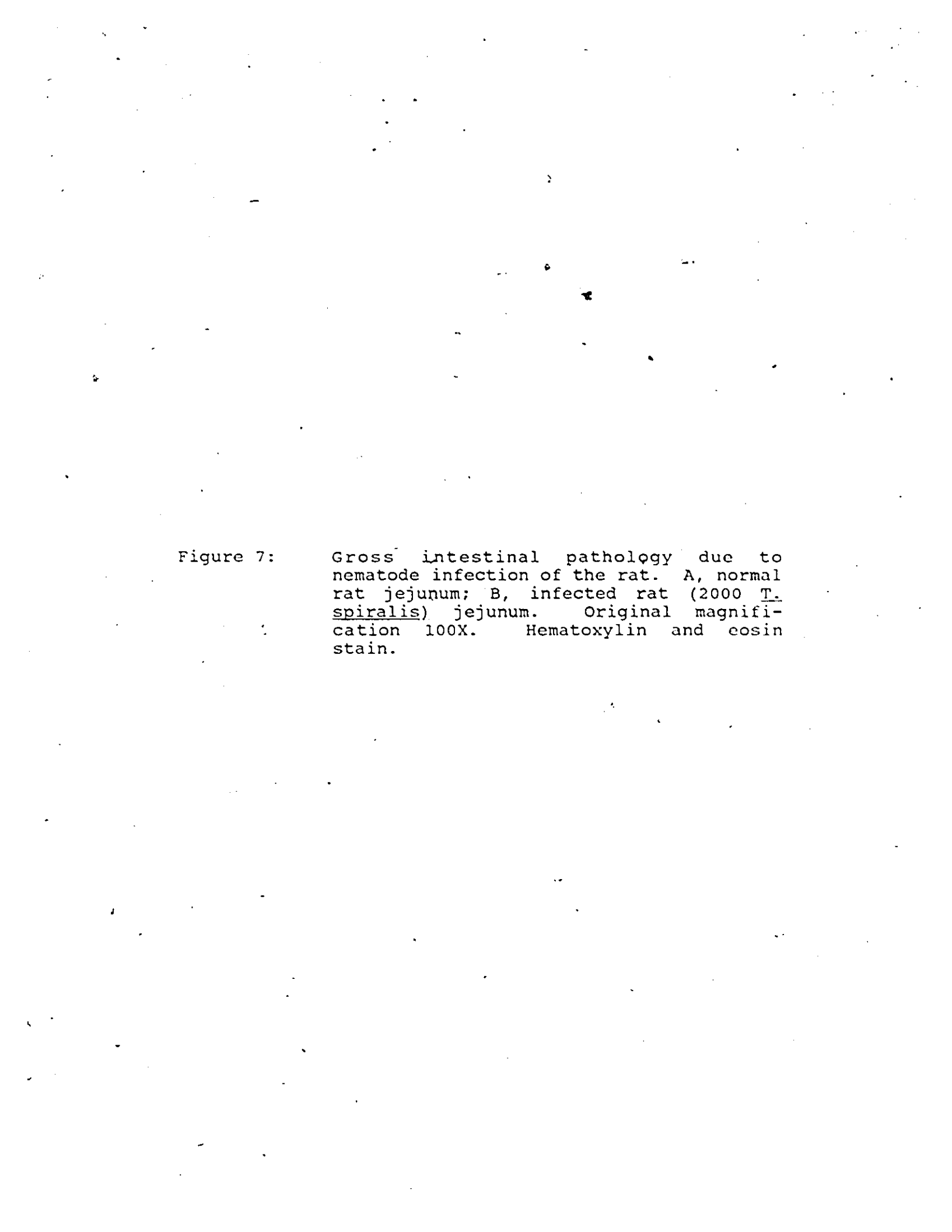


Figure 7: Gross intestinal pathology due to nematode infection of the rat. A, normal rat jejunum; B, infected rat (2000 T. spiralis) jejunum. Original magnification 100X. Hematoxylin and eosin stain.

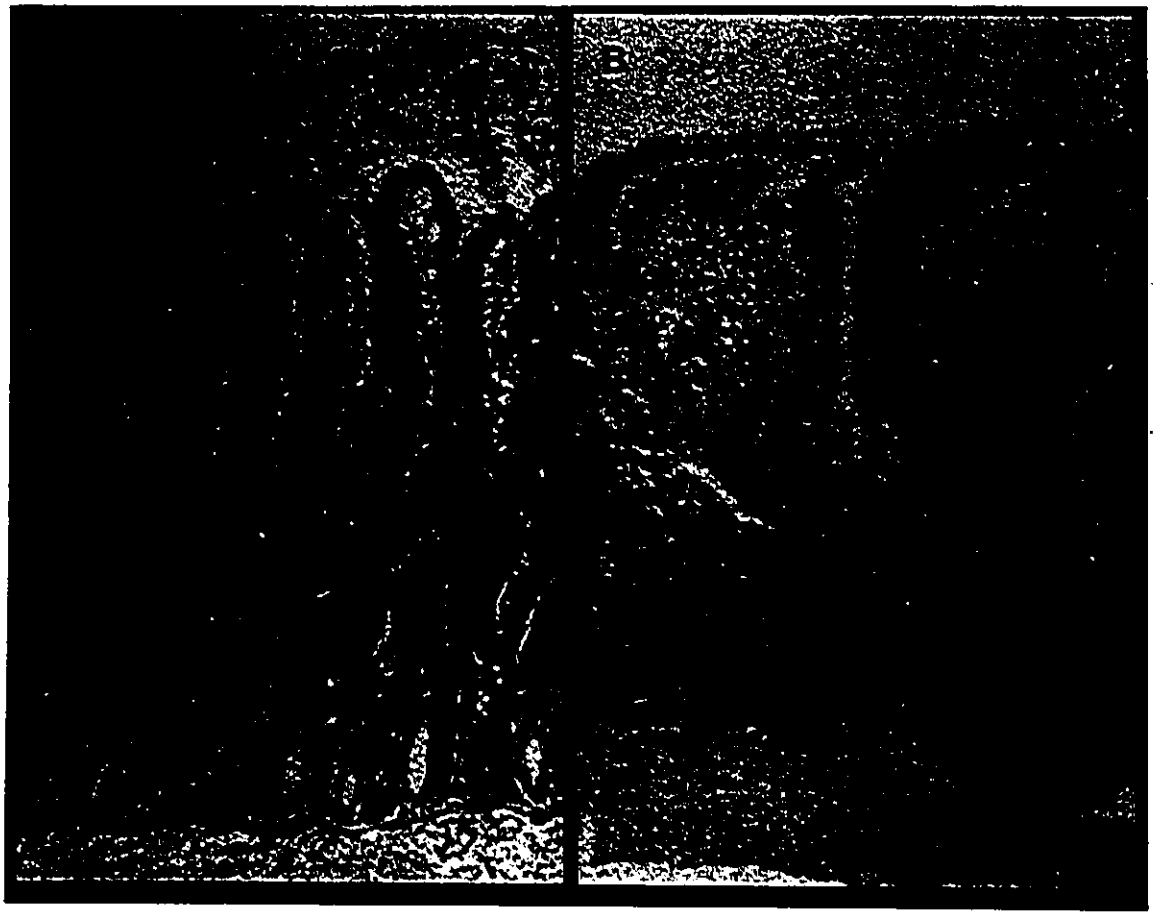
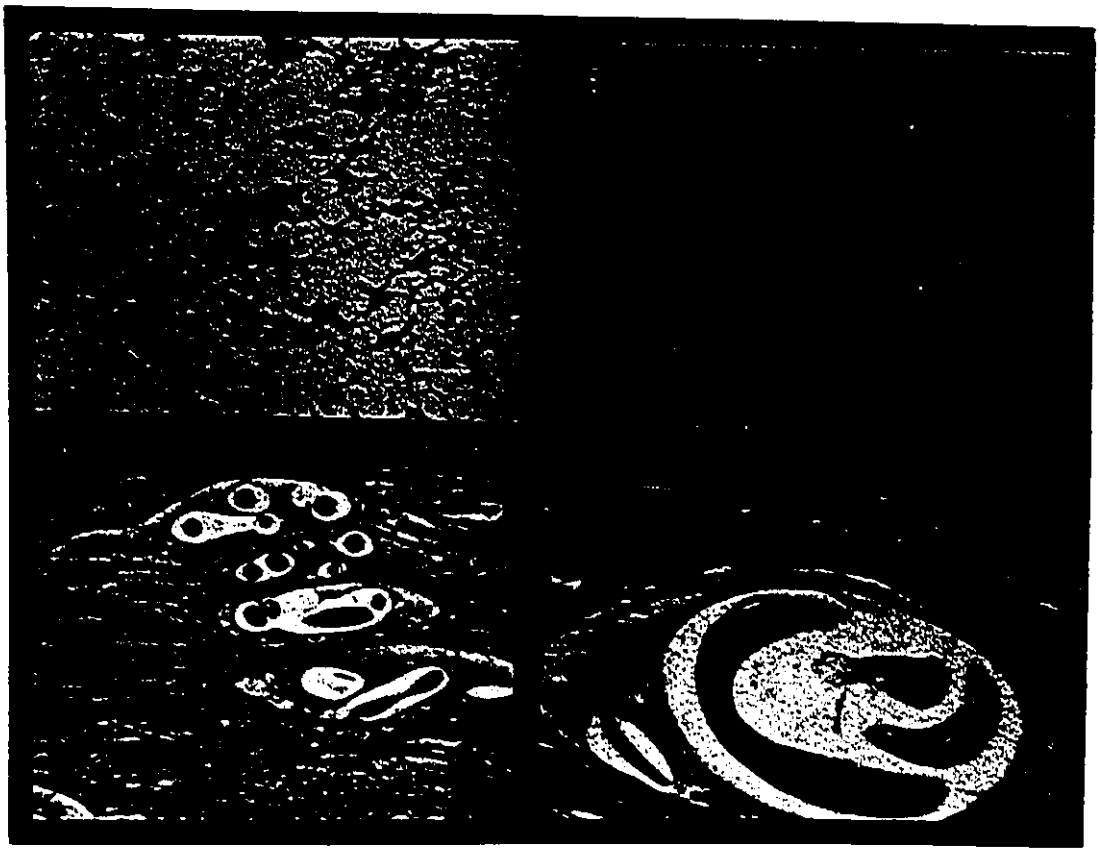

B

Figure 8: Systemic pathology associated with nematode infection of the rat. A and B, rat lung infected with N. brasiliensis (day 2 PI); C and D, rat diaphragm infected with T. spiralis (day 30 PI). Rats encounter systemic infective N. brasiliensis before the intestinal stage but following the intestinal stage during infection by T. spiralis. Hematoxylin and eosin stain. Original magnification, A and C, 100X; B and D, 250X.



infection, highlighted by increased neutrophils (Egwang, Gauldie and Befus, 1984). T. spiralis larvae are delivered to skeletal muscle, where they encyst, following their birth in the intestinal epithelium (Figure 8). A granulomatous reaction consisting of macrophages and lymphocytes ensued in the infected diaphragm. Eosinophils were also abundant in the skeletal muscle at the time of encystment. Thus the infections differ by the timing of the systemic exposure of the host to the parasite.

A number of systemic physiological and immunological changes have been documented in the plasma of infected rat. Changes were seen in circulating gastrointestinal hormones (Ovington, Bacarese-Hamilton and Bloom, 1985), albumin (Ash, Crompton and Lunn, 1985), mast cell proteases (Woodbury et al., 1984), antibodies (Ogilvie and Jones, 1971) and blood-born leukocytes (Roth and Levy, 1980; Lee and Best, 1983b). With the possible exception of mast cells and RMCP II levels, the cells responsible for eliciting most of these plasma changes have not been identified. Moreover, it is not known whether inflammatory mediators arise as a result of the direct contact of effector cells with the parasite or are non-specific events typical of gastrointestinal homeostasis.

3.2.2 Serum Acute Phase Protein Response of infected rats

Another measure of the systemic inflammatory response to intestinal infections was the serum Acute Phase Protein Response, shown in Figures 9 and 10. The concentions of positive Acute Phase Proteins; α_1CPI, α_1AGP, haptoglobin and α_2M (not shown) all increased during infection of rats by N. brasiliensis but not T. spiralis. The changes seen during N. brasiliensis were biphasic, the first increase maximal at day 2 and the second maximal between days 8 and 9 PI. These periods of increased Acute Phase Protein concentrations corresponded to the periods of pathology in the lung and intestine, respectively.

The magnitude of the increases observed during N. brasiliensis infection differed between the different glycoproteins. The absolute magnitude of the changes varied between experiments. The greatest increases were observed with α_1CPI with 5 to 8 fold changes followed by α_1AGP (4 to 5 fold), in constrast with the changes due to turpentine. Haptoglobin gave the lowest changes of the positive proteins with 2 to 3 fold changes over normal circulating concentrations. Serum α_2M became detectable during the second peak and represented greater than a 10 fold increase over normal levels.

Infection of rats by 2000 T. spiralis failed to elicit any significant serum changes in any positive Acute Phase Protein (Figure 10). The efficacy of the T. spiralis

Figure 9: Changes in the concentrations of serum Acute Phase Proteins during *N. brasiliensis* infection of Sprague-Dawley rats. □ , 3000 L_3 *N. brasiliensis*; ○ , uninfected control rats. Results are the mean ± standard error of 5 rats bled via the retro-orbital plexus. * , indicates a significant difference ($P \leq 0.05$) between infected and non-infected animals bled on the same day.

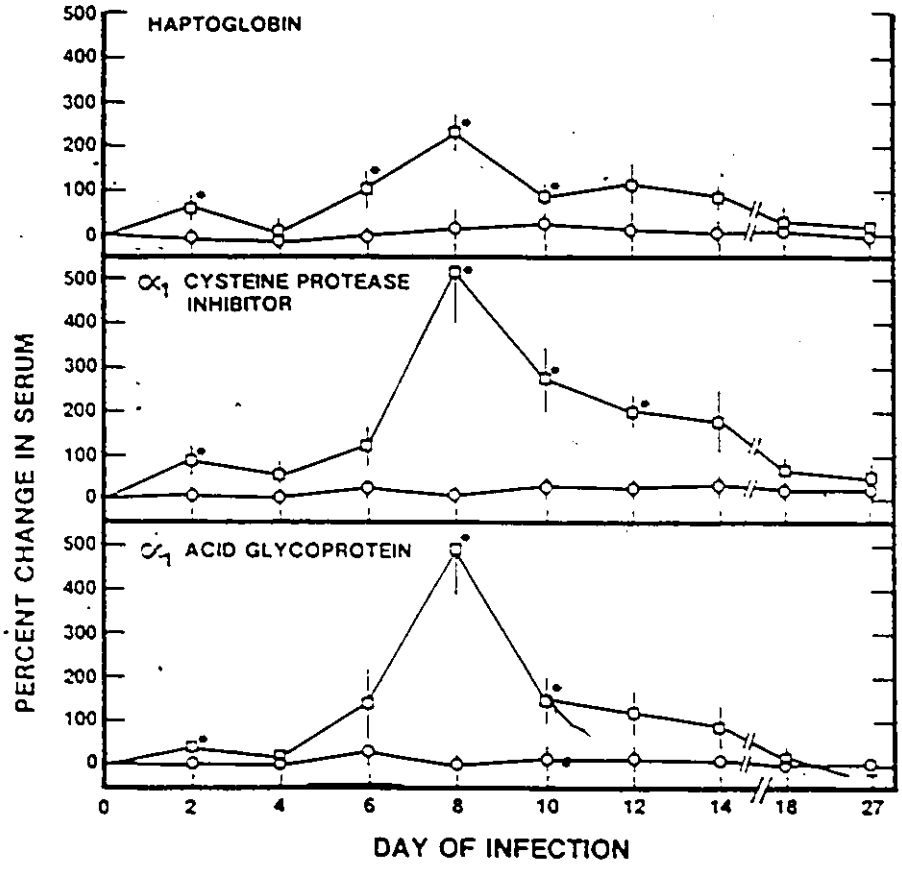
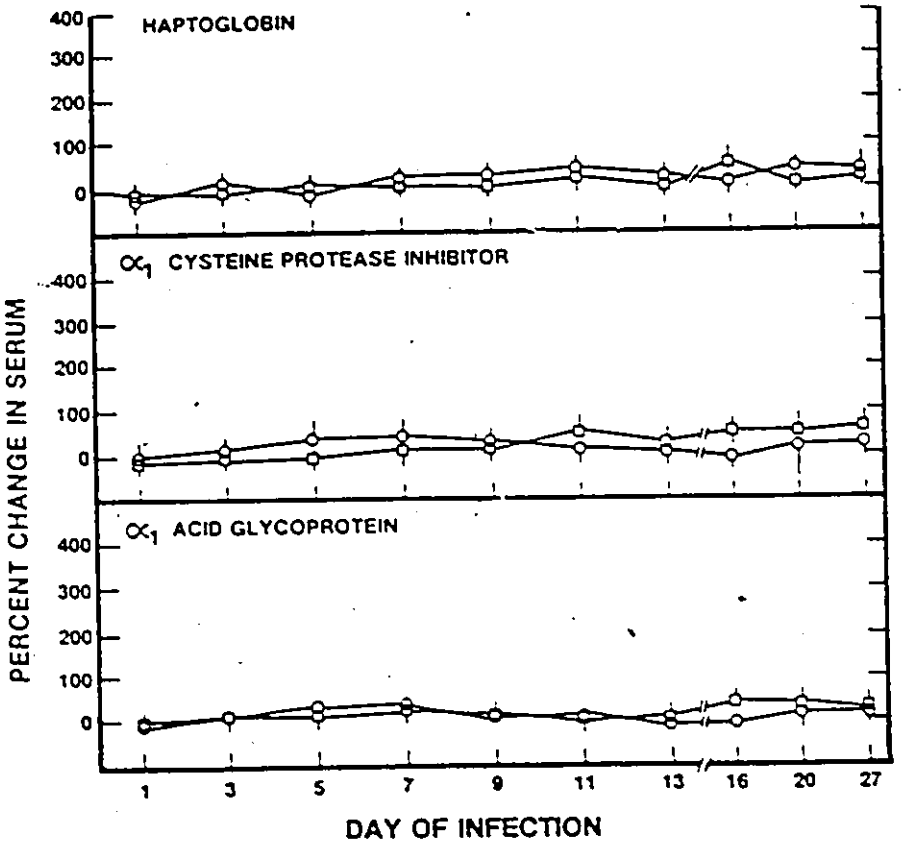

HAPTOGLOBIN
α_1 CYSTEINE PROTEASE INHIBITOR
α_1 ACID GLYCOPROTEIN
PERCENT CHANGE IN SERUM
DAY OF INFECTION
0
100
200
300
400
500
2
4
6
8
10
12
14
18
27

Figure 10: Changes in the concentrations of serum Acute Phase Proteins during *T. spiralis* infection of Sprague-Dawley rats. □, 2000 *T. spiralis* infective larvae; ○, uninfected controls. Results are the mean ± standard error of 5 rats bled via the retro-orbital plexus.


HAPTOGLOBIN
400
300
200
100
0
α_1 CYSTEINE PROTEASE INHIBITOR
400
300
200
100
0
α_1 ACID GLYCOPROTEIN
400
300
200
100
0
PERCENT CHANGE IN SERUM
1
3
5
7
9
11
13
16
20
27
DAY OF INFECTION

infection was checked by killing the animals after 30 days and examining their diaphragms for encysted larvae (Figure 8). All animals were infected. It is interesting to note that at no time during the first 4 weeks of a T. spiralis infection were there obvious changes in positive Acute Phase Protein concentrations which included the period during which newborn larvae were encysting in skeletal muscle.

Serum concentrations of α_1CPI showed the greatest variability between uninfected control animals and even rose steadily in one animal. Otherwise uninfected controls in both experiments failed to show statistically significant changes in protein concentration indicating the effect of repeated bleeding was minimal.

The choice of infectious dose was somewhat arbitrary but was based on the dose commonly used by other authors. An infection with only a few worms (as few as 5 females, Kassai, 1982) will immunize a rat against subsequent infections becoming patent. From Figure 6 it can be seen that about 80% of an N. brasiliensis infection, given subcutaneously, reach and mature in the host's intestine. Considering all the worms do not reach the intestine from an infection of 3000 N. brasiliensis, a dose of 2000 T. spiralis was chosen as the routine infection for this parasite. The fact that T. spiralis is ovoviviparous was also taken into account when choosing the dose, as newborn larvae deposited into the lamina propria may further

stimulate pathology.

With these considerations in mind, the effect of the infectious dose of each species was examined. An infection with as few as 500 L_3 N. brasiliensis gave significant increases in positive Acute Phase Proteins, during the second peak and for haptoglobin during the first (day 2) peak (Figure 11). An infection with 6000 L_3 resulted in a response that began on day 2 and continued until day 8 when it began to decline. A significant decline in albumin levels was obvious in rats which had received 6000 N. brasiliensis larvae (Figure 11).

Rats which had received large doses of T. spiralis also showed positive changes in haptoglobin and α_1CPI and a significant decline in circulating albumin (Figure 12). The changes were of a magnitude equal to the first peak of increases during N. brasiliensis infection. A dose of 5000 infective T. spiralis larvae leads to considerable pathology and likely result in perforation of the small bowel and larval migrans. The expulsion kinetics differ in animals with infections of 5000 T. spiralis- considerable numbers of adults remain in the intestine beyond day 14, possibly due to immunosuppression by the worms (Bell et al., 1983).

A challenge infection by the homologous parasite leads to accelerated expulsion of the worms, the so-called "rapid expulsion" phenomena (Love, Ogilvie and McLaren,

Figure 11: Changes in the concentrations of serum Acute Phase Proteins during infection of Sprague-Dawley rats with different doses of *N. brasiliensis*. ○, infection with 500 L_3; ●, infection with 6000 L_3. Each point represents the mean ± standard error of 5 animals. *, indicates a significant difference ($P \leq 0.05$) between day 0 and the day indicated, for the same animals.

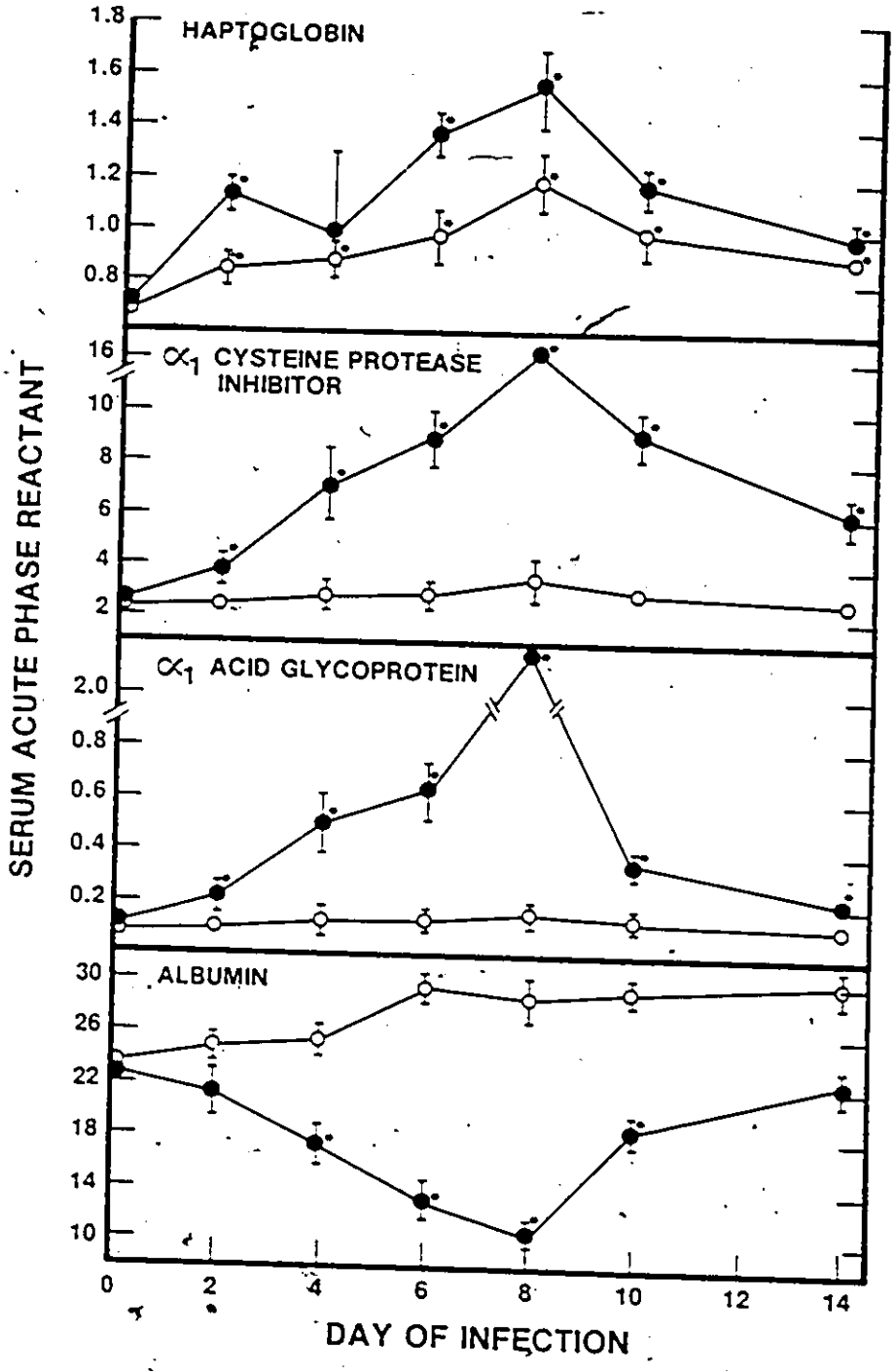
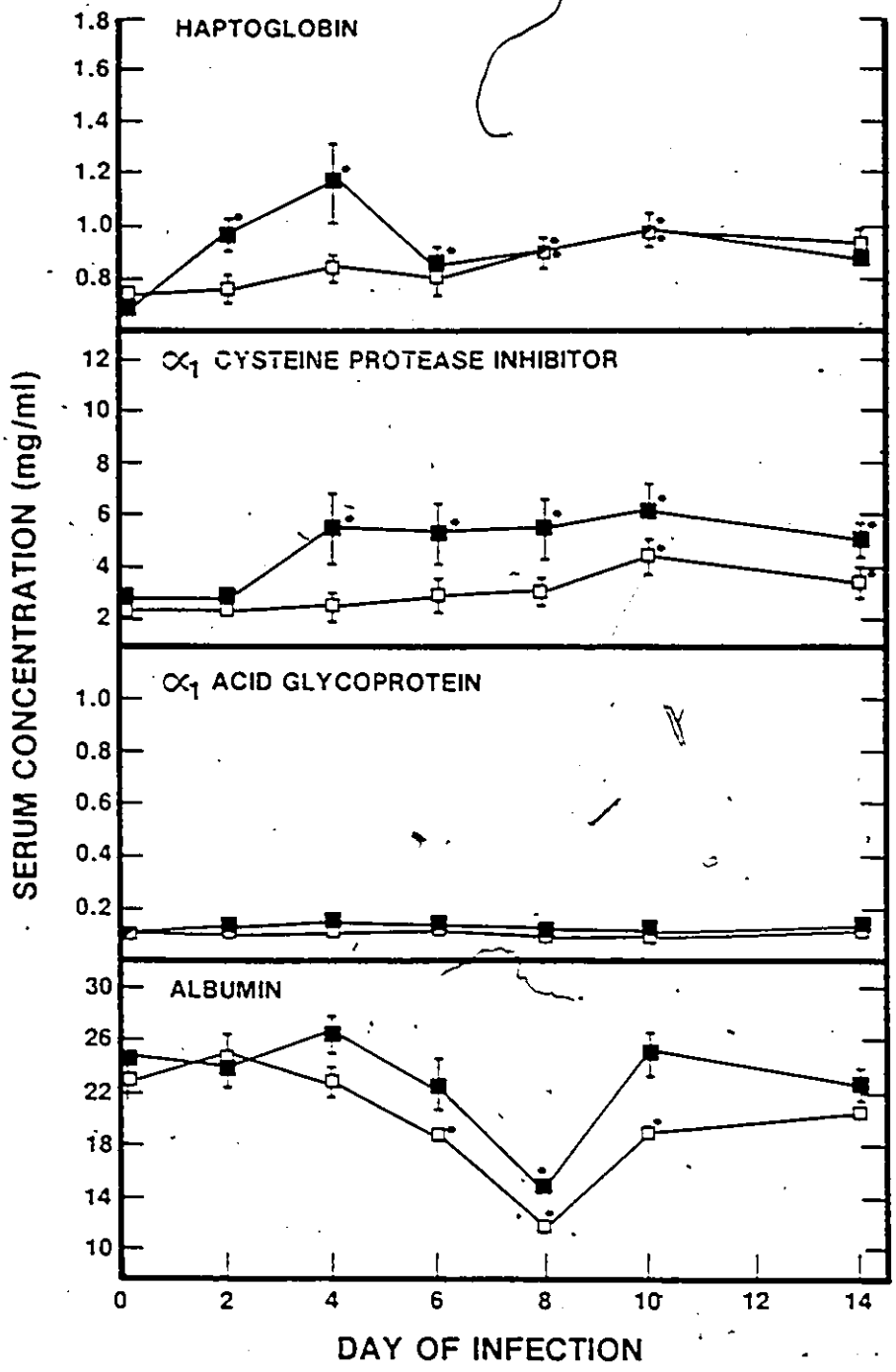

HAPTOGLOBIN
α_1 CYSTEINE PROTEASE INHIBITOR
α_1 ACID GLYCOPROTEIN
ALBUMIN
SERUM ACUTE PHASE REACTANT
DAY OF INFECTION
1.8
1.6
1.4
1.2
1.0
0.8
16
10
8
6
4
2
2.0
0.8
0.6
0.4
0.2
30
26
22
18
14
10
0
2
4
6
8
10
12
14

Figure 12: Changes in the concentrations of serum Acute Phase Proteins during infection of Sprague-Dawley rats with different doses of *T. spiralis*. □, 2500 infective larvae; ■, 5000 infective larvae. Each point represents the mean ± standard error of 5 animals. *, indicates a significant difference ($P \leq 0.05$) between day 0 and the day indicated, for the same animals.

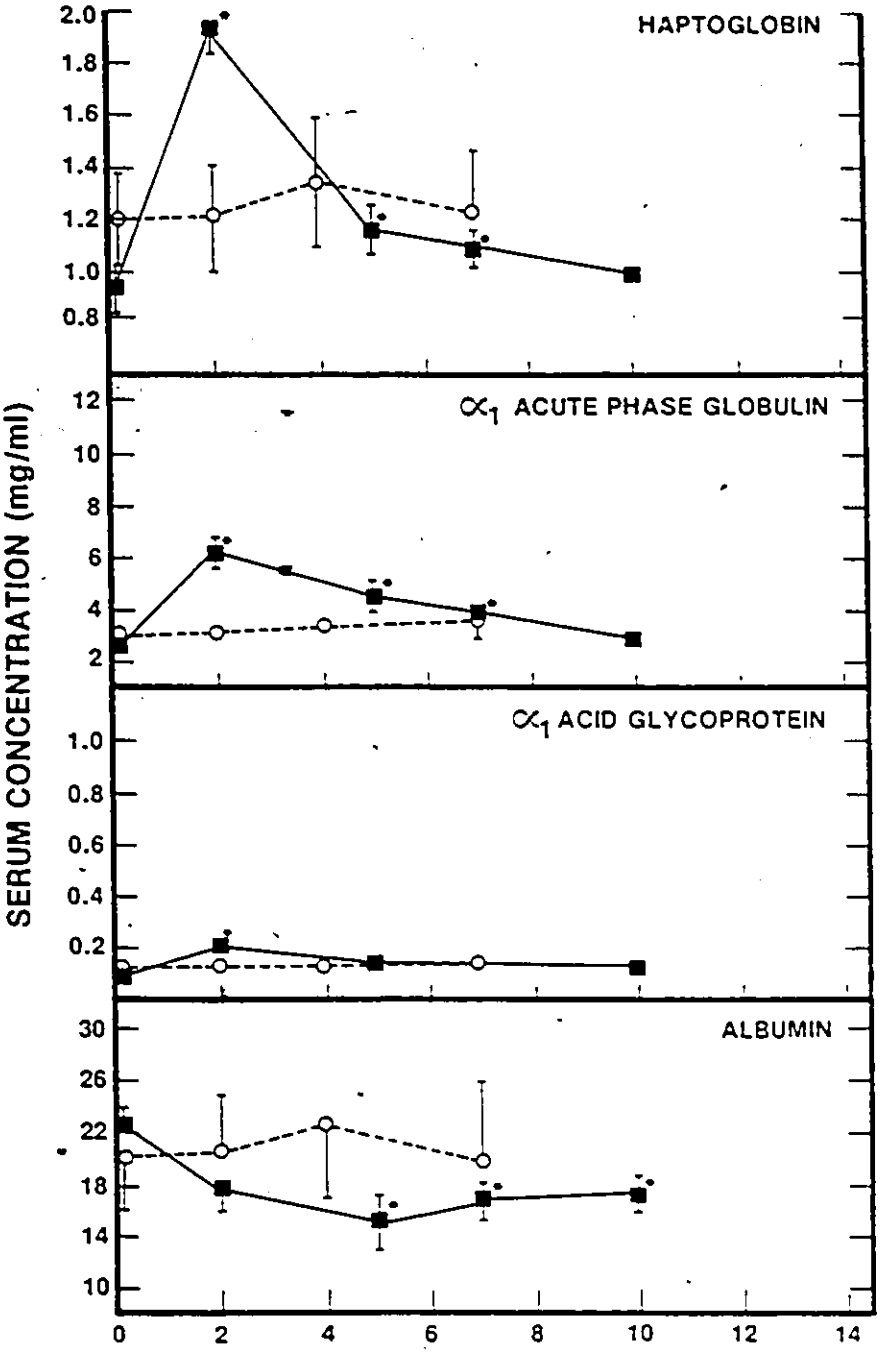

HAPTOGLOBIN
1.8
1.6
1.4
1.2
1.0
0.8
α_1 CYSTEINE PROTEASE INHIBITOR
12
10
8
6
4
2
α_1 ACID GLYCOPROTEIN
1.0
0.8
0.6
0.4
0.2
ALBUMIN
30
26
22
18
14
10
SERUM CONCENTRATION (mg/ml)
0
2
4
6
8
10
12
14
DAY OF INFECTION

1976; Miller, Huntley and Wallace, 1981). Rapid expulsion was complete by day 6 in both infections and few worms remained in the intestine beyond this time. It has been suggested that the larvae fail to establish in their normal site in the intestine in a challenge infection (Miller et al., 1981). Few of the challenge infection of N. brasiliensis ever reach the intestine and considerable worm attrition occurs in the host's skin and lungs (Taliaferro and Sarles, 1939). Figure 13 shows the consequences of challenge infections using the homologous parasite and the serum Acute Phase Protein Response. There was a single peak of increased positive reactants during N. brasiliensis but not T. spiralis infection. The increase was maximal by day 2 PI and concentrations recovered to normal by day 7. A decline in albumin levels was also evident in the N. brasiliensis infected rats.

3.2.3 Liver Acute Phase Protein Response of infected rats

The results from the titred-turpentine model of inflammation indicated the changes in liver activity due to the infections may be too subtle to be detected in the in vitro assay. Indeed, only 8 day-N. brasiliensis infected rat liver showed consistent evidence for in vivo activation when analyzed using the in vitro assay. It was still important to determine whether the failure to detect changes during infection by T. spiralis was matched by a lack of

Figure 13: Changes in the concentrations of serum Acute Phase Proteins during challenge infections with the homologous parasite in Sprague-Dawley rats. ■, N. brasiliensis challenged rats; ○, T. spiralis challenged animals. Both challenge infections were given on day 30 post primary infection. *, indicates a significant difference ($P \leq 0.05$) between day 0 and the day indicated, for the same animals.


HAPTOGLOBIN
α_1 ACUTE PHASE GLOBULIN
α_1 ACID GLYCOPROTEIN
ALBUMIN
SERUM CONCENTRATION (mg/ml)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
12
10
8
6
4
2
1.0
0.8
0.6
0.4
0.2
30
26
22
18
14
10
0
2
4
6
8
10
12
14

stimulation of liver protein synthesis.

The availability of molecular probes for the Acute Phase Proteins allowed for the direct assay of numbers of mRNA copies of each particular protein. For this method of measuring hepatocyte activity, the liver was excised from the infected animal and the RNA isolated immediately; there was no culture period during which the cells became stimulated.

In order to quantify the amount of specific mRNA for each protein from infected animals, total RNA was prepared and used in dot/blot hybridization analysis. This method involved fixing known amounts of RNA to nitrocellulose filters and hybridizing the filters with a known amount of specific, radiolabelled cDNA. Differing amounts of the probe hybridized to each RNA sample depending on the number of copies of the RNA message. This difference was detected by exposing the radioactive filters on photographic film (Figure 14) or by cutting-out the spots of RNA and counting the β-radioactivity of each sample. Figure 14 shows two blots; one probed for the positive Acute Phase Protein α_1AGP, and the second, for the negative protein $\alpha_{2\mu}$globulin (Faict et al., 1983). The positive (turpentine-treated) control showed the greatest increase in specific RNA message. The increase in message on day 2 PI for the T. spiralis infected rat was not consistent on a repeat blot (Figure 15). The changes in $\alpha_{2\mu}$globulin message paralleled

Figure 14: Dot/blot hybridization of total rat liver RNA from N. brasiliensis and T. spiralis infected animals. Dilutions of RNA were denatured in formalin and applied to nitrocellulose, the filter dried, baked, and hybridized using the respective cDNA. Each point represents a single animal. For the turpentine control, rats were given 100µl turpentine/100 g weight. A, RNA probed for α_1AGP; B, RNA probed for $\alpha_{2\mu}$globulin.

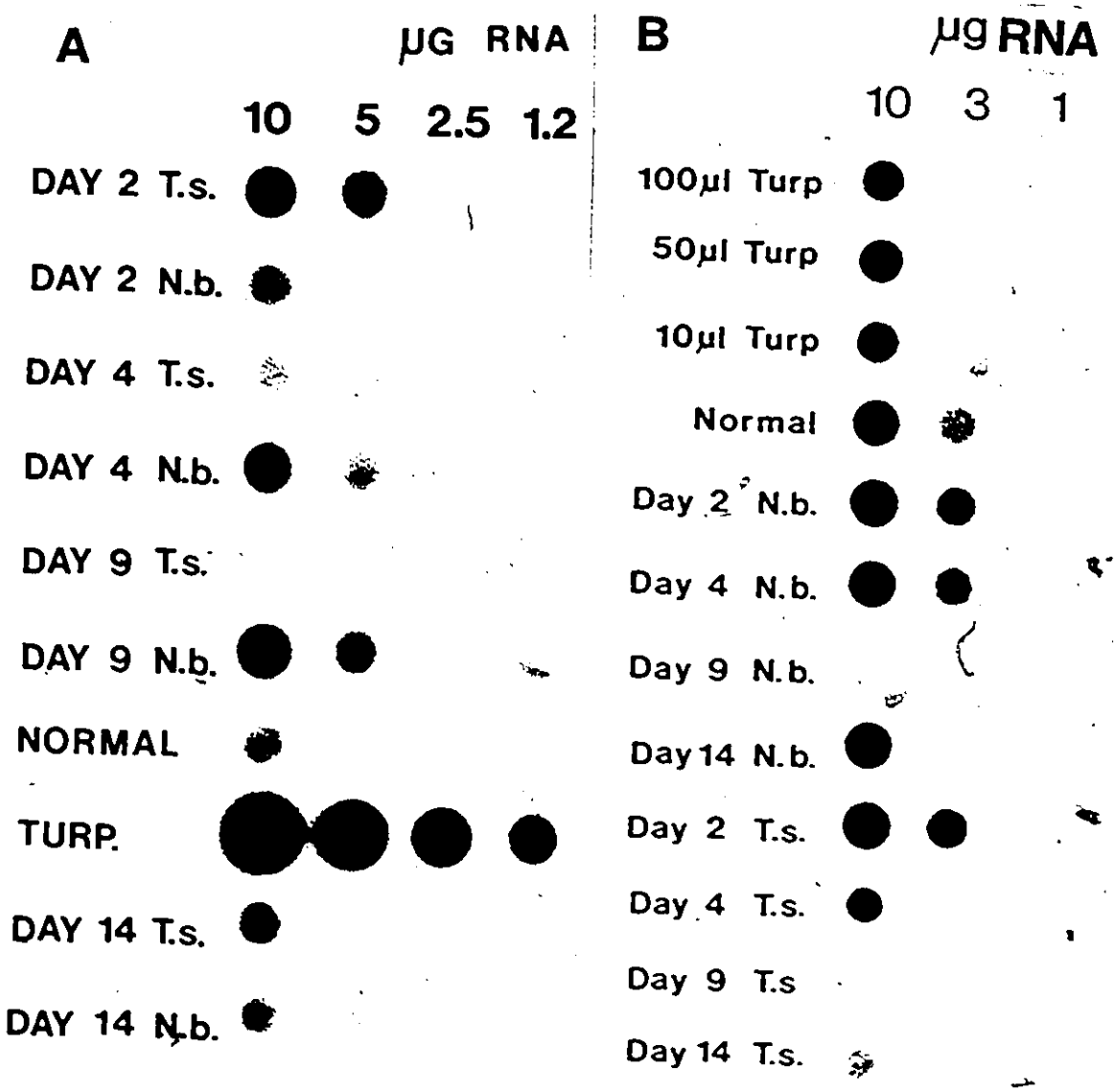
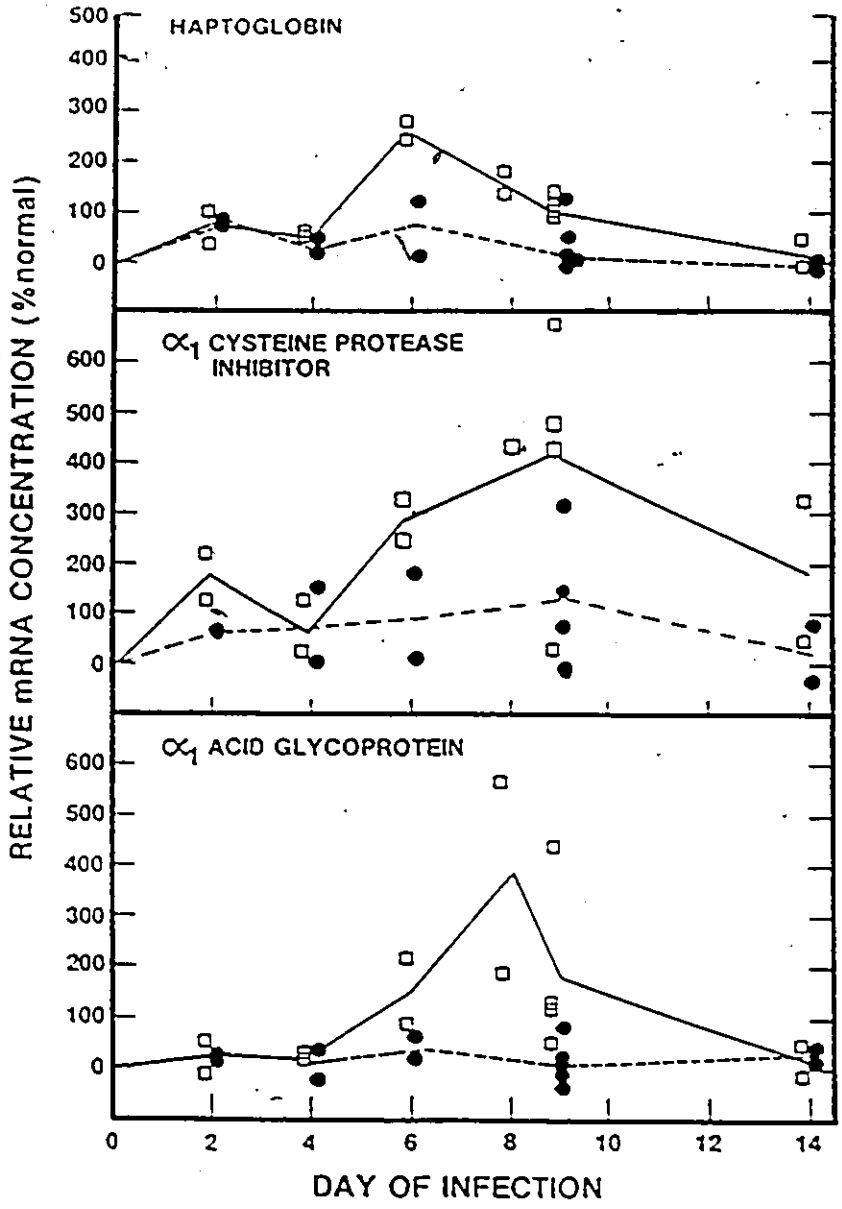

A
µG RNA
10
5
2.5
1.2
DAY 2 T.s.
DAY 2 N.b.
DAY 4 T.s.
DAY 4 N.b.
DAY 9 T.s.
DAY 9 N.b.
NORMAL
TURP.
DAY 14 T.s.
DAY 14 N.b.
B
µg RNA
10
3
1
100µl Turp
50µl Turp
10µl Turp
Normal
Day 2 N.b.
Day 4 N.b.
Day 9 N.b.
Day 14 N.b.
Day 2 T.s.
Day 4 T.s.
Day 9 T.s
Day 14 T.s.

Figure 15: Summary of molecular hybridizations of specific liver RNA for Acute Phase Proteins in N. brasiliensis and T. spiralis infected Sprague-Dawley rats. □, N. brasiliensis infected liver; ●, T. spiralis infected liver. Each point represents a single animal; the line is drawn through the mean of the points. A total of five experiments were performed using different days of infection.


HAPTOGLOBIN
α_1 CYSTEINE PROTEASE INHIBITOR
α_1 ACID GLYCOPROTEIN
RELATIVE mRNA CONCENTRATION (%normal)
DAY OF INFECTION
0
100
200
300
400
500
600
2
4
6
8
10
12
14

changes in another negative Acute Phase Protein, albumin, declining about day 9 during both infections.

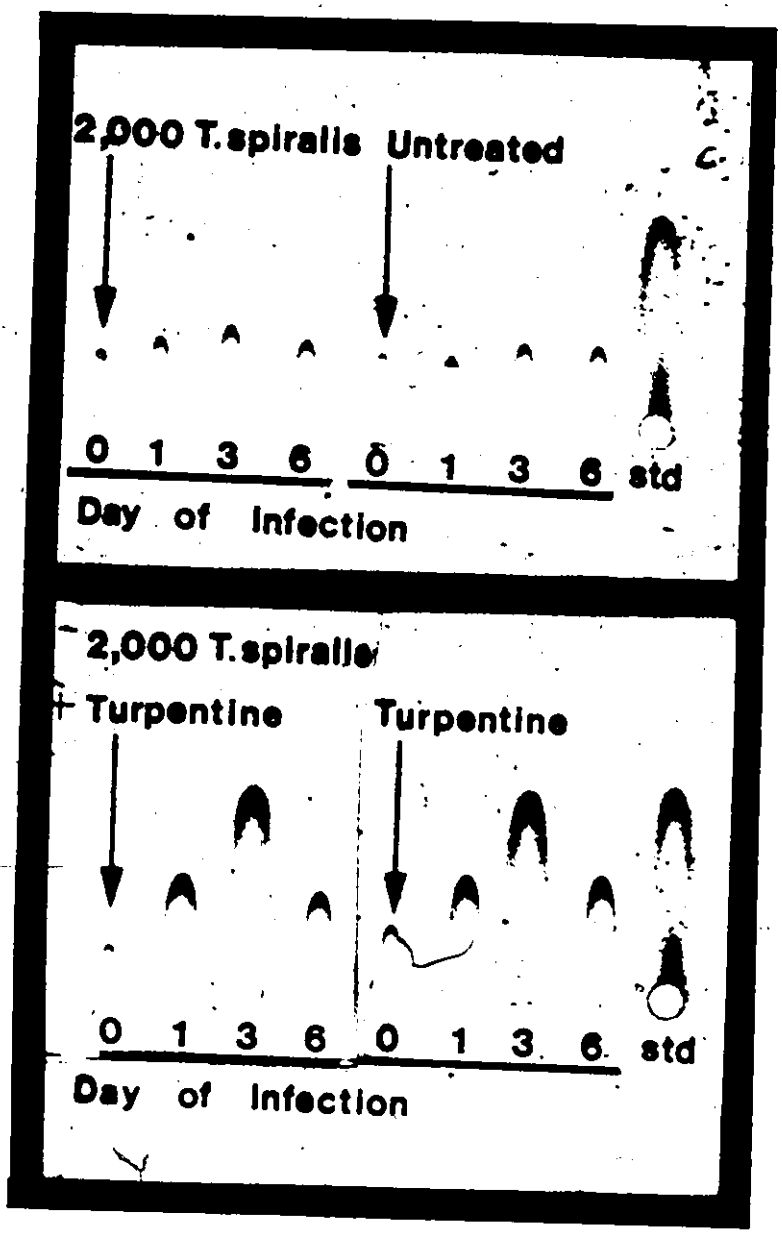
The absolute amount of RNA loaded onto each dot on the nitrocellulose was determined using either actin or 18Sribosomal cDNA probes, neither of which are Acute Phase molecules. The normalized RNA concentrations were used to compare radioactivity on the dots between samples on the same nitrocellulose filter. Figure 15 shows the summary of five separate dot/blot experiments, each with a normal, uninfected rat serving as a control against which the radioactivity of infected RNA samples was compared. Each point represents a single animal and the line was drawn through the mean of all the animals of one infection. The greatest increases in mRNA were seen during days 6-9 in the N. brasiliensis infected rats. There were changes in the RNA for haptoglobin, on day 2 of infection due to T. spiralis which were at least equal to changes in N. brasiliensis infected rats. Otherwise the failure to detect significant and consistent changes in mRNA for the Acute Phase Proteins during infection with T. spiralis eliminates the possibility that the lack of serum changes was due to increased catabolism of the proteins.

3.2.4 Non-inflammatory nature of T. spiralis infection

The failure to detect Acute Phase Protein changes during adult T. sprialis occupation of the rat intestine led to the consideration of whether the infection was anti-inflammatory or non-inflammatory. The infection has been shown to be anti-inflammatory by others (Castro, Malone and Smith, 1980). To address the issue of whether infection with T. spiralis suppressed the systemic Acute Phase Protein Response, infected animals were challenged with a second inflammatory stimuli. Figure 16 shows the consequences of turpentine injection in T. spiralis-infected rats. The rats reacted to the turpentine (50µl/100g) with a typical serum response, indicating that their liver was able to respond to systemic inflammatory messages. This dose of turpentine gave serum changes similar to those evoked by N. brasiliensis. As a second "positive" inflammatory stimuli, T. spiralis-infected rats were given a single dose of N. brasiliensis. The concurrent infection of rats gave a serum protein response similar to that of animals undergoing primary infection with N. brasiliensis alone (Figure 17). These results indicate that the liver Acute Phase Protein-synthetic capacity of T. spiralis- infected rats was normal and that infection by T. spiralis was not anti-inflammatory.

In contrast with the evidence for systemic anti-inflammatory events are data which shows reticuloendothelial cells are activated during infection by T. spiralis. The

Figure 16: Changes in the concentrationa of serum Acute Phase Proteins during concurrent turpentine- and T. spiralis induced inflammation of Sprague-Dawley rats. The rockets shown are for α_1AGP. Rats were infected with 2000 T. spiralis and given 50μl/100 g weight turpentine on day 0 and bled on the days indicated.


2,000 T.spiralis
Untreated
0 1 3 6 0 1 3 6 std
Day of Infection
2,000 T.spiralis
+ Turpentine
Turpentine
0 1 3 6 0 1 3 6 std
Day of Infection

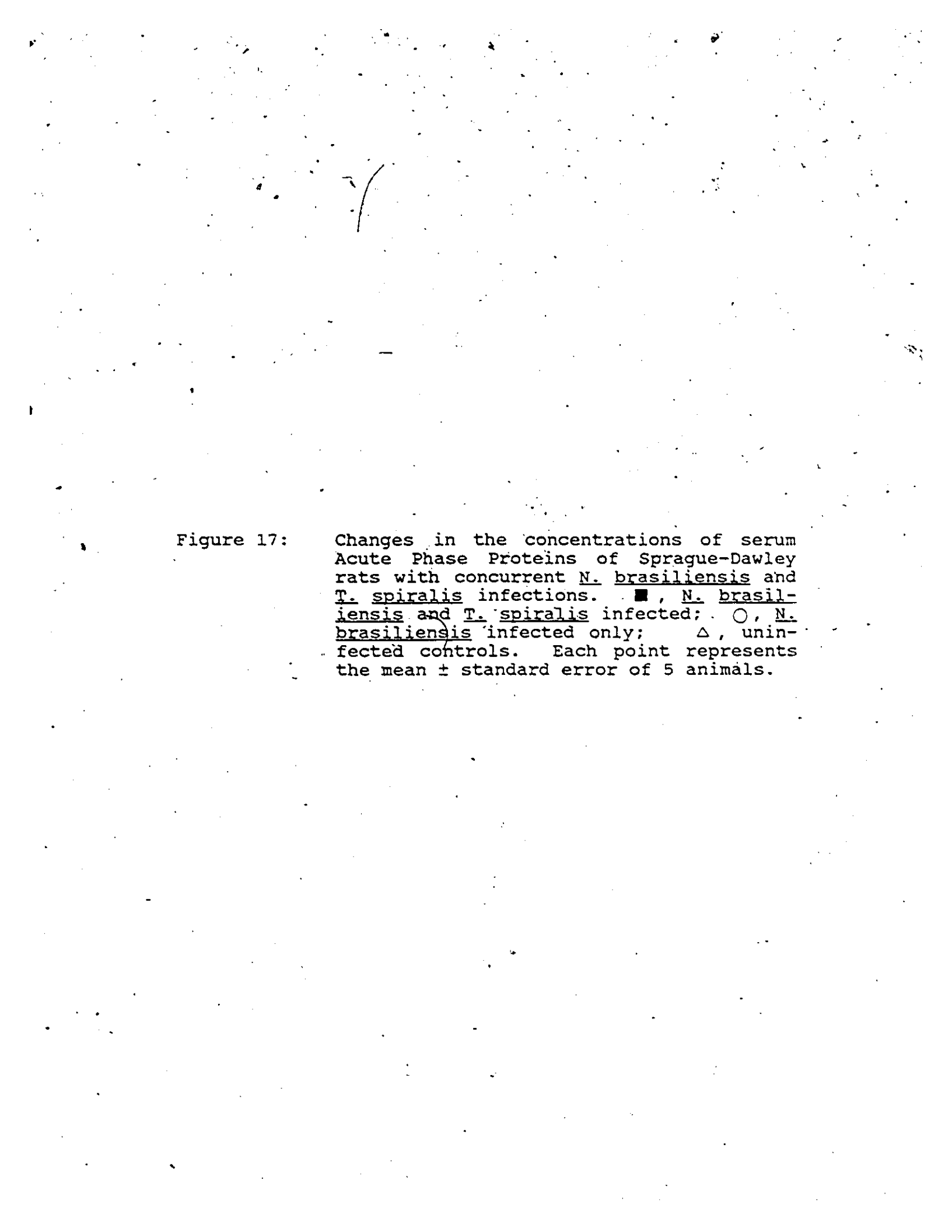
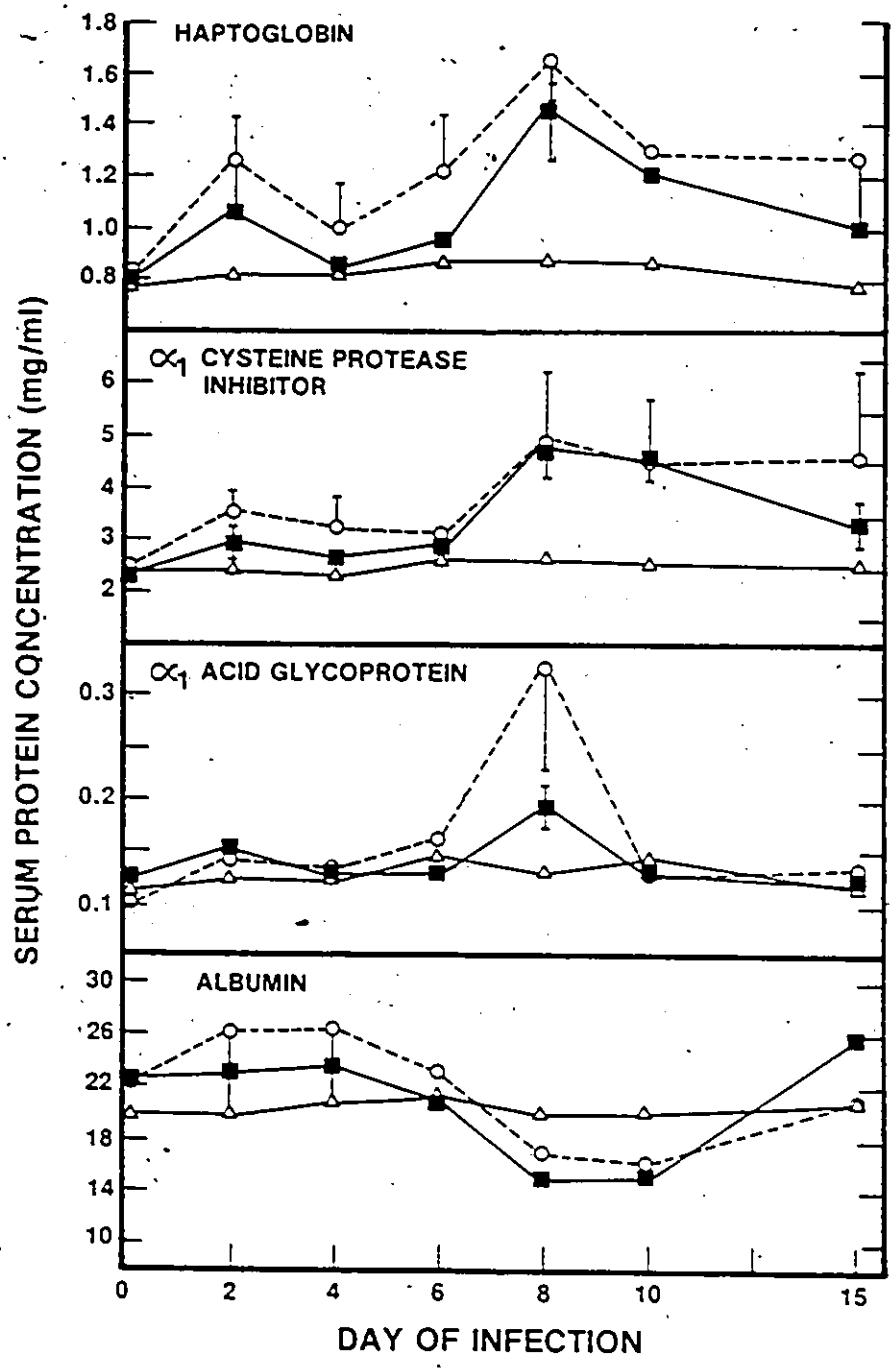


Figure 17: Changes in the concentrations of serum Acute Phase Proteins of Sprague-Dawley rats with concurrent *N. brasiliensis* and *T. spiralis* infections. ■, *N. brasiliensis* and *T. spiralis* infected; ○, *N. brasiliensis* infected only; △, uninfected controls. Each point represents the mean ± standard error of 5 animals.


HAPTOGLOBIN
1.8
1.6
1.4
1.2
1.0
0.8
α_1 CYSTEINE PROTEASE INHIBITOR
6
5
4
3
2
α_1 ACID GLYCOPROTEIN
0.3
0.2
0.1
ALBUMIN
30
26
22
18
14
10
0
2
4
6
8
10
15
SERUM PROTEIN CONCENTRATION (mg/ml)
DAY OF INFECTION

affected cells were not in the intestine and usually the peritoneal macrophages were shown to be activated (Lee and Best, 1983b). Such experiments led to the supposition of a role for macrophages in the resistance against infection by T. spiralis and a considerable amount of in vitro work showing mechanisms of parasite killing by macrophages (Mackenzie et al., 1980; Ruitenberg et al., 1983; Egwang, Gauldie and Befus, 1984b).

In order to determine whether the macrophages' capacity to elicit the serum Acute Phase Protein Response was compromised by infection with T. spiralis, hepatocyte-stimulating activities in culture supernatants of cells recovered from 4 and 7 day infected animals were assayed. Table 11 shows the activity of (plastic adherent) alveolar and peritoneal cell (24 hr) supernatants in the primary hepatocyte culture system and LAF (IL-1) assay. It can be seen in Table 11, that the cells from infected animals were not secreting more of either activity than cells from control rats but that their in vitro factor production was enhanced by the addition of LPS to the cultures, since LPS does not act directly on the hepatocyte. The results in Table 11 indicate that macrophage function was not compromised by the infection, and further, that the cells were not stimulated to constitutively secrete mediators important in the serum Acute Phase Protein Response.

TABLE 11

Hepatocyte-stimulating activity and Lymphocyte-activating factor activity (IL-1) of macrophages recovered from T. spiralis infected Sprague-Dawley rats

MACROPHAGE SOURCE[1]	STIMULATING ACTIVITY Hepatocyte-[2] -LPS	+LPS	Lymphocyte-[3] -LPS	+LPS
normal alveolar	0.23	0.64	16349 ± 1961	35447 ± 11166
4 day infected alveolar	0.24	0.80	10738 ± 953	26052 ± 13084
7 day infected alveolar	0.23	1.14	20878 ± 3104	26662 ± 12458
positive control[4]	----	1.60	-----	140607± 2841
normal peritoneal	0.12	0.17	13995 ± 4882	39059 ± 1755
4 day infected peritoneal	0.14	0.14	16649 ± 4253	23967 ± 2260
7 day infected peritoneal	0.13	0.16	17176 ± 3639	27021 ± 4399
positive control	----	1.60	-----	123913± 5406

[1] Alveolar cells were pooled from 4 animals and peritoneal cells from 3 animals. Plastic adherent cells were cultured for 24 hr.

[2] Results shown are the ratio of $\alpha_2$macroglobulin rocket peak height/albumin peak height (Koj et al., 1985).

[3] Results shown are the radioactive "counts per minute" determined from a PHA co-mitogenic assay using C3H/HeJ thymocytes and are mean ± standard error of three replicates.

[4] Human peripheral blood mononuclear cells cultured in the presence of LPS (Koj et al., 1984).

The above results argue that the T. spiralis was non-inflammatory when the infected hosts' potential to respond to other inflammatory signal was measured. Both liver and macrophage function were intact and the infected rat responded with a normal serum Acute Phase Protein Response due to a second inflammatory agent.

3.2.5 Non-inflammatory nature of intestinal infection

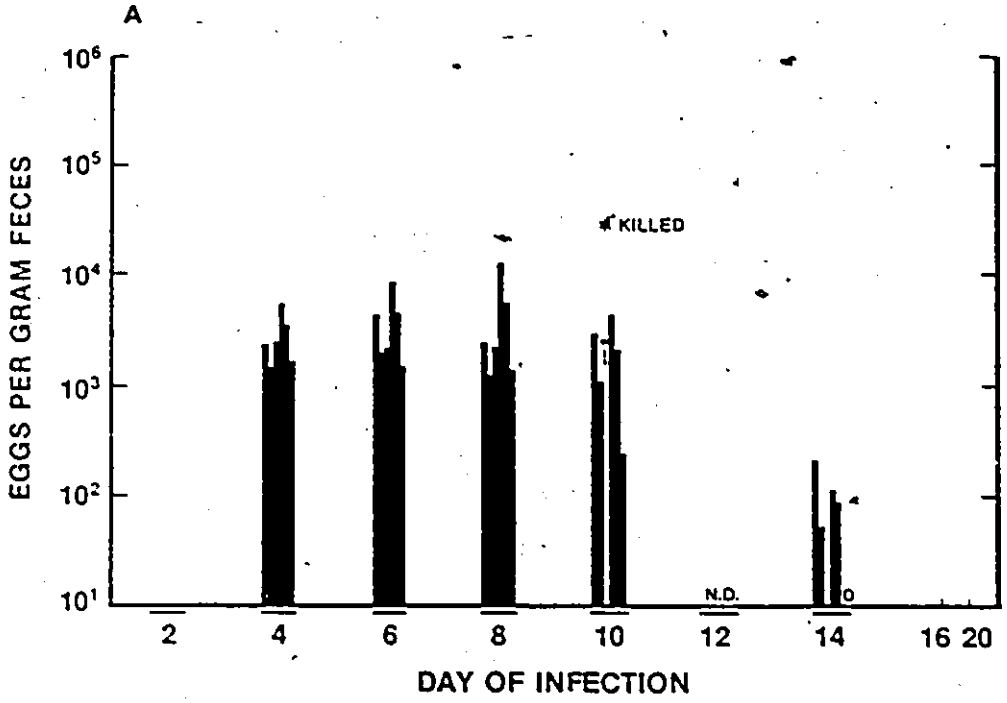
The evidence above indicated that an infection by T. spiralis may be non-inflammatory- the infection failed to elicit the serum Acute Phase Protein Response. It was not obvious whether intestinal infection by N. brasiliensis adults alone also were non-inflammatory. Important consideration must be made of the fact that N. brasiliensis larvae pass through (and molt in) the host's lungs before reaching the intestine. Breaching of a mucosal surface has important consequences for the host, including activation of alveolar macrophages and possibly stimulating defense mechanisms in the "common mucosal" system (Befus and Bienenstock, 1980). A number of events occur in the host on day 2 of the N. brasiliensis infection that are repeated about day 8 including the serum Acute Phase Protein Response, changes in lung permeability (Irving et al., 1986), and loss of host appetite (Ovington, 1985). Thus the postulate that the second peak of increased liver protein synthesis was due in part to the fact that the worms had passed through the lungs was

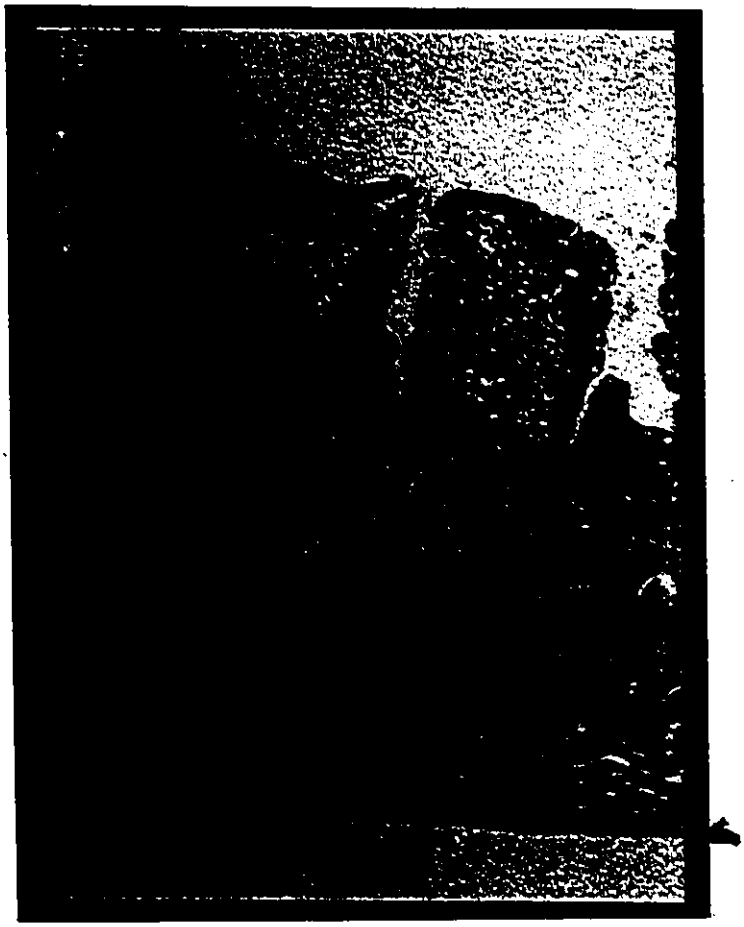
examined. Two approaches were taken to examine the hypothesis that the intestinal stage of N. brasiliensis was non-inflammatory and that worm migration through the lungs was necessary for the second peak of serum changes.

One approach utilized an "intestinal only" Nippostrongylus infection. Transfer of adult worms into a naive host results in the generation of specific immunity with similar characteristics to that generated by a normal infection (Kassai, 1982). Donor rats were infected with 3000 L_3 and killed on day 3 of infection. By 60 hr over 90% of the larvae complete the migration from the lungs to the intestine and by about 100 hr worms have begun their final molt (Yokogawa, 1921; Haley, 1962). Thus 3 day-old larvae are likely to be L_4. Worms of this age were chosen because they may be less susceptible to the digestive enzymes of a second animal's stomach. Between 1500 and 3000 worms were delivered to rats using the gavage tube. Figure 18 shows the worm-egg production by 6 individual infections. A noteworthy point was the significant egg production by day 4 by the transferred worm population- two days earlier than a subcutaneous infection by L_3. The egg production declined with kinetics similar to that of a normal infection and few worms remained in the intestine beyond day 14 PI. Figure 18 also shows a section of the inflamed jejunum of rats infected with the transferred worms.

Figure 19 shows the serum Acute Phase Protein Res

Figure 18: Infection of Sprague-Dawley rats with transferred populations of N. brasiliensis. Donor rats were killed on day 3 of infection, the worms collected and transferred in 0.2% agar into naive rats. A, egg production by transferred worms; B, histology of rat jejunum on day 10 PI with transferred worms, stained with hematoxylin and eosin. Oringinal magnification, 100X.


A
EGGS PER GRAM FECES
10^6
10^5
10^4
10^3
10^2
10^1
KILLED
N.D.
2
4
6
8
10
12
14
16 20
DAY OF INFECTION



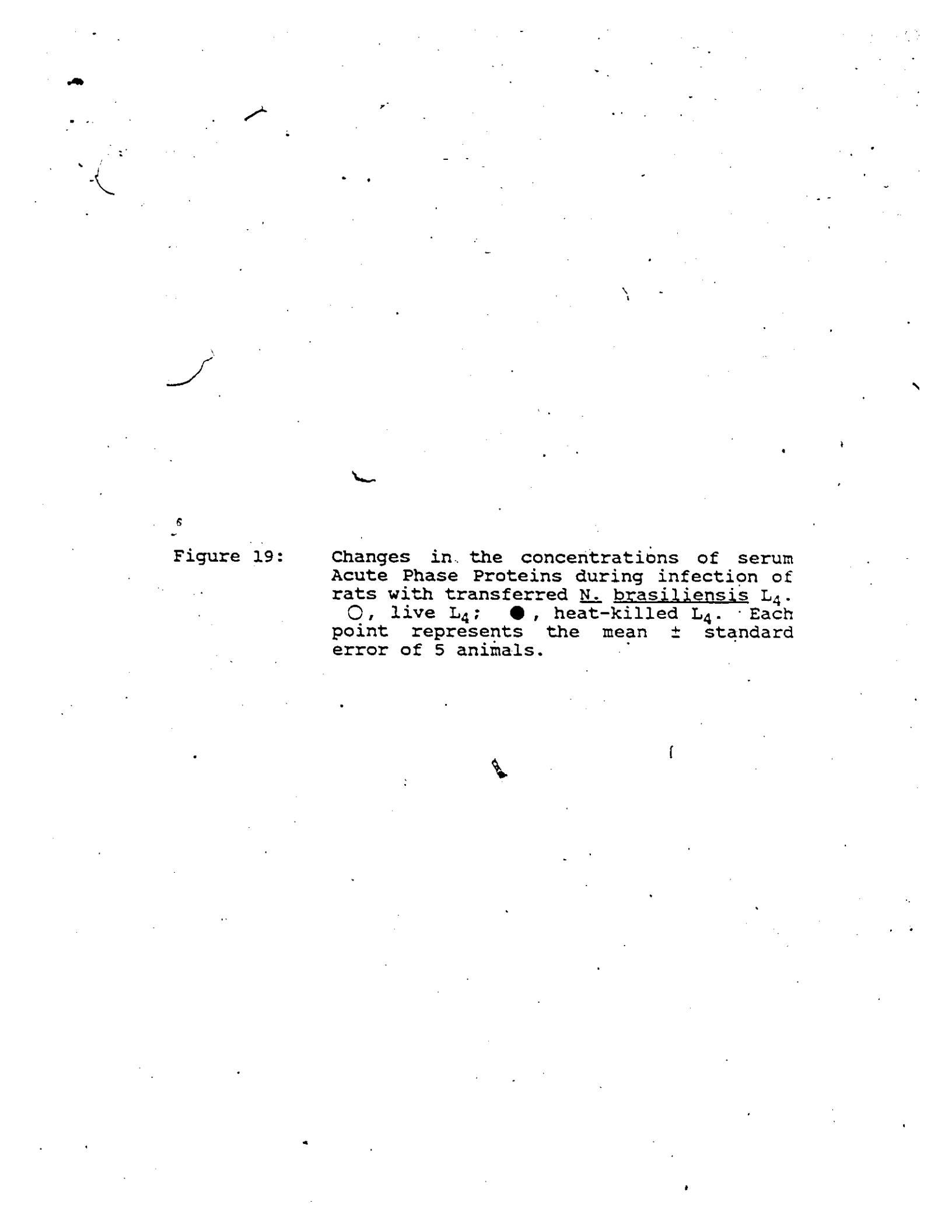
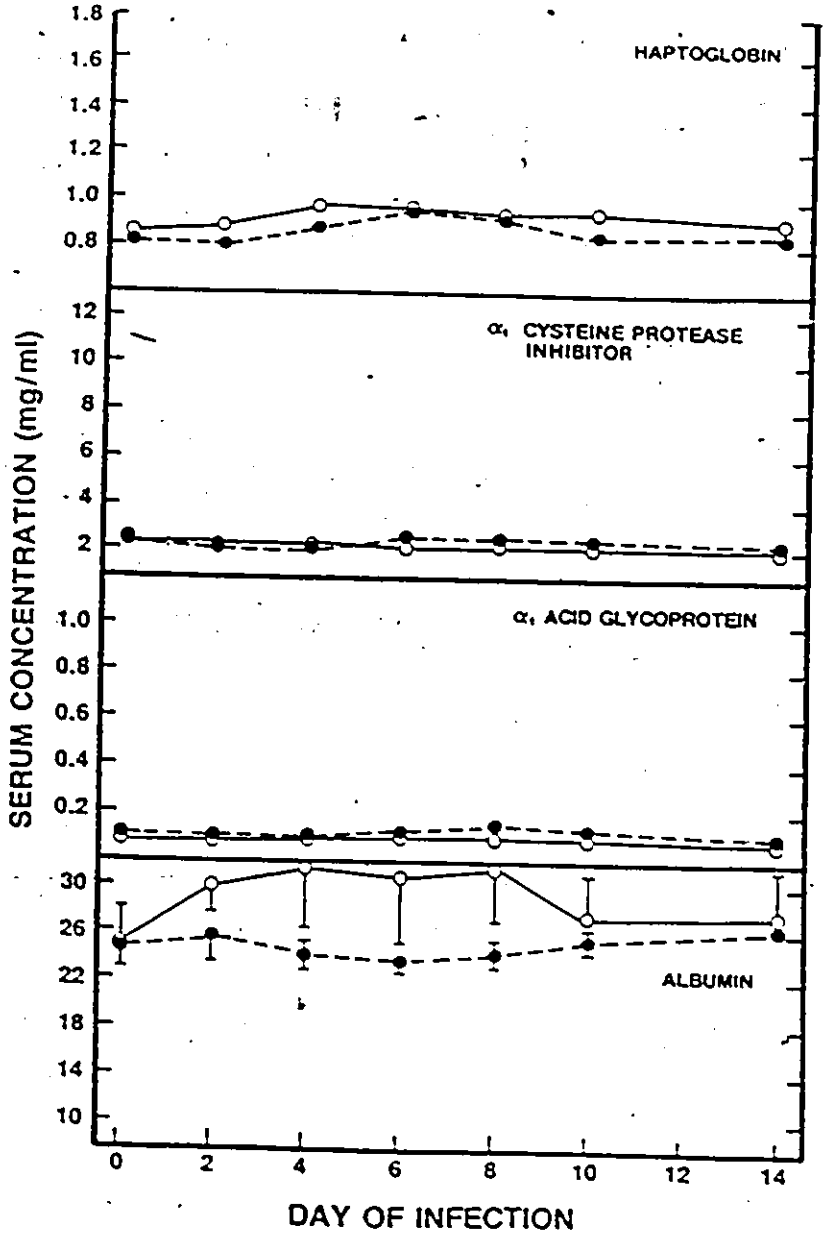


Figure 19: Changes in the concentrations of serum Acute Phase Proteins during infection of rats with transferred N. brasiliensis L_4. ○, live L_4; ●, heat-killed L_4. Each point represents the mean ± standard error of 5 animals.


HAPTOGLOBIN
α1 CYSTEINE PROTEASE INHIBITOR
α1 ACID GLYCOPROTEIN
ALBUMIN
SERUM CONCENTRATION (mg/ml)
DAY OF INFECTION
1.8
1.6
1.4
1.2
1.0
0.8
12
10
8
6
4
2
1.0
0.8
0.6
0.4
0.2
30
26
22
18
14
10
0
2
4
6
8
10
12
14

ponse of the rats which had received transferred L_4. There were no changes in circulating concentrations of any proteins during the infection of rats by the transferred worm population. However, upon challenge with infectious N. brasiliensis L_3, these animals did show serum Acute Phase Protein changes similar in quality to those seen during challenge of L_3-immunized animals (Figure 20). These results imply that the transferred population successfully immunized the recipient rats yet failed to elicit the systemic Acute Phase Protein Response.

A second experiment, involving anthelminthic abbreviation of the N. brasiliensis intestinal stages, was examined as a model of "lung only" infection. It was expected that an infection by the worms could be limited to the lungs, in order to examine the consequences of lung pathology, if the intestinal infection was terminated early using the anthelminthic Mebendazole. However the results of this experiment were uninterpretable because treatment of rats with the anthelminthic alone gave a positive Acute Phase Protein Response (Figure 21). Although this effect of the anthelminthic was an interesting finding, the reasons for the response were not pursued further.

The experiments involving intestinal infections of rats by a single nematode species, or concurrent infections by both species, are evidence that intestinal infections are non-inflammatory. However, an interesting observation was

Figure 20: Changes in the concentrations of serum Acute Phase Proteins during challenge infection with N. brasiliensis L_3, of rats immunized by transferred L_4. Rats were challenged on day 30 of the primary infection.

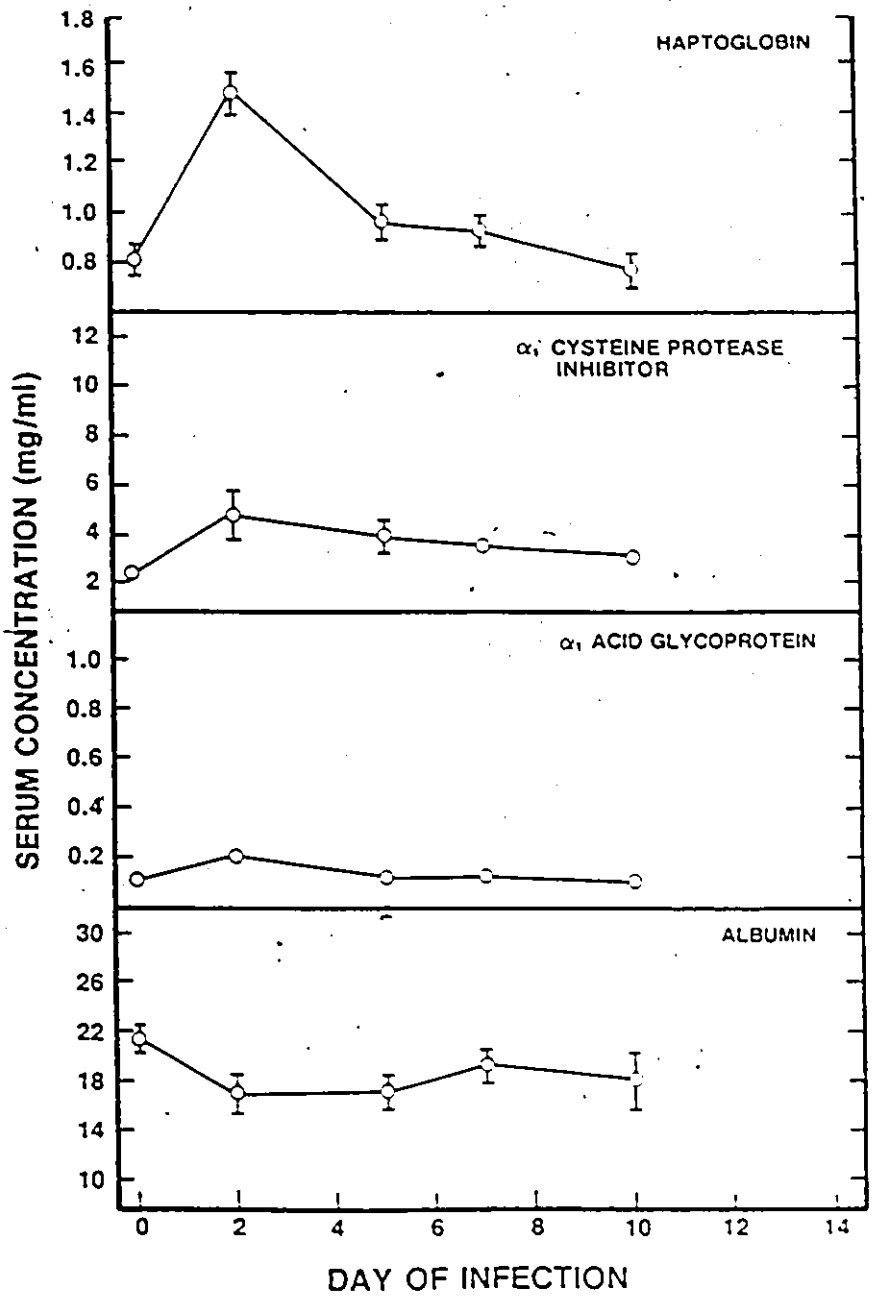
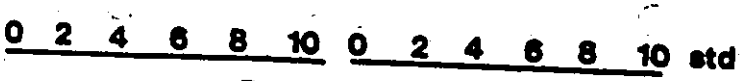
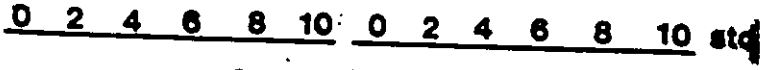

HAPTOGLOBIN
α_1 CYSTEINE PROTEASE INHIBITOR
α_1 ACID GLYCOPROTEIN
ALBUMIN
SERUM CONCENTRATION (mg/ml)
DAY OF INFECTION
1.8
1.6
1.4
1.2
1.0
0.8
12
10
8
6
4
2
1.0
0.8
0.6
0.4
0.2
30
26
22
18
14
10
0
2
4
6
8
10
12
14

Figure 21: Changes in the concentrations of serum Acute Phase Proteins during N. brasiliensis infection of Sprague-Dawley rats treated with Mebendazole. Rocket immunoelectrophoresis showing haptoglobin levels of rats which had received L_3 and Mebendazole (1g in 240 ml water, ad libitum, days 0-6) or Mebendazole only.

3,000 L_3 + Mebendazole



Mebendazole

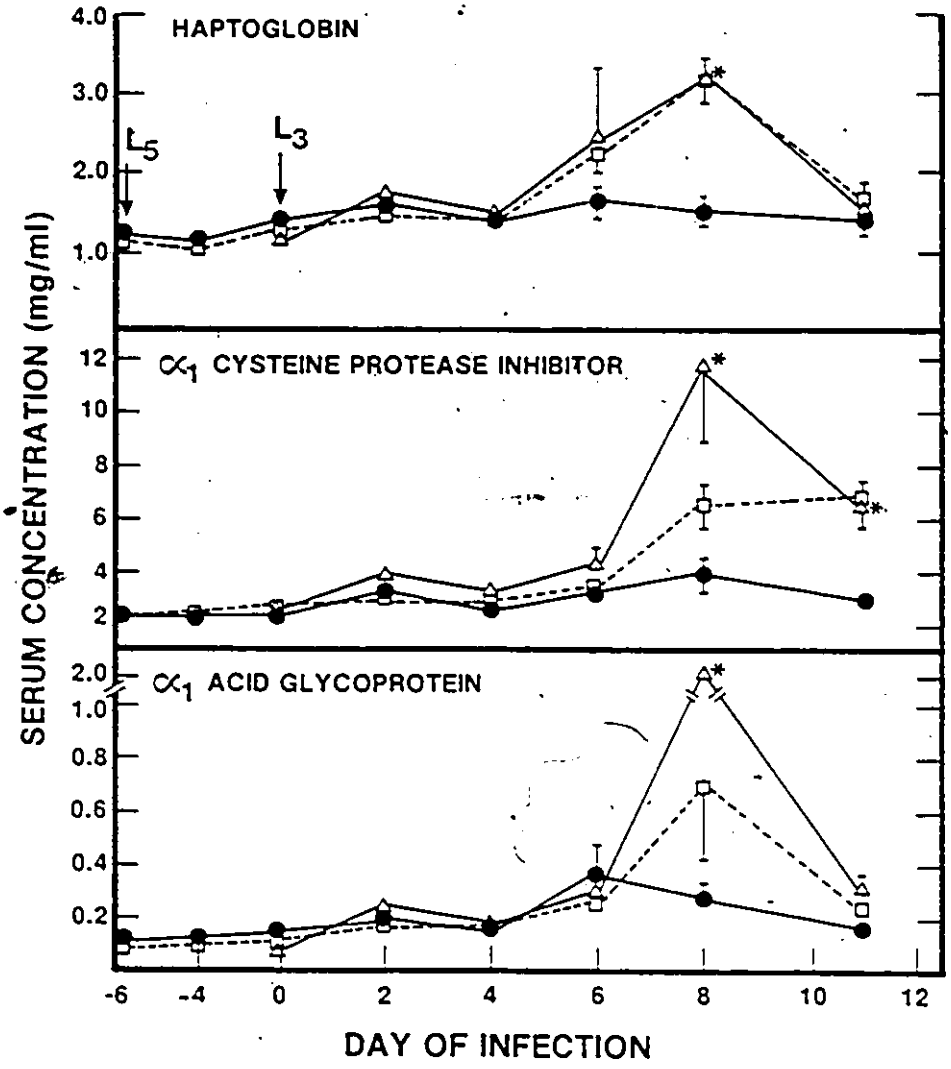


made when the timing of the concurrent infections was staggered such that the period of worm expulsion of one infection coincided with day 8 PI of a second, N. brasiliensis, infection. Rats that had received transferred N. brasiliensis L_4 were challenged with N. brasiliensis L_3, on day 6 PI of the transferred infection. It was estimated that the immune response would have begun in the intestine of the L_4-infected rat by the time the challenge infection reached the host's lungs. The challenge infection arrived in the intestine during the period of expulsion of the transferred worms. Figure 22 shows that the outcome of this experiment was the suppression of the expected (day 8) response due to the challenge infection. The presence of the initial intestinal infection clearly abolished the predicted, second peak of Acute Phase Protein changes due to the challenge N. brasiliensis infection. The specific events in the infected intestine which are responsible for worm expulsion, are not clearly understood, but it seems that the host reaction may either suppress the Acute Phase Protein Response or induce such large changes in the tissue distribution of serum proteins or intestinal permiability that increases in Acute Phase Proteins are not detectable.

3.2.6 Serum Acute Phase Protein Response in other infectious models

The results showing there was no serum Acute Phase

Figure 22: Changes in the concentrations of serum Acute Phase Proteins during concurrent infection of Sprague-Dawley rats by transferred L_4 and L_3 N. brasiliensis. ●, live L_4 and L_3; □, heat-killed L_4 and live L_3; △, live L_3 only. Three-day-old N. brasilensis were transferred into rats on day -6. All rats were infected with L_3 on day 0. Each point is the mean ± standard error of 3 or 5 animals. *, indicates a significant difference ($P \leq 0.05$) between animals with two infections versus animals with one infection, bled on the same day.


HAPTOGLOBIN
α_1 CYSTEINE PROTEASE INHIBITOR
α_1 ACID GLYCOPROTEIN
L_5
L_3
SERUM CONCENTRATION (mg/ml)
DAY OF INFECTION
4.0
3.0
2.0
1.0
12
10
8
6
4
2
2.0
1.0
0.8
0.6
0.4
0.2
-6
-4
0
2
4
6
8
10
12

Protein Response during T. spiralis infection or during infection by the transferred N. brasiliensis led to a question of whether there was a role for the response in host resistance and the expulsion of the worms during the immune response. To determine more closely whether there is a relationship between the inflammatory response and the subsequent immune response, models of infection in which the worms were not expelled by the host were used. Two such models were available; infection of young or newborn rats and infection of congenitally athymic (nude) rats. In both models either nematode species establishes a persistent population of mature and sexually active worms in the host intestine. N. brasiliensis adults continue to lay eggs and in T. spiralis infected animals, larvae continue to be born. Using these model infections the observation that T lymphocytes are essential for the specific immunity and the development of the pathology generated during the nematode infections was made.

Circulating concentrations of α_1CPI and α_2M are increased in newborn rats and decline to normal levels during the first few weeks of life. Turpentine-induced inflammation stimulated further serum increases in the young rats, despite the high levels of these proteins (Thomas and Schreiber, 1985).

Young Sprague-Dawley rats, aged less than 28 days, failed to expel an infection of 500 or 1000 N. brasiliensis

TABLE 12

Intestinal worm counts from Sprague-Dawley rats infected at 21 days-of-age with *N. brasiliensis*

	DAY OF INFECTION[1]				
	7	28	42	49	56
worm numbers	69	77	122	92	154
standard error	16	8	61	10	27

[1] 21-day-old rats, weighing about 60 g, were infected with 500 L_3 in 0.5 ml PBS, 3 animals per time point.

(Table 12). This choice of infectious dose amounted to a greater infection in the young rats than routinely used for mature rats, when the dose was calculated as "worms per gram body weight". The serum Acute Phase Protein Response of the infected young rats is shown in (Figure 23). The finding of no significant increases is in contradiction to the reports showing a serum response in young animals due to turpentine. The lack of a serum response in these animals was interpreted to imply there was a role for T lymphocytes or another mature cell-type, in the generation of the Acute Phase Protein Response.

Infection of athymic animals with N. brasiliensis leads to a protracted infection and continued egg production by the worms (Jacobson and Reed, 1974). The "nude" mutation results in congenitally athymic and therefore T lymphocyte-deficient, animals. These T lymphocyte-deficient animals fail to generate an antibody response or cell-mediated immunity against the worms and hence do not expel the infection. An examination of the serum protein response of infected nude rats showed they indeed undergo similar changes as their euthymic littermates (Figure 24). This result argues against T cell involvement in the generation of the Acute Phase Response.

Figure 23: Changes in the concentrations of serum Acute Phase Proteins of 21-day-old Sprague-Dawley rats infected with *N. brasiliensis*. △, infected rats; □, uninfected controls. Rats were infected with 1000 L_3. Each point represents the mean ± standard error of 5 animals.

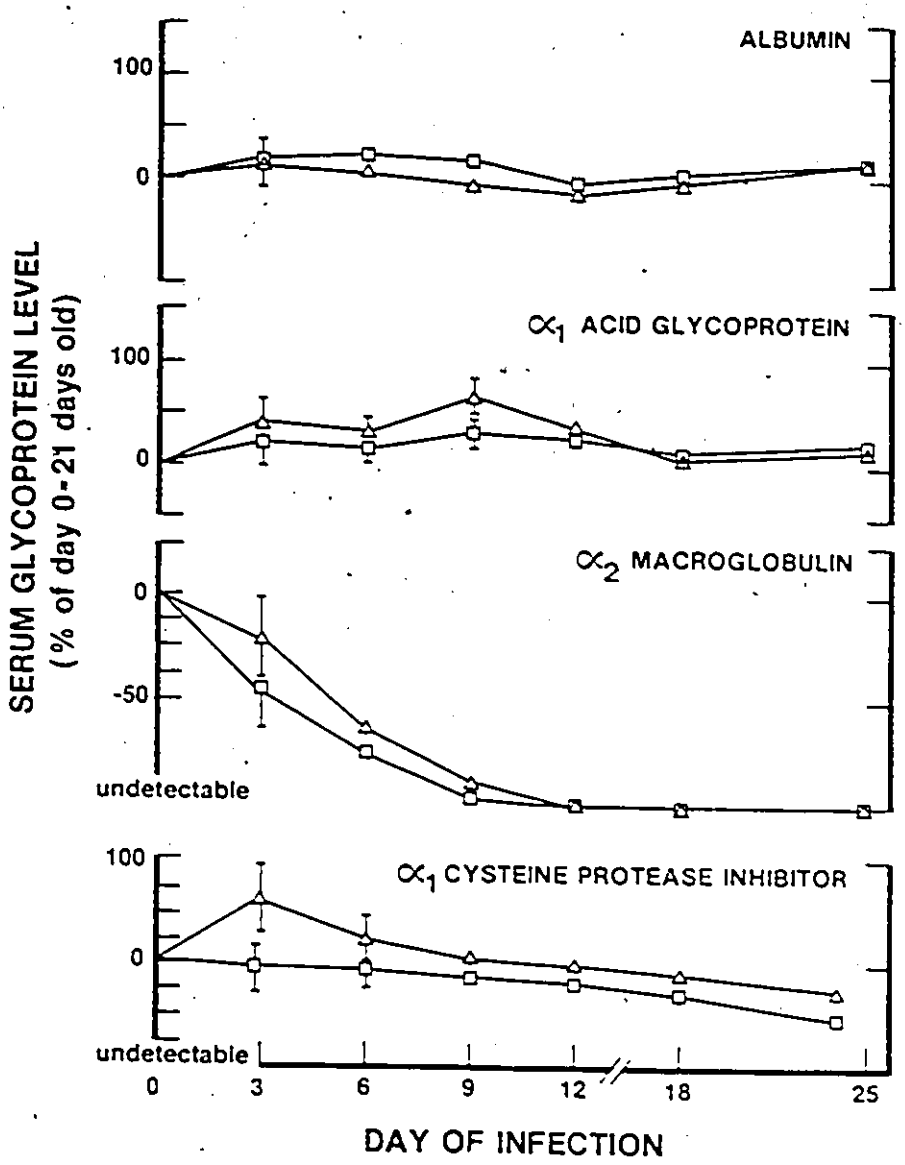
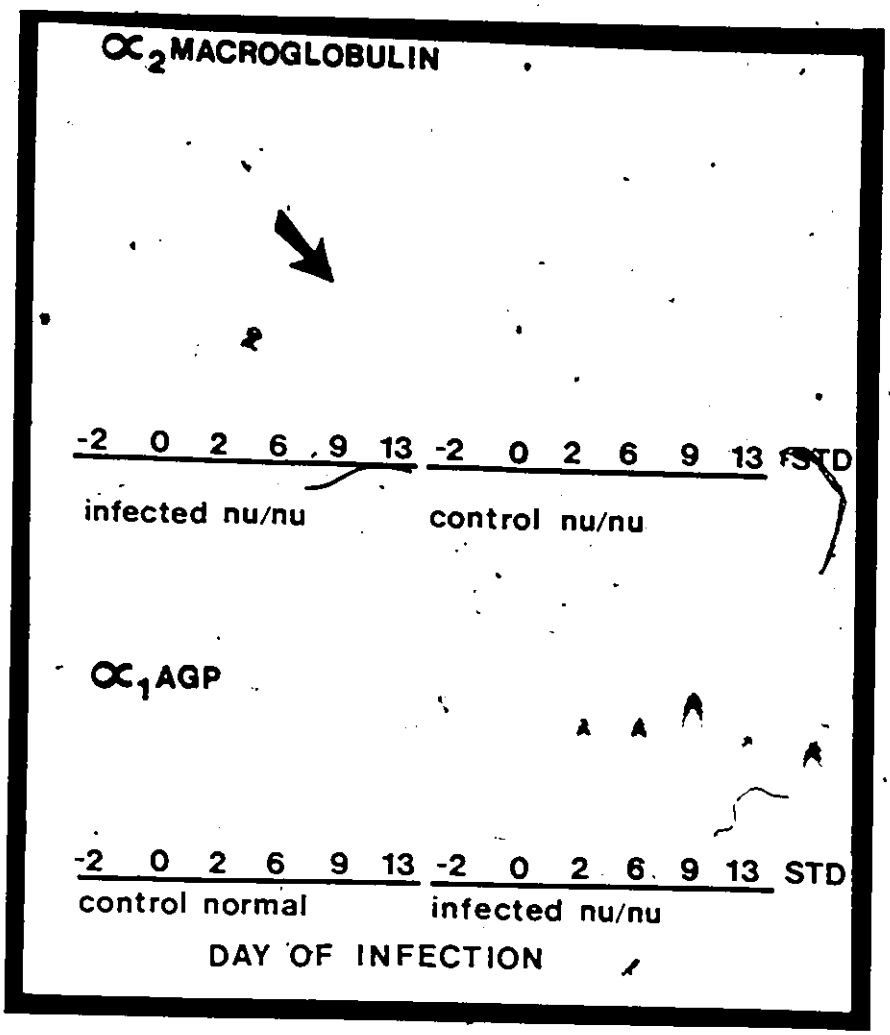

ALBUMIN
100
0
α_1 ACID GLYCOPROTEIN
100
0
α_2 MACROGLOBULIN
0
-50
undetectable
100
α_1 CYSTEINE PROTEASE INHIBITOR
0
undetectable
0
3
6
9
12
18
25
DAY OF INFECTION
SERUM GLYCOPROTEIN LEVEL
(% of day 0-21 days old)

Figure 24: Rocket immunoelectrophoresis of serum from uninfected and N. brasiliensis infected nude rats. A total of five nude and five euthymic littermates were used in the experiment.


α_2MACROGLOBULIN
-2 0 2 6 9 13 -2 0 2 6 9 13 STD
infected nu/nu
control nu/nu
α_1AGP
-2 0 2 6 9 13 -2 0 2 6 9 13 STD
control normal
infected nu/nu
DAY OF INFECTION

3.3 Isolation and enrichment of rat intestinal macrophages

The events in the parasitized rat intestine could be better characterized if the cells from the intestine were isolated and their functions defined. Little work has been performed using isolated intestinal cells from either normal or infected rats. Most of the work concerning cell function in the intestine of most species is conjecture based on histological studies, label up-take, and using cells which traffic from the intestine (Deane, 1964; Sawicki et al., 1977; LeFevre, Hammer and Joel, 1979; Verspaget and Beekan, 1985; Takahashi-Iwanaga and Fujita, 1985; Mayrhofer, Holt and Papadimitriou, 1986). A single publication has identified macrophages in the rat intestine using monoclonal antibodies (Sminia and Jeurissen, 1987).

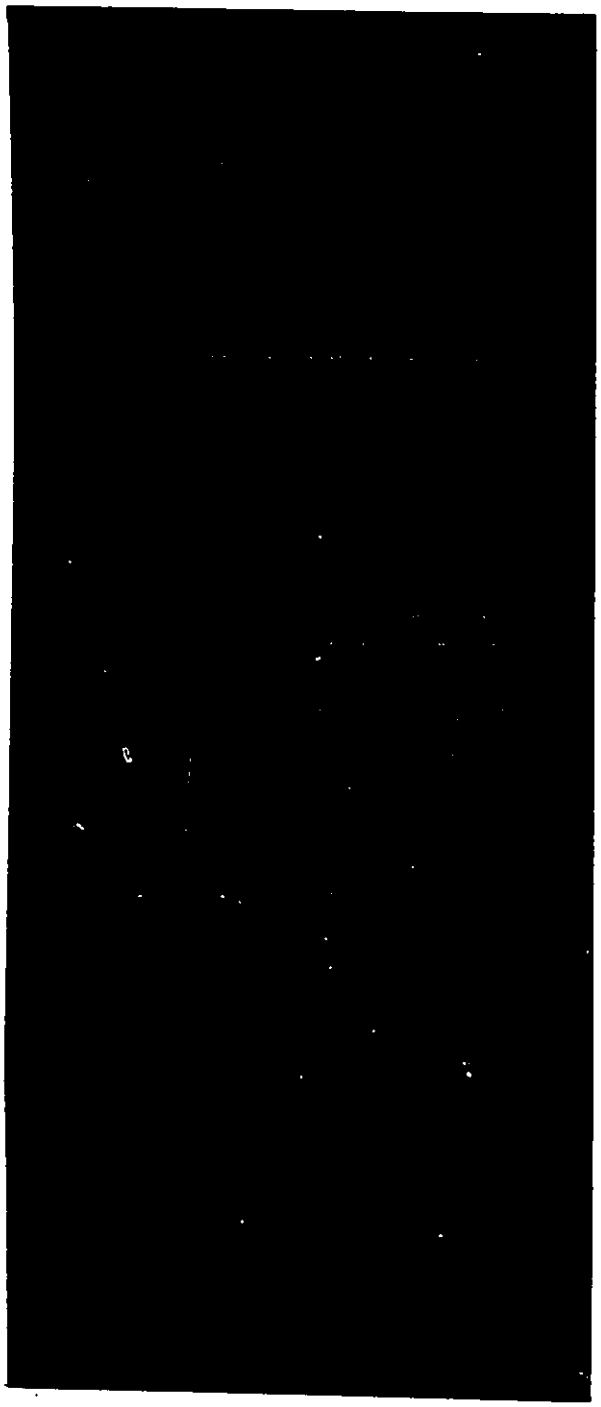
A paucity of information on the function of intestinal macrophages is acutely obvious. Considering work alluded to earlier in which a role for macrophages in the host response to N. brasiliensis was implied and the evidence from other laboratories that macrophages are involved in the host response to T. spiralis, it was postulated that macrophages functioned in a similar capacity in the intestine. First, it was important that the characteristics of macrophages from normal rat intestine be documented.

3.3.1 Isolation of intestinal cells from uninfected rats

Isolation of intestinal cells may be performed in two ways; scraping-off the lamina propria (Winter et al., 1983) and enzyme digestion (Befus et al., 1982; Golder and Doe, 1983). Scraping the lamina propria has achieved less popular usage than enzyme digestion because the cells are often clumped together in mucus. On the other hand, the success of enzyme digestions requires substantial work to optimize the conditions for the enzymes yet minimize the damage to the cells. These conditions have been satisfactorily achieved for the isolation of mast cells from the rat intestine and was used to isolate macrophages (Befus et al., 1984).

The digestion procedure began with serial incubations in iso-osmotic media at 37°C, with the Ca^{2+} and Mg^{2+} chelator, EDTA. Following three 10 min incubations, each in fresh media, most of the epithelium was removed from the intestinal wall (Figure 25). No steps were taken at this stage to eliminate the epithelial cells from contaminating the remaining tissues, which were then exposed to two periods of digestion using collagenase (or in two experiments, dispase). Each collagenase digestion continued for 1 hr and the media was changed between digestion. The cells collected from the first collagenase digestion were referred to as "coll I" and the second as "coll II". Cells from both collagenase digestion were immediately washed through a

Figure 25: Low power histological micrographs of enzyme-digested rat intestine. A,B and C, following 1st, 2nd and 3rd EDTA incubation, respectively; D and E, following 1st and 2nd collagenase digestions. Original magnification 100X.



nylon wool column to remove mucus and centrifuged through a 30% Percoll solution to enrich for viable cells. The two digestion were kept separate in some experiments because it was not clear whether the macrophages would be more abundant in one wash over the other, as is the case with mast cells (Lee et al., 1985). It was expected that the digestion would progressively release cells from deeper inside the lamina propria (Figure 25) and this may be a rough measure of macrophage location in the rat intestine.

Table 13 gives a summary of the outcome of the digestive procedure in terms of cell recoveries and cell viability (determined using trypan blue exclusion) from several representative experiments. The single-step 30% Percoll was used, effectively, to enrich for viable cells immediately following their isolation from the intestine. It was found that the digestion media was insufficient for the simultaneous digestion of two rat intestines. This was an unfortunate finding because it was very difficult to process more than two rat intestines, independently, in one day. It is also evident from Table 13 that Dispase was of limited utility as a digestive enzyme for rat intestinal tissue.

The viability of the isolated cells, as determined by trypan blue exclusion and light microscopic observation, was consistently greater than 75% for coll I and greater than 70% for coll II, although 90% viability was achieved

TABLE 13

Cell yield and viabilities of enzyme-digested rat intestine

TREATMENT	YIELD[1] (viable X 10^6)	VIABILITY[2] (%)	NUMBER OF EXPERIMENTS
Pre-30% Percoll			
Coll I	16.8 ± 0.4	52 ± 3	5
Coll II	30.9 ± 6.5	58 ± 6	
Post-30% Percoll			
Coll I	7.4 ± 0.8	83 ± 3	8
Coll II	17.3 ± 0.8	71 ± 5	
Post-30% Percoll, 2 rats[3]			
Coll I	6.2	81	2
Coll II	10.4	69	
Post-30% Percoll, Post-18% Metrizamide			
Coll I	1.8 ± 0.8	81 ± 5	3
Coll II	4.2 ± 1.7	81 ± 11	
Dispase digestion[4], Post-30% Percoll	1.6 ± 0.6	47 ± 6	3

[1] Mean ± standard error.
[2] Determined by trypan blue exclusion.
[3] Digested together in a single batch of media.
[4] 40 mg Dispase (Boehinger Mannheim) per 100 ml HANKS, stirring for 90 min at 37°C.

in both. However when the cells were viewed following cytocentrifugation (May-Grunwald Giemsa stain) the viability was determined to be less than 70% for both collagenase preparations (Figure 26). This discrepancy was likely due to the fragility of many cells and the consequential damage due to the centrifugation, but also to the use of trypan blue as the viability marker. While many of the cells in Figure 26 probably exclude trypan blue, it was obvious even at the light microscope level that cells were breaking-down, with pycnotic, homogeneous-staining nuclei and incomplete cell membranes. These characteristics of dying cells were more apparent with the discriminating resolution of the electron microscope (Figure 27). Greater than one half all the eosinophilic cells were non-viable. They appeared as small buttons with a collapsed nucleus and homogeneous pink-staining cytoplasm and blue nuclei. These small cells were likely of two types; eosinophils (confirmed using the electron microscope) and paneth cells, which are thought to stain eosinophilic (Trier and Madara, 1981). It seemed that some cell types suffered greater damage due to the isolation procedure than other cell types.

The leukocyte differential of the typical intestinal cell population is given in Table 14. The cell identity was decided entirely on the basis of its morphology, using peritoneal wash-out cells as the prototype. Intestinal (mucosal) mast cells were smaller and stained slightly different

Figure 26: Light micrographs of dissociated intestinal cells of the rat. Cells were isolated by collagenase digestion of the entire small intestine and prepared for staining by cytocentrifugation. m, mast cell; gl, granulated lymphoctye; d, dead cell; mø, macrophage; n, neutrophil; e, eosinophil. May-Grunwald-Giemsa stain.

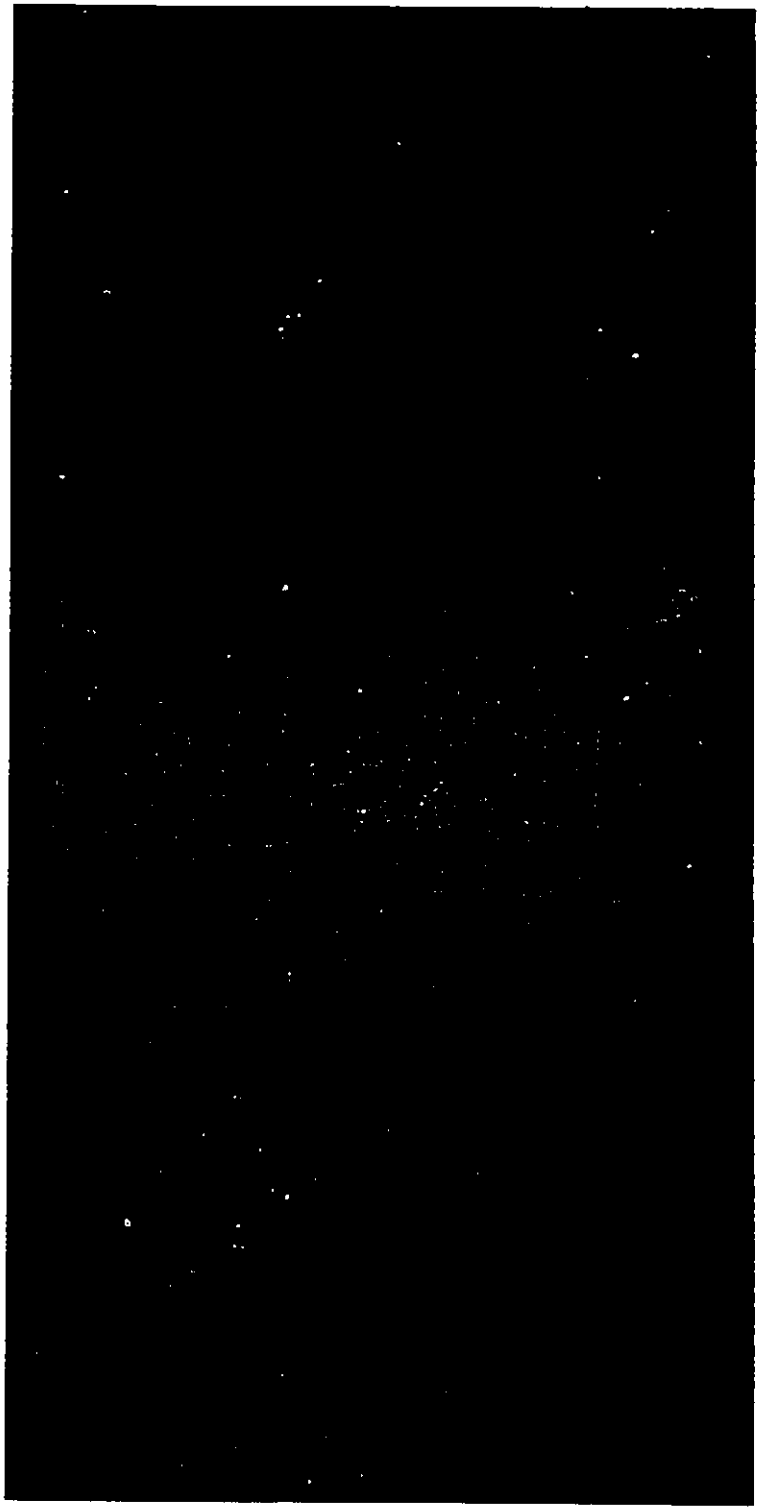
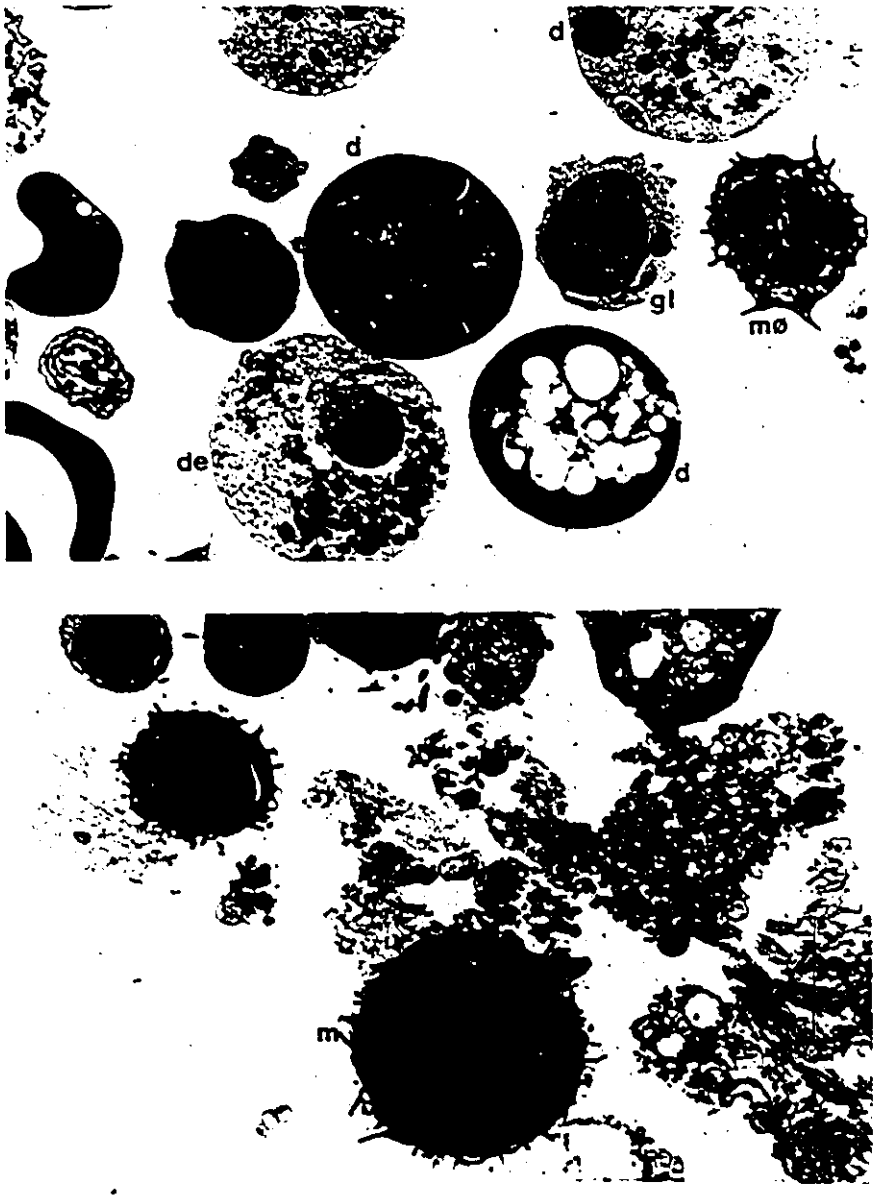


Figure 27: Transmission electron micrographs of single cells isolated from normal Sprague-Dawley intestine. the cells were fixed in gluteraldehyde following their isolation, and prepared for microscopy by McMaster University electron microscope services. d, dead cell; de, dead eosinophil; gl, granulated lymphocyte; mø, macrophage; m, mast cell.


d
d
gl
mø
de
d
m

than the peritoneal (connective) mast cell (Lee et al., 1985). Epithelial cells did not exist in the peritoneal population and there was no good prototype for identifying this cell. Few of the cells in gut preparations were columnar. Therefore the identity of epithelial cells was based on the morphology of the cells recovered from the EDTA digestion. These cells resembled macrophages by their relatively large size and by having a low nucleus to cytoplasm volume and "foamy" cytoplasm. The "dead cell" category of Table 14 includes all those cells with disintegrating and homogeneous-staining nuclei, including the dead eosinophils (Figures 26 and 27). The cells of the rat intestine have been poorly characterized and certainly some of the cells are not white blood cells (for example goblet cells, paneth cells, fibroblast and veiled cells; Deane, 1964; Trier and Madara, 1981). From Table 14 it is evident that macrophages constituted a minor proportion of the cells recovered by enzyme digestion and that it would be necessary to enrich for them in order to study their function.

3.3.2 Characterization and histochemistry of intestinal cells from uninfected rats

Table 14 shows the outcome of the first step in purifying macrophages away from other cell types using a discontinuous Percoll gradient. Table 15 gives the results of a control (peritoneal) population separated by the same

TABLE 14

Leukocyte differential of normal Sprague-Dawley intestine and separation of cells on a discontinuous Percoll gradient

Gradient interface	Recovered viable cells[2] (%)	viability[3] (%)	Cell type[1] lymphocyte	eosinophils	macrophages	mast cells	epithelial	dead[4]
Total gut n = 10	--	--	21 ± 2	18 ± 1	5.9 ± 1.0	1.3 ± .3	1.7 ± .4	53 ± 2
Med-30% n = 3	5.2 ± 2.0	55	25 ± 5	11 ± 4	6 ± 3	12 ± 6	1 ± .6	43 ± 4
30%-50% n = 4	21 ± 6.6	76	23 ± 7	11 ± 1	7.5 ± 3.3	1.7 ± .5	< 1	55 ± 9
50%-70% n = 3	28 ± 1	95	20 ± 5	22 ± 4	6.7 ± 3	< 1	< 1	50 ± 8
PELLET n = 3	11 ± 2	97	4 ± 1	47 ± 1	2.3 ± .7	5.6 ± 2.9	0	44 ± 2

1. Mean ± standard error.
2. 65% of cells loaded onto PERCOLL were recovered.
3. Viability determined by trypan blue exclusion.
4. Viability determined by morphology following centrifugation.

TABLE 15

Leukocyte differential of normal Sprague-Dawley peritoneal cells and separation of cells on a discontinuous Percoll gradient

GRADIENT INTERFACE	CELL TYPE[1] lymphocytes	eosinophils	macrophages	mast cell
Total population n = 6	2 ± 0.3	14 ± 2	66 ± 4	18 ± 2
30%-50% n = 5	5 ± 2	6 ± 2	78 ± 10	11 ± 9
50%-70% n = 4	4 ± 2	19 ± 3	72 ± 5	5 ± 2
Pellet n = 4	0	8 ± 5	3 ± 2	89 ± 7

[1] Mean ± standard error.

technique. It is evident from both Tables 14 and 15 that the macrophages of each tissue were heterogeneous with respect to their density. Although eosinophils collect at the higher densities, it is interesting to note that more of the eosinophilic cells in the intestine traveled into the pellet of the tube. Mast cells usually penetrated to the pellet of the gradient. The dead cells isolated from the intestine showed heterogeneity of density comparable to macrophages. The discontinuous Percoll gradient proved to be ineffective at eliminating the dead cells from the viable population or from macrophages.

A widely-used method for the enrichment of macrophages is by cell adherence to some substrate, usually plastic or glass. The cells from the intestinal digestions were cultured in plastic dishes for two hours in media supplemented with 10% FBS. Following this incubation the dishes were washed with several changes of pre-warmed media until only the adherent population remained. The adherent cells were then removed by a wash with pre-cooled PBS (4°C). Only 10% of the initial cell population adhered to the plastic representing a slight enrichment for macrophages (Table 16). It was unlikely that the eosinophils and lymphocytes were plastic adherent but rather were lumped together with adherent cells and were not washed off. From Table 16 it can be seen that there was no enrichment for (non-specific, neutral red) phagocytic cells among the

TABLE 16

Properties of plastic-adherent cells isolated from rat small intestine

Cells	Viability[2]	Percent recovered	Cell type[1]						
			Neutral Red phagocytic	lymphocytes	eosinophils	macrophages	mast cells	epithelial	dead[3]
TOTAL n = 3	78	100	34	21 ± 3	27 ± 8	5.5 ± 1.3	< 1	< 1	45 ± 3
PLASTIC[4] ADHERENT	89	10 ± 2.7	31	33 ± 15	34 ± 13	15 ± 2	< 1	< 1	17 ± 6

1. Mean ± standard error.
2. Viability determined using trypan blue.
3. Viability determined by morphology.
4. Total small intestinal cells were cultured for 2 hr at 37^0 C, 5% CO_2. The adherent cells were washed repeatedly using PBS.

plastic adherent population.

In order to facilitate in the identification of macrophages a cell-marker, non-specific esterase (NSE), was characterized among the intestinal cells. The technique utilized endogenous esterases of the cell to cleave an exogenous substrate which then complexes with hexatozized pararosanalin. The complex precipitates in the cell imparting a unique colour to those cells possessing the esterases. The incubation conditions under which the esterases operate were manipulated such that only macrophage-specific enzymes ought to react with the substrate. These conditions included the choice of the substrate and the presence of the inhibitor NaF (Stadnyk, Befus and Gauldie, 1987).

When the intestinal cell population was stained for NSE using the substrate α-naphthyl acetate, a considerable number of cells were positive (Figure 28 and Table 17). The NSE positive cells clearly outnumbered the expected number of macrophages. Positive-staining cells were not enriched at any single band on the Percoll gradient (Table 17). When the reaction substrate was changed to α-naphthyl butyrate an equally large number of cells stained positive from the intestine as had stained using α-naphthyl acetate. Two other populations of macrophages, alveolar and peritoneal, served as controls for macrophage esterases and equal numbers of these cells stained positive for the two naphthyl substrates. Finally the inhibition of the esterase activity

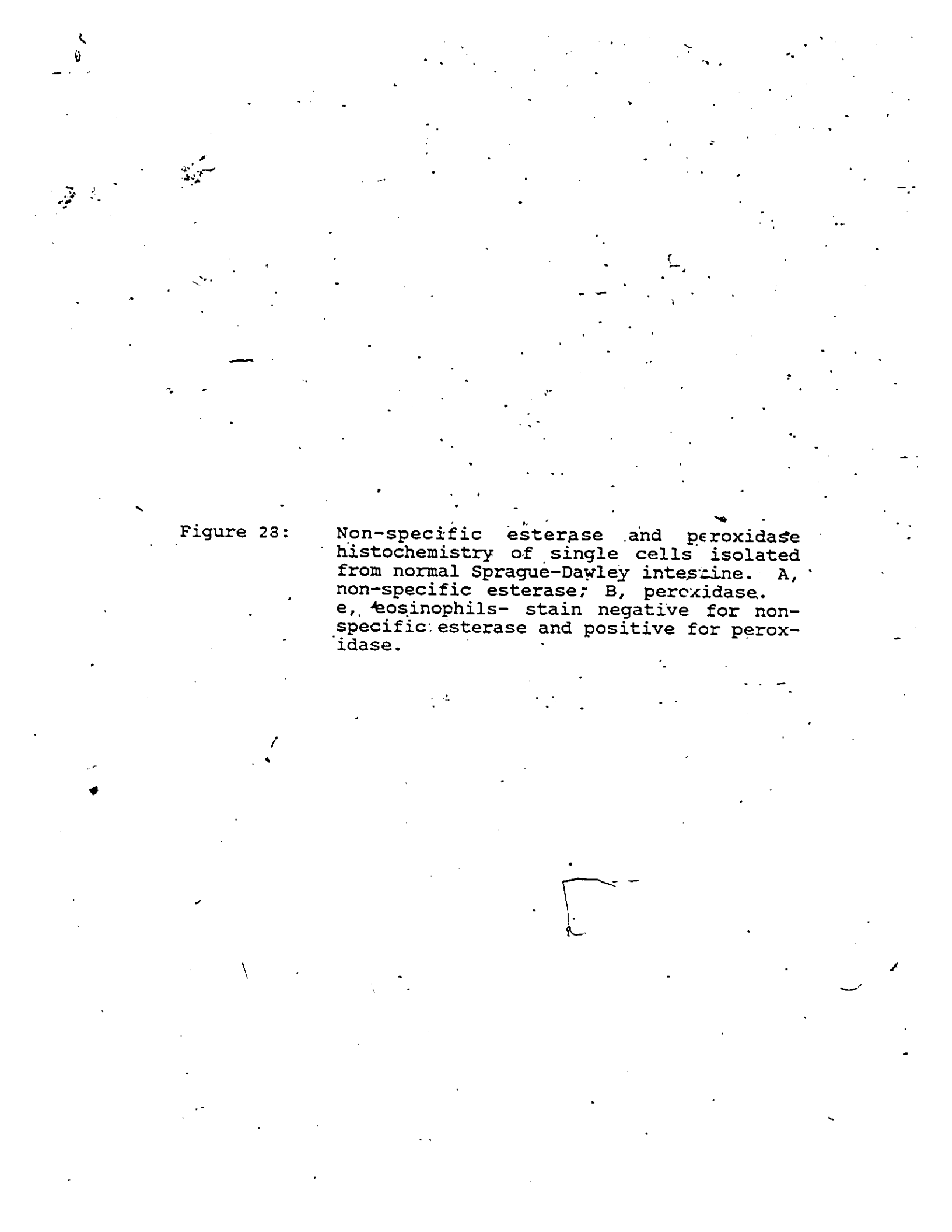


Figure 28: Non-specific esterase and peroxidase histochemistry of single cells isolated from normal Sprague-Dawley intestine. A, non-specific esterase; B, peroxidase. e, eosinophils- stain negative for non-specific esterase and positive for peroxidase.

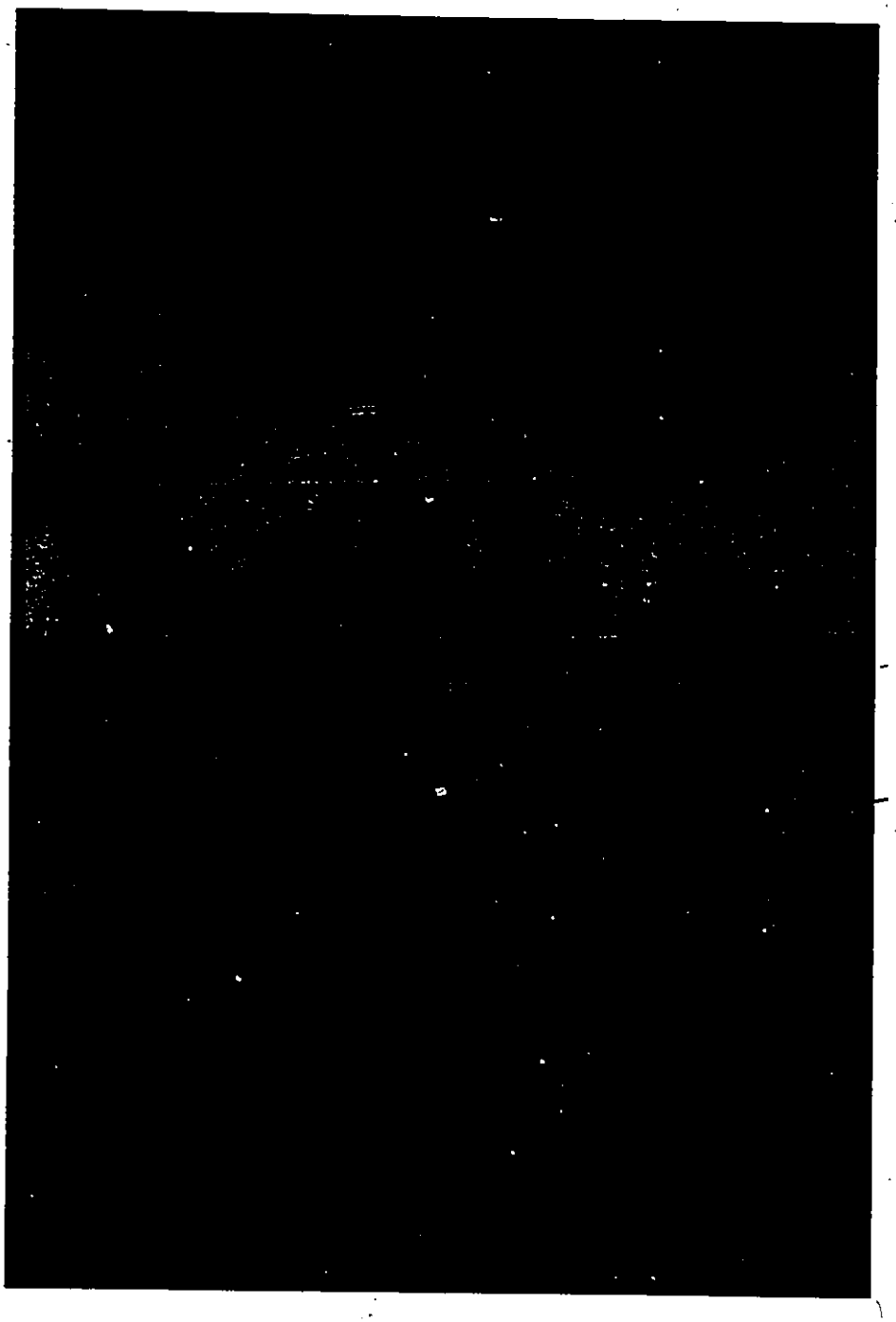


TABLE 17

Histochemical staining properties of intestinal cells isolated from Sprague-Dawley rats

CELLS	NON-SPECIFIC ESTERASE (%)	PEROXIDASE (%)
Coll I	70 ± 5[1]	15 ± 2
Coll II	58 ± 3	14 ± 4
Percoll separated[2]		
30%-50%	45	nd[3]
50%-70%	49	nd
Pellet	69	nd

[1] Mean ± standard error of 5 experiments
[2] Coll I and II were pooled.
[3] Not determined.

by NaF of all three population was examined. Figure 29 shows that the NSE activity of all three cell populations could be inhibited by increasing concentrations of NaF, indicating the esterases were similar.

The number of peroxidase positive cells approximately equals the number of viable eosinophils determined in the differential (Table 17).

It also can be seen, in Table 17, that the number of NSE staining cells closely resembles the total number of macrophages and "dead" cells. The identity of the dead cells was therefore suspected to be epithelial. The NSE stain was performed on sections of rat intestine. Figure 30 shows that the entire rat intestinal epithelium was positive for NSE. In fact little positive activity was discernable elsewhere in the intestine, indicating that NSE-positive macrophages were probably sub- or intra-epithelial.

3.3.3 Isolation of intestinal cells from infected rats

The isolation of cells from the normal rat intestine showed that the numbers of macrophages was low and difficult to selectively enrich away from other cell types, particularly the epithelial cells. Work with infected rats presented new problems including a significant increase in mucus and changes in the cellularity of the lamina propria. A neutrophil infiltration was characteristic of the local inflammatory response and eosinophil, mast cell and goblet

Figure 29: Inhibition of non-specific esterase staining in various populations of rat cells by sodium fluoride. □, coll I; ■, coll II; △, alveolar macrophages; ○, peritoneal cells. One representative experiment.

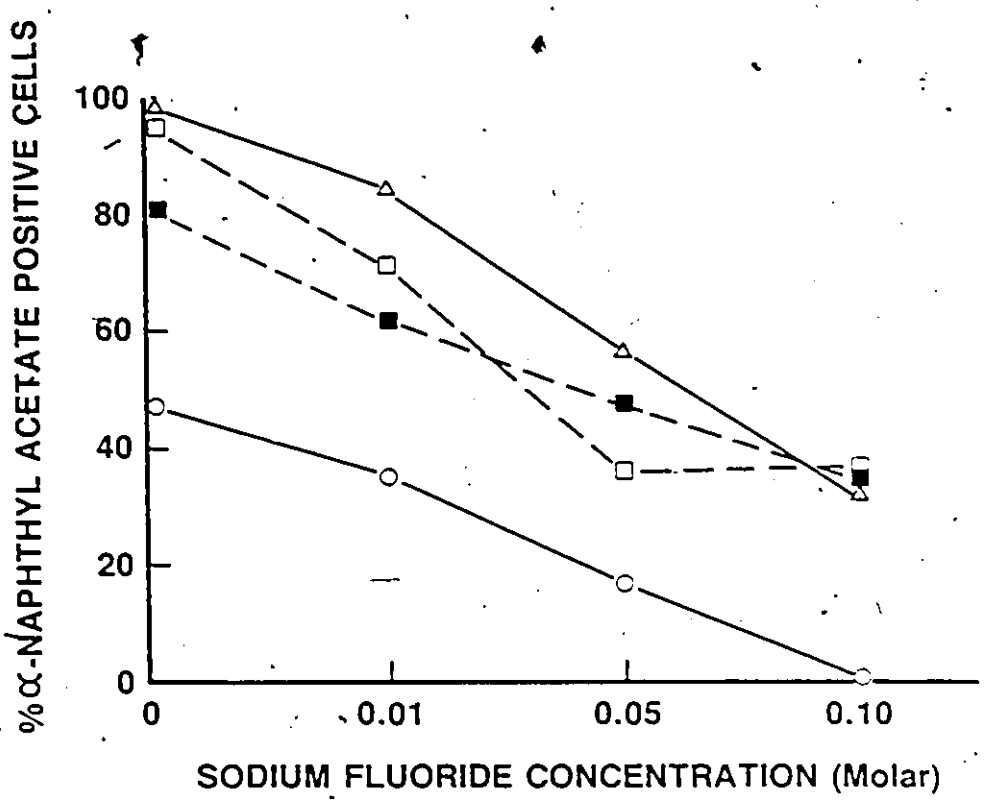

%α-NAPHTHYL ACETATE POSITIVE CELLS
100
80
60
40
20
0
0
0.01
0.05
0.10
SODIUM FLUORIDE CONCENTRATION (Molar)

Figure 30: Non-specific esterase staining in normal Sprague-Dawley intestine. Rat jejunum was snap-frozen and embedded in paraffin before sectioning. Tissue sections were stained for NSE similar to cells.

cell hyperplasias are characteristic of nematode infections.

Isolation of intestinal cells from infected rats usually gave lower yields of cells. There was no convincing evidence for increased numbers of macrophages in the intestines of infected animals. There were significant increases in neutrophils isolated from the intestine as early as day 4 post infection. The mast cell hyperplasia occurs concurrent with worm expulsion in Sprague-Dawley rats (Denburg, Befus and Bienenstock, 1980) and no increase in this cell type was seen during the days examined.

3.3.4 Hepatocyte-stimulating activity of intestinal cells

With the present difficulties of enriching for macrophages from the intestine, the total digested population was examined for hepatocyte-stimulating activities. Following overnight cell culture, in media supplemented with 10% FBS, considerable amounts of hepatocyte-stimulating activity was detectable in the culture supernatant (Table 18). This constitutive secretion was dependent on the presence of the FBS as there was no activity in parallel cultures of the same cells in incomplete media. Additionally, the intestinal cell activity was enhanced by the presence of LPS in the cultures (Table 18).

It seemed unlikely that the activity detected in the supernatant of the intestinal cells was due entirely to macrophages considering the few cells that had been identified

TABLE 18

Hepatocyte-stimulating and Lymphocyte-activating factor accivity of normal Sprague-Dawley intestinal cells in the presence or absence of fetal bovine serum

CELL CULTURE CONDITIONS	STIMULATING ACTIVITY[1] Hepatocyte-ratio	Hepatocyte-units[3]	Lymphocyte-
Experiment 1			
No cells	0.21	--	52744 ± 7994
Intestine -FBS	0.23	<1	72343 ± 3037
Intestine -FBS,+LPS[2]	0.20	<1	81376 ± 1232
Intestine +10% FBS	0.47	30	100515 ±16880
Intestine +FBS,+LPS	1.45	60	177559 ± 3508
Positive control[4]	1.96	1000	250000
Experiment 2			
No cells	0.29	--	34857 ± 1084
IEC-18[5]	0.41	12	27703 ± 6755

[1] See legend for Table 11.
[2] LPS alone has no effect on HSF units.
[3] These data represent values from a single experiment. Unit measurements have a variability of <10% in any singly determination.
[4] Human peripheral blood mononuclear cell supernatant.
[5] Rat intestinal epithelial cell line, courtesy of Drs. M. Perdue and A. Hanglow, Intestinal Disease Research Unit, McMaster University; see Quaroni and May, 1980.

as macrophages. This fact and additional recent evidence that other cells may synthesize and secrete hepatocyte stimulating factors led to the hypothesis that other intestinal cell populations may be responsible for the activity. The culture supernatants from a rat intestinal epithelial cell-line, IEC-18 (kindly provided by Drs. M. Perdue and A. Hanglow, McMaster University), were tested for hepatocyte-stimulating activity. As can been seen in Table 18 there was considerable hepatocyte-stimulating but no lymphocyte-activating factor activity in the IEC-18 supernatants. Since epithelial cells were the principle cell type in the intestinal cell preparations, it was speculated that a significant amount of the hepatocyte-stimulating activity was due to these cells, in addition to macrophages.

Following the successful detection of hepatocyte-stimulating factor(s) in the intestinal cell culture supernatants, cells isolated from parasitized animals were examined for similar activities. Table 19 shows that intestinal cell supernatants prepared from rats infected with N. brasiliensis had greater constitutive secretion of the hepatocyte-stimulating activities than uninfected cells. Cells recovered from N. brasiliensis-infected rats were maximally stimulated as LPS had little enhancing potential for increased hepatocyte-stimulating activity. Interleukin-1 secretion, by the same cells, changed in parallel to the changes in hepatocyte-stimulating activity (Table 19).

TABLE 19

Hepatocyte-stimulating and Lymphocyte-activating factor activity of intestinal cells from N. brasiliensis infected Sprague-Dawley rats

Cell Culture	STIMULATING ACTIVITY [1]					
	Ratio		Units [3]			
	Hepatocyte		Hepatocyte		Lymphocyte	
	-LPS	+LPS	-LPS	+LPS	-LPS	+LPS
No cells	0.46		--		3349 ± 201	
Normal intestine	1.63	1.74	60	129	13801 ± 947	22265 ± 736
4 day infected						
Rat 1	1.89	2.00	85	85	14154 ± 1902	23046 ± 811
Rat 2	1.95	2.23	117	129	13733 ± 1851	27895 ± 4395
8 day infected						
Rat 1	2.63	3.14	109	140	28884 ± 3140	39278 ± 1993
Rat 2	3.07	3.62	285	259	42535 ± 2147	38172 ± 339
Positive Control [2]	--	1.66	--	209		39262 ± 8347

1. See legend from Table II.
2. Human peripheral blood mononuclear cell supernatant.
3. These data represent values from a single experiment. Unit measurements have a variability of 10% in any single determination.

To the contrary, in all animals examined, and at two times points of the infection, the hepatocyte-stimulating activity of the cells isolated from T. spiralis-infected rats was less than that from the uninfected control rats (Table 20). The addition of LPS to cultures of cells from the infected rats gave variable hepatocyte-stimulating results including a failure to stimulate further secretion in culture. Addition of LPS did lead to increased secretion of IL-1 in the absence of changes in hepatocyte-stimulating activity (Table 20).

Intestinal cells from T. spiralis-infected animals show decreased, constitutive, hepatocyte-stimulating factor secretion while cells from N. brasiliensis infected rats, which do undergo significant serum Acute Phase Protein increases, showed increased constitutive secretion.

TABLE 20

Hepatocyte-stimulating and Lymphocyte-activating factor activity of intestinal cells from T. spiralis infected Sprague-Dawley rats

Cell Culture	STIMULATING ACTIVITY [1]					
	Hepatocyte				Lymphocyte	
	Ratio		Units [3]			
	-LPS	+LPS	-LPS	+LPS	-LPS	+LPS
[illegible] cells	0.29	---	---	---	38857 ± 1084	---
Normal Intestine	1.05	1.26	45	73	160344 ± 2046	164123 ± 6012
4 day infected Rat 1	0.70	1.11	<1	9	74138 ± 1575	105406 ± 947
Rat 2	0.86	0.68	18	13	88851 ± 4825	102445 ± 1783
7 day infected Rat 1	0.81	0.90	13	21	75230 ± 149	94249 ± 3335
Rat 2	0.31	0.75	<1	18	49109 ± 1492	90581 ± 3072
[illegible] SITIVE C [illegible]	---	1.86	---	1474	------ [4]	161111 ± 3366

1. See Legend from Table 11.
2. Human peripheral blood mononuclear cell supernatant.
3. These data represent values from a single experiment. Unit measurements have a variability of 10% in any single determination.

DISCUSSION

4.1. The Acute Phase Protein Response of the rat

The development of a sterile abscess and the ensuing inflammation, induced by the injection of turpentine, is a useful experimental model of acute inflammation. Delivery of a single subcutaneous dose of turpentine, to rats, resulted in significant and reproducible serum increases in haptoglobin, α_1AGP, α_1CPI and α_2M, and decreased albumin, similar to the findings of others (Esnard and Gauthier, 1983; Bohannon, Kiorpes and Wolf, 1979; Mahu and Feldmann, 1984; Urban, Chan and Schreiber, 1979). Increases in Acute Phase Protein concentrations were evident by 8 hr following turpentine administration, maximal by 36 hr, and were returning to normal by 48 hr. The concentrations of the proteins appeared to change in parallel.

To what extent does the Acute Phase Protein Response caused by noxious inflammatory agents resemble the response caused by a natural infection? To address this question it was necessary to reduce the dose of turpentine; to give rise to changes in protein concentrations comparable to those seen with parasitic infection. It was shown that infection of rats with nematodes induced changes equal to those changes seen with a relatively low dose of turpentine, about

10-fold less than the commonly used dose of 500µl/100g body weight.

In studies of the effects of the turpentine titer, it was observed that high doses of turpentine made the detection of increased Acute Phase Protein synthesis by *in vitro* cultures of hepatocytes possible. However, similar increases were not detectable in cells isolated and cultured from animals which had received low doses of turpentine, despite detectable serum increases. It was inferred that stimulation of the hepatocytes by the isolation procedure (use of sodium pentobarbitol and collagenase digestion) and the culturing conditions (presence of dexamethasone in the culture media) likely accounted for this lack of a detectable effect of the lower doses of turpentine. Therefore a molecular assay for detecting changes in Acute Phase Protein mRNA concentration was used to quantify *in vivo* hepatocyte stimulation due to low turpentine doses and the nematode infections.

4.1.1 The Acute Phase Protein Response due to N. brasiliensis infection

The serum Acute Phase Proteins of rats infected with *N. brasiliensis* changed in a manner that was predictable from the temporal sequence of inflammation. There is inflammation in the host's skin, as the infectious larvae migrated to the lungs (Taliaferro and Sarles, 1939). Con-

sidering that liver mRNA changes may occur as early as 24 hr before maximal serum changes, it was reasonable to expect that skin inflammation resulted in some Acute Phase changes. Inflammation of the skin became more evident following repeated inoculations in the same site, and the rats developed a subcutaneous welt. Only a single peak of increased Acute Phase Proteins occurred during a challenge infection. At this time a significant number of larvae remain at the inoculation site (Taliaferro and Sarles, 1939; Weinstein, 1955) suggesting the skin inflammation has a greater Acute Phase impact in challenge infections.

Lung inflammation

The contribution of the skin reaction to the first peak of Acute Phase Protein changes was complemented by the reaction in the host's lungs. Worms reached the lungs in significant numbers by 30 hr and pathology was obvious by 48 hr. By that time worms had breached the lung capillaries and entered into the alveolar spaces. Damage to the lungs is hallmarked by increased lung permeability to small (^{99}MTc-DTPA, molecular weight 420) and large (albumin) molecules (Irving et al., 1986) and an increase in the total number of leukocytes recoverable by bronchoalveolar lavage (Eqwang, Gauldie and Befus, 1984). Over the course of the infection, alveolar macrophages have been shown to adopt characteristics of activated cells, measured by increased C3- and IgG- phagocytosis. While these changes were detec-

table by day 2 PI in the rat, they were not significant until day 8 (Egwang, Befus and Gauldie, 1985). On day 8 PI the alveolar cells were able to kill infectious larvae in vitro (Egwang, Gauldie and Befus, 1984b).

An important aspect of the state of activation of the alveolar cells was their constitutive secretion of factors having hepatocyte-stimulating properties, and therefore the capability of eliciting the Acute Phase Protein Response. Monokines capable of mediating Acute Phase changes were evident by day 2 PI. While 2 day-infected alveolar cells secreted greater than normal amounts of hepatocyte-stimulating factors, day 8 infected cells secreted even higher levels, and considerable levels of IL-1 (Egwang, Befus and Gauldie, 1985). The timing of the changes in macrophage secretion of hepatocyte-stimulating factors, correspond with the periods of increased Acute Phase Proteins in the serum. Secetion of hepatocyte-stimulating molecules by 8-day infected alveolar macrophages overlaps with the period of intestinal inflammation.

Intestinal inflammation

Intestinal inflammation became obvious by day 6 PI, although worms were found in the host intestine as early as 40 hr PI and maximal numbers were achieved by 72 hr. During the intestinal phase of the infection the intestine became fluid-filled and dilated. During infection there is greater loss of epithelial cells, the villi hypertrophy and adopt a

flattened architecture, and adjacent villi often fuse together, even in sites where worms are absent (Symons, 1965; Symons, 1978).

Peak cellular changes in the intestine occur during days 7 through 10 PI with deep crypt and goblet cell hyperplasia and a neutrophil infiltrate (Miller, Nawa and Parish, 1979). Concurrent with the goblet cell hyperplasia is increased production and turnover of mucus (Miller and Huntley, 1982). Mast cell numbers initially decline (days 5-6) but then undergo hyperplasia in concordance with the eosinophil numbers (Kelly and Ogilvie, 1972). Smooth muscle cells hypertrophy and there are changes in cell contractility (Fox-Robichaud and Collins, 1986). Local physiological changes due to the infection include reduced absorption of Na^+, Cl^- and water, which lead to the accumulation of fluids in the intestine and a reduction of brush border enzymes (Symons and Fairbairn, 1962). Absorption of carbohydrates, amino acids and proteins is depressed and the infection results in a protein-losing enteropathy (Lunn et al., 1986). As much as 0.3 ml of blood was reported lost through the intestine every 24 hours, between days 8 and 10 of an infection with 3000 worms (Cummins et al., 1986).

The second peak of serum Acute Phase Protein changes occurred during the period of intestinal inflammation. Although all the proteins changed in parallel, the liver mRNA for haptoglobin increased to maximum levels by day 6

PI. Liver mRNA for α_1CPI and α_1AGP peaked between days 8 and 9. A significant decline in serum albumin and liver mRNA for $\alpha_{2\mu}$globulin supported the contention that there was inhibition of synthesis of some proteins, in addition to losses due to bleeding. Therefore, the liver was stimulated into increased Acute Phase Protein production at the time when the pathological changes were initiated.

The second Acute Phase Protein increase was likely due to contributions of hepatocyte-stimulating factors from three sources. First, there was a contribution from the lung as discussed earlier. The failure of transferred N. brasiliensis to elicit a response underscores the importance of the lung pathology for the second peak of increased protein concentrations. Secondly, there was constitutive secretion, by normal intestinal cells, of hepatocyte-stimulating factors; detectable by culturing the cells in vitro. Considering that LPS was likely in the normal small intestine, in the bacterial flora, constitutive secretion of hepatocyte-stimulating factors in the intestine, was expected from macrophage and perhaps non-macrophages sources. In support of the idea that non-macrophage cells may be involved in the constitutive secretion was the finding that an intestinal epithelial cell line secreted an hepatocyte-stimulating activity which was not IL-1. Thirdly, during infection of rats by N. brasiliensis, an increase in hepatocyte-stimulating activity was detected in

the intestine. This increase was greater than the LPS-enhanced secretion by normal cells and was likely due to the infiltration of the intestine by cells secreting the stimulating activity.

The final description of events leading to the systemic Acute Phase Protein Response during N. brasiliensis infection awaits further characterization of the hepatocytes-stimulating factors derived from intestinal cells. In light of the architectural changes in the intestine (due to the infection) and the recent evidence that fibroblasts secrete HSF, it was reasonable to expect non-macrophage sources of this factor. As molecular probes for the various hepatocyte-stimulating factors become available, the particular cell types secreting the molecules may be determined by in situ hybridization studies.

The kinetics of the N. brasiliensis life stages and the parasite's migration pattern is similar in mice and rats and most mouse strains also expel their intestinal worm burden by day 14 PI. Acute Phase Protein changes have been documented in infected mice, which are similar to the findings for rats (Lamontagne et al., 1984). Serum amyloid A and serum amyloid P concentrations (SAA and SAP, respectively) increased on day 1 PI, with the SAP response protracted out to day 4. $Alpha_1$protease inhibitor and the third component of Complement (C3) rose in concentration beginning on day 8 and returned to normal levels shortly

after worm expulsion. It was shown that the liver was synthesizing increased amounts of α_1PI despite the lack of a detectable serum increase on day 2 PI (Lamontagne et al., 1984). It seems that in the mouse this Acute Phase Protein was sequestered in the animal's lungs and in fact was found inside the alveolar macrophages (Lamontagne, Gauldie and Stadnyk, 1985). The alveolar macrophages of the infected mouse are activated and secreting greater than normal amounts of hepatocyte-stimulating factor activities, similar to the findings in the rat (Lamontagne et al., 1985). Thus infection of either rodent species by N. brasiliensis gives similar Acute Phase changes, although the same proteins were not examined.

T lymphocytes are intimately involved in the genesis of the pathology seen during the infection and worm expulsion, yet it was not clear whether these cells were necessary for the Acute Phase Protein Response. Young animals, which fail to expel the parasite, failed to show an Acute Phase Protein Response. Adoptive transfer of mesenteric lymph node cells (MLN) from infected young animals failed to transfer immunity to adult rats, but young rats which received immune adult MLN (Dineen and Kelly, 1973; Love and Ogilvie, 1975), and non-immune MLN (Keller and Keist, 1972) reject as great as 70% of their worm burden. Young rats may respond to N. brasiliensis infection with suppressor T lymphocytes as the animals remain susceptible to re-in-

fection later in life (Kassai and Aitken, 1967). It would be interesting to examine whether the Acute Phase Protein Response is restored in passively-immunized young animals; however, transfer experiments must be performed between syngeneic animals. In contrast to the lack of a response in young rats, the serum response due to N. brasiliensis infection was present in athymic (nude) animals. Athymic animals lack T lymphocytes, do not display the intestinal pathology characteristic of the infection, and fail to expel the adult worms. It has been suggested that a lack of T lymphocyte suppression of macrophage function leaves the macrophages in athymic animals in a chronic state of activation (Sharp and Colston, 1984). While T lymphocytes may be important in certain aspects of the cellular inflammatory response they may influence the outcome of the Acute Phase Response through interactions with macrophages.

The findings of a positive Acute Phase Protein Response during infection by N. brasiliensis is compatible with the report of increased serum proteins in nematode infected humans. As was seen with N. brasiliensis infected rats, the role of the macrophage and the nature of events leading to the activation of macrophages are important considerations when examining the occurrence of the Acute Phase Protein Response. In the case of the infected humans, the life stages of Ascarids and hookworms pass through the patients' lungs and probably activate the alveolar macro-

phages in a similar manner as N. brasiliensis in the rat. The characterization of macrophages from infected humans and patients with inflammatory bowel diseases suggests that these cells are also activated and are likely participating in the intestinal response in these patients.

4.1.2 The Acute Phase Protein Response due to T. spiralis infection

Infection of the rat by T. spiralis leads to many pathologic and physiologic changes in the host's intestine that resemble changes due to infection by N. brasiliensis. However, some differences in host response are detectable between T. spiralis and N. brasiliensis infected rats. For example, the intestinal transit time and plasma gastrin levels are increased during infection by T. spiralis (Castro et al., 1976; Castro et al., 1976b) whereas they decrease during infection by N. brasiliensis (Ovington, Bacarese-Hamilton and Bloom, 1985). The two infections also differed with respect to the Acute Phase Protein Response they elicited.

A moderate infective dose of T. spiralis failed to elicit the Acute Phase Protein Response in rats. The lack of a systemic Acute Phase Protein Response due to T. spiralis must be explained in view of the positive findings during the N. brasiliensis infection. The obvious decline in the negative Acute Phase Protein albumin and mRNA for

$\alpha_{2\mu}$globulin, indicate that some Acute Phase mediators were indeed increased during the T. spiralis infection. It is likely that the constitutive secretion of certain hepatocyte-stimulating factors was diminished in the face of similar pathophysiology.

Adult T. spiralis live in the epithelium and are directly cytotoxic for the cells they pass through (Dunn and Wright, 1985). Epithelial cells killed by the parasite do not necessarily migrate to the villus tip and may slough-off from the base of the villi. An epithelial cell line was shown to secrete an hepatocyte-stimulating activity; thus epithelial cells may contribute a major proportion of the hepatocyte-stimulating factor activity from the intestine. T. spiralis may restrict the constitutive factor secretion by these cells. It has not been reported if these worms may selectively destroy or adsorb molecules such as hepatocyte-stimulating factor or IL-1, nor have secreted products of the adult worms been assayed for inhibitory activity.

T. spiralis adults failed to suppress the host serum response during a concurrent N. brasiliensis and T. spiralis infection. There was no complimentary effect, nor an inhibitory effect, of the T. spiralis on protein changes due to N. brasiliensis during the concurrent infection. The lack of suppression substantiates the idea the T. spiralis only inhibits constitutive hepatocyte-stimulating factor secretion in the intestine and that the infection was not

anti-inflammatory. N. brasiliensis larvae have passed through the host's lungs even during the concurrent infection, before any pathology occurs in the intestine. The lung pathology was critical in order for the second peak of serum protein increases.

It was noted that a large infective dose of T. spiralis led to an Acute Phase Protein Response, the magnitude of which were considerably lower than the magnitude of changes due to the normal dose of N. brasiliensis. Such large doses of T. spiralis may result in perforation of the intestine and perhaps the escape of worms into the peritoneal cavity where they stimulate the response. The lack of an Acute Phase Protein Response during infection by moderate doses of T. spiralis suggests that the response is not necessary for the expulsion of the worms from the intestine.

4.2 Systemic activition of macrophages in inflamed animals

The necrosis due to the toxic effects of turpentine probably led to the release of specific chemotactic factors and emigration of neutrophils and monocytes. There was considerable swelling at the subcutaneous injection site 24 hr following the administration of turpentine; typical changes in vascular permeability due to factors such as histamine and vasopressin. However, the specific cells which elicit the Acute Phase Protein Response in the presence of turpentine have not been identified. Taking

into consideration the cell types that have been shown to secrete IL-1 and the various hepatocyte-stimulating factors, a number of candidate cells are suggested, including Langerhans cells, keratinocytes and fibroblasts.

During a local inflammatory response a systemic phenomena commonly called "counter-irritation" occurs. Counter-irritation refers to the anti-inflammatory activity exerted by a local inflammatory response, usually measured as decreased plasma exudate into a second site of inflammation (Atkinson and Hicks, 1975). More recently, counter-irritation was shown to affect the emigration of cells, including monocytes, into a second inflammatory site (Norman, Schardt and Sorkin, 1985). The inhibitory effect of the first inflammatory agent also manifests if a second noxious agent is administered into the same site as the first agent, but at a later time (Lu, Scragg and Williams, 1978). The mechanism leading to reduced leukocyte emigration during counter-irritation, is poorly understood and no evidence exists to suggest that the tissue-resident cells or cells affected by counter-irritation are anergic in any way.

An examination of macrophage function from turpentine-inflamed animals showed that the cells' capacity to elicit the Acute Phase Protein Response was intact. Alveolar and peritoneal macrophage populations isolated from inflamed animals, were capable of secreting hepatocyte-stimulating factors, including IL-1, in response to <u>in vitro</u>

activation signals. Neither of the two cell populations were activated, *in vivo*, by subcutaneous turpentine. This result indicated that macrophages at sites different from the site of turpentine inflammation, were capable of eliciting the Acute Phase Protein Response.

An examination of the function of macrophages recovered from *T. spiralis*-infected rats showed that these cells were not inhibited in their capacity to synthesize and secrete hepatocyte-stimulating factors. The infected animals were able to undergo an Acute Phase Protein Response when given turpentine. *T. spiralis* has been reported to be anti-inflammatory by Castro *et al.* (1980) who showed suppression of granuloma formation, in infected rats, at a site distant from the intestine. Granuloma measurements are an indication of the cellularity of an inflammatory foci, and the anti-inflammatory activity was likely due to counter-irritation, as observed by others (Cygeilman and Robson, 1963) and not a specific anti-inflammatory effect of the worms.

4.3 Inflammatory mediators and the Acute Phase Protein Response

The mediators responsible for the Acute Phase Protein Response, observed during nematode infection of rats, have not been identified. Various monokines have hepatocyte-stimulating properties, including IFN-β_2, IL-1

and TNF; however, the definitive action of each of these factors has not been fully elucidated using rat hepatocytes and rat monokines. Much of the reported literature entails species cross-reactivity; for example, the assay for IL-1 utilizes mouse thymocytes. Furthermore, macrophages may not be the only cell source of these factors. Despite these shortcomings it is interesting to speculate on which factors may be acting during the infections, based on the occurrence of other systemic events.

4.3.1 Tumor Necrosis Factor

A loss of host appetite occurs in N. brasiliensis, in a remarkably clear biphasic pattern; at 2 and 8 days PI (Ovington, 1985). A loss of appetite (or the state of cachectia) during periods of infections has been confidently traced to the monokine, Tumor Necrosis Factor (TNF)(Swedlund and Gorelick, 1987). If the loss of appetite was indeed due to TNF, the timing of the bursts of activity correspond with the peaks of serum Acute Phase Protein changes. Human TNF has some hepatocyte-stimulating properties when tested on human hepatoma cells (Perlmutter, 1987) and stimulates C3, haptoglobin and α_1AGP but not fibrinogen or anti-protease production by a rat hepatoma cell line (Baumann et al., 1987). Tumor Necrosis Factor also exerts an antiviral state through the selective enhancement of IFN-β_2 secretion by fibroblasts (van Damme et al., 1987; Le and Vilcek, 1987).

As was discussed earlier, IFN-β_2 has potent hepatocyte-stimulating properties (Gauldie et al., 1987). Thus tumor Necrosis Factor may be involved in the generation of the second peak of Acute Phase Protein changes by stimulating IFN-β_2 secretion from cells in the intestine.

4.3.2 Interferons

The roles of the various Interferons are becoming increasingly important in inflammation and immunity and Interferons (IFN) are detectable in nematode infected animals (Grencis, Riedlinger and Wakelin, 1987). The major hepatocyte-stimulating component of rat lung macrophage culture supernatants is probably IFN-β_2 (Hepatocyte Stimulating Factor; HSF). Human lung fibroblasts also have been shown to secrete IFN-β_2 and secretion by these cells may be enhanced by the addition of LPS (Gauldie et al., 1987). The increased secretion of this molecule, by lung cells and possibly the intestine, by day 8 of an N. brasiliensis infection, was compatible with the magnitude of Acute Phase Protein changes observed during the intestinal stage of the infection. However, the secretion of IFN-β_2 by intestinal epithelial cells or intestinal fibroblasts has not been confirmed, although they do secrete an hepatocyte-stimulating activity.

It is unclear which IFN is prominent in the intestine but IFN-γ is probably secreted by T lymphocytes in the

infected animal. Interestingly, IFN-γ was shown to directly stimulate C4 in human hepatoma cell-lines (Perlmutter and Miura, 1987). Interferons are also thought to be involved in the suppression of the hepatic microsomal drug-metabolizing system, as these enzymes are inhibited by the in vivo administration of IFN-inducing agents (Renton and Mannering, 1976). Infection by N. brasiliensis also inhibits these liver enzymes (Tekwani et al., 1987); whether interferon are involved remains to be examined. A concentrated appraisal is necessary to determine if IFNs are important in the Acute Phase Protein Response or whether they act directly or indirectly through the activation of other cells.

4.3.3 Interleukin-1

There are no published reports documenting increased intestinal IL-1 during infection. Constitutive IL-1 secretion was detected from the intestine in this study, and the constitutive secretion was enhanced by the addition of LPS to cell cultures. The constitutive activity was probably derived from several sources, including macrophages. For example, (human) endothelial cells synthesize and secrete IL-1, which is LPS- and IFN-γ- inducible (Miossec and Ziff, 1986). During infection by N. brasiliensis the detectable IL-1 activity was increased and during infection by T. spiralis it was depressed. Intestinal cells from 8-day N. brasiliensis-infected rats were constitutively

secreting greater amounts of IL-1 than normal cells stimulation in culture by LPS. To achieve this increase there must have been a recruitment of cells synthesizing IL-1 into the intestine or stimulation of cells by novel mechanisms. Interleukin-1 has hepatocyte-stimulating potential and may affect the significant declines in albumin concentrations, observed during either infection. Human IL-1 is a potent inhibitor of albumin synthesis by the liver (Moshage et al., 1987). Recently human IL-1 was shown to cause histamine release from mast cells (Subramanian and Bray, 1987) which may account for the early decline in histamine typical of the N. brasiliensis- but not T. spiralis-infected rat intestine (Befus, Johnston and Bienenstock, 1979).

4.3.4 Leukotrienes and Prostaglandins

Leukotrienes (LT) and Prostaglandins (PG) are important multifunctional inflammatory mediators that are generated by a variety of stimuli (Kuehl and Egan, 1980; Hawkey and Rampton, 1985). Leukotriene levels have been examined during the first 20 hr of infection by T. spiralis in rats. Levels of LTD_4 rose immediately following infection while LTC_4 and LTB_4 rose 10-fold over normal levels during the first hour of a challenge infection, in both intestinal perfusate and homogenate (Moqbel et al., 1987). Similar findings were noted in N. brasiliensis-infected rats, challenged intravenously with worm antigen (Moqbel et

al., 1987b). These LTs presumably were derived from mast cells and eosinophils.

Prostaglandin E, the prototype PG, has vasodilatory properties, and PGE_2 and PGI_2 both potentiate the effects of bradykinin. Elevated levels of PGE were detected on day 7 PI of an N. brasiliensis infection (Dineen and Kelly, 1976).

Prostaglandins and LTs likely affect the Acute Phase Response indirectly through the regulation of phagocytic cells (Kunkel and Chensue, 1985). Prostaglandin synthesis may be an avenue whereby some phagocytic cells produce bioactive oxidants (Kuehl and Egan, 1980). Direct evidence for the effects of PGs on the Acute Phase Protein Response comes from experiments where rats show increased α_2M levels following intravenous administration of PGE_1 (Anbalagan and Sadique, 1984). Using rat liver slices from normal rats, it was shown that high doses (0.2µg/ml) of PGE_1 suppressed α_2M synthesis while lower doses enhanced synthesis. All doses of prostaglandin E_1 inhibited α_2M synthesis by inflamed rat liver slices, indicating that there may be a threshold effect of the molecule on liver protein synthetic activity. Whether changes in intestinal PG levels directly effect liver function has not been determined in these studies.

4.3.5 Hormones

Changes in host hormones (particularly in the pituitary-adrenal axis), during the inflammatory response,

may affect Acute Phase protein production. Plasma corticosteroid levels rose slightly, but not significantly, during infection of the rat by 3000 N. brasiliensis. Corticosteroid levels were significantly greater in pair-fed control animals than in infected animals (Ovington, 1987). In protein malnourished animals infected with N. brasiliensis, plasma corticosteroid declined until day 10 PI, then, returned to uninfected levels by day 20 PI (Ash, Crompton and Lunn, 1985b). This finding suggests there is an active suppression of the hormone during the infection. In the same protein-deprived animals, adrenocorticotrophic hormone (ACTH) rose sharply on day 1 PI but declined (in parallel to uninfected pair-fed controls) to one-tenth normal levels by day 5 and remained low for the remainder of the infection. (Ash, Crompton and Lunn, 1985b). The changes in the levels of these hormones do not correspond with changes that would be predicted by the Acute Phase Protein Response.

Corticosteroids enhance liver protein synthesis and inhibit both macrophage function and the immune response. It is interesting that macrophages have been shown to secrete ACTH and directly stimulate in vitro ACTH production by a pituitary cell-lines (Woloski et al., 1985). Hence, these cells are involved in their own negative feedback loop, although the significance of this finding during nematode infection remains to be determined.

4.4 Characterization of intestinal cell types

Various cells have been isolated from the rat intestine or recovered from the afferent intestinal lymphatics. Intraepithelial lymphocytes, dendritic cells, and veiled cells all have been described in the rat intestine (Pugh, MacPherson and Steer, 1983; Mayrhofer and Whately, 1983; Mayrhofer, Holt and Papadimitriou, 1986). Characterization of these cells has shown that many intestinal cells share physical and functional properties with macrophages. Intestinal epithelial cells bear Class II Major Histocompatibility Antigens (Mayrhofer, Pugh and Barclay, 1983) in addition to non-specific esterase. Non-lymphoid cells have been identified in the afferent intestinal lymphatics which are capable of serving as accessory cells in the mitogenic and antigen-induced proliferation of T lymphocytes (Mayrhofer, Holt and Papadimitriou, 1986). These cells were heterogeneous in their morphology, non-specific esterase-staining characteristics, glass adherence, and had lower levels of IgG and Complement receptors than other macrophages (Pugh, MacPherson and Steer, 1983). They also increased in absolute numbers following intestinal inflammation (Steer, 1981). It is not clear whether "veiled cells" are the same as dendritic cells which are thought responsible for antigen presentation in Peyer's Patches. There is some dispute whether veiled cells occur in the lamina propria of the rat (Wilders, et al., 1985).

4.4.1 Intestinal macrophages

Although macrophages have not been characterized from the rat intestine, both rat and mouse intestinal macrophages have been described through the use of several monoclonal antibody-specific antigens (Hume, Perry and Gordon, 1984; Sminia and Jeurissen, 1986). The availability of multiple monoclonal antibodies for macrophage antigens is testament to the heterogeneity of these cells. For example, rat intestinal macrophages were described as either ED-1, 2, or 3 positive, yet all three antibodies were reported to be specific for macrophages. The most abundant cell in the lamina propria of the intestine was ED-2 positive, identifying them as connective tissue macrophages (Sminia and Jeurissen, 1986).

Intestinal macrophage heterogeneity becomes further complicated by the use of acid phosphatase as a marker and Class II Major Histocompatibility Antigen-staining in addition to monoclonal histochemistry. Intestinal cells have been described which stain with the same monoclonal antibody but with different cytoplasmic acid phosphatase distributions and weak or strong Class II antigen expression. Considering macrophage heterogeneity and the heterogeneity of cells in the intestine, it cannot be definitively concluded that the cells seen in tissue section are indeed macrophages.

Intestinal cells identified as macrophages have been

prepared in single cell suspensions from the Guinea pig and human small intestine (Sawicki et al., 1977; Winter et al., 1983) and human colon (Bull and Bookman, 1977; Golder and Doe, 1983). An average of 12% of intestinal cells from the Guinea pig intestine were esterase positive and no cells possessed C3 receptors (Winter et al., 1983). An equally low number of human cells from the small intestine were shown to possess complement or IgG receptors. A review of the studies made of human intestinal cells revealed a consistent macrophage differential of about 15% (Verspaget and Beeken, 1985).

Using morphology as the selection criteria, macrophages averaged 20% of the dissociated cell population from the human colon and were enriched to 30% through the use of Percoll (Golder and Doe, 1983). This number closely resembled the number of esterase positive and phagocytic cells at each stage of the isolation. All the studies of human intestinal macrophages relied on non-specific esterase in their physical characterization.

Human intestinal macrophages also have been characterized from inflamed tissues. Using $\alpha_1$antitrypsin as a macrophage marker in tissue section, Geboes et al., (1983) showed reduced numbers of macrophages in inflamed (Crohn's, ischemic and Ulcerative Colitis) colon. In normal human intestinal tissue $\alpha_1$antitrypsin was detected along the luminal border of the surface epithelial cells with some

cytoplasmic epithelial staining. In contrast, a greater number of macrophages were routinely isolated, per gram of tissue, from inflamed intestine (Foster et al., 1983; Verspaget and Beeken, 1985). Grossly normal, non-involved cancerous intestine served as the control tissue in these studies. Associated with increased numbers of isolated intestinal macrophages was decreased β-glucuronidase and lysosomal activity; the decrease thought to be due to in vivo secretion (Wang et al., 1983). Intestinal macrophages recovered from Crohn's diseased intestine had a greater phagocytic capacity than peripheral blood monocytes from the same patient. In addition to increases in numbers of macrophages, increases in the number of veiled cells have been observed in Crohn's diseased colon (Wilders et al., 1984).

The evidence that intestinal macrophages exist is incontrovertible. Increases in the numbers of macrophages and their activity during inflammatory diseases implicate these cells in the intestinal inflammatory response. Patients with inflammatory bowel diseases often have increased concentrations of Acute Phase Proteins. It is likely that the intestinal macrophages are involved in the genesis of this response.

The regulation of intestinal pathophysiological events are an enigma, in animals and humans, due to the difficulties in procuring and studying the different cells.

As isolation procedures improve, more information will be forthcoming. An important consideration stemming from this work, will be to determine the extent to which macrophage function is duplicated or replaced by other cell types in the intestine. Epithelial cells seem particularily important in this respect. For example, epithelial cells are phagocytic and specialized epithelial cells occur over Peyer's patches for the purpose of sampling luminal contents. The permeability of the epithelial layer may determine whether the lymphoid cells are stimulated to react against particular antigens. The intestinal epithelium bears Class II antigens and may participate in antigen presentation. Epithelial cells contain esterases and phosphatases similar to macrophages. Epithelial cells may be among other cells that participate in the local and systemic inflammatory response in the intestine through the secretion of various mediators including hepatocyte-stimulating factors.

4.5 Intestinal disease and the Acute Phase Protein Response

Nematode infections of rodents have been used to elicit intestinal inflammation in a model study of potential systemic indicators of local inflammatory disease. An interesting paradigm was exposed by following the Acute Phase Protein Response due to two infectious nematode

species. Infection of rats by N. brasiliensis led to activation of macrophages in the lungs of the host, and macrophages and possibly other cell types in the intestine. These periods of cell activation were consistent with increases in the concentrations of the serum Acute Phase Proteins. Rats infected with T. spiralis failed to show evidence of macrophage activation or a positive Acute Phase Protein Response. The effect of the infection seemed limited to diminishing the constitutive hepatocyte-stimulating factor secretion by the intestinal cells, probably by interfering with epithelial cell function. The failure to register a positive Acute Phase Protein Response implies that inflammation did not occur. However, changes were observed in the negative Acute Phase Proteins during infection of rats by T. spiralis, underscoring the need to examine a number of proteins if the Acute Phase Protein Response is to serve as a window to local inflammatory events. It should, however, be recognized that down regulation of negative Acute Phase Proteins might be elicited by different mediators and/or mechanisms.

It is feasible that studying these two models of intestinal inflammation will contribute to our understanding of the human inflammatory bowel diseases. The positive relationship between the dose of N. brasiliensis and the magnitude of the Acute Phase Protein levels is compatible with the staging of inflammatory bowel diseases being

related to the levels of the same proteins in humans. Based on the findings of a requisite systemic injury, previous to the period of inflammation in the intestine, and the positive serum response, it is interesting to speculate that those humans which present with Acute Phase Protein changes have also suffered a systemic injury. This was certainly the case in humans suffering from multiple intestinal parasites. The model infection may permit the identification of the cells responsible for signalling the Acute Phase Response of the intestine and the specific events leading to their activation.

Important considerations should be made of the conditions under which inflammatory bowel diseases fail to elicit the Acute Phase Protein Response, and the rodent infection by T. spiralis may be important in this respect. High doses of this parasite did induce small changes, which may indicate that in the absence of a systemic injury, there must be considerable damage in the intestine before the response is elicited. This may be an indication of the limited efficacy of the response to serve as a prognostic tool for the prediction of relapses.

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