STUDIES ON THE IMMUNOPATHOLOGIC MECHANISMS OF INTESTINAL LESION FORMATION IN TURKEY POULTS INFECTED WITH HEMORRHAGIC ENTERITIS VIRUS

By

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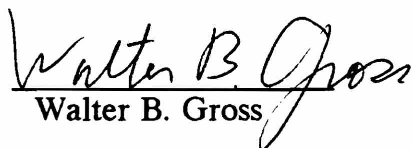

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Veterinary Medical Science

(ABSTRACT)

The immunopharmacologic and immunopathologic mechanisms through which hemorrhagic enteritis virus (HEV) induces intestinal lesion formation associated with clinical infection is unknown. These studies were designed to 1) determine differences in age susceptibility to HEV infection and parameters which best indicate severity of virus infection, 2) determine the involvement of specific inflammatory mediators in the formation of intestinal lesions by using anti-inflammatory compounds which directly or indirectly inhibit either the synthesis or the mechanism of action of the mediators, and 3) determine the involvement of immunologically-active cells -- basophils, connective tissue mast cells (CTMC), and mucosal mast cells (MMC) -- in the formation of HEV-induced lesions. Seven-week-old poults were more susceptible to viral infection when compared to four-week-old poults as judged by HEV antigen presence within the spleen, spleen weight/body weight ratio, heterophil to lymphocyte ratio, and changes in serum lipid and albumin concentration. Of those anti-inflammatory agents used, corticosterone, vitamin E, and indomethacin significantly reduced lesion scores. FPL 55712 and FPL 57231, specific leukotriene receptor blockers, markedly increased lesion scores. Inoculated birds had significantly higher MMC counts than uninoculated birds (120 ± 64 vs. 55 ± 39, respectively). CTMC and basophils were unaffected by viral challenge. In addition to the increase of MMC within the lamina propria of the duodenal villus, there was also a concurrent increase in vascular permeability within the lamina propria which was demonstrated using colloidal carbon and

vascular permeability within the lamina propria which was demonstrated using colloidal carbon and ferritin as vascular markers. The results of these studies indicate that vasoactive mediators, such as histamine and the eicosanoids, play a role in lesion formation associated with HEV infection, and that a source of at least some of these compounds appears to be the MMC within the lamina propria of the duodenal villus. Finally, some of the other clinical manifestations of HEV infection, such as decreased serum lipid, protein, and albumin, may be a result of increased vascular permeability which results from vasoactive mediator release and action on the vessels of the lamina propria of the intestinal villus.

With love and appreciation to my parents and grandparents

Arlene, Alfred, Betty, Irving, Louis and Clara

You have given me my deeper thirsting after life.
Surely there is no greater gift to a man than that which turns all his aims into parching lips and all life into a fountain.
And in this lies my honour and my reward,--
That whenever I come to the fountain to drink I find the living water itself thirsty;
And it drinks me while I drink it.

-- Kahil Gibran
"The Prophet"

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Chapter I

Introduction

Adenoviruses: Their Structure and Role in Disease

The description of adenoviruses by Rowe and his colleagues in 1953 (110) and later by Hilleman and Werner (60), received much attention at the time of their discovery since no new etiologic agent of acute respiratory disease had been identified since the isolation of the influenza virus (115). Adenoviral infection, at that time, was associated with influenza-like illnesses, especially among army recruits where it had reached epidemic proportions and was known as "acute respiratory disease of recruits" (51, 60). Since the first description, there have been at least 41 serotypes of human adenoviruses which have been associated with acute respiratory, ocular, urinary, and gastrointestinal diseases (Table 1). Adenoviruses also affect many different body systems among many non-human animal species (Tables 2 and 3). Although the first description of human adenovirus infection was in 1953, the first non-human adenovirus outbreak recorded was in 1930 (53). The etiologic agent in this case, fox encephalitis virus, was later identified as canine adenovirus type I, the cause of infectious canine hepatitis.

Adenoviruses have been classified as one family, the *Adenoviridae*, based on many similar physical, chemical and structural properties. Serologic tests have enabled further their characterization based on cross-reacting antigenic determinants located on the hexon protein, the major capsid protein (50, 84).

Table 1. Human adenovirus infections[a].

Type	Prototype strain[b]	Major associated disease[c]
1	Ad71	respiratory
2	Ad6	respiratory
3	G.B.	respiratory
4	RI67	respiratory
5	Ad75	respiratory
6	Ton99	respiratory
7	Gomen	respiratory
8	Trim.	ocular
9	Hicks	--[d]
10	J.J.	--
11	Slobitski	cystitis
12	Huie	--
13	A.A.	--
14	DeWitt	respiratory
15	Ch38	--
16	Ch79	--
17	Ch22	--
18	D.C.	--
19	587	ocular
20	931	--
21	1645	respiratory
22	2711	--
23	2732	--
24	3153	--
25	BP-1	--
26	BP-2	--
27	BP-4	--
28	BP-5	--
29	BP-6	--
30	BP-7	--
31	1315163	--
32	H.H.	--
33	D.J.	--
34	Compton	--
35	Holden	--
36	275	--
37	G.W.	--
38	No assignment	ocular, genital?
39	D335	--
40	Dugan	enteric
41	Tak	enteric

[a]Modified from Kasel, 1979 (74).
[b]Type status of recently identified adenoviruses according to ATCC nomenclature.
[c]Major infectious syndrome associated with each immunotype.
[d]Human disease not proved to be associated with immunotype.

Table 2. Animal diseases associated with mastadenoviruses[a].

Animal diseases	Number of serotypes	Diseases
Equine	2	asymptomatic or mild respiratory disease, generalized disease of foals with congenital immunodeficiency
Bovine Porcine Ovine Caprine	9	asymptomatic or mild respiratory disease
Canine	2	infectious canine hepatitis infectious canine tracheobronchitis

[a] Modified from Fenner *et al.*, 1987 (46).

Table 3. Adenoviruses affecting avian species[a].

Species	Adenovirus serogroup	Serotypes/ type strains	Diseases
Chicken	I	FAV 1-12 (85, 86)	CELO, IBH, decreased egg shell quality, decreased feed consumption, respiratory disease
	II	AASV (30, 38)	Splenomegaly, pulmonary congestion
	III(?)	EDS-76 (128)	Precipitous decline in egg production
Turkey	I	FAV (120)	Mild respiratory disease, diarrhea, depressed egg production
	II	HEV (30, 49, 107)	Hemorrhagic enteritis, splenomegaly, immunosuppression
Duck	I	GR (12)	
Goose	I	HS, N1, 569 (142)	
Quail	I	FAV 1 (101, 136)	Bronchitis
Pheaseant	II	MSDV (68, 140)	Splenomegaly, pulmonary congestion

[a] Modified from McFerran, 1991 (85).

These viruses contain 11.6-13.5% double- stranded DNA with the genetic material having a molecular weight of approximately 20-30 x 10^6(99). There are at least 11 species of polypeptides within the virion identified by specific sizes, locations, and proposed functions (Table 4 and Figure 1). Adenoviruses are non-enveloped and icosahedral in shape. The diameter and density of these viruses is fairly consistent. The type II avian adenovirus, for example, reportedly has a diameter of 70-90 nm (17, 64, 65, 124) and a density of 1.34 g/ml (15).

The capsid of these viruses contains 240 hexon proteins and 12 pentons (125) (Figures 2 and 3). Grutter and Franklin (57) determined the molecular weight of the hexon to be 360k and concluded that there were three identical polypeptides per hexon. Computer simulation employed by Nermut and Perkins (100) confirmed the unique structural properties of the hexon. These studies showed that the hexon was composed of three polypeptides which were linked to form a trimer. The composite was rounded at the bottom, hexagonal at the middle, and triangular at the top. This model was later redefined (13, 109) to include just a triangular top and a pseudo-hexagonal base. The hexagonal base is thought to facilitate hexon trimer interaction with other capsid hexons and pentons. Hexons also have the characteristic of forming structural conglomerates known as "group of nine" (GON) hexons (Figure 4). The significance of GONs, however, has not yet been determined. The non-structural importance of hexons has been studied. It has been reported to carry an antigen which is responsible for virus neutralization (102, 135). In fact, the hexon has been identified as the carrier of the group specific antigen (α) (2), as well as the type specific antigen (ϵ) (103).

The other major capsid protein, the penton, is composed of two structurally distinct

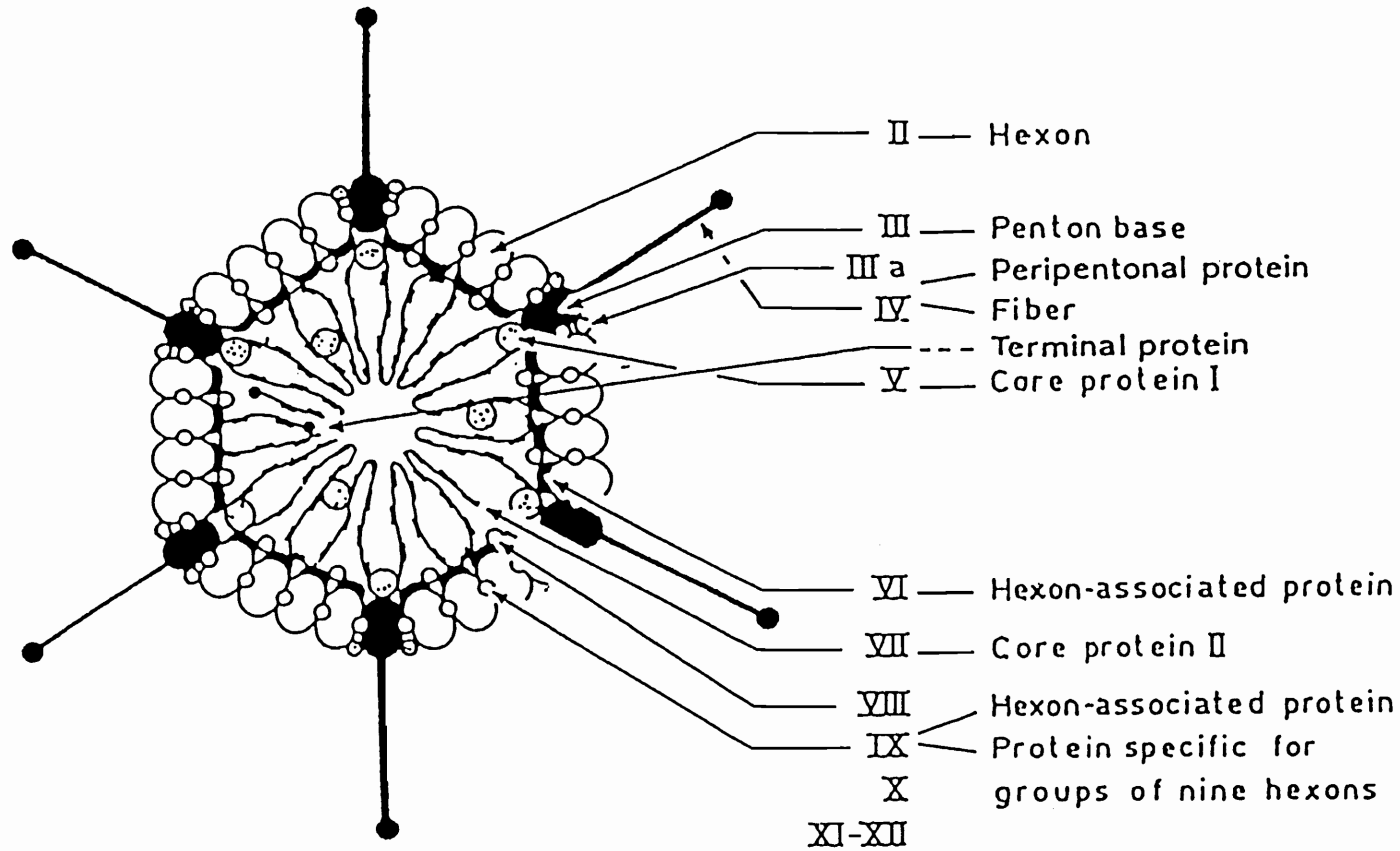


Figure 1. Tentative model for the location of the proteins in the adenovirus type 2 virion. Modified from Everitt *et al.*, 1977 (40).

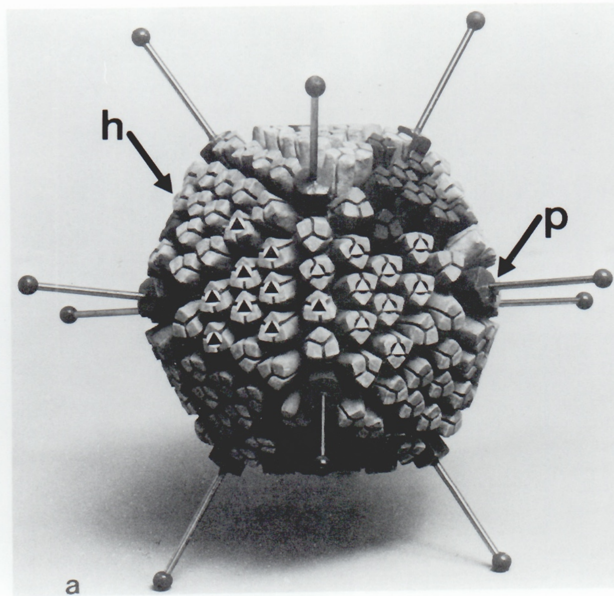


Figure 2. A schematic representation of an adenovirion containing hexons (h), penton bases (p), and the hexon-penton interaction. Taken from Nermut, 1984 (99).

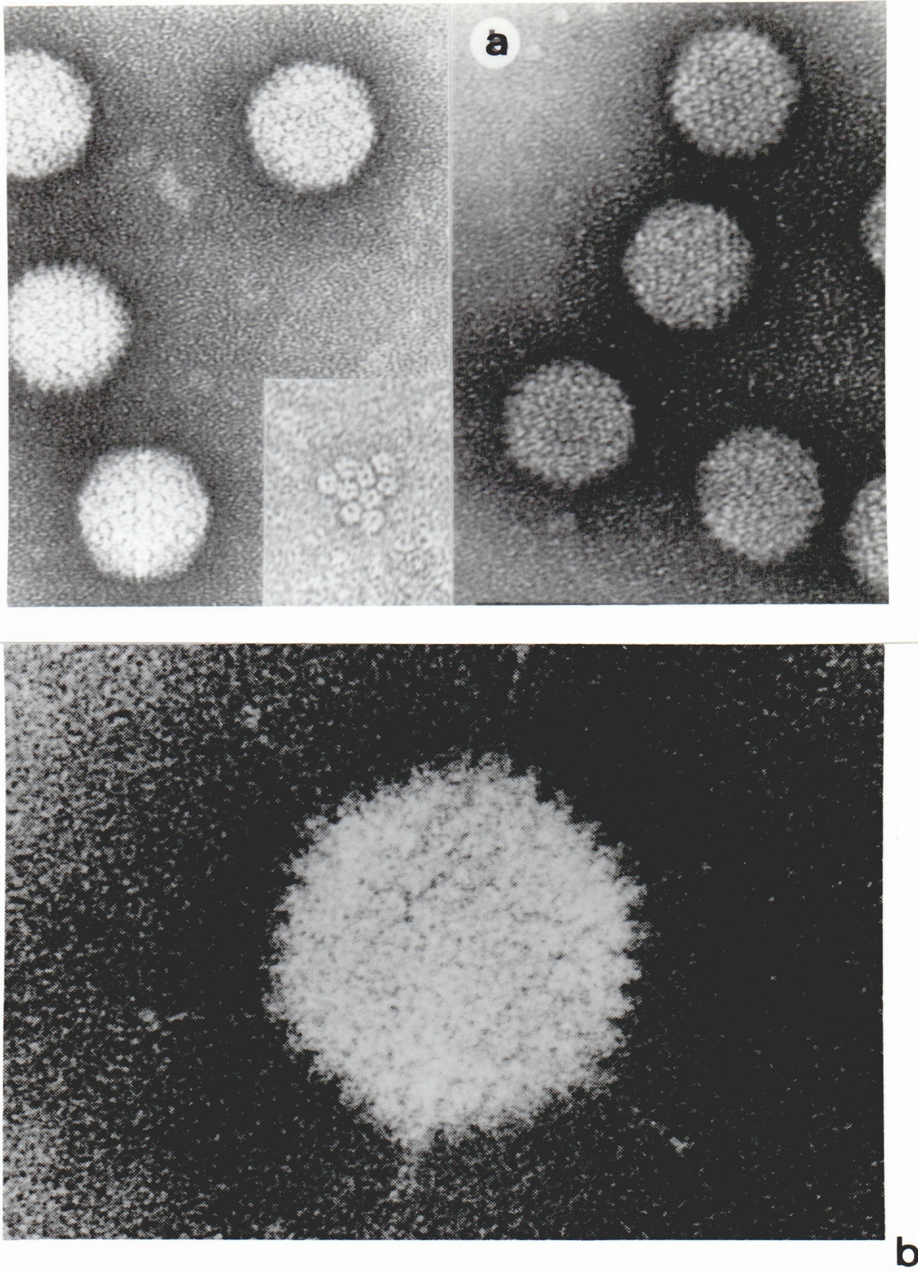


Figure 3. Electron micrographs of adenoviruses. a) Lower power view of four virus particles. Inset: one group of nine (GON). b) High power view of virus particle containing surface hexons, penton bases (vertices), and fibers. From (a) Van den Hurk, 1988 (126) and (b) Fraenkel-Conrat and Kimball, 1982 (47).

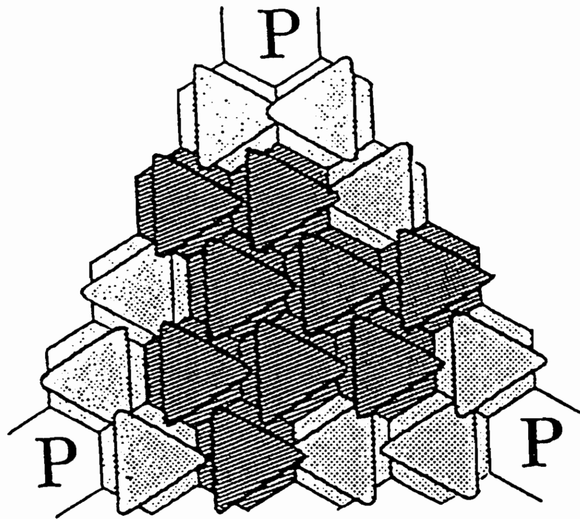


Figure 4. Diagram of one triangular facet of an adenovirus capsid. penton (P), tops of hexons (△), lower part of hexons (◯). Dark hexons represent one group of nine (GON). Taken from Nermut and Perkins, 1979 (100).

subunits, the base and the fiber. The penton has been associated with the induction of cytopathic changes, i.e. rounding of cells, increased granularity of cell cytoplasm, and detachment of cells from their surface (125). Purified penton base has been shown to induce early cytopathic changes (11, 104), while the administration of anti-penton base sera neutralized the cytopathic effects induced by free pentons. The same was not true after administration of anti-fiber sera (104, 130). This suggested that the penton base was responsible for the induction of cytopathic changes. The penton fiber (Figure 5) is believed to be involved in recognition of specific receptors on the plasma membrane of infectable cells, and is thereby important during the early stages of infection (79, 105). Other adenoviral proteins and their characteristics have been summarized in Table 4.

Although mastadenoviruses and aviadenoviruses share many characteristics, the finding by Sharpless (113) that these two groups did not share the same group antigen eventually led to the description of two separate genera, mastadenoviruses (those adenoviruses which infect mammals), and aviadenoviruses (those adenoviruses which infect birds). Kawamura *et al.* (75) first demonstrated the existence of a common group antigen among eight serologically related fowl adenoviruses, but not among mastadenoviruses.

Aviadenoviruses are currently classified into three groups. Group I avian adenoviruses (GpIaa) contain isolates from chickens, ducks, geese, and turkeys (Table 3). These viruses are widely distributed throughout the world and do not appear to be species or age specific. The role of GpIaa in disease is not well defined in most cases although they are frequently associated with upper and lower respiratory disease of birds (87). It has been suggested that different serotypes and perhaps different strains of the same serotype may vary in their

Table 4. Adenovirus proteins and their characteristics[a,b].

Name	Number	Mol wt	Copies/virion	Location	Designation	Specificity	Remarks
Hexon	II	108,000	720	capsid	α ? ϵ	Group Subgroup Type	3 polypeptides per hexon
Penton base	III	85,000	36(?)	capsid; vertices	β	Group Subgroup	Carries toxin activity
--	IIIa	66,000	60(?)	peripentonal	-	Group	Phosphorylated
Fiber	IV	62,000	24(?)	capsid; vertices	γ ? δ	Type Intersubgroup Intrasubgroup	Glycosylated, reacts with HI antibody
--	IVa_2	50,000	---(?)	core associated			DNA binding
DNA terminal protein		55,000	2	5' end of DNA			--
Core protein 1	V	48,000	180	core shell			DNA binding
--	VI	23,400	420(?)	hexon assoc.			DNA binding
Core protein 2	VII	18,000	1,070	nucleocapsid	-	Group and type	DNA binding
--	VIII	13,000	---	hexon assoc.			--
--	IX	11,500	300	GON assoc.	-	Group and type	--
--	X	7,000[c]	50	internal			--
--	XI	4,500[c]	125	internal			--
--	XII	3,000[c]	---	internal			--

[a] Adapted from Philipson *et al.*, 1975 (106) and Nermut, 1984 (99).
[b] Based mainly on human adenovirus types 2 and 5.

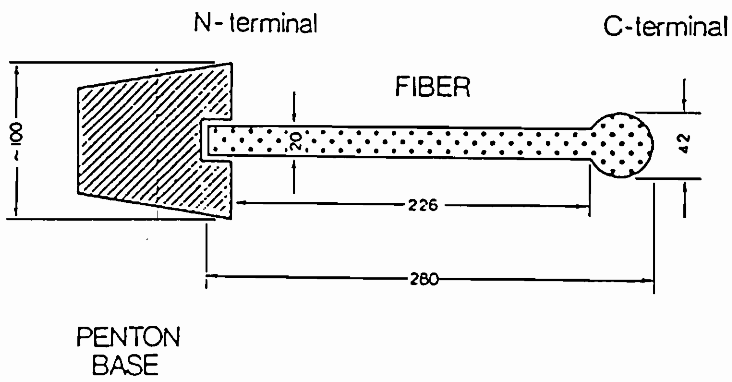


Figure 5. Diagram of a human adenovirus group 2 (Ad2) penton base and fiber. The dimensions are in angstrom units. Taken from Nermut, 1984 (99).

ability to produce disease (21, 27, 86). It is generally agreed that GpIaa cause more problems as secondary pathogens following primary exposure to other immunosuppressive infectious agents such as infectious bursal disease (IBD) or chick anemia agent (CAA) (84). An excellent example of this type of problem is inclusion body hepatitis (IBH). This disease is typically associated with an IBD outbreak or vaccination failure and is characterized by an acute onset of spiking mortality which usually peaks after several days. Diagnosis is usually made on flock history (IBD problems, spiking mortality) and typical lesions which include pale, friable, swollen livers and intranuclear inclusions within hepatocytes (61).

Both horizontal (fecal/oral contact or aerosol exposure) and direct vertical (*in ovo*) transmission are important in the spread of GpIaa infections (22). There is also evidence that GpIaa infections can remain latent in breeder flocks (44). The incubation period of GpIaa is relatively short, approximately 24-48 hours. Many syndromes have been associated with these viruses including depressed egg production (20), inferior egg shell quality (138), decreased feed consumption (23), respiratory disease (75), and tenosynovitis (70).

Quail bronchitis (QB) is considered to be the only primary pathogen among the GpIaa. It is an acute, highly contagious respiratory disease of bobwhite quail (*Colinus virginianus*) (101). It is now considered to be the same etiologic agent as chick embryo lethal orphan (CELO) (39) and is characterized by sneezing, coughing, conjunctivitis, huddling, and depression in quail less than four weeks of age. Morbidity can be as high as 100% while mortality can range from 0-100%, usually averaging around 50% (137). Control of the disease is usually done through biosecurity and vaccination.

The virus causing egg drop syndrome-1976 (EDS 76) is serologically related to GpIaa but is presently considered a separate group. Although the virus causing EDS 76 is considered not to be a pathogen of ducks and geese (6), it is responsible for major losses in chicken egg production throughout the world and is characterized by the production of thin-shelled and shell-less eggs in otherwise healthy birds (128). These problems usually develop as the hens reach peak egg production. Gross lesions include inactive ovaries, atrophied oviducts, and uterine edema (82). Prevention and control is usually accomplished by using birds from EDS 76-free flocks, proper biosecurity, and inactivated vaccines.

Group II aviadenoviruses (GpIIaa) include hemorrhagic enteritis virus of turkeys (HEV), marble spleen disease virus of pheasants (MSDV), and aviadenovirus group II splenomegaly of chickens (AASV), all of which are serologically indistinguishable (38). Recently, however, strain differences have been identified using restriction enzyme digestion (141).

Aviadenovirus group II of chickens occurs in chickens less than seven weeks of age. These birds are clinically normal, although the viral infection does produce splenomegaly (36). Older chickens infected with the virus can have splenomegaly as well as pulmonary congestion (37). Mortality can occur in these birds and is usually associated with asphyxiation secondary to pulmonary congestion and edema (37).

Marble spleen disease virus of pheasants has been more extensively studied than AASV. MSD most commonly occurs in pheasants three to eight months of age (14, 17). Clinical signs include enlargement and marbling of the spleen. Pulmonary congestion associated with MSDV can result in asphyxiation (17, 140). Mortality occurs approximately six days post-infection and generally does not exceed 2-3% (17, 140).

The causative agent of hemorrhagic enteritis of turkeys (HEV) is also a type II aviadenovirus. The importance of this disease to the turkey industry is highlighted by losses incurred by the industry due to the disease prior to the development of an effective vaccine. These have been estimated to be approximately $3,000,000 per year (30).

Hemorrhagic enteritis first occurred in Minnesota in 1937 (107). The disease was not described again in the literature for twenty years until it occurred in Ohio (49). By the mid-1960s, HEV became endemic in Virginia and Texas. Today, HEV is endemic in all major turkey producing areas of the world (30).

Hemorrhagic enteritis, as well as MSD, is thought to replicate primarily within the lymphocytes of the spleen (15, 17, 48, 124, 126, 140), although the exact population(s) of cells within which it replicates has not yet been determined. It generally affects turkeys older than four-weeks of age (8), although two-and-one-half-week old turkeys were reported to have been infected with HEV (58). This age related resistance is thought to be limited by presence of maternal antibody (42).

Mortality in field outbreaks of HE has reached 60% (56), but usually averages around 10-20%. Because of the availability and the efficacy of the HEV vaccine, mortality due to the disease is minimal. Most of the mortality associated with HEV infection is due to secondary colibacillosis. Losses due to colibacillosis generally occur in flocks approximately seven to fourteen days post-HEV exposure (Figure 6). Bacterial septicemia is thought to occur as a result of the immunosuppression associated with HEV and possibly the disruption of the intestinal mucosa following HEV infection.

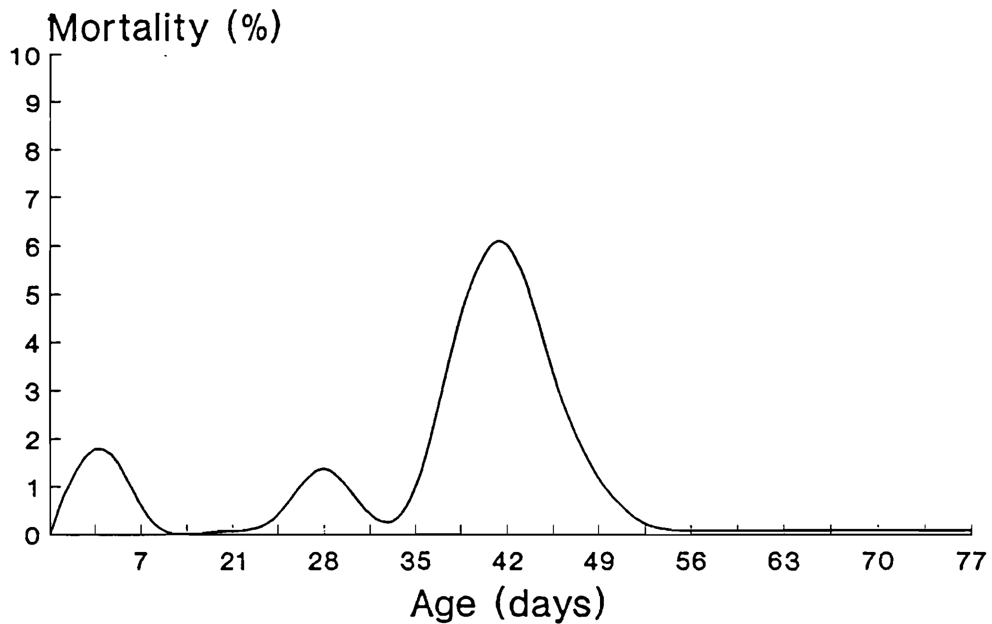


Figure 6. Characteristic mortality due to colibacillosis in a flock of young turkeys following exposure to HEV at 21-28 days. Note the peak in mortality approximately 7-14 days following HEV exposure. The peak in mortality during the first week is due to starve-outs and die off of weak poults.

which allows the entrance of intestinal *E. coli* into circulation or the inhalation of *E. coli*-containing dust with subsequent penetration of impaired lung or air sac tissue.

The incubation period for HEV/MSD is approximately 5-6 days after oral or cloacal inoculation (33, 34) and its clinical course is usually limited to seven to ten days in a flock of susceptible turkeys (56). Clinical HEV is characterized by an acute onset of depression, anorexia, and dehydration. Birds with severe enteritis may have bloody droppings (Figure 7a) and mortality (30, 49, 107). Gross lesions characteristically include enlarged, marbled spleen (Figures 8 and 9) and a congested, cyanotic intestine often beginning in the duodenal loop and extending distally throughout the entire length of the intestine (Figure 10). The intestinal mucosa is congested, hemorrhagic, and often has a yellow, fibrinous pseudo-membrane (Figure 11).

Histologically, the spleens of HEV-infected turkeys contain multi-focal areas of lymphoid necrosis within the areas of the white pulp (48, 55). The red pulp appears congested and contains increased numbers of reticuloendothelial (RE) cells, areas of lymphoid depletion and intranuclear inclusion bodies (Figure 12). These inclusion bodies are also found in the reticular cells which proliferate around the sheathed arteries of the red pulp (67). The inclusions, often stained pale pink to pale purple (Figure 13), occupy the majority of the nucleus and cause margination of the nuclear chromatin. This often gives these nuclei a "signet ring" appearance. Ultrastructurally, these inclusions appear as a crystalline array of virions, both as empty capsids and complete virions (Figures 14 and 15) (124, 140).

Histologically, the villi of the intestinal mucosa are shortened, blunted, congested, and infiltrated with lymphocytes (Figure 16). The tips of the intestinal villi, within the lamina propria, are often congested and swollen (Figure 17). It has been suggested that the characteristic mucosal

Figure 7. a) Characteristic bloody feces from an HEV infected bird. b) A dead bird with blood-stained vent feathers indicative of the severe enteritis associated with HEV infection. Pressure exerted upon the abdomen often forces bloody feces out of the cloaca. Provided by C. H. Domermuth.

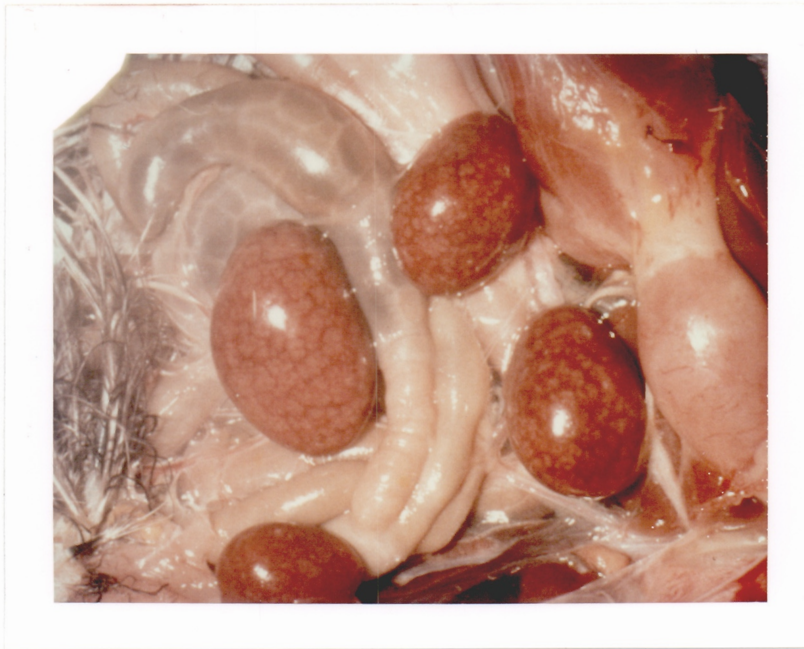


Figure 8. Three spleens from HEV infected turkeys showing the characteristic marbling pattern (top). Note the one uninfected, smaller, less marbled spleen for comparison (bottom). Provided by C. H. Domermuth.

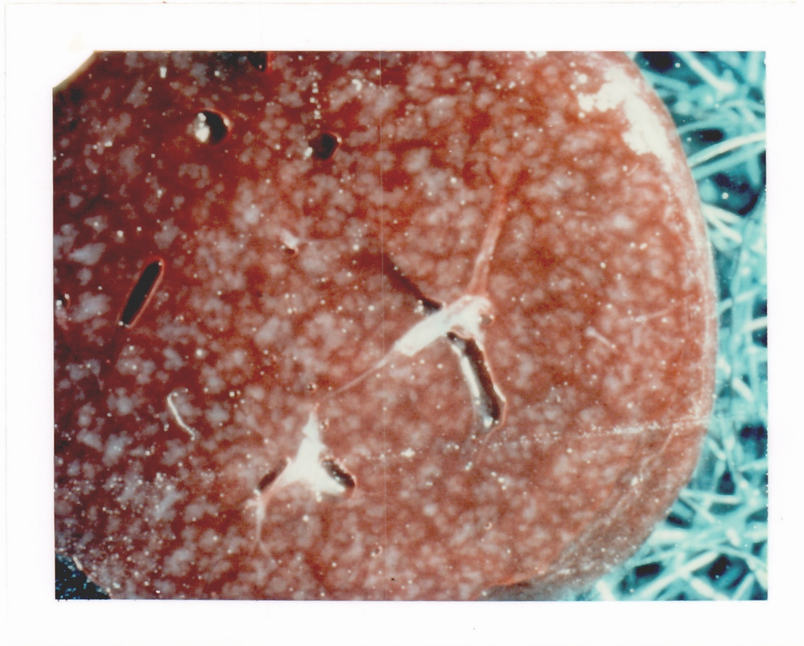


Figure 9. A cross section of an HEV infected spleen showing the characteristic marbling pattern. Provided by C. H. Domermuth.

Figure 10. The duodenal loop of an HEV infected turkey which demonstrates the congested, hemorrhagic nature of the intestinal tract. Provided by C. H. Domermuth.

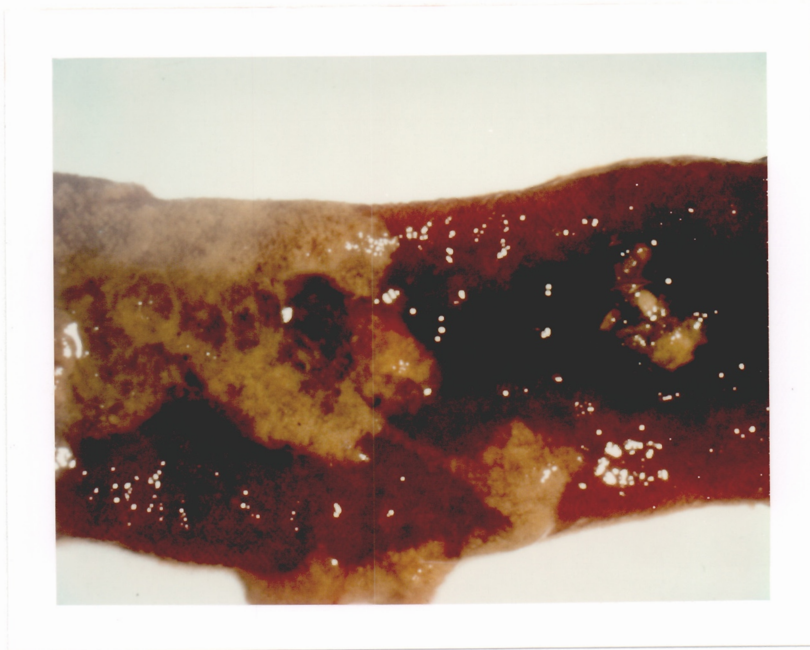


Figure 11. The congested, hemorrhagic intestinal mucosa characteristic of HEV infection. Note the yellow, fibrinous material covering part of the mucosa. Provided by C. H. Domermuth.

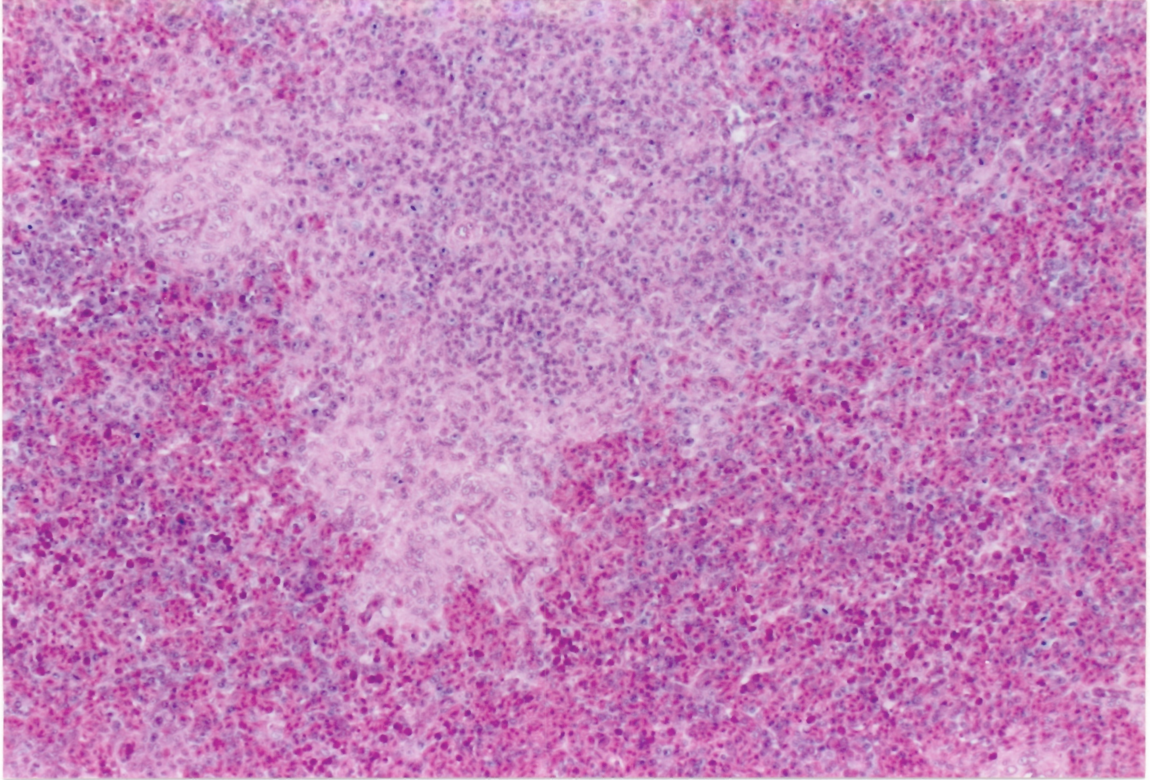


Figure 12. An area of reticuloendothelial cell hyperplasia and lymphoid depletion within the white pulp of the spleen in an HEV infected turkey. Note the mild splenic congestion.

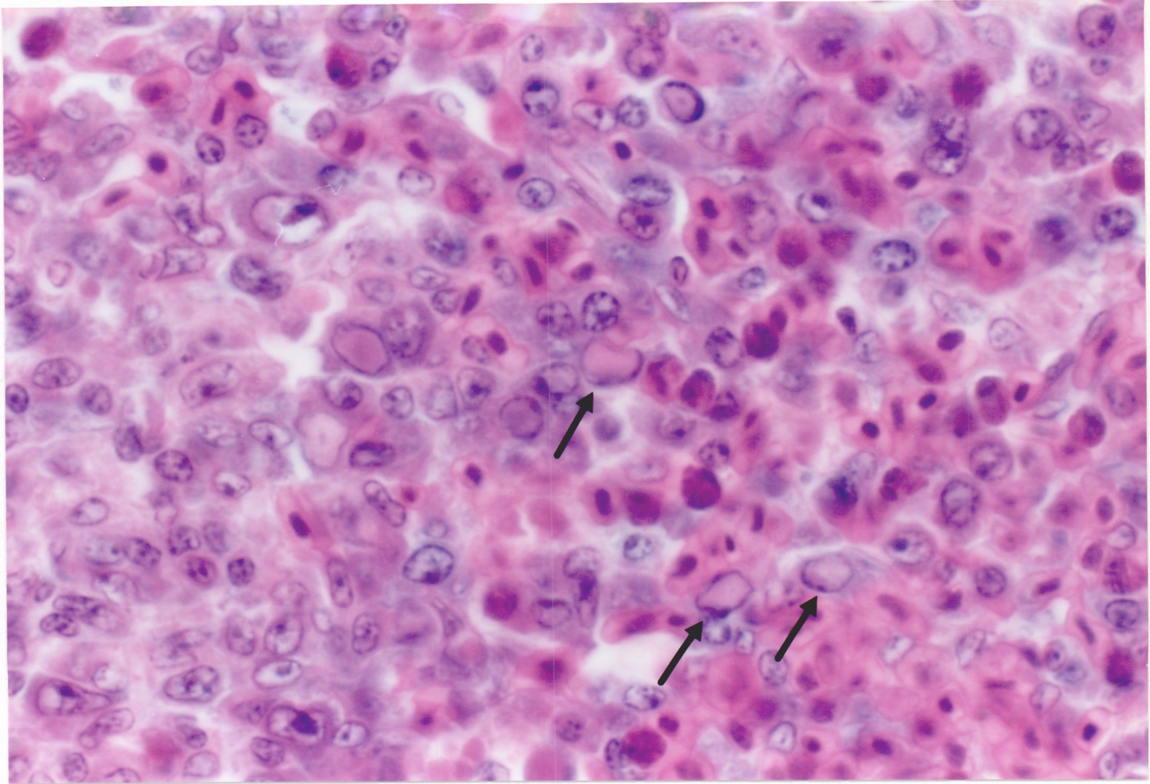


Figure 13. Intranuclear inclusion bodies in the cells of the spleen which indicates HEV infection (arrows). Note the margination of the nuclear chromatin and the resulting "signet ring" appearance of the cell.

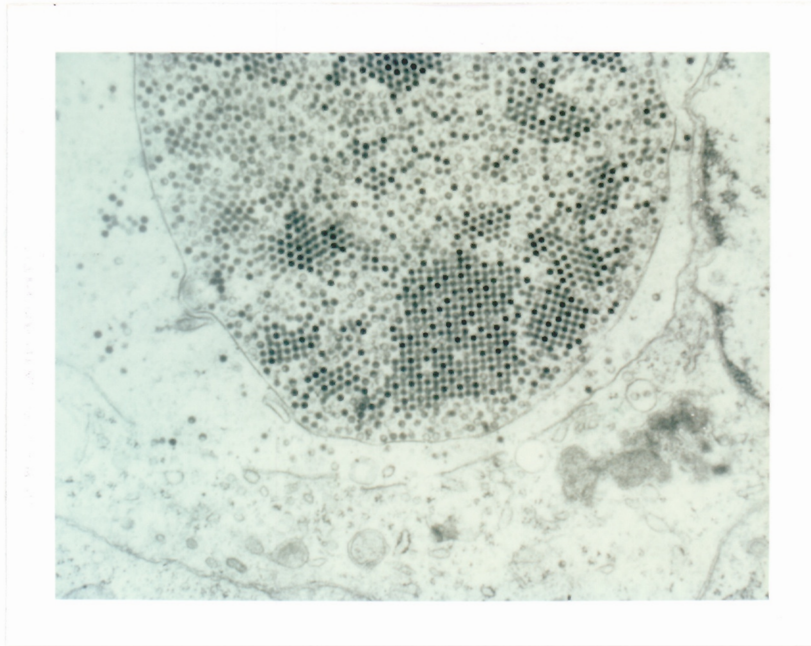


Figure 14. An electronmicrograph of an HEV infected cell within the spleen of a turkey. Note the crystalline array of adenovirions within the nucleus of the cell which compose the inclusion body.

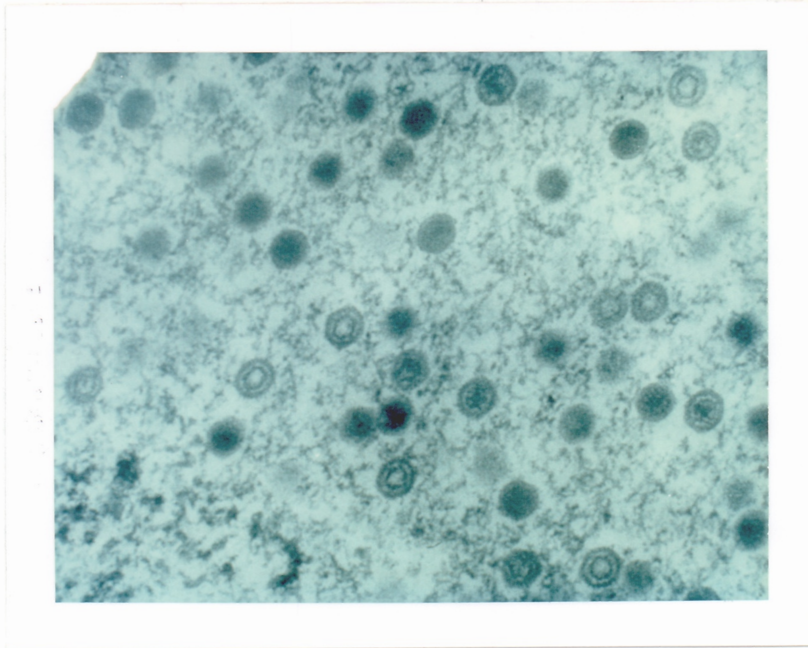


Figure 15. Higher power electronmicrograph of adenovirions within the nucleus of the cell. Note the complete (solid) and incomplete (empty) virions. Incomplete replication (the presence of capsids without DNA) is common in adenoviruses. Provided by C. H. Domermuth.

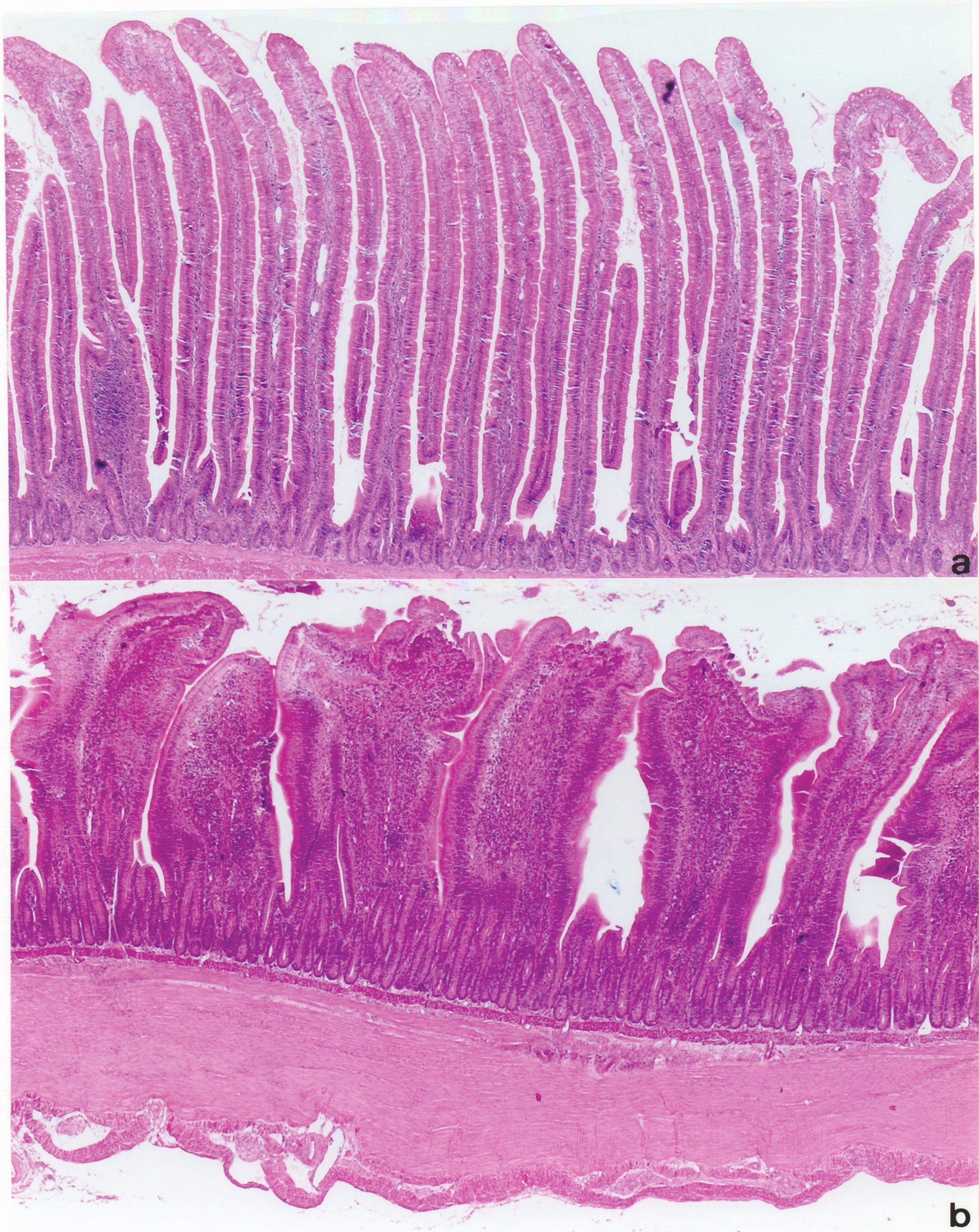


Figure 16. The duodenal villi from an uninfected (a) and HEV infected (b) turkey. Note the shortening and blunting of the villi as well as the lymphocytic infiltration in the infected duodenal section.

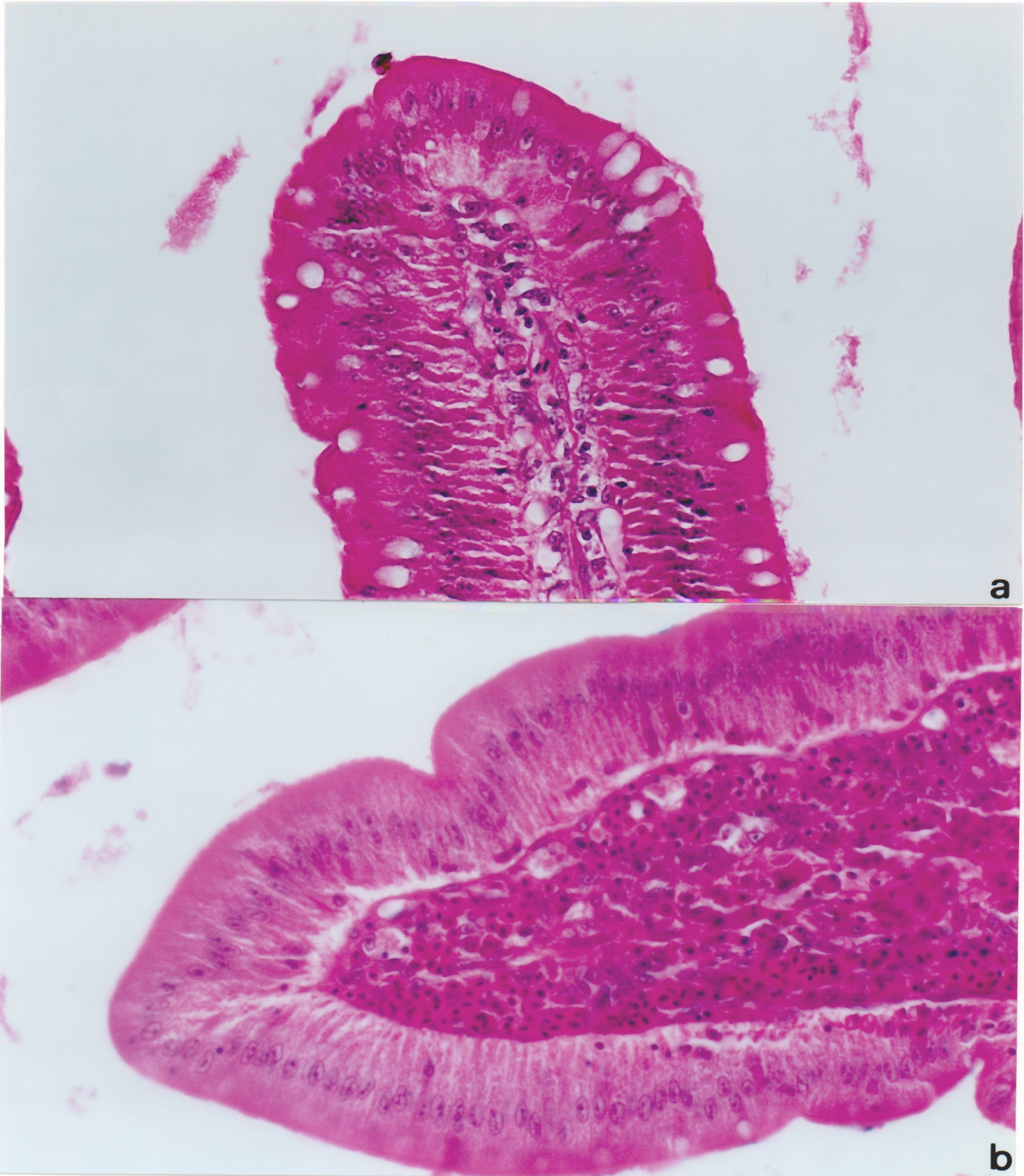


Figure 17. The villus tip from an uninfected (a) and HEV infected (b) turkey. Note the severe congestion in the villus tip of the infected bird and the absence of congestion in the healthy bird.

congestion and edema associated with HEV may lead to necrosis of the villus tip, separation of the epithelium from the basement membrane, and eventual rupture of the villus tip (54, 107). This rupture results in the release of free erythrocytes into the intestinal lumen (Figure 18). Although the virus has been localized in the intestine, it is not believed to be a major site of viral replication. The question of why a virus which replicates primarily in the spleen can cause such a severe enteritis remains an enigma.

Congestion of the lungs has been reported as a feature of HEV infection but is considered more characteristic of MSD infection (14, 17, 140). Hepatomegaly has also been associated with HEV infection as have lesions of the proventriculus, ventriculus, ceca, large intestine, thymus, and bursa of Fabricius (15, 45, 48, 54, 66, 68, 134), but these lesions are generally inconsistent.

Poults which are infected with HEV either naturally or experimentally become resistant to re-infection. Resistance to infection does not appear to be strain specific as vaccination with an avirulent HEV isolate or a MSD isolate will protect against virulent HEV challenge (31). As mentioned earlier, passive immunity is also believed to be of importance in the age-related resistance to HEV infection of poults less than four weeks of age (29).

The most important immunologic aspect of HEV is the immunosuppressive nature of the virus. HEV infection has been associated with secondary colibacillosis (78, 119), rhinotracheitis and chlamydia infection (3), as well as decreased antibody response to Newcastle disease vaccination (94). Additionally, lymphocyte function tests have indicated decreased immune function *in vitro* following HEV exposure (93, 95). Although the exact mechanism by which HEV causes immunosuppression is unknown many possible explan-

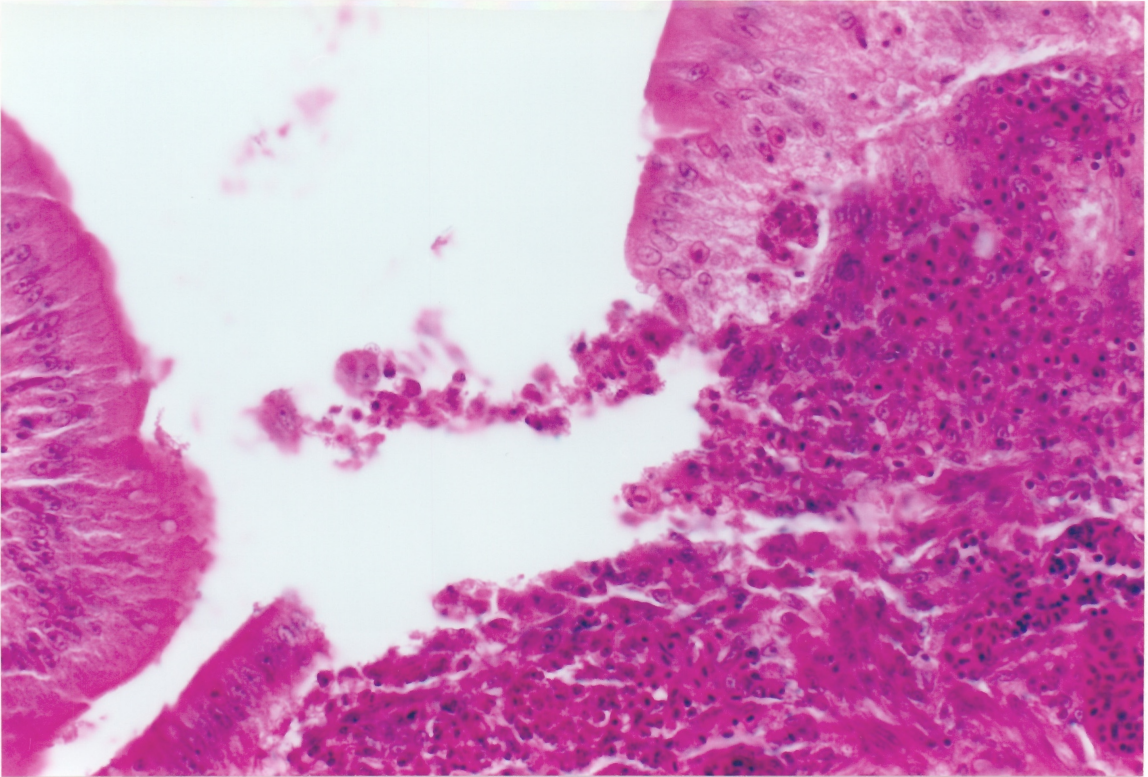


Figure 18. The rupture of a duodenal villus tip following HEV infection. This follows villus congestion as seen in Figure 17a. It appears that the normal columnar epithelium has fractured thus allowing free leakage of erythrocytes into the intestinal lumen.

-ations have been offered (Figure 19).

Presence of the virus can be demonstrated using the agar gel precipitin test (32, 33), ELISA test (26, 62, 97, 127), or immunoperoxidase and immunofluorescence staining (1). The virus can be recovered from intestinal contents or the spleen of infected birds approximately five days post-inoculation (56, 67). Bioassays can also be used to demonstrate the presence of the virus. Susceptible poults can be inoculated orally, cloacally, or intravenously. Characteristic clinical signs and lesions will develop five to six days (oral or cloacal inoculation) or three days (intravenous inoculation) post infection if the suspect inoculum contains HEV (63, 140). HEV antibody can be detected at least two weeks post- exposure using either agar gel precipitin (32, 33) or ELISA (26, 62, 97, 127).

Control of HEV infection is accomplished with the use of an avirulent HEV vaccine. This vaccine is produced in two ways. Production can include inoculation of susceptible turkeys with an avirulent strain of HEV, and then harvesting the spleens five days post-inoculation. These spleens are processed and re-administered to susceptible poults in the drinking water (31, 35, 122). Alternatively, the avirulent HEV vaccine may be produced in a Marek's Disease derived turkey lymphoblastoid cell line RP-19 (43, 98). Efficacy and lateral transmissability are still being evaluated.

Although there is no practical treatment for HEV, administration of convalescent antisera to susceptible birds in the midst of an outbreak has prevented further spread

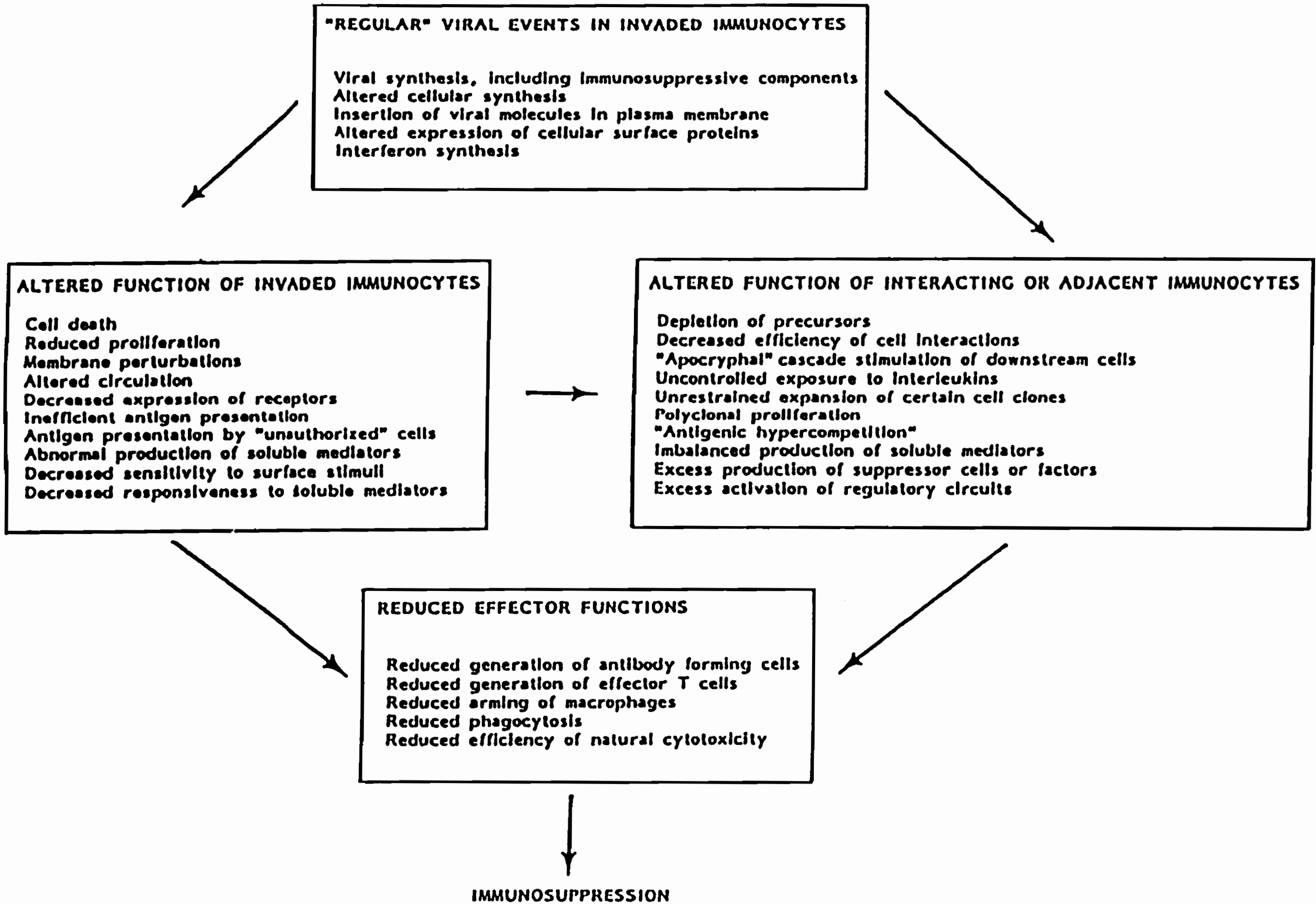


Figure 19. Mechanisms by which viral infection of immunocytes may lead to immunosuppression. Taken from Specter *et al.*, 1989 (117).

the disease (29). Antibiotics have also been employed with varying success following HEV exposure in an attempt to prevent secondary bacterial infections.

The Inflammatory Response

Inflammation is classically described as the response of living tissue to local injury (108). This response typically includes the local accumulation of blood cells and fluid and its primary significance is thought to be a protective mechanism against microscopic invaders and foreign material. Because injury and inflammation are generally directly related, the two terms are often confused. "Injury" is more aptly used to describe changes induced by noxious agents or trauma which can effect cells and extracellular components, while "inflammation", on the other hand, is comprised of the chemical and physical signals which arise as a result of the injury (112).

As long as there have been living organisms on this planet, there has been injury and inflammation. The earliest known references to inflammation have been found in the cuneiform writings of Mesopotamia. "Ummu", for example, has been translated as "hot thing" and was used in a context which suggested generalized heat (inflammation or fever) (83, 111, 112). Ancient Egyptians around 1650 B.C. used the terms "seref" and "shememet" for inflammation (83, 111, 112). Greeks around the time of Hippocrates (400 B.C.) referred to inflammation as "phlegmone", "the fiery thing" (112). The first classical definition for "inflammatio" was offered during Roman times (first century A.D.) by Cornelius Celsus (108). This definition was so precise that little has been done since that time in history to alter the definition "rubor et tumor cum calore et dalore" or "redness and swelling with heat and pain" (83, 108, 111, 112). It was not until 1858 when Rudolph Virchow, the founder of modern cellular pathology, added the fifth cardinal sign of inflammation, "functio laesa" or "loss of function" (111, 112). These five cardinal signs of inflammation comprise the very

basic yet comprehensive definition used today.

Inflammation is a series of events, a continuous process, not a static condition. In 1888 Sir John Burdon-Sanderson wrote:

> The process of inflammation is the succession of changes which occurs in a living tissue when it is injured, provided that the injury is not of such a degree as at once to destroy its structure and vitality (111).

Much of the research done since the time of Burdon-Sanderson has been an attempt at elucidating the processes of inflammation including the key cells and chemical mediators involved.

Inflammation is typically characterized by its severity and duration. John Hunter, in 1793, wrote that "inflammation is not a disease but a non-specific response that has a 'salutary' effect on its host (83). This immediate response (seconds to minutes) acts primarily on microcirculation. Following a transient vasoconstriction of local arterioles there is a subsequent vasodilatation. This leads to increased blood flow to the area which results in two important phenomena. Firstly, delivery of white blood cells (primarily polymorphonuclear leukocytes or PMNs) to combat invading organisms. Secondly, exudation of protein-rich plasma which causes edema and swelling (92, 111, 112). Following the onset of the vascular events described above, more PMNs, eosinophils, monocytes, and platelets (if vascular endothelial injury has occurred) adhere to the vessel wall (marginate) and begin to emigrate into the surrounding tissues (121, 123). These cells then respond to characteristic signals produced at the site of bacterial growth or tissue injury and begin to destroy and phagocytize foreign material or tissue debris.

While these cellular events are occurring, there are simultaneous alterations in vascular flow and permeability which are of prime importance in the determination of the amount of

exudate that is produced. Permeability in acute inflammation is thought to increase in two stages (Figure 20). Almost immediately, there is an increase mediated by vasoactive mediators released by damaged tissues. The second phase occurs several hours after the initial onset of inflammation during the period of leukocyte emigration (111, 112, 121).

As a consequence of extravascular plasma exudation and vascular endothelial injury three enzyme cascades are activated by Hageman factor also known as factor XII (Figure 21). Activated Hageman factor initiates the fibrinolytic system. Plasmin, a potent fibrinolytic enzyme, is generated following activation of plasminogen activator (72). The release of fibrin-derived peptide fragments serves as a chemoattractant for PMNs (73).

Additionally, Morawitz in 1905 found that, following vascular injury, large quantities of thrombin are produced by the action of thromboplastin on prothrombin, a reaction indirectly controlled by Hageman factor (121). Thrombin, the main clotting enzyme, cleaves fibrinogen to produce insoluble fibrin strands which are laid down in inflammed tissues and vessels (19). This is of primary importance in limiting the spread of infectious agents.

The third cascade system triggered by Hageman factor is the kinin system. Kallikrein, which is generated from a pre-enzyme by the action of Hageman factor (139), plasmin, and some PMN and macrophage enzymes, cleaves kininogens (α globulins) to form kinins (112). These compounds are highly potent vasodilators and cause an increase in vascular permeability.

With the induction of endothelial damage, a variety of preformed or newly synthesized inflammatory mediators released to aid the early cellular defense of the body (Table 5) (123).

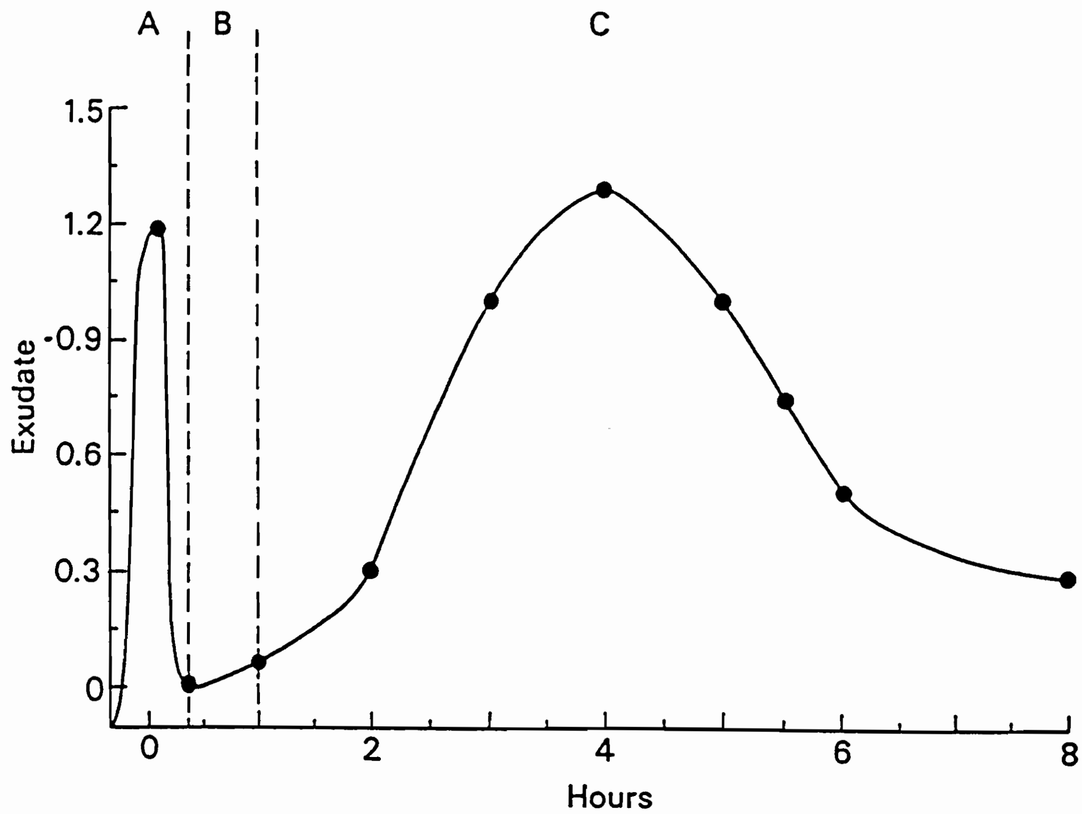


Figure 20. Diphasic permeability change in acute inflammation. A. Immediate phase, mediated by histamine and 5-HT. B. Latent period. C. Delayed phase, mediated by kinins, prostaglandins, and leukotrienes. Taken from Taussig, 1984 (121).

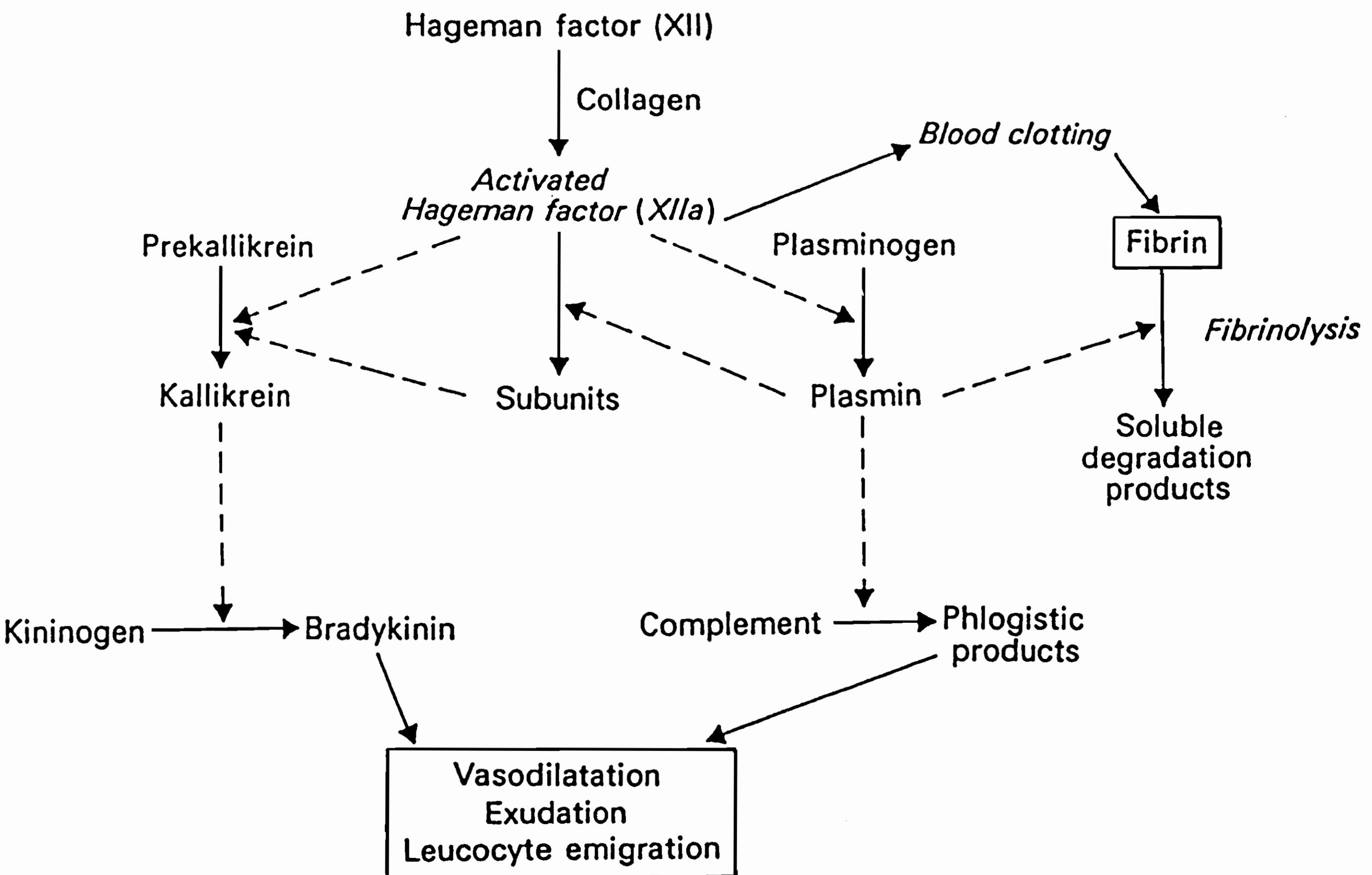


Figure 21. The involvement of Hageman factor in the production of mediators of inflammation. Taken from Taussig, 1984 (121).

Perhaps the best known of the inflammatory mediators is histamine, a product of histidine decarboxylation, most of which is stored, preformed, in the granules of mast cells and basophils (9). Histamine release can be triggered in response to physical injury, chemical agents, immunologic processes, or exposure to anaphylatoxins (9, 112). The biologic activity of histamine includes effects on blood vessels, smooth muscle, and exocrine glands. Most microvessels (capillaries and venules) dilate in response to histamine release (24, 25), although some specific larger vessels contract, i.e. pulmonary vessels of herbivores and hepatic vessels of dogs (41). Histamine also induces smooth muscle contraction of the airway, gastrointestinal tract, uterus, and bladder and is a potent stimulator of bronchial mucus secretion, lacrimation and salivation (9).

5-hydroxytryptamine (5-HT) or serotonin, a derivative of the amino acid tryptophan, is also stored within mast cells and platelets of some species. 5-HT, like histamine, has a complex action and may induce vasoconstriction or vasodilatation and increased vascular permeability (111, 112), but neither vasoactive amine appears to be chemotactic for PMNs (133). Histamine, however, has been reported to be chemotactic for eosinophils (18, 77, 112).

Prostaglandins (PGs) were first discovered in the 1930s by von Euler (121) who believed them to be products of the prostate gland. They are now known to be a universally distributed group of compounds which mediate a wide variety of physiologic effects. Unlike histamine and 5-HT, PGs are not stored in cells, but are newly synthesized as they are needed (123). Damage or stimulation of the cell membrane enables phospholipase A_2 to

Table 5. Inflammatory mediators synthesized by mast cells and other immuno-active cells. Taken from Tizard, 1987 (123).

Agent	Stored	Newly Formed
Vasoactive factors		
Histamine	+	
Serotonin (5-HT)	+	
Leukotrienes C_4 and D_4		+
Prostaglandins		+
Activators		
Platelet activating factor		+
Tryptase	+	
Kallikreins (kinins)	+	
Chemoattractants		
ECF-A[a]	+	
NCF[b]	+	
Leukotriene B_4		+
Anticoagulants		
Heparin	+	
Chondroitan sulfate E	+	

[a]Eosinophil chemotactic factor of anaphylaxis
[b]Neutrophil chemotactic factor

convert membrane phospholipids to free fatty acids (69). Of great importance is the production of the fatty acid arachidonic acid, the commom precursor for the PGs (Figure 22).

Similarly, another group of biologically active lipid compounds are produced from arachidonic acid. These compounds produced by the action of 5-lipoxygenase on arachidonic acid precursors (Figure 22) are known as leukotrienes, so named because they were first isolated from white blood cells and they contain a conjugated triene (121). Leukotrienes, although structurally distinct from PGs, are also unsaturated fatty acids.

The functions of leukotrienes and PGs in inflammation are summarized in Table 6. These compounds also seem to be involved principally in the delayed, prolonged phase of acute inflammation (28) as described in Figure 19. Interestingly, certain classes of PGs, PGE_1 and PGE_2, have also been found to inhibit inflammatory changes by preventing mast cell mediated histamine release and lysosomal release by PMNs. This phenomenom appears to be related to PG concentration in that low levels stimulate inflammation while excessive levels are inhibitory (80). This is a classical, self-regulating "feedback" phenomenon.

Several classes of polypeptides have either potent vasoactive or chemotactic properties and for these reasons are important in the inflammatory reaction. As mentioned earlier, kinins are produced by the action of kallikreins on kininogens. In addition to the initiation of production of kallikreins by Hageman factor, these compounds may also be produced directly from mast cells and basophils or indirectly from activated platelets (123). The most widely researched kinin is bradykinin which may be responsible for extravascular smooth muscle contraction, vasodilation, increased vascular permeability, pain, and possibly

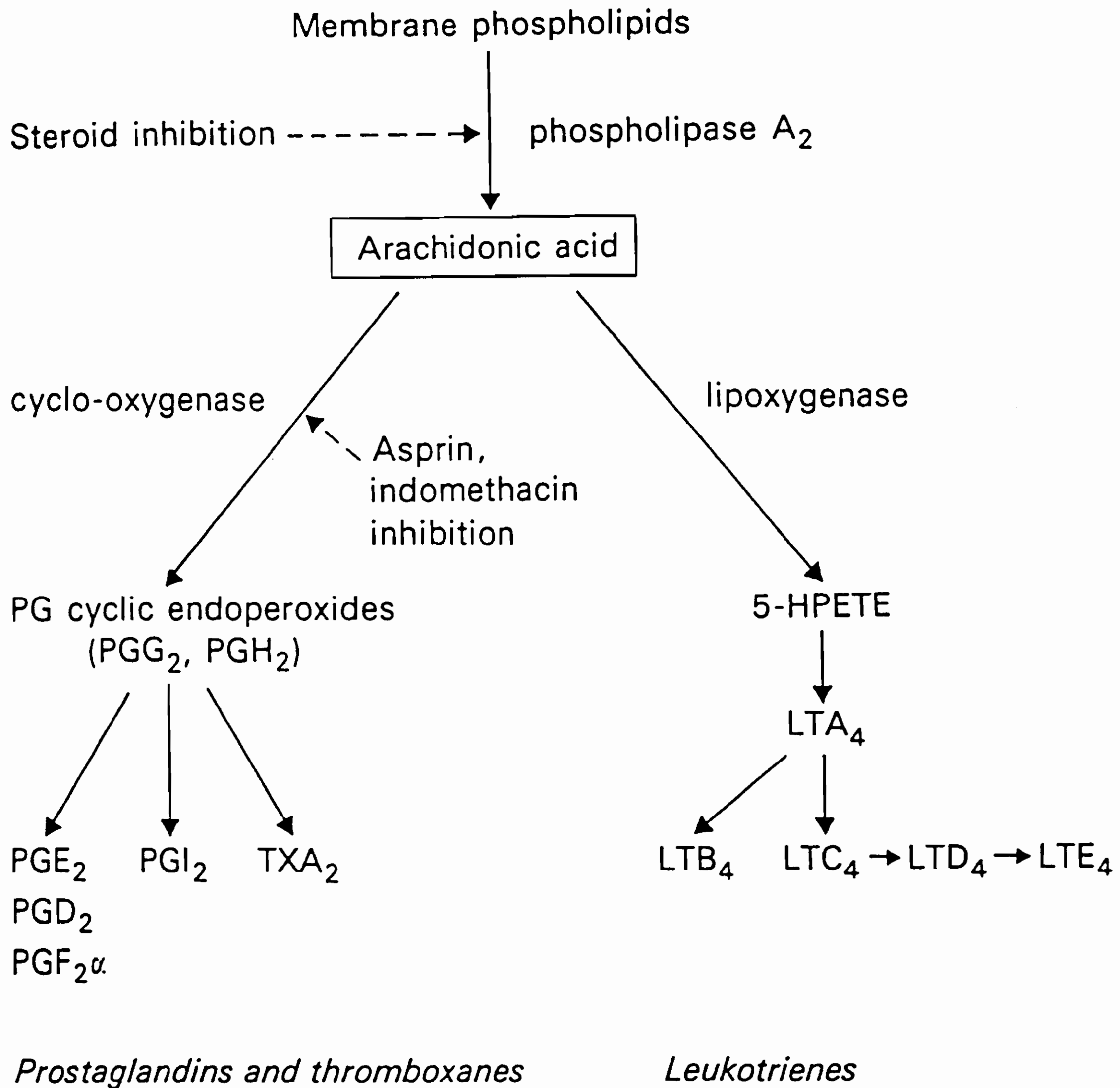


Figure 22. Products of arachidonic acid metabolism. Note the steps at which exogenous pharmacologic inhibition can occur. Taken from Taussig, 1984 (121).

Table 6. The role of prostaglandins and leukotrienes in inflammation. Taken from Tizard, 1987 (123).

Activity	Mediators
Vasodilatation	PGE_1, PGE_2, PGI_2, LTC_4, LTD_4
Increased permeability	PGE_2, PGI_2, LTC_4, LTD_4, LTE_4
Pain	PGE_2, PGI_4
Neutrophil migration	LTB_4
Vasoconstriction	$PGF_{2\alpha}$, TxA_2
Smooth muscle constriction	$PGF_{2\alpha}$, TxA_2
Inhibition of platelet aggregation	PGE_1, PGI_2, $PGF_{2\alpha}$
Promotion of platelet aggregation	PGE_2, TxA_2
Promotion of mast cell degranulation	$PGF_{2\alpha}$

margination and emigration of leukocytes (136). At least some of these peptide effects are thought to be mediated secondarily through the eicosanoids (PGs and leukotrienes) (129).

Anaphylatoxins, polypeptides derived from the cleavage of two complement components, C3 and C5, are known as C3a and C5a. These compounds act indirectly on blood vessel permeability by promoting histamine release from mast cells (112). Additionally, C5a is a very potent chemoattractant for PMNs (132), mononuclear cells (59, 116), basophils (76) and eosinophils (131).

The margination, emigration, and subsequent release of lysosomal contents by PMNs into an area undergoing injury provides another source of inflammatory mediators. Polymorphonuclear leukocytes are a source of phospholipase, an essential enzyme in arachidonic acid production (the precursor of the eicosanoids). Lysosomes within PMNs contain kallikrein which generates kinins from kininogens. Cationic proteins released from PMNs cause mast cells and basophils to degranulate. Finally, proteases, like plasmin, are able to convert C3 and C5 to the anaphylatoxins. In summary, PMNs have an important role in promoting and regulating acute inflammation, as well as their own migration and action to the site (92).

Although platelets are the smallest cellular component in the blood, they play an important role in clotting and hemostasis, as well as thrombus formation (Figure 23). Platelets contain a variety of inflammatory mediators and are capable of synthesizing a number of PGs. The α granules of platelets contain proteolytic enzymes as well as cationic proteins capable of inducing increases in vascular permeability. Dense bodies, on the other hand, contain ADP and 5-HT (52). The release of these compounds occurs after adherence

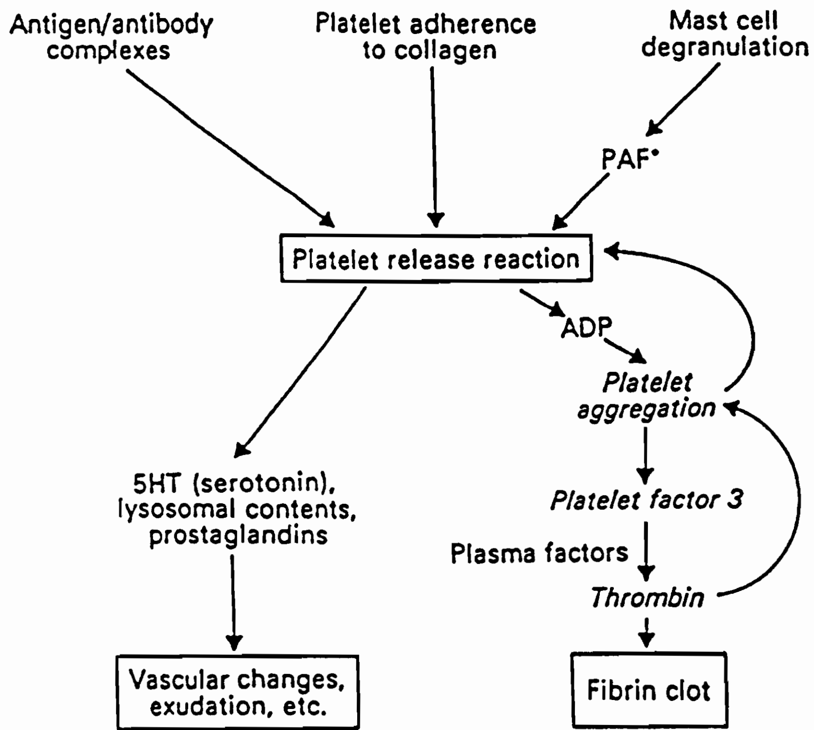


Figure 23. The role of platelets in inflammation. PAF - platelet activating factor. Taken from Taussig, 1984 (121).

of platelets to a foreign substance, such as the collagen of an exposed basement membrane in an injured vessel. The release of ADP from dense bodies enables the platelets to aggregate (121). As platelets aggregate, platelet factor 3, a platelet-associated phospholipid, is expressed in a high enough concentration to facilitate generation of thrombin. Thrombin then, as a positive feedback mechanism, causes further platelet aggregation (52). Additionally, mast cells (89), basophils (114), macrophages (88), and PMNs (81) contain acetyl glyceryl ether phosphorylcholine or platelet activating factor (PAF) which is released upon degranulation and further stimulates platelet secretion.

The acute inflammatory reaction in the avian species has not been as thoroughly investigated as that of mammals. Results from previous studies indicate that, as in mammals, irrespective of the stimulus applied, the qualitative nature of the cellular reaction remains almost identical, especially in the early stages. As in the inflammatory response of mammals, there is an initial, extensive migration of heterophils (avian PMNs) and monocytes into the area of inflammation. Avian heterophils have been shown to be the prominent cell in the early inflammatory reaction (4, 5, 16, 96). Several studies have confirmed the phagocytic ability of these cells (16, 96), while the content of their granules has been an area of less agreement. Some studies have reported no or low activity of
alkaline or acid phosphatase in heterophils (16, 90), while others have demonstrated acid phosphatase, β-glucuronidase, and arylsulphatase within lysosomes (96).

Basophils, not frequently described as part of the mammalian inflammatory response, are present in relatively high numbers in the avian response (16). These cells are present for a short period of time, very early in the inflammatory response (96). Although mast cells constitute the source of much of the histamine and other vasoactive mediators which affect

vascular flow and permeability in mammals (118), basophils are thought to be mainly responsible for these effects in the bird. In fact, there appears to be an inverse relationship between mast cell number and basophil number in that those species with high mast cell numbers (mammals) have low basophil numbers and those with high basophils (amphibians and birds) have low mast cell numbers (10, 91).

The most constant and characteristic observation on the avian inflammatory response, and perhaps the observation most different from descriptions of mammalian inflammation (16, 96), is the rate of appearance of lymphocytic perivascular cuffing. Lymphocyte presence is evident around vessels from 6-12 hours post-injury and becomes a dominant feature at 24-46 hours (16, 71, 96).

The immunopathology of HEV infection, the immunologic mechanisms by which intestinal lesions are produced, remains unclear. Likewise there is no clear explanation why a virus which replicates primarily in the spleen induces severe enteritis as one of its primary lesions. The occurrence of acute inflammation (that which occurs in HEV infection by description and definition) is a non-specific, stereotypical response. Additionally, the populations of immunocytes within the intestinal mucosa and submucosa are well defined (Figure 24). By pharmacologically manipulating inflammatory mediators and examining selected immunologically active cell populations we have attempted to elucidate the immunopharmacologic and immunopathologic processes which may be involved in the production of the intestinal lesions characteristic of HEV infection. In other words, which of the inflammatory mediators or cells discussed previously affect lesion development and what is the sequence of events with which this occurs?

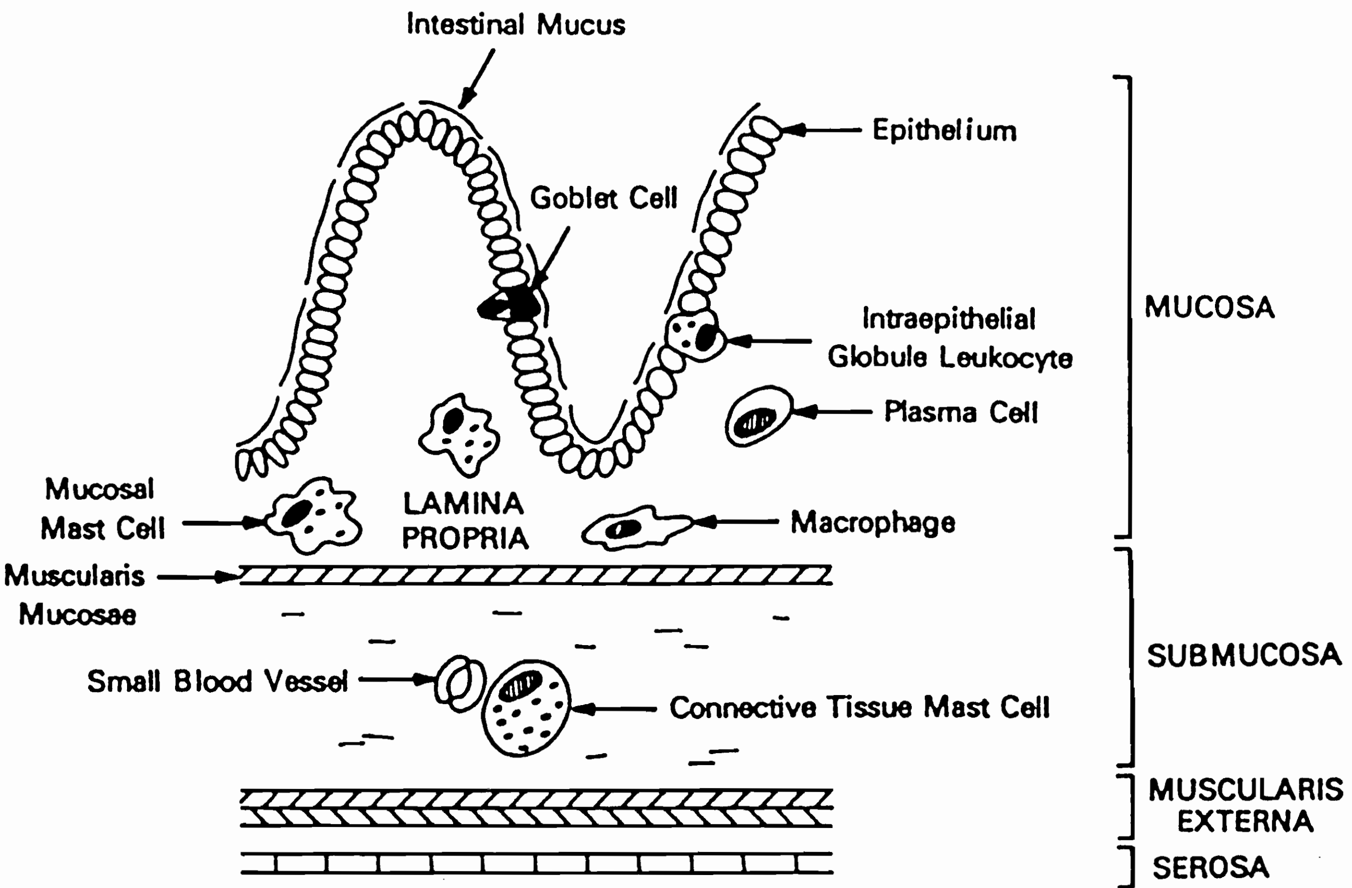


Figure 24. Diagrammatic representation of immunocyte location within the intestinal tract. Taken from Barrett and Metcalfe, 1984 (7).

References

1. Allan, G.M., M.S. McNulty, and B.M. Adair. A comparison of four methods for the detection of turkey hemorrhagic enteritis virus antigen in experimentally infected turkeys. Avian Pathol. 26:107-113. 1987.

2. Allison, A.C., H.G. Pereire, and C.P. Forthing. Investigation of adenovirus antigens by agar gel diffusion techniques. Virol. 10:316-328. 1960.

3. Andral, B., M. Metz, D. Toquin, J. LeCoz, and J. Newman. Respiratory disease (rhinotracheitis) of turkeys in Brittany, France. III. Interaction of multiple infecting agents. Avian Dis. 29: 233-243. 1985.

4. Awadhiya, R.P., J.L. Vegad, and G.N. Kolte. Studies on acute inflammation in the chicken using mesentery as a test system. Res. Vet. Sci. 29:172-180, 1980.

5. Awadhiya, R.P., J.L. Vegad, and G.N. Kolte. A microscopic study of increased vascular permeability and leukocyte emigration in thermal injury in the chicken skin. Avian Pathol. 10:313-319. 1981.

6. Bartha, A.J., J. Meszaros, and J. Tanyi. Antibodies against EDS 76 avian adenovirus in bird species before 1975. Avian Pathol. 11:511-513. 1982.

7. Barrett, K.E. and D.D. Metcalfe. The mucosal mast cell and its role in gastrointestinal allergic diseases. Clin. Rev. Allergy. 2:39-53. 1984.

8. Beasley, J.N., and J. Wisdom. Studies on the pathogenesis of hemorrhagic enteritis of turkeys. Avian Dis. 22:313-319. 1978.

9. Beavan, M.A. Histamine: Its role in physiological and pathological processes. In: Monographs in allergy. Vol. 13. Karger. N.Y., N.Y. pp. 1-114. 1978.

10. Boseila, A.A. The basophil leukocyte and its relationship to the tissue mast cell. Munksgaard. Copehagen. pp. 1-59. 1959.

11. Boudin, M., M. Moncany, J.C. D'Halluin, and P. Boulanger. Isolation and characterization of adenovirus type 2 vertex capsomer (penton base). Virol. 92:125-138. 1979.

12. Bouquet, J.F., Y. Moreau, J.B. McFerran, and T.J. Connor. Isolation and characterization of an adenovirus isolated from muscoavy ducks. Avian Pathol. 11:301-307. 1982.

13. Burnett, R.M., M.G. Grutter, and J. L. White. The structure of the adenovirus capsid. An envelope model of hexon at 6A resolution. J. Mol. Biol. 185:105-123. 1985.

14. Bygrave, A.C., and M. Pattison. Marble spleen disease in pheasants (*Phasianus colchicus*). Vet. Rec. 92:534-535. 1973.

15. Carlson, H.C., F. Al-Sheikhly, J.R. Pettit, and G.L. Seawright. Virus particles in spleens and intestines of turkeys with hemorrhagic enteritis. Avian Dis. 18:67-73. 1974.

16. Carlson, H.C., and J.R. Allen. The acute inflammatory reaction in chicken skin: Blood cellular response. Avian Dis. 13:817-833. 1969

17. Carlson, H.C., J.R. Pettit, R.V. Hemsley and W. R. Mitchell. Marble spleen disease of pheasants in Ontario. Com. J. Comp. Med. 37:281-286. 1973.

18. Clark, R.A.F., J.I. Gallin, A.P. Kaplan. The selective eosinophil chemotactic activity of histamine. J. Exp. Med. 142:1462-1476. 1975.

19. Cochrane, C.G., S.D. Revak, K.D. Wuepper, A. Johnston, D.C. Morrison, R. Ulevitch. Soluble mediators of injury of the microvasculature: Hageman factor and the kinin forming, intrinsic clotting and the fibrinolytic systems. Microvasc. Res. 8:112-121. 1974.

20. Cook, J.K.A. Avian adenovirus alone or followed by infectious bronchitis virus in laying hens. J. Comp. Pathol. 82:119-128. 1972.

21. Cook, J.K.A. Pathogenicity of avian adenoviruses for day-old chicks. J. Compar. Pathol. Ther. 84:505-515. 1974.

22. Cook, J.K.A. Spread of avian adenovirus (CELO virus) to uninoculated fowls. Res. Vet. Sci. 16:156-161. 1974.

23. Cowan, B., B.W. Calnek, N.A. Melendez and R.R. Ball. Avian adenoviruses - effect on egg production, shell quality, and feed consumption. Avian Dis. 22: 459-470. 1978.

24. Dale, H.H., and P.P. Laidlaw. The physiological action of ß-imidazolyl-ethylamine. J. Physiol. (London). 41:318-344. 1910.

25. Dale, H.H., and P.P. Laidlaw. Further observations on the action of ß-imidazolylethylamine. J. Physiol. (London). 43:182-195. 1911.

26. Davidson, I., A. Arnovici, Y. Weisman, and M. Malkinson. Enzyme immunoassay studies on the serological response of turkeys to hemorrhagic enteritis virus. Avian Dis. 29:43-52. 1985.

27. Dhillon, A.S., and R.W. Winterfield. Pathogenicity of various avian adenovirus serotypes in the presence of *Escherichia coli* in chickens. Avian Dis. 28:147-153. 1984.

28. Dirosa, M., J.P. Giroud, D.A. Willoughby. Studies of the mediators of the acute inflammatory reponse induced in rats in different sites by carrageenan and turpentine. J. Pathol. 104:15-29. 1971.

29. Domermuth, C.H., and W. B. Gross. Hemorrhagic enteritis of turkeys. Antiserum-efficacy, preparation, and use. Avian Dis. 19:657-665. 1975.

30. Domermuth, C.H., and W.B. Gross. Hemorrhagic enteritis and related infections. In: Diseases and poultry. 9th ed. Calnek, B.W., H.J. Barnes, C.W. Beard, W.M. Reid, H.W. Yoder, Jr., eds. Iowa St. University Press. Ames, IA. pp. 567-572. 1991.

31. Domermuth, C.H., W.B. Gross, C.S. Douglass, R.T. DuBose, J.R. Harris, and R.B. Davis. Vaccination for hemorrhagic enteritis of turkeys. Avian Dis. 21:557-565. 1977.

32. Domermuth, C.H., W.B. Gross, and R.T. DuBose. Microimmunodiffusion test for hemorrhagic enteritis of turkeys. Avian Dis. 17:439-444. 1973.

33. Domermuth, C.H., W.B. Gross, R.T. DuBose, C.S. Douglass, and C.B. Reubush, Jr. Agar gel diffusion precipitin test for hemorrhagic enteritis of turkeys. Avian Dis. 16:852-857. 1972.

34. Domermuth, C.H., W.B. Gross, R.T. DuBose, and E.T. Mallinson. Experimental reproduction and antibody inhibition of marble spleen disease of pheasants. J. Wildlife Dis. 11:338-342. 1975.

35. Domermuth, C.H., W.B. Gross, L.D. Schwartz, E.T. Mallinson, and R. Britt. Vaccination of ring-necked pheasants for marble spleen disease. Avian Dis. 23:30-38. 1979.

36. Domermuth, C.H., J.R. Harris, W.B. Gross, and R.T. DuBose. A naturally occurring infection of chickens with a hemorrhagic enteritis/marble spleen disease type of virus. Avian Dis. 23:479-484. 1979.

37. Domermuth, C.H., L. Van der Heide, and G.P. Faddoul. Pulmonary congestion and edema (marble spleen disease) of chickens produced by group II avian adenovirus. Avian Dis. 26:629-633. 1982.

38. Domermuth, C.H., C.R. Weston, B.S. Cowen, W.M. Colwell, W.B. Gross, and R.T. DuBose. Incidence and distribution of "avian adenovirus group II splenomegaly of chickens". Avian Dis. 24:591-594. 1980.

39. DuBose, R.T., and L.C. Grumbles. The relationship between quail bronchitis virus and chicken embryo lethal orphan virus. Avian Dis. 3:321-344. 1959.

40. Everitt, E., L. Lutter, and L. Philipson. Structural proteins of adenoviruses. XII. Location and neighbor relationship among proteins of adenovirus type 2 as revealed by enzymatic iodination, immunoprecipitation and chemical cross-linking. Virol. 67:197-208. 1975.

41. Eyre, P. The biochemical pharmacology of allergic reactions in domestic animals. Vet. Rev. 23:3-16. 1972.

42. Fadly, A.M., and K. Nazerian. Hemorrhagic enteritis of turkeys: Influence of maternal antibody and age at exposure. Avian Dis. 33:778-786. 1989.

43. Fadly, A.M., K. Nazerian, K.V. Nagaraja and G. Below. Field vaccination against hemorrhagic enteritis of turkeys by a cell-culture live virus vaccine. Avian Dis. 29:768-777. 1985.

44. Fadly, A.M., B.J. Riegle, K. Nazerian, and E.A. Stephens. Some observations on an adenovirus isolated from specific pathogen-free chickens. Poult. Sci. 59:21-27. 1980.

45. Fasina, S.O., and J. Fabricant. Immunofluorescence studies on the early pathogenesis of hemorrhagic enteritis virus infection in turkeys and chickens. Avian Dis. 26:158-163. 1982.

46. Fenner, F., P.A. Bacaran, E.P.J. Gibbs, F.A. Murphy, M.J. Studdert, D.O. White. Adenoviruses. In: Veterinary virology. Academic Press, Inc. Orlando, FL. pp. 329-338. 1987.

47. Fujiwara, H.S., M. Tanaami, M. Yamaguchi, and T. Yoshino. Histopathy of hemorrhagic enteritis in turkeys. Nat. Inst. Anim. Health Q. (Tokyo). 15:68-75. 1975.

48. Fraenkel-Conrat, H., and P.C. Kimball. Medium and large DNA viruses. In: Virology. Prentice-Hall, Inc. Englewood Cliffs, N.J. p. 199-248. 1982.

49. Gale, C., and J.W. Wyne. Preliminary observations on hemorrhagic enteritis of turkeys. Poult. Sci. 36:1267-1270. 1957.

50. Ginsberg, H.S. Adenovirus structural proteins. In: Comprehensive virology. Vol 13. Fraenkel-Conrat, H., and R. R. Wagner, eds. Plenum Press. N.Y. pp 409-457. 1979.

51. Ginsberg, H.S., G.F. Badger, J.H. Dingle, W.S. Jordan, Jr., and S. Katz. Etiologic relationship of the RI-67 agent to acute respiratory disease (ARD). J. Clin. Invest. 34:1077-1086. 1954.

52. Gordon, J.L., and A.J. Milner. Blood platelets as multifunctional cells. In: Platelets in biology and pathology. Gordon, J.L., ed. North Holland Publishing Co. N.Y. pp. 3-22. 1981.

53. Green, R.G., N.R. Ziegler, B.B. Green, and E.T. Dewey. Epizootic fox encephalitis. I. General description. Am. J. Hyg. 12:109-129. 1930.

54. Gross, W.B. Lesions of hemorrhagic enteritis. Avian Dis. 11:684-693. 1967

55. Gross, W.B. and C. H. Domermuth. Spleen lesions of hemorrhagic enteritis of turkeys. Avian Dis. 20:455-466. 1975.

56. Gross, W.B., and W.E.C. Moore. Hemorrhagic enteritis of turkeys. Avian Dis. 11:296-307. 1967.

57. Grutter, M., and R. M. Franklin. Studies on the molecular weight of the adenoviruses type 2 hexon and its subunit. J. Mol. Biol. 89:163-178. 1974.

58. Harris, J.R., and C.H. Domermuth. Hemorrhagic enteritis in two-and-one-half-week-old turkey poults. Avian Dis. 21:120-122. 1977.

59. Hausman, M.S., R. Snyderman, and S.W. Mergenhagen. Humoral mediators of chemotaxis of mononuclear leukocytes. J. Infect. Dis. 125:595-602. 1972.

60. Hilleman, M.R., and J. R. Werner. Recovery of a new agent from patients with acute respiratory illness. Proc. Soc. Exp. Biol. Med. 85:183-188. 1954.

61. Howell, J., B.W. McDonald, and R.G. Christian. Inclusion body hepatitis in chickens. Can. Vet. J. 11:99-101. 1970.

62. Ianconescu, M., E.J. Smith, A.M. Fadly, K. Nazerian. An enzyme-linked immunosorbent assay for detection of hemorrhagic enteritis virus and associated antibodies. Avian Dis. 28:677-692. 1984.

63. Iltis, J.P., R.M. Jakowski, and D.S. Wyand. Transmission of marble spleen disease in turkeys and pheasants. Am. J. Vet. Res. 36: 97-101. 1975.

64. Iltis, J.P., and D.S. Wyland. Indications of a viral etiology for marble spleen disease in pheasants. J. Wildlife Dis. 10:272-278. 1974.

65. Itakura, C., and H.C. Carlson. Electron microscopic findings of cells with inclusion bodies in experimental hemorrhagic enteritis of turkeys. Can. J. Comp. Med. 39:299-304. 1975.

66. Itakura, C., and H.C. Carlson. Pathology of spontaneous hemorrhagic enteritis of turkeys. Can. J. Comp. Med. 39:310-315. 1975.

67. Itakura, C., H.C. Carlson, and G.N. Long. Experimental transmission of hemorrhagic enteritis of turkeys. Avian Pathol. 3:279-292. 1974.

68. Jakowski, R.M., and D.S. Wyand. Marble spleen disease in ring-necked pheasants: Demonstration of agar gel precipitin antibody in pheasants from an infected flock. J. Wildlife Dis. 8:261-263. 1972.

69. Johnston, M.G., J.B. Hay, H.Z. Movat. The role of prostaglandins in inflammation. Cur. Topics. Pathol. 68:259-287. 1979.

70. Jones, R.C., and K. Georgiou. Experimental infection of chickens with adenoviruses isolated from tenosynovitis. Avian Pathol. 13:13-23. 1984.

71. Jortner, B.S., and W.R. Adams. Turpentine-induced inflammation in the chicken. A light and electron microscope study with emphasis on the macrophage, epitheloid cll and multinucleated giant cell reaction. Avian Dis. 15:533-550. 1971.

72. Kaplan, A.P., and K.F. Austen. The fibrinolytic pathway of human plasma. Isolation and characterization of the plasminogen proactivator. J. Exp. Med. 136:1378-1392. 1972.

73. Kaplan, A.P., E.J. Goetzl, and K.F. Austen. The fibrinolytic pathway of human plasma. II. The generation of chemotactic activity by activation of plasminogen proactivator. J. Clin. Invest. 52:2591-2595. 1973.

74. Kasel, J. A. Adenovirus. In: Diagnostic procedures for viral, rickettsial and chlamydial infections. 5th ed. Lennette, E. H., and N. J. Schmidt, eds. American Public Health Assoc. Washington, D. C. p. 229. 1979.

75. Kawamura, H., T. Sato, H. Tsubahara, and S. Isogai. Isolation of CELO virus from chicken trachea. Nat. Inst. Anim. Health Q. (Tokyo). 4:183-193. 1963.

76. Kay, A.B., and K.F. Austen. Chemotaxis of human basophil leukocytes. Clin. Exp. Immunol. 11:557-563. 1972.

77. Keller, H.V., and E. Sorkin. Studies on chemotaxis. XIII. Differences in the chemotactic response of neutrophil and eosinophil polymorphonuclear luekocytes. Int. Arch. Allergy. 35:279-287. 1969.

78. Larsen, C.T., C.H. Domermuth, D.P. Sponenberg, and W.B. Gross. Colibacillosis of turkeys exacerbated by hemorrhagic enteritis virus. Laboratory studies. Avian Dis. 29:729-732. 1985.

79. Levine, A.J., and H.S. Ginsberg. Mechanism by which fiber antigen inhibits multiplication of type 5 adenovirus. J. Virol. 1:747-757. 1967.

80. Lewis, G.P. Immunoregulatory activity of metabolites of arachidonic acid and their role in inflammation. Brit. Med. Bull. 39:243-248. 1989.

81. Lotner, G.Z., J.M. Lynch, S.J. Betz, and P.M. Henson. Human neutrophil-derived platelet-activating factor. J. Immunol. 124:676-684. 1980.

82. Lu, Y.S., D.F. Lin, H.J. Tsai, Y.L. Lee, S.Y. Chui, C. Lee, and S.T. Huang. Outbreaks of egg drop syndrome-1976 in Taiwan and isolation of the etiological agent. J. Chin. Soc. Vet. Sci. 11:157-165. 1985.

83. Majno, G. The healing hand: Man and wound in the ancient world. Harvard University Press. Boston, MA. 1975.

84. Matthews, R.E.F. Classification and nomenclature of viruses. Intervirol. 17:59-61. 1982.

85. McFerran, J.B. Adenovirus infections. In: Diseases of poultry. 9th ed. Calnek, B.W., H.J. Barnes, C.W. Beard, W.M. Reid, and H.W. Yoder, Jr., eds. Iowa State University Press. Ames, IA. pp. 552-564. 1991.

86. McFerran, J.B., and B. McC. Adair. Avian adenoviruses - a review. Avian Pathol. 6:189-217. 1977.

87. McFerran, J.B., W.A.M. Gordon, S.M. Taylor, and P.J. McParland. Isolation of viruses from 94 flocks of fowl with respiratory disease. Res. Vet. Sci. 12:565-569. 1971.

88. Mencia-Huerta, J.M., and J. Benveniste. Platelet-activating factor and macrophages. I. Evidence for the release from rat and mouse peritoneal macrophages and not from mastocytes. Eur. J. Immunol. 9:409-415. 1979.

89. Mencia-Huerta, J.M., R.A. Lewis, E. Razin, and K.E. Austen. Antigen-initiated release of platelet-activating factor (PAF-aceter) from mouse bone marrow-derived mast cells sensitized with monoclonal IgE. J. Immunol. 131:2958-2964. 1983.

90. Merkel, R.S., and E.C. Mora. Cytochemistry of erythrocytes and leukocytes of the white leghorn chicken. Exp. Mol. Pathol. 1:497-508. 1962.

91. Michels, N.A. The mast cells. In: Handbook of hematology. H. Downey, ed. Hafner Publishing Co. N.Y. pp. 235-372. 1965.

92. Movat, H.Z. The vascular phenomena of acute inflammaton. In: The inflammatory reacton. Elsevier. N.Y., N.Y. pp. 43-76. 1985.

93. Nagaraja, K.V., D.J. Emery, B.L. Batel, B.S. Pomeroy, and J.A. Newman. *In vitro* evaluation of B-lymphocyte function in turkeys infected with hemorrhagic enteritis virus. Am. J. Vet. Res. 43:502-504. 1982.

94. Nagaraja, K.V., S.Y. Kang, J.A. Newman. Immunosuppressive effects of virulent strain of hemorrhagic enteritis virus in turkeys vaccinated against Newcastle Disease. Poult. Sci. 64:588-590. 1985.

95. Nagaraja, K.V., B.L. Patel, D.A. Emery, B.S. Pomeroy, and J.A. Newman. *In vitro* depression of the mitogenic response of lymphocytes from turkeys infected with hemorrhagic enteritis virus. Am. J. Vet. Res. 43:134-136. 1982.

96. Nair, M.K. The early inflammatory reaction in the fowl. Acta. Vet. Scand. 42:1-103. 1973.

97. Nazerian, K., and A.M. Fadly. Further studies on *in vitro* and *in vivo* assays of hemorrhagic enteritis virus (HEV). Avian Dis. 31:234-240. 1987.

98. Nazerian, K., and A.M. Fadly. Propagation of virulent and avirulent turkey hemorrhagic enteritis virus in cell culture. Avian Dis. 26:816-827. 1982.

99. Nermut, M.V. The architecture of adenoviruses. In: The adenoviruses. H.S. Ginsberg, ed. Plenum Press. N.Y. pp 5-34. 1984.

100. Nermut, M.V., and W.J. Perkins. Consideration of the three dimensional structure of the adenovirus hexon from electron microscopy and computer modeling. Micron. 10:247-266. 1979.

101. Olson, N.O. A respiratory disease (bronchitis) of quail caused by a virus. Proc. 54th Annu. Meet. U.S. Livest. Sanit. Assoc. pp. 171-174. 1950.

102. Pereira, H.G., and W.G. Laver. Comparison of adenovirus types 2 and 5 hexons by immunological and biochemical techniques. J. Gen. Virol. 9:163-170. 1970.

103. Pettersson, U., Structural components of adenovirus. VI. On the antigenic determinants of the hexon. Virol. 43:123-136. 1971.

104. Pettersson, U., and S. Hoglund. Structural proteins of adenoviruses. III. Purification and characterization of the adenovirus type 2 penton antigen. Virol. 39:190-203. 1969.

105. Philipson, L., K. Lonberg-Holm, and U. Pettersson. Virus receptor interaction in an adenovirus system. J. Virol. 2: 1064-1075. 1968.

106. Philipson, L.K., U. Pettersson, and H. Lindberg. Molecular biology of adenoviruses. In: Virology monographs. Vol. 14. Gard, S., and C. Hallaver, eds. Springer-Verlag. N.Y. pp. 1-115. 1979.

107. Pomeroy, B.S., and R. Fenstermacher. Hemorrhagic enteritis of turkeys. Poult. Sci. 16:378-382. 1937.

108. Robbins, S.L., and R.S. Cotran. Inflammation and Repair. In: Pathological basis of disease. W.B. Saunders Co. Philadelphia, PA. pp. 55-106. 1979.

109. Roberts, M.M., J.L. White, M.G. Grutter, and R.M. Burnett. Three dimensional structure of the major coat protein hexon. Science. 232:1148-1151. 1986.

110. Rowe, W.P., A.J. Huebner, L.K. Gilmore, R.N. Pariot, T.G. Ward. Isolation of a cytopathic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exp. Biol. Med. 84: 570-573. 1953.

111. Ryan, G.B., and G. Majno. Inflammation. The Upjohn Co. Kalamazoo, MI. 1977.

112. Ryan, G.B., and G. Majno. Acute Inflammation. Am. J. Pathol. 86:184-276. 1977.

113. Sharpless, G.R. GAL virus. Ann. N.Y. Acad. Sci. 101: 515-519. 1962.

114. Siragenian, R.P., and A.G. Osler. Destruction of rabbit platelets in the allergic response of sensitized leukocytes. II. Evidence for basophil involvement. J. Immunol. 106:1252-1259. 1971.

115. Smith, W., C.H. Andrewes, and P.P. Laidlaw. A virus obtained from influenza patients. Lancet. 2:66-68. 1933.

116. Snyderman, R., H.S. Shin, and M.S. Hausman. A chemotactic factor for mononuclear leukocytes. Proc. Soc. Exp. Biol. Med. 138:387-390. 1971.

117. Specter, S., M. Bendinelli, and H. Friedman. Viruses and immunosuppression. In: Virus-induced immunosuppression. Plenum Press. N.Y. pp. 1-18. 1989

118. Spector, W.G., and D.A. Willoughby. The inflammatory response. Bact. Rev. 27:118-154. 1963.

119. Sponenberg, D.P., C.H. Domermuth, and C.T. Larsen. Field outbreaks of colibacillosis of turkeys associated with hemorrhagic enteritis virus. Avian Dis. 29:838-842. 1985.

120. Sutjipto, S., S.E. Miller, D.G. Simmons, R.C. Dillman. Physiochemical characterization and pathogenicity studies of two turkey adenovirus isoants. Avian Dis. 21:549-556. 1977.

121. Taussig, M.J. Inflammation,. In: Processes in pathology and microbiology. Blackwell Scientific Publications. Boston, MA. pp. 1-68. 1984.

122. Thorsen, J., N. Weninger, L. Weber, and C. Van Dijk. Field trials of an immunization procedure against hemorrhagic enteritis of turkeys. Avian Dis. 26:473-477. 1982.

123. Tizard, I. Inflmmation. In: Veterinary immunology. 3rd ed. W.B. Saunders Co. Philadelphia, PA. pp. 267-250. 1987.

124. Tolin, S.A., and C.H. Domermuth. Hemorrhagic enteritis of turkeys. Electron microscopy of the causal virus. Avian Dis. 19:118-125. 1975.

125. Valentine, R.C., and H.G. Pereira. Antigens and structure of adenoviruses. J. Mol. Biol. 13:13-21. 1965.

126. Van den Hurk, J.V., J.M. Development of a vaccine for the prevention of hemorrhagic enteritis in turkeys. Ph.D. Dissertation. Veterinary Infectious Disease Organization. Saskatoon, Saskatchewan. 1988.

127. Van den Hurk, J.V. Quantitation of hemorrhagic enteritis virus antigen and antibody using enzyme-linked immunosorbent assays. Avian Dis. 30:662-671. 1986.

128. Van Eck, J.H.H., F.G. Davelaar, T.A.M. Van den Hevvel-Plasman, N. Van Kol, B. Kouwenhoven and F.H.M. Guldie. Dropped egg production, soft shelled and shell-less eggs associated with appearance of precipitins to adenovirus in flocks of laying fowl. Avian Pathol. 5:261-272. 1976.

129. Vane, J.R, and S.H. Ferreira. Interaction between bradykinin and prostaglandins. In: Chemistry and biology of the kallikrein-kinin system in health and disease. DHEW No. 76-791. Pisano, J.J., and K.F. Austen, eds. U.S. Government Printing Office. Washington, D.C. pp. 255-266. 1976.

130. Wadell, G., and E. Norby. Immunological and other biological characteristics of pentons of human adenoviruses. J. Virol. 4:671-680. 1969.

131. Ward, P.A. Chemotaxis of human eosinophils. Am. J. Pathol. 54:121-128. 1969.

132. Ward, P.A. Complement-derived leukotactic factors in pathological fluids. J. Exp. Med. 134:109s-113s. 1971.

133. Ward, P.A., C.G. Cochrane, H.J. Muller-Eberhard. Further studies on the chemotactic factor of complement and its formation *in vivo*. Immunol. 11:141-153. 1966.

134. Wilcock, B.P. and H.L. Thacker. Focal hepatic necrosis in turkeys with hemorrhagic enteritis. Avian Dis. 20:205-208. 1976.

135. Wilcox, W.C., and H.S. Ginsberg. Production of specific neutralizing antibody with soluble antigens of type 5 adenovirus. Proc. Soc. Exp. Biol. Med. 114:37-44. 1963.

136. Wilhelm, D. L. Kinins in human disease. Annu. Rev. Med. 22:63-84. 1971.

137. Winterfield, R.W., and R.T. DuBose. Quail bronchitis. In: Diseases of poultry. 9th ed. Calnek, B.W., H.J. Barnes, C.W. Beard, W.M. Reid, H.W. Yoder, Jr., eds. Iowa St. Univ. Press. Ames, IA. pp 564-566. 1991.

138. Winterfield, R.W., A.M. Fadly, and A.M. Gallina. Adenovirus infection and disease. I. Some characteristics of an isolate from chickens in Indiana. Avian Dis. 17:334-342. 1973.

139. Wuepper, K.D., and C.G. Cochrane. Plasma prekallikrein: Isolation characterization, and mechanism of action. J. Exp. Med. 135:1-20. 1972.

140. Wyland, D.S., R.M. Jakowski, and C.N. Berke. Marble spleen disease in ring-necked pheasants: Histology and ultrastructure. Avian Dis. 16:319-329. 1972.

141. Zhang, C., and K.V. Nagaraja. Differentiation of avian adenovirus type II strains by restriction endonuclease fingerprinting. J. Am. Vet. Med. Assoc. 192:1790. 1988.

142. Zsak, L., and J. Kisary. Characterization of adenoviruses isolated from geese. Avian Pathol. 13:253-264. 1984.

Chapter II

Effects of Age on Infectivity and Clinical Manifestation of Hemorrhagic Enteritis Virus Infection in Turkey Poults

Abstract

The pathological, biochemical and hematological effects of hemorrhagic enteritis virus inoculated in dilutions ranging from 10^{-1} to 10^{-8} in four-and seven-week-old commercial turkey poults were examined to determine differences in age susceptibility to infection and parameters which best indicate severity of virus infection. Four-week-old poults were less sensitive to clinical infection than seven-week-old poults in all parameters measured. The presence of hemorrhagic enteritis virus antigen in the spleen was considered the most sensitive indicator of infection. Poult infectious $dose_{50}$ (PID_{50}) of the standard, lyophilized hemorrhagic enteritis virus inoculum used in this experiment, as determined by hemorrhagic enteritis virus antigen detection in the spleen, was $10^{2.75}$/ml in four-week-old birds and $10^{4.10}$/ml in seven-week-old birds. Seven-week-old poults had a greater incidence of and more severe lesions than four-week-old birds. Other significant indicators of infection in younger birds were spleen enlargement and increased spleen weight/body weight ratio. In older birds, significant indicators of infection were spleen enlargement, increased heterophil-lymphocyte ratio and a concurrent decrease in serum lipid and albumin concentration.

Introduction

Hemorrhagic enteritis (HE), a Group II avian adenovirus of turkeys (1), produces mortality ranging from less than 1 percent to over 60 percent (6). The disease, first reported in 1937 (8), is characterized by enlarged, marbled spleens; distended, dark intestines; and markedly congested intestinal mucosa (1). In the most severe cases, the birds hemorrhage intra-luminally until they bleed to death. Some birds make full recoveries, rapidly progressing to a normal state. This study was undertaken to examine the effects of graded dilutions of pathogenic hemorrhagic enteritis virus inoculum on age susceptibility to infection and on clinical disease in four- and seven-week-old turkey poults, and to determine which of several parameters examined are the best indicators of the presence and severity of viral infection. This was done prior to utilizing these parameters to study the effects of anti-inflammatory drugs on lesions of HEV infection.

Materials and Methods

Forty-five seven-week-old and 45 four-week-old commercial large white female turkeys were assigned to eighteen treatment groups. Five birds were randomly placed in each group. Poults in each group were inoculated *per os* with 0.5 ml of a dilution of splenic suspension reconstituted from a master seed of lyophilized spleens harvested from susceptible turkeys which had been infected with pathogenic HEV (2). Dilutions of splenic (viral) suspension ranged from 10^{-1} to 10^{-8}. Poults in the two negative control groups each received

0.5 ml of physiologic saline.

Five ml of blood was taken from each bird by brachial venipuncture 5 days post inoculation. Packed cell volume was determined. Blood smears were prepared from whole blood and were stained with May-Grunwald-Giemsa stain to enable determination of heterophil lymphocyte ratios (7). A total of sixty heterophils or lymphocytes were counted from each slide and the heterophil to lymphocyte ratio (HLR) was determined by dividing total numbers of heterophils by lymphocytes. Serum was collected from the remainder of the whole blood by allowing the blood to coagulate for 12 hr and then centrifuging for 10 min. Diagnostic kits for total protein (Sigma Diagnostics No. 540), albumin (Sigma Diagnostics No. 630), and lipids (American Dade No. B5244-10) were used to colorimetrically determine the concentration of these components. Colorimetric determination was made using a Gilford Response UV-VIS spectrophotometer.

On the sixth day post-inoculation the body weight of each bird was recorded, and the spleen removed, weighed, and frozen. Each spleen was later assayed for HEV antigen using agar gel precipitin (AGP) test described by Domermuth *et al.*, (3). Spleen to body weight ratios were calculated by dividing spleen weight by body weight and multiplying by 1000.

The intestinal tract was opened from the pancreatico-duodenal junction posteriorly for approximately 20 cm. Gross intestinal lesions were scored on a scale of 1 to 5; 1 representing marked congestion < 1 cm in length, 2 representing marked congestion 1-10 cm in length, 3 representing marked congestion > 10 cm in length, 4 representing sanguineous material present within the intestinal tract, and 5 representing death caused by the infection. Lesion scores for each group were calculated by dividing the total score for each group by the number of birds per group.

All data were analyzed by analyses of variance using a 2 (age) by 9 (dilution) factorial arrangement of treatments. Differences among means were determined by Duncan's multiple-range test. Significance of the non-parametric data presented in Tables 2a and 2b was determined using Fisher's exact test.

Results

Effect of Age and Dilution on Lesion Score. Four-week-old birds were generally more resistant to the formation of lesions than seven-week-old birds (Tables 1a and 1b). Because of the possibility of false positive lesion scores due to mild congestion and petechiation of the intestinal mucosa for other reasons, columns A and B of Tables 2a and 2b compare the number of poults with (A) and without (B) lesion scores of 1 included. The seven-week-old birds receiving 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions of viral suspension all had lesion scores significantly greater ($P \leq .05$) than the controls-3.80, 3.20, 3.60, and 1.60, vs. 0.00, respectively. Mortality was lower and lesions formed were fewer (Tables 2a and 2b) and less severe in poults inoculated with greater dilutions of virus. This accounts for the lower lesion scores of these groups (Tables 1a and 1b).

Effect of Age and Dilution on Spleen Weight and Spleen Weight/Body Weight Ratio. Both the four and seven-week-old birds showed splenomegaly characteristic of HEV infection (Tables 1a and 1b). Once again the four-week-old birds were more resistant to clinical infection since only the least dilute inoculum, 10^{-1}, produced significant ($P \leq .05$) enlargement of the spleen compared to the controls (2.64 g vs. 1.00 g). The older birds responded more

actively to viral inoculation. Dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} caused significant enlargement of the spleens of the seven-week-old birds compared to the seven-week-old controls. Additionally, there was a lower correlation between lesion formation and spleen enlargement in four versus seven-week-old birds (Tables 2a and 2b).

In order to standardize spleen size as it relates to body weight, spleen weight to body weight ratios (SW/BW) were employed. Generally speaking these results followed those of spleen weight alone. In the four-week-old birds, only the least dilute inoculum caused a significant increase in the SW/BW ratio. Both the 10^{-1} and the 10^{-2} dilutions caused a significant increase in SW/BW ratio in the seven-week-old birds compared to the controls. The 10^{-3} and 10^{-4} dilutions, however, did not reflect the significantly enlarged spleens found when measuring spleen weight only at the same dilution level.

Effect of Age and Dilution on Biochemical Parameters. Total serum protein, albumin and lipid were measured in order to examine some of the biochemical mechanisms involved in HEV infection. Biochemical parameters of younger birds were also less affected by viral challenge compared to the older birds. Although not significantly different, mean total serum protein was lower in the seven-week-old groups receiving less dilute inoculums and may have revealed significance if the sample size had been increased. The values for the 10^{-1}, 10^{-2}, and 10^{-3} dilutions, 1.91, 2.23, and 1.34 g/dl, respectively, were all lower than the control value of 2.51 g/dl. The 10^{-1} dilution of viral suspension did cause a significant ($P \leq .05$) decrease in serum albumin compared to the controls (0.35 g/dl vs. 0.88 g/dl). Serum lipids significantly decreased from 74.40 mg/dl (control) to 39.07, 44.73, and 40.60 mg/dl in the 10^{-1}, 10^{-2}, and 10^{-3} dilutions, respectively.

Effect of Age and Dilution on Heterophil to Lymphocyte Ratios (HLR). Only the least dilute inoculum of virus caused a significant increase in HLR in the younger birds (Table 1a). The seven-week-old birds, however, again responded to more dilute viral inoculums showing significantly higher HLRs in response to the 10^{-1}, 10^{-2}, and 10^{-3} dilutions. The birds receiving the more dilute inoculums in both age groups had HLRs which were not significantly different from the controls.

Effect of Age on Poult Infectious Dose$_{50}$ (PID$_{50}$). PID$_{50}$/ml as determined by the presence or absence of HEV antigen in the spleen was $10^{2.75}$/ml and $10^{4.10}$/ml for four- and seven-week-old birds, respectively (9). Because these birds were of the same genetic stock and received identical inocula, the difference in PID$_{50}$ may be attributed to differences in age susceptibility of the birds to HEV infection. Significant differences ($P \leq .05$) in antigen presence and detection were found in seven-week-old poults receiving 10^{-1}, 10^{-2} and 10^{-3} dilutions of HEV inoculum (Table 2b). Similar differences in antigen detection were also found in four-week-old poults but these were not significant, probably due to insufficient group size.

Discussion

HEV antigen was detected in the spleens of younger birds less frequently than in the spleens of older birds but in groups of birds receiving identical dilutions of inoculum (Tables 2a and 2b). The younger birds were not as severely affected by the clinical manifestations of HE. These findings indicate that HE virus was infective regardless of poult age, but was

able to cause splenic enlargement or intestinal lesions only in the seven-week-old birds.

Since poults used in this study were from breeding stock which are consistently HEV seropositive, it is probable that the age-related resistance to infection observed in this study is due to maternal antibody. The highly protective effect of HEV maternal antibody and vaccination has been reviewed (1). Most recently, Fadly and Nazerian (5) have also shown that maternal antibody to HEV can last as long as six weeks. They also have shown that maternal antibody, although undetectable in most turkeys between 4 and 6 weeks of age, still provides protection from HEV infection.

The significant decrease in serum lipid found in this study appears to correlate well with HEV infection. It has been suggested that this effect may result from either loss of lipid through compromised intestinal mucosa or decreased intestinal absorption (10). Serum lipid is normally conjugated with serum proteins such as albumin. Any decrease in albumin, such as that seen in HEV infection, would also manifest itself as a concurrent decrease in measurable lipids. In contrast to the work of Soback *et al.* (10), our study showed that total serum lipid, and not total protein, was a better biochemical indicator of HEV infection. The decrease in serum lipid concentration, concurrent with HEV infection, was more closely related to lesion score and spleen enlargement than either total protein or albumin.

Stress, due to any cause, including viral infection, will cause the heterophil to lymphocyte ratio (HLR) to increase (7). This is due to several factors. First, steroids released in response to a stressful incident cause a decrease in heterophil margination and emigration, thus increasing the circulatory pool of heterophils. Second, acute systemic viral infections with generalized disbursement of antigen, such as HE, cause entrapment of circulating lymphocytes in lymphoid tissue, and thus a decrease in circulating numbers of

lymphocytes (4).

Our data indicate that HEV antigen detection in the spleen is the most sensitive indicator of infection in both four- and seven-week-old birds. Of those clinical parameters measured in this study, lesion score, spleen weight, SW/BW ratio, serum lipid concentrations and HLR were the most sensitive indicators of viral infection as determined by comparison with HEV antigen detection in the spleen.

References

1. Domermuth, C. H. and W. B. Gross. Hemorrhagic enteritis and related infections. In: Diseases of poultry. 9th ed. Calnek, B.W., H.J. Barnes, C.W. Beard, W.M. Reid, and H.W. Yoder, Jr., eds. Iowa State University Press, Ames, IA. pp. 567-572. 1991.

2. Domermuth, C. H., W. B. Gross, C. S. Douglass, R. T. Dubose, J. R. Harris, and R. B. Davis. Vaccination for hemorrhagic enteritis. Avian Dis. 21:557-565. 1977.

3. Domermuth, C. H., W. B. Gross, and R. T. Dubose. Microimmunodiffusion test for hemorrhagic enteritis of turkeys. Avian Dis. 17:430-444. 1973.

4. Duncan, R. J., and K. W. Prasse. Erythrocytes. In: Veterinary laboratory medicine. 2nd ed. Iowa State University Press, Ames, IA. pp. 3-30. 1986.

5. Fadly, A. M., and K. Nazerian. Hemorrhagic enteritis of turkeys. Influence of maternal antibody and age of exposure. Avian Dis. 33:778-786. 1989.

6. Gross, W. B., and W. E. C. Moore. Hemorrhagic enteritis of turkeys. Avian Dis. 11:296-307. 1967.

7. Gross, W. B., and H. S. Siegel. Evaluation of heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27:972-979. 1983.

8. Pomeroy, B. S., and R. Fenstermacher. Hemorrhagic enteritis in turkeys. Poul. Sci. 16:378-382. 1937.

9. Reed, L. J., and H. Muench. A simple method of estimating fifty percent endpoints. Amer. J. Hyg. 27:493-497. 1938.

10. Soback, S., E. Bogin, and Y. Weisman. Biochemical changes in the blood, spleen, and duodenum of turkeys experimentally and naturally exposed to hemorrhagic enteritis virus. Avian Pathol. 14:189-197. 1985.

Table 1a. The effect of dilution of hemorrhagic enteritis virus on four-week-old turkeys: Lesion score, spleen weight, SW/BW ratio, serum lipid, total protein and albumin concentrations, and heterophil to lymphocyte ratio[1].

HEV Dilution	Lesion Score	Spleen Wt.(g)[2]	SW/BW Ratio[2]	[Lipid] (mg/dl)	[Protein] (g/dl)	[Albumin] (g/dl)	H/L Ratio	% Infected[3]
Control[4]	0.00^{a}	1.00^{a}	1.25^{a}	57.10^{ab}	2.82^{ab}	0.99^{a}	0.58^{a}	0^{a}
10^{-1}	0.20^{ab}	2.64^{b}	3.43^{b}	59.08^{ab}	2.85^{ab}	1.01^{a}	3.34^{b}	60^{a}
10^{-2}	0.00^{a}	1.10^{a}	1.42^{a}	70.80^{b}	1.44^{b}	0.93^{a}	0.66^{a}	60^{a}
10^{-3}	0.00^{a}	0.71^{a}	0.92^{a}	65.20^{ab}	2.28^{ab}	0.95^{a}	0.70^{a}	20^{a}
10^{-4}	0.60^{b}	0.67^{a}	0.92^{a}	47.15^{a}	3.04^{a}	0.80^{a}	1.10^{a}	20^{a}
10^{-5}	0.20^{a}	0.74^{a}	0.96^{a}	49.53^{ab}	2.38^{ab}	1.04^{a}	0.68^{a}	0^{a}
10^{-6}	0.00^{a}	0.81^{a}	1.07^{a}	60.93^{ab}	2.53^{ab}	0.94^{a}	0.86^{a}	20^{a}
10^{-7}	0.20^{ab}	0.81^{a}	1.04^{a}	66.46^{ab}	2.33^{ab}	0.89^{a}	0.74^{a}	0^{a}
10^{-8}	0.00^{a}	1.02^{a}	1.27^{a}	62.90^{ab}	2.63^{ab}	0.92^{a}	0.74^{a}	20^{a}

[1]Means within age groups with different superscripts are significantly different from each other ($P \leq .05$).
[2]Means do not include spleens of birds succumbing to the viral infection.
[3]Infection as determined by presence or absence of HEV antigen in the spleen.
[4]Control birds received no virus inoculum.

Table 1b. The effect of dilution of hemorrhagic enteritis virus on seven-week-old turkeys: Lesion score, spleen weight, SW/BW ratio, serum lipid, total protein and albumin concentrations, and heterophil to lymphocyte ratio[1]

HEV Dilution	Lesion Score	Spleen Wt.(g)[2]	SW/BW Ratio[2]	[Lipid] (mg/dl)	[Protein] (g/dl)	[Albumin] (g/dl)	H/L Ratio	% Infected[3]
Control[4]	0.00^{a}	3.49^{a}	1.18^{a}	74.40^{b}	2.51^{ab}	0.88^{ab}	1.18^{a}	0^{a}
10^{-1}	3.80^{c}	9.09^{b}	2.97^{b}	39.07^{a}	1.91^{ab}	0.35^{a}	2.60^{b}	80^{b}
10^{-2}	3.20^{c}	8.69^{b}	2.77^{b}	44.73^{a}	2.23^{ab}	0.99^{ab}	2.70^{b}	80^{b}
10^{-3}	3.60^{c}	4.76^{a}	1.61^{a}	40.60^{a}	1.34^{a}	0.79^{ab}	3.20^{b}	80^{b}
10^{-4}	1.60^{b}	5.02^{a}	1.69^{a}	51.76^{ab}	3.78^{ab}	1.05^{ab}	1.08^{a}	60^{ab}
10^{-5}	0.60^{ab}	3.81^{a}	1.37^{a}	50.25^{ab}	3.22^{ab}	1.11^{b}	1.09^{a}	0^{a}
10^{-6}	0.80^{ab}	4.11^{a}	1.33^{a}	53.23^{ab}	4.12^{b}	1.15^{b}	0.70^{a}	0^{a}
10^{-7}	0.60^{ab}	3.32^{a}	1.09^{a}	52.37^{ab}	3.36^{ab}	1.18^{b}	0.82^{a}	20^{a}
10^{-8}	0.60^{ab}	3.88^{a}	1.35^{a}	52.89^{ab}	2.18^{ab}	1.13^{b}	1.15^{a}	0^{a}

[1]Means within age groups with different superscripts are significantly different from each other ($P \leq .05$).
[2]Means do not include spleens of birds succumbing to the viral infection.
[3]Infection as determined by presence or absence of HEV antigen in the spleen.
[4]Control birds received no virus inoculum.

Table 2a. The response of four-week-old turkey poults to infection with HEV.

HEV Dilution	no. poults with enlarged spleen/no. tested [1]	no. poults with intestinal lesions/no. tested[2]		no. died/ no. tested	positive spleens/ no. tested[3]
		A	B		
Control[4]	0/5	0/5	0/5	0/5	0/5
10^{-1}	5/5*	1/5	0/5	0/5	3/5
10^{-2}	2/5	0/5	0/5	0/5	3/5
10^{-3}	0/5	0/5	0/5	0/5	1/5
10^{-4}	0/5	3/5	0/5	0/5	1/5
10^{-5}	0/5	1/5	1/5	0/5	0/5
10^{-6}	0/5	0/5	0/5	0/5	1/5
10^{-7}	0/5	2/5	1/5	0/5	0/5
10^{-8}	1/5	0/5	0/5	0/5	1/5

[1]Spleen weights ≥ the means of the negative controls + 1/2 S.D.
[2]Columns A and B represent the number of poults with (A) and without (B) lesion scores of 1 included.
[3]Infection as determined by presence or absence of HEV antigen in the spleen.
[4]Control birds received no inoculum.
*Significantly different than the controls ($P \leq .05$).

Table 2b. Response of seven-week-old turkey poults to infection with HEV.

HEV Dilution	no. poults with enlarged spleen/no. tested[1]	no. poults with intestinal lesions/no. tested[2]		no. died/ no. tested	positive spleens/ no. tested[3]
		A	B		
Control[4]	0/5	0/5	0/5	0/5	0/5
10^{-1}	5/5*	5/5*	5/5*	2/5	4/5*
10^{-2}	4/5*	5/5*	5/5*	1/5	4/5*
10^{-3}	2/5	4/5*	4/5*	3/5	4/5*
10^{-4}	5/5*	3/5	3/5	0/5	3/5
10^{-5}	2/5	1/5	1/5	0/5	0/5
10^{-6}	3/5	3/5	2/5	0/5	0/5
10^{-7}	1/5	1/5	1/5	0/5	1/5
10^{-8}	1/5	2/5	1/5	0/5	0/5

[1]Spleen weights ≥ the means of the negative controls + 1/2 S.D.
[2]Columns A and B represent the number of poults with (A) and without (B) lesion scores of 1 included.
[3]Infection as determined by presence or absence of HEV antigen in the spleen.
[4]Control birds received no inoculum.
*Significantly different than the controls ($P \leq .05$).

Chapter III

The Effects of Steroidal and Non-steroidal Anti-inflammatory Drugs on the Manifestation of Hemorrhagic Enteritis Virus in Turkeys

Abstract

Anti-inflammatory agents with specifically defined mechanisms of action were administered to 6-week-old turkeys inoculated with hemorrhagic enteritis virus (HEV) to determine which inflammatory mediators may be involved in the formation of intestinal lesions associated with HEV. Corticosterone, vitamin E and indomethacin considerably reduced intestinal lesions formation, FPL 55712, FPL 57231, and specific leukotriene blockers markedly increased lesion scores. Chlorpheniramine maleate (histamine H_1 antagonist) decreased lesions scores, whereas cimetidine (histamine H_2 antagonist) increased lesion severity. The results of this study indicate that histamine and the eicosanoids may be important contributors to the formation and severity of intestinal lesions associated with HEV infection.

Introduction

Hemorrhagic enteritis (HE), a disease of young turkeys, is characterized by enlarged, mottled spleens; distended, cyanotic intestines; and markedly congested intestinal mucosa (47, 52, 94). Mortality associated with HE can range from less than 1% to greater than 60% (52). In these instances, death is usually a result of intraluminal intestinal hemorrhage which is often characterized ante-mortem by the passing of bloody droppings, depression, and blood-stained feathers encircling the vent (29).

The immunopathological mechanisms through which hemorrhagic enteritis virus (HEV) causes the inflammation associated with HE are unknown. Many substances have been found which mediate inflammation and congestion -- bradykinin, histamine, prostaglandins (PG), and leukotrienes are but a few. The objective of this study was to treat HE inoculated birds with "selective" pharmacological agents which are known to inhibit certain components of inflammation and congestion, namely corticosterone, vitamin E, indomethacin, the histamine receptor antagonists chlorpheniramine (H_1) and cimetidine (H_2), aminophylline, disodium cromoglycate, and experimental leukotriene antagonists FPL 55712 and FPL 57231. Because of the relative specificity of these drugs, their ability to effect measurable parameters of the disease should provide preliminary evidence of the chemical mediation of the inflammatory processes associated with the virus.

Materials and Methods

Three-hundred-and-five 5-week old large white female turkeys were randomly assigned to 38 treatment groups. Two-hundred and ninety-five of these birds were inoculated

per os with 0.5 ml of a splenic suspension reconstituted from a master seed of lyophilized spleens infected with a pathogenic HEV isolate propagated in susceptible turkeys, $PID_{50}=10^{4.10}$ (31). Twenty of the inoculated birds received no drug treatment and were denoted as positive (+) controls. Twenty uninoculated birds also received no drug treatment and were denoted as negative (-) controls. The remaining 275 birds were divided among twenty-eight drug treatment groups: corticosterone, vitamin E, indomethacin, cimetidine, chlorpheniramine, aminophylline, disodium cromoglycate, FPL 55712 and FPL 57231. Ten birds from each group received the middle drug dose level (see below) and no virus inoculum and served as drug control groups.

Corticosterone (Sigma Chem. Co., No. C-2505) was administered in the feed by dissolving the four dose levels (15, 25, 35, and 45 mg/kg feed) in 100 ml ethanol and thoroughly mixing with 1 kg of mash feed. These doses were within the range of those used by others to increase resistance of chickens to *E. coli* and Newcastle Disease (54).

Vitamin E (dlα-tocopherol, Sigma Chem. Co., No. T 3634) in doses of 25, 50, 100 and 200 mg/kg feed was also administered in the feed and prepared in a similar manner. These dose rates were calculated as 2, 4, 8 and 16 times the daily vitamin E requirements as established by the National Research Council (85).

Indomethacin (Indocin-oral suspension, Merck Sharp & Dohme) was given in doses of 0.78, 1.56, 3.12 and 6.24 mg/kg body weight and was administered b.i.d. *per os* with a Gilson precision microliter pipette. These dosages, as well as others in this study, were extrapolated from information available on the use of these drugs in humans and other species since there has been essentially no work done with these compounds in birds.

Chlorpheniramine maleate tablets (Cord Laboratories, Inc.) administered orally three

times daily were given in dosages of 1, 2, and 4 mg/kg.

Cimetidine (Smith, Kline and French) was given *per os* t.i.d. at a dose rate of 25, 50, and 100 mg/kg with a tuberculin syringe.

Aminophylline (Elkins and Sinn) was administered orally b.i.d. in 5, 10, and 20 mg/kg doses.

Disodium cromoglycate (Fisons Corp.) was placed in gelatin capsules and administered *per os* in 5, 10, and 20 mg/kg doses three times daily.

Experimental leukotriene antagonists, FPL 55712 and FPL 57231 (Fisons Corp), were also placed in gelatin capsules and given orally b.i.d. in doses of 0.5, 1, 2 (FPL 57231) and 20 mg/kg (FPL 55712).

Drug treatments were initiated 48 hr post inoculation to allow the virus to thoroughly invade the body and to ensure, as far as possible, that drugs were acting on the effects of the virus and not the viability of the virus itself.

Five days post inoculation, 5.0 ml of blood was taken from each bird by brachial venepuncture. Packed cell volume was determined and blood smears were prepared from whole blood. Blood smears were stained with May-Grunwald-Giemsa stain to enable determination of heterophil lymphocyte ratios (53). A total of sixty heterophils or lymphocytes were counted from each slide and the heterophil lymphocyte ratio was determined by dividing total numbers of heterophils by lymphocytes. Serum was collected from the remainder of the whole blood by allowing the blood to clot for 12 hr and then centrifuging for 10 min. Diagnostic kits for total protein (Sigma Diagnostics, No. 540), albumin (Sigma Diagnostics, No. 630), and lipids (American Dade, No. B5244-10) were used to determine the concentration of each serum component calorimetrically, using a Gilford

Response UV-VIS spectrophotometer. Quantitative determination of creatine phosphokinase (CPK) and alkaline phosphatase (ALKPHOS) activity in serum was performed using the CentrifiChem CK reagent set (Baker Instrument Corp., No. 27-017-350-000) and the CentrifiChem ALP reagent set (Baker Instruments Corp., No. 27-017360-009) with a CentrifiChem 500 Analyzer. Serum Na^+, K^+, Cl^- and $HC0_3^-$ were measured using the Photovolt Electrolyte Analyzer and PVA4 reagents (Seragen Diagnostics, No. 00-160-10).

On the sixth day post inoculation, the body weight of each bird was recorded, and the spleen removed, weighed, and frozen. The spleen was later assayed for HEV antigen using the agar gel precipitin (AGP) test described by Domermuth *et al.* (30). Spleen weight/body weight ratios were calculated by dividing spleen weight by body weight and multiplying by 1000.

The intestinal tract was opened from the pancreatico-duodenal junction posteriorly for approximately 20 cm. Gross intestinal lesions were graded on a scale of 1 to 5;1 - representing marked congestion < 1 cm in length, 2 - representing marked congestion 1-10 cm in length, 3 - representing marked congestion > 10 cm in length, 4 - representing sanguineous material present in the intestinal tract, and 5 - representing death caused by the infection.

Non-orthogonal contrasts were used to make comparisons of measured parameters among control and treated birds. Bonferroni F values ($P \leq .05$) were used in determining significance.

Results and Discussion

Corticosterone (Tables 1a and 1b): Adrenocorticosteroids are powerful biochemical cellular regulators produced in the adrenal cortex. This group of compounds includes adrenal sex hormones, mineralocorticoids, and corticosteroids. Corticosteroids (CS) affect carbohydrate and protein metabolism. Additionally, these compounds have powerful anti-inflammatory and immunologic properties. Generally CS exert their effect on the immune system by influencing leukocyte circulation and activity (33, 45, 49), lymphocyte availability, antigen processing, and antibody production (36, 46), and inflammatory mediator production and release (21). More specifically, CS act to stabilize lysosomes in leukocytes and damaged tissue while also impeding the access of neutrophils and macrophages to the site of inflammation (80). Kindahl (67) also reported that CS can interfere with prostaglandin (PG) biosynthesis. Steroids, however, do not act on specific enzymes of PG synthesis as is the case of the non-steroidal anti-inflammatory drugs (NSAIDs), like indomethacin. CS inhibit phospholipase A_2 and thus stabilize cell membranes by retarding the release and conversion of cellular phospholipids to arachidonic acid, the precursor of PGs. Others (12, 28, 39) have described the production of polypeptides, collectively known as lipocortins, by phospholipase containing cells which have been exposed to anti-inflammatory concentrations of glucocorticoids. Lipocortin is thought to inhibit the enzyme phospholipase A_2, normally responsible for arachidonic acid release. The inhibition of eicosanoid synthesis at the initial step may be one reason why CS has such a general anti-inflammatory effect compared to those drugs which inhibit PG or leukotriene (LT) pathways alone.

Others (51, 54) have described an immunostimulatory role of CS and have reported increased resistance to bacterial infection in chickens given exogenous corticosterone, while

Kass (66) has reported similar findings in mice. This experiment was designed to determine the effect of CS on the pathogenesis of HEV in turkey poults.

Hematocrit was significantly higher ($P \leq .05$) in the 15, 25, and 35 mg/kg groups when compared to the (+), (-) and drug control, and the 45 mg/kg treatment group. Corticosterone was the anti-inflammatory agent that had the most pronounced effect on lesion score. The 15, 25, and 35 mg/kg groups all had lesion scores significantly less than the (+) control (0.50, 0.40, 0.30 vs 2.21), respectively. The 45 mg/kg treatment, however, was not significantly different from the (+) control. This finding is in agreement with that found by Kass (66) and Gross *et al.* (54) who found increased resistance to bacterial challenge in chickens given 5 to 40 mg/kg CS. These researchers showed that optimal resistance developed, resulting in almost complete protection, while further increases in the level of CS resulted in increasing susceptibility to infection. This might explain what was seen in this study when comparing the 15, 25, and 35 mg/kg groups to the 45 mg/kg group. Perhaps this is the point at which CS changes from an anti-inflammatory-protective agent to one which produces marked immunosuppression resulting in increased viral replication and lesion severity. It is also important to point out that the pronounced effect seen on lesion score with administration of CS is related to its ability to inhibit production of many inflammatory mediators-histamine, PGs, LTs, etc.

Spleen weight and SW/BW ratio were significantly lower in the 15, 25, and 35 mg/kg groups compared to the (+) controls. This was the only drug of those used in this study which reduced spleen weight. Others (54, 100) have also demonstrated a decrease in lymphoid mass with dietary supplementation of CS. If this were the case, the 45 mg/kg group should not have been significantly enlarged. As discussed earlier, CS at lower doses

may be providing resistance to infection by preventing viral replication in the spleen. Beyond an optimal dose, this protective effect is diminished and viral replication is able to proceed.

Total serum lipid significantly decreased in the groups receiving the 15 and 25 mg/kg CS treatment and approached significance in the 35 mg/kg treatment groups compared to the drug control and the (-) control. This suggests that the decrease is a result of viral infection, which is in agreement with Soback *et al.* (108) who hypothesized this to be due to loss through intestinal bleeding or decreased absorption. The 45 mg/kg group, however, had the most severe intestinal damage and a significantly higher lipid concentration. This may have been due to the severe stress of the viral infection and resultant combined effect of exogenous and endogenous steroids causing increased fat mobilization and a subsequent rise in circulating fatty acids (80).

Total serum protein and albumin were significantly higher in the drug control group and those birds in the 15, 25, and 35 mg/kg groups than the (-) control group. This may have been due to the catabolic effects of CS on proteins causing increased amino acid blood levels. The 45 mg/kg CS group had significantly lower protein and albumin levels than even the (+) controls indicating that this treatment may have exacerbated protein loss. This could have resulted from massive bleeding into the intestine or loss of serum proteins through the damaged intestinal epithelium (108).

Corticosterone administered to chickens has been shown to increase heterophil lymphocyte ratios (HLR) (53, 54). This can be observed when the drug control and (-) control groups are compared (1.73.0.34 vs. 0.88$\pm$0.33). Again when looking at the 15, 25, and 35 mg/kg groups, it appears that these levels of CS afford protection against infection and subsequent stress related events. Only in the 45 mg/kg group (that which had

significantly higher lesion scores and spleen weights) was the HLR significantly higher than the other groups with the exception of the (+) controls. This again demonstrates the immunosuppressive effects of high levels of CS and the protective effect of lower doses.

Serum alkaline phosphatase (ALKPHOS) in poults arises mainly from bone and intestine (108). ALKPHOS was shown to decrease in poults infected with HE (108). This decrease was attributed to reduced leakage of ALKPHOS from the intestine, which is illustrated in this study by comparing (+) and (-) controls. Treating with CS confounds this generalization as CS induces hepatic ALKPHOS and thus increases serum activity (37). This accounts for the significantly higher ALKPHOS levels in the drug control group. Those birds receiving CS and a virus inoculum show a gradual decline in ALKPHOS as CS dose increases. The 45 mg/kg group has an ALKPHOS concentration which approaches significance ($P \leq .05$). This indicates a balance of increased ALKPHOS levels due to CS administration and decreased AP levels from HEV infection. Serum creatine phosphokinase (CPK) did not differ between treated and control groups. Although significant differences existed among groups in electrolyte values, there was no obvious pattern to these differences.

Vitamin E (Tables 2a and 2b): Vitamin E, a biologically active tocopherol, is an antioxidant which inhibits production of free oxygen radicals. Freeman and Crapo (44) found that the production of free radicals can result in a disruption of mitochondrial energy production, DNA structure, protein function, cell membrane integrity and eicosanoid production. Vitamin E (VE) was first shown to stabilize cell membranes by Tappel (109), who proposed "The Biological Antioxidant Theory". The stabilization of the polyunsaturated fatty acids of biomembranes in the disease state by vitamin E results in a decrease in the release of cellular

inflammatory substances. Tengerdy (112) also reported that pharmacological levels of VE potentiate the immune response to viral and bacterial challenges by increasing IgG and IgM titers and phagocytic activity. Nockels (86) also reported that an excess of VE above the requirement significantly increased disease resistance, and others (59, 113, 114) have shown an increase in humoral immune response and phagocytosis. Additionally, Machlin (78) showed that VE was an effective inhibitor of PG synthesis. The mode of action of VE is, however, different from that of indomethacin and other NSAIDs. Vitamin E acts to prevent oxidation of arachidonic acid, thus inhibiting prostaglandin (PG) synthesis at an early stage (88). The modulation of PG synthesis by VE may counteract or inhibit the modulation of PG caused by the disease state, thus stimulating the defense mechanisms of the host (74).

Vitamin E did not have a specific effect on hematocrit. Lesion score, however, did show the positive effects of VE administration. Although the administration of 25 mg/kg VE did not show a significant improvement in lesion score compared to the (+) controls (2.10 vs. 2.21, respectively), the 50 and 100 mg/kg doses did produce a protective effect. Lesion scores were reduced to 1.00 and 0.60, respectively. These findings are in agreement with Nockels (86) who found increased resistance to *E. coli* in chicks and poults when supplemented with VE at 100 and 300 mg/kg ration. Others (59) have demonstrated similar findings. This study, however, showed a detrimental effect of VE on lesion formation at the 200 mg/kg dose level. Lesion scores produced were significantly greater than even the (+) controls (4.40 vs 2.21, respectively). There is no evidence in other studies of the development of toxicity at this level, nor has anyone described exacerbation of infectious disease with VE administration. This result, therefore, is difficult to explain and perhaps paradoxical, and will have to be investigated further.

Spleen weight significantly increased in those poults receiving the 25, 50, 100 and 200 mg/kg dosages. Increased spleen weight was indicative of HEV infection. SW/BW ratio paralleled spleen weights within the same treatment group.

Biochemically, the VE treated birds reacted similarly to untreated infected birds studied by Soback *et al.* (108). HEV infection, regardless of VE treatment level significantly reduced serum lipid concentration. The 25, 50, 100, and 200 mg/kg VE treated groups had lipid concentrations of 6.4±5.1, 10.1±4.1, 12.1±3.9 and 8.1±6.2 mg/dl, respectively. This compares to the (+) control which also significantly decreased lipid concentration (15.0±2.8) compared to the (-) control (25.3±3.2). Total serum protein and albumin were also affected by HEV infection. The group with the most severe lesions (200 mg/kg) correspondingly had the lowest serum protein (1.04±.52 g/dl) and albumin (0.26±.21 g/dl). Other groups were not significantly different from either the (+) or (-) controls.

Soback *et al.*, (108) found a decrease in serum ALKPHOS highly correlated with a decrease in serum protein and the presence of HEV infection. The birds in the 200 mg/kg group had significantly lower ALKPHOS levels than the (-) controls (632±257 vs. 1653±182 U/ml). Other biochemical parameters measured (CPK, Na^+, K^+, Cl^- $HC0_3^-$) were not specifically affected by the influence of VE on HEV infection.

HLR appeared to be good a indicator of severity of infection as judged by lesion score. The birds in the 200 mg/kg group had lesion scores and HLRs significantly greater than the other treated and control groups. This suggests a relationship between severity of infection and HLR rather than viral infection and HLR.

Indomethacin (Tables 3a and 3b): Many theories have been proposed about the mechanism

by which non-steroidal anti-inflammatory drugs (NSAIDs) affect inflammation. Adams and Cobb (2) proposed the disassociation of oxidative phosphorylation as the primary mechanism of action of these NSAIDs. This "uncoupling" supposedly deprives the tissues of the necessary energy required for active inflammation to continue. This theory was later disproved, but others were soon to follow. Douwes (35) and Nakanishi and Goto (84) claimed that indomethacin (IND) and other NSAIDs inhibit inflammation by stabilization of lysosomes. Others have proposed the action of NSAIDs to be related to their fibrinolytic activity (99), their ability to accelerate sulfhydryl group interchange (48), or their ability to stabilize membrane proteins (82). Phelps and McCarty (90) suggested that IND decreased neutrophil motility while Blackham and Owen (11) modified this theory to include the inhibition of synthesis of chemotactic PGs, secondarily leading to a decrease in number of migratory leukocytes reaching the inflamed site. These researchers were approaching the currently accepted action of NSAIDs, which is the inhibition of PG synthesis (38, 116). Indomethacin and other aspirin-like drugs are potent inhibitors of PG cyclooxygenase (14).

Indomethacin has properties in itself which might be beneficial in the symptoms of HEV infection. It has both analgesic and anti-inflammatory properties. It is unfortunate that one of the other effects is to increase the risk of gastric bleeding and ulcer formation in the gastrointestinal tract (27).

In these experiments, the dose of IND had an inverse relationship to hematocrit. The two low doses of IND (0.78 and 1.56 mg/kg) significantly increased hematocrit compared to the (+) controls, while the two higher doses (3.12 and 6.24 mg/kg) had hematocrits which approached significance. As IND dose increased from 0.78 to 6.24 mg/kg, hematocrit declined in a stepwise manner. This effect of IND on hematocrit has previously been

undescribed, but Mizushima *et al.* (82) and Brown *et al.* (18) conversely reported the stabilization of erythrocyte membranes by IND which might lead to reduced RBC destruction and a subsequent rise in hematocrit.

Lesion score was beneficially affected by IND administration at the 0.78, 1.56, and 3.12 mg/kg doses (1.70, 1.50, and 1.30, respectively). These values all approached significance when compared to the (+) controls. The 6.24 mg/kg dose, however, increased lesion score (3.00) which approached being significantly greater than the (+) controls (2.21). It has been hypothesized that low doses of IND may inhibit normal physiologic tissue (mucosal) repair mediated by PGs. Robert (98) described the "cytoprotective" effect of PGs following IND administration. Inhibition of cytoprotection may well potentiate the severity of lesions rather than inhibit lesion formation. As the disease state progresses, PGs released in response to inflammation may become more important, and inhibition of PG synthesis by IND become more beneficial in preventing the development of more severe lesions. It seems, therefore, that there might be a dual mechanism at work, one which potentiates lesion formation and another which prevents it, making interpretation of these data difficult. Further complicating this is the fact that at higher doses IND may be ulcerogenic by markedly inhibiting PG synthesis and thus causing loss of tissue integrity (106). Additionally, any compound which has highly selective anti-PG activity may indirectly cause an increase of arachidonic acid metabolism through the lipoxygenase pathway after stimulation of phospholipase activity, resulting in an increase in leukotriene levels (71). Others (73, 111) have reported that blockade of PG synthesis accentuates histamine release. Perhaps it is also possible at this level of PG inhibition (6.24 mg/kg IND), that histamine or leukotrienes become more important mediators of inflammation.

Spleen weight and SW/BW ratio followed the same pattern described for CS and VE. Those groups having the greatest lesion scores generally had significantly heavier spleens, indicating the effect of IND is directly on the intestinal mucosa and not on virus replication in the spleen.

Biochemically, there were similar patterns to those seen in other drug groups. Those groups having the higher lesion scores generally had the lowest total serum lipid, protein, and albumin, despite treatment with IND. Enzymatically, as expected from comparison with other drug treated groups, ALKPHOS was significantly lower in the group with the most severe lesion scores. CPK and electrolytes were not specifically affected by the use of IND during HEV infection.

Heterophil lymphocyte ratios correlated well with lesion score. Those birds in the 6.24 mg/kg IND group which had significantly higher lesion scores than the other treated groups or the (-) controls also had significantly greater HLRs. Those groups receiving 0.78, 1.56, or 3.12 mg/kg IND had HLRs which were not significantly different from either the drug control or the (-) control groups indicating that IND may have played a role in reducing some of the stress associated with HEV infection.

Histamine receptor antagonists [chlorpheniramine (H_1) and cimetidine (H_2)] (Tables 4a and 4b, 5a and 5b): Histamine is a vasoactive substance which has been identified in almost all mammalian, reptilian and avian tissues. Synthesized from l-histidine and stored in mast cells (in tissue) and basophils (in circulation), it is released by energy-dependent mechanisms (cAMP) in response to both immunological (IgE-antigen-mast cell mediated immune reactions) and nonimmunological reactions (trauma, toxin) (7). Histamine (H) was first

described by Dale and Laidlaw (24, 25) to cause a generalized dilatation of capillaries in peripheral organs, a resultant pooling of blood in capillary beds, and a subsequent fall in systemic blood pressure. Dale and Laidlaw (26) also reported loss of plasma through capillary epithelium producing edema and hemoconcentration. This effect is thought to occur at the postcapillary venule where H induces contraction of the endothelial cell leading to the formation of intercellular gaps between cells. It is between these cells that plasma protein may pass (79).

With the development of the first group of antihistamines in the late 1930s and early 1940s, it was soon realized that these compounds did not block all of the manifestations of histamine-induced shock and allergic reaction. Ash and Schild (5) proposed the name "H_1" for receptors blocked by the known antihistaminic drugs and presented evidence for a second type of H receptor not blocked by H_1 antagonists. Black *et al.*, (10) were later able to describe and antagonize this second type of histamine receptor and name it the H_2 receptor.

As stated earlier, the majority of stored H in the body is within mast cells and basophils. Generally a reciprocal relationship exists between tissue mast cell number and basophil number. Species which are known to have high basophil counts, like birds, have low mast cell counts (89). This work also indicated that basophils can be rapidly mobilized within the circulation and may play an important role in localized delayed immune reactions in tissue. Riley (97) found that H concentrations remained normal or rose in gastric or intestinal mucosa following depletion by chemical releasing agents or antigen origin. Lack of this response prompted Riley to refer to this as "non-mast cell" histamine. Similarly, Schayer (102) described a marked increase in histidine decarboxylase activity (the enzyme responsible for H production) in certain tissues following exposure to infectious agents or

irritants and referred to this as "inducible histamine". Schayer (102) suggested that this "inducible histamine" may play a role in regulation of microcirculation as it is located in or near vascular endothelial cells. Histamine is continually synthesized but not stored in endothelial cells. Cyclic accumulation and release of H could account for spontaneous opening and closing of small blood vessels in the vascular beds. Schayer suggested that tissue stress might lead to an induction of histidine decarboxylase. These theories may explain a source of H at the pancreatico-duodenal junction of HEV infected poults in which a cellular infiltrate (basophils or mast cells) is usually not seen.

The variability of the effects of H in tissues and organs has been attributed to differences in distribution of H_1 and H_2 receptors and the fact that these receptors generally mediate opposing effects. H_1 receptors are found predominantly in smooth muscle and large blood vessels where they produce contraction and constriction (5). H_1 receptors also act in smaller vessels where stimulation acts in a synergystic manner with H_2 receptors to induce vasodilation and cause an increase in vascular permeability. Stimulation of H_2 receptors in smooth muscle typically results in relaxation. H_2 receptors are also present in gastric mucosa, cardiac muscle, basophils and lymphocytes (7).

The application of histamine receptor antagonists will help to evaluate the possible dual effect of H in the manifestation of HEV infection. In this study chlorpheniramine and cimetidine were used as representatives of selective H_1 and H_2 receptor antagonists, respectively.

The blocking of H_1 receptors with chlorpheniramine potentiates the effects of H at H_2 receptor sites by eliminating H_1 effects. The fact that lesion score was significantly lower in the 2 mg/kg group and approached significance in the 4 mg/kg group (0.80 and 1.50 vs.

2.21, respectively) indicates that the action of H at H_1 receptors is important in the mediation of duodenal inflammation. It also implies that H_2 receptor activation may be beneficial in protection against duodenal lesion formation. This effect is contradictory to other studies in mammals where blockade of H_2 receptors inhibits gastric acid secretion and thus decreases the incidence and severity of duodenal ulcers (9, 13, 55, 60, 61, 96). The pathogenesis of these two types of lesions are different and may account for the variation in results.

H_2 receptors function in the immune system as well. Their stimulation further inhibits release of H, leukotrienes, and eosinophil chemotactic factor from leukocytes involved in immediate hypersensitivity reactions (75, 76). In the delayed hypersensitivity reaction, H_2 receptors have been shown to inhibit T-cell cytolysis (92). Histamine-dependent inhibition of eosinophil chemotaxis is also mediated through H_2 receptors (20) as is basophil chemotaxis to complement (72). In addition to beneficial immune effects of H_2 receptors possibly accounting for the improvement of lesion scores, the antagonism of the H_1 receptor may also add to the effect. This may be manifested as a decrease in edema and capillary permeability, usually attributed to the actions of H_1 receptors.

Predictably, when the H_2 receptors were antagonized with cimetidine, there was a significant ($P \leq .10$) increase in lesion score in the 50 and 100 mg/kg groups compared with the (+) control group (3.10 and 3.10 vs. 2.21, respectively). This effect may be due to the blocking of the protective effects of H_2 receptors described above, as well as to the direct inflammatory effects of H_1 receptors (increased vascular permeability, vasoconstriction) not inhibited by H_2 receptor antagonism.

Spleen weight and SW/BW ratio were significantly greater in inoculated treated birds

(all drug levels) than either the (-) or the drug controls. No significant difference existed in either the H_1 or H_2 drug control groups when compared with the (-) controls. This suggests that neither H_1 nor H_2 are responsible for splenic enlargement due to pooling of blood. More likely, this enlargement is due to lymphoid hyperplasia associated with HEV replication. H_1 antagonism at 2 and 4 mg/kg chlorpheniramine significantly increased spleen weight when compared with the (+) controls (5.16 and 5.65 vs 4.11, respectively). Perhaps at this level H action on the H_2 receptor affords intestinal protection while also producing a vasodilatory effect in the spleen causing pooling of blood.

The characteristic decrease in serum lipid reported by Soback *et al.*, (108) did not occur in either the chlorpheniramine or cimetidine treated groups as it did in the (+) control group. In fact, the lipid concentrations in each inoculated, treated group were close to the (-) control. It seems then that both H_1 and H_2 receptors may be responsible for maintaining serum lipid levels in the face of HEV infection. Protein levels in the chlorpheniramine treated groups also were maintained near (-) control levels. This again may indicate either that H_2 receptors maintain intestinal endothelial integrity (thus preventing intestinal protein loss) or that H_1 receptors disrupt the endothelial barrier and cause protein loss characteristic of HEV infection. Antagonism of H_2 receptors with cimetidine, produces lower protein levels than even the (+) controls, indicating exacerbation of protein loss by selective inhibition of H_2 (stimulation of H_1) receptors. Albumin follows a similar pattern but appears to be less specifically affected by H antagonist type and concentration.

As with other drugs, HLRs appeared to be good indicators of infection increasing significantly compared to (-) controls. Those birds, however, in the drug control groups (chlorpheniramine and cimetidine) had HLRs significantly greater than the (-) controls, but

less than the (+) controls. This indicates, as expected, that the t.i.d. administration of H antagonists was more stressful than the b.i.d. administration of other drugs.

Biochemically, chlorpheniramine prevented the characteristic decline in ALKPHOS described by Soback *et al.*, (108), while administration of cimetidine did not. This again suggests that H_1 antagonism protects intestinal integrity. CPK in both groups was not significantly affected. Sodium, chloride, and bicarbonate were unaffected by H antagonism. Potassium, however, was significantly increased over both (+) and (-) controls when cimetidine was administered. Chlorpheniramine alone (drug control group), significantly increased K^+ compared with (+) and (-) controls, but did not maintain these concentrations when HEV infected birds were treated. In this case, serum K^+ concentrations fell to the level of the (+) controls.

Aminophylline (Tables 6a and 6B): The role of cyclic nucleotides (cAMP and cGMP) in the onset and/or maintenance of disease is a consequence of their involvement in cellular activity and metabolism. The intracellular production and actions of these nucleotides represents a combined biochemical signal of hormonal, neural, and other control mechanisms. In disease, the abnormal levels of these second messengers may indicate abnormal responsiveness of the tissue to normal biological control (4).

By blocking their synthesis or degradation, the effects of these cyclic nucleotides in the disease state may be examined. Among the earliest drugs which were shown to affect cyclic nucleotides, specifically cAMP, was theophylline. Aminophylline (AP), a derivative, prevents cAMP degradation by inhibiting the phosphodiesterase enzyme responsible for normal catabolism. The fact that cAMP is so ubiquitous within different body systems might

suggest a very general effect when concentrations are altered. It has been shown, however, that certain aspects of inflammation and the immune response are highly cAMP dependent processes (15). Examples of these include the inhibition of histamine release from human leukocytes *in vitro* (16, 77) and inhibition of T cell induced cytolysis (93), both processes also involving H_2 receptors. In fact, when other effects of H_2 receptors were examined (cardiac acceleration, increased cardiac contractility, and increased gastric secretion) the close relationship between cAMP and H_2 receptor activation was revealed (8, 32, 62, 81). Dousa and Code (34) and Scholes *et al.*, (103) have also shown in gastric mucosa that activation of the cAMP system is blocked by H_2 receptor antagonism. It may be that the effects of cAMP in the pathogenesis of HE are mediated through H_2 receptors. If this were the case, however, the same effects on lesion score as seen with the administration of an H_1 antagonist or an H_2 agonist would be observed. This was not what was found in this study. Antagonism of H_1 receptors actually produced a beneficial effect on lesion score while H_2 antagonism exacerbated lesion formation. Aminophylline did not have a beneficial or deleterious effect on lesion formation. These scores (2.70-2.80) were not significantly different ($P \leq .05$) from the (+) control which received only a virus challenge with no drug therapy. The efficacy (bioavailability and dose range) of AP in birds, however, has not been well investigated, nor has its relationship to H_2 receptors and cAMP. These are all possible reasons for the failure of AP to affect lesion score. Spleen weights of AP treated birds were significantly greater than the (+) control in the face of HEV infection ($5.11 \pm .39$, $5.95 \pm .37$, $4.91 \pm .37$ vs. $4.11 \pm .22$). This is an interesting effect since AP, a sympathomimetic, causes constriction of the spleen and a subsequent reduction of splenic weight in mammals. This also adds doubt to AP effectiveness, at least as far as known effects in birds.

Heterophil lymphocyte ratios also indicated that the stress on the birds was not alleviated with the administration of AP. The birds receiving the lowest dose of AP (5 mg/kg) had an HLR significantly greater than the (+) control, while those receiving 10 and 20 mg/kg were not significantly greater than the (+) control.

Total serum lipid and protein were unaffected by AP administration. Serum albumin showed the characteristic decline in infected treated birds. These concentrations were significantly lower than the (-) controls. ALKPHOS was also characteristically lowered in infected birds receiving 5 mg/kg AP. Potassium also dropped precipitously in infected treated birds as well as in the (+) control group.

Disodium Cromoglycate (Tables 7a and 7b): The mechanism of action of disodium cromoglycate (DSCG), a drug which has received much attention for its prophylactic treatment of human bronchial asthma, is still incompletely known. The original mechanism of action of DSCG was postulated to be associated with the interference of release of vasoactive substances like histamine and leukotrienes by selectively inhibiting immunological reactions involving mast cells, surface antibodies (IgE), and antigen (22). Others (63, 64) have reported the inhibition of mast cell degranulation by DSCG following administration of polyamine 48/80, a classic mast cell secretagogue. This suggests that the action of DSCG is not restricted to antigen-evoked mast cell secretion. Wilson and McPhillips (117) speculated that DSCG may act as a mast cell membrane stabilizer while Foreman and Garland (41) and Foreman *et al.*, (42) suggested that it may facilitate closure of Ca^{++} channels in mast cell membranes. Lack of Ca^{++} mobilization would thus decrease the secretory response. Theoharides *et al.*, (115) has reported that DSCG may inhibit mast cell

degranulation by regulating the phosphorylation of a mast cell membrane protein, which may also be related to Ca^{++} mobilization. DSCG has also been reported to have the same effect as aminophylline adminstration, i.e. inhibition of phosphodiesterase and a resultant increase in cAMP levels. As discussed earlier this rise in cAMP levels would inhibit release of histamine from granulocytes (43, 70, 77, 110). In summary, DSCG does not prevent or modify mast cell-IgE binding or antigen-IgE interaction (69), but it does inhibit a subsequent step (or steps) in the reaction sequence which would otherwise result in the release of inflammatory mediators from mast cells (22).

DSCG was not effective in controlling lesion formation. Lesion scores of 2.10, 2.60, and 2.40 for the 5, 10, and 20 mg/kg doses of DSCG were not significantly different than the (+) control group. If histamine is as important in lesion formation as the histamine antagonist data suggests, it would follow that DSCG should have some effect since it inhibits both basophil and mast cell degranulation. Possible explanations for its ineffectiveness include inappropriate dosage because of lack of knowledge on the pharmacokinetics of DSCG in birds. There is also some question about the importance of mast cells in the normal immunological response in birds. As mentioned previously, Parwaresch (89) found that birds as a group have low tissue mast cell counts and correspondingly high blood basophil counts. Riley (97) also reported that some tissues, particularly, gastric and intestinal mucosa have few mast cells but high histamine levels. Because this histamine resists the depleting action of polyamine 48/80, Riley referred to this as "non-mast cell" histamine. Whatever the reason, DSCG was ineffective in the control of lesion formation in these poults.

Spleen weight and SW/BW ratio were characteristically enlarged in infected, DSCG

treated birds. Spleen weights for the 5, 10, and 20 mg/kg groups were 5.60±.39, 5.94±.41, and 5.15±.39, respectively. These weights were significantly greater than both (+) and (-) control groups (4.11±.22 and 1.74±.26, respectively). SW/BW ratio reflected a similar pattern.

Serum lipid concentrations in the 5 and 10 mg/kg treated groups were significantly reduced. Total serum protein was unaffected by treatment or infection. Albumin, however, was significantly reduced compared to the (+) and (-) controls in these groups.

HLRs were significantly increased compared to the (-) controls (3.23±.48, 4.57±.48, 3.88±.83, vs. 0.88±.33, respectively). The drug control dose also had an HLR significantly greater than the (-) controls (2.16±.48 vs. 0.88±.33). This suggests that DSCG may in itself cause a rise in HLR.

Alkaline phosphatase, creatine phosphokinase, sodium, chloride, and bicarbonate were all unaffected by DSCG treatment. Potassium, however, showed a characteristic decline in infected birds in the 5 and 10 mg/kg groups. The drug control group also had a low K^+ (2.9±.7 mEq/l) suggesting that DSCG may play a role in reducing serum K^+.

Leukotriene receptor antagonists [FPL 55712 AND FPL 57231] (Tables 8a and 8b): Leukotrienes (LTs) are members of the "eicosanoid" family which include all biologically active metabolites of arachidonic acid. Other eicosanoids include prostaglandins and thromboxanes. LTs are formed by the action of 5-lipoxygenase on arachidonic acid (58). The first step in the formation of an eicosanoid is the liberation of arachidonic acid from membrane phospholipids. This can be initiated by the binding of exotoxin, endotoxin, complement, aggregate IgE molecules, or other compounds to specific receptors (17, 19, 68).

The sulfidopeptido-leukotrienes, LTC_4, LTD_4, and LTE_4, collectively, probably account for the biologic activity of what used to be commonly known as slow-reacting substance of anaphylaxis (SRS-A). These compounds represent some of the most potent inflammatory mediators described. Dahlen (23) found LTC_4 and LTD_4 to be 1000 times more potent than histamine as a contractile agent in human bronchi *in vitro*. Other actions of these LTs include the stimulation of mucus secretion from bronchial mucus glands, contraction of microvasculature, and increasing microvascular permeability (91). LTB_4, however, exerts its major effect on cells derived from the bone marrow. Stimulation of LTB_4 receptors on human neutrophils and eosinophils increases chemokinesis (motion in response to a chemical stimulus), expression of complement receptors, complement dependent cytotoxicity, aggregation, adherence to endothelium, and lysosomal enzyme release (40, 65, 83, 87, 104). *In vitro*, LTB_4 has pronounced effects on macrophages and lymphocytes as well. LTB_4 has been shown to enhance or suppress lymphocyte proliferation, cytokine production, and cytotoxicity (57). Additionally, LTs may interact with platelet activating factor (PAF), a phospholipid released from platelets, lymphocytes, basophils, neutrophils, macrophages, and endothelial cells (107). This compound, the production of which is closely related to LT production during inflammation (95), has also been associated with the manifestations of the acute inflammatory response, i.e. mononuclear cell regulation, neutrophil and eosinophil activation, and increased vascular permeability (107). The effects of LTs described above give reason to speculate that they might be involved in the pathogenesis of intestinal lesions produced by HEV in turkeys.

To test this hypothesis, two selective competitive receptor antagonists (6, 105), FPL 55712 and FPL 57231, were used. FPL 57231 is a propionic acid analog of FPL 55712 and

retains selective "anti-SRS-A" activity, but has little general anti-allergic activity unlike its parent compound (105). In sheep these compounds have been shown to antagonize both the bronchoconstrictor effects of inhaled LTD_4 and the pulmonary pressor effects of infused LTD_4 (1,3). However, these results have not yet been confirmed in various tissues from different species and care must be taken in concluding that their sole action is that of an LT antagonist (50).

The LT antagonist FPL 55712 significantly increased hematocrit compared to the (+) and (-) controls (35.0 ± 1.9 vs. 28.5 ± 0.8 and 31.2 ± 1.0). FPL 57231, however, did not have an effect on hematocrit. Hammarstrom *et al.*, (56), however, reported that systemic administration of sulfidopeptide LTs to guinea pigs caused extravasation of plasma into tissues leading to a loss of circulating volume and a subsequent rise in hematocrit. Whether the effect seen in this study was due to the actual antagonism of LTs or another action of the antagonist is difficult to determine.

The results from this study show that LTs may play a role in the protection of, or at least maintenance of, intestinal mucosal integrity. Administration of either antagonist exacerbated lesion formation. In each case lesion scores were significantly increased ($P\leq.10$) over the (+) controls. Therefore LTs appear to be important in mucosal protection in the midst of HEV infection.

As expected, spleen weight and SW/BW ratio were significantly increased in infected birds compared to both drug and (-) controls. LTs apparently do not affect viral replication within the spleen. The decline in serum lipid concentration characteristic of HEV infection was absent as it was with indomethacin administration. Again, as suggested earlier, perhaps a change in the normal pathways of lipid metabolism may somehow influence total serum

lipid. Total serum protein and albumin, however, were significantly lower than the (-) control and in most cases the (+) control. This again suggests that the absence of LTs at the mucosal level may lead to increased vascular permeability and protein loss.

HLRs reflected the high degree of stress these birds underwent as a result of HEV infection. Each group, with the exception of the group receiving 2.0 mg/kg FPL 57231, had HLRs significantly greater than the (-) controls. The 2.0 mg/kg group, however, only contained one observation and it is believed that this was the reason for the lower value. Biochemically, there was no parameter, enzyme or electrolyte, that was specifically affected by LT antagonist administration.

Each table contains results of the agar gel precipitin test (AGP) performed on spleens from each bird. Antigen detection from infected groups ranged from 30-100%. There was no correlation between those birds having lesion scores of 0 and those birds having a (-) AGP result. In fact, birds with lesion scores of 4 and significant splenic lymphoid hyperplasia occasionally had (-) AGP results. Although a very specific test, the varying degree of antigen detection from splenic tissue may be attributed to the sensitivity of the test or, perhaps, because spleens were collected 1 day after what is considered optimal for detection of antigen.

Conclusion: The pathogenesis of intestinal lesions as a result of HEV infection has remained a mystery since its first description by Pomeroy and Fenstermacher (94). In as far as can be ascertained, this study, using both wide-acting anti-inflammatory drugs and selective receptor antagonists in an attempt to prevent lesion formation, appears to be the first biochemical-pharmacological investigation of lesion formation. The inherent limitation of a

pharmacologic study like this is the possibility that the anti-inflammatory agents employed may have acted at sites or had effects other than those that they were specifically implemented for. Recognition of this fact and the inclusion of at least three points on each drug curve should address this issue and provide results that are more easily interpreted.

Corticosterone and vitamin E, both non-specific anti-inflammatory agents, greatly reduced lesion formation. Their ability to stabilize biomembranes and affect the immune system are well documented. The eicosanoids were antagonized with the use of indomethacin (prostaglandins) and FPL 55712 and 57231 (leukotrienes). This data suggested that leukotrienes may be important in maintaining intestinal integrity in the face of HEV infection. Antagonizing LT action exacerbated lesion formation, while blocking PG synthesis, and thus theoretically increasing LT production, decreased lesion score.

Finally, selective antagonism of both H_1 and H_2 receptors revealed that H_1 receptors may be important in lesion formation while H_2 receptors may be important in intestinal protection. Assuming proper pharmacokinetic values, the failure of DSCG to effect lesion formation suggests, tentatively, that mast cell histamine may not be the major source and that the histamine derived from other cells may play a role in pathogenesis. It appears from this study that lesion formation may be induced by several chemical mediators. However, further research will be necessary to understand the pathogenesis of their release and the likely involvement of other immunological mediators. The virus' predilection for the pancreatico-duodenal junction is yet another intriguing question which remains to be investigated.

References

1. Abraham, W.M.E., A. Russi, J. Wanner, J.C. Delehunt, L.D. Yerger, G.A. Chapman. Production of early and late pulmonary responses with inhaled leukotriene D_4 in allergic sheep. Prostaglandins. 29:715-726. 1985.

2. Adams, S.S., and R. Cobb. A possible basis for the anti-inflammatory activity of salicylates and other non-hormonal anti-rheumatic drugs. Nature. 181:773-775. 1958.

3. Ahmed, T., B. Marchette, A. Wanner, L. Yerger. Direct and indirect effects of leukotriene D_4 on the pulmonary and systemic circulations. Am. Rev. Resp. Dis. 131:554-558. 1985.

4. Amer, M.S., Cyclic nucleotides as targets for drug design. In: Advances in drug research. Harper, N.J., and A.B. Simmonds, eds. Academic Press. New York. pp. 1-38. 1977.

5. Ash, A.S.F., and H.O. Schild. Receptors mediating some actions of histamine. Br. J. Pharmacol. 27:427-439. 1966.

6. Augstein, J., J.B. Farmer, T.B. Lee, P. Sheard, M.L. Tattersall. Selective inhibition of slow reacting substance of anaphylaxis. Nature. 245:215-216. 1973.

7. Beaven, M.A. Histamine: Its role in physiological and pathological processes. In: Monographs in allergy. Vol 13. Karger. New York. pp. 1-114. 1978.

8. Bieck, P.R., J.A. Oates, G.A. Robinson, R.B. Adkins. Cyclic AMP in the regulation of gastric secretion in dogs and humans. Am. J. Physiol. 224:158-164. 1973.

9. Binder, H.J., A. Cocco, R.J. Crossley, W. Finkelstein, R. Font, G., Friedman, J. Groarke, W. Hughes, A.F. Johnson. Cimetidine in the treatment of duodenal ulcer: a multicenter double blind study. Gastroenterol. 74(supp):380-388. 1978.

10. Black, J.W., W.A.M. Duncan, C.J. Durant, C.R. Ganellin, E.M. Parsons. Definition and antagonism of histamine H_2-receptors. Nature. 236:385-390. 1972.

11. Blackham, A., and R.T. Owen. J. Prostaglandin synthetase inhibitors and leukocytic emigration. J. Pharmacy and Pharmacology. 27:201-203. 1975.

12. Blackwell, G.J., R. Carnuccio, M. DiRosa, R.J. Flower, L. Parente, P. Persico. Macrocortin: a polypeptide causing the antiphospholipase effect of glucocorticoids. Nature. 287:147-149. 1980.

13. Blackwood, W.S., R.G. Pickard, D.P. Maudgal, D. Lawrence, T.C. Northfield. Cimetidine in duodenal ulcer: a controlled trial. Lancet. 2:174-176. 1976.

14. Booth, N.E. Non-narcotic analgesics. In: Veterinary pharmacology and therapeutics. 5th ed. Booth, N.E. and L.E. McDonald, eds. Iowa State University Press. Ames, IA. pp. 329-362. 1982.

15. Bourne, H.R., L.M. Lichtenstein, K.L. Melmon, C.S. Henney, Y. Weinstein, G.M. Shearer. Modulation of inflammation and immunity by cyclic AMP. Science. 184:19-28. 1974.

16. Bourne, H.R., K.L. Melmon, L.M. Lichtenstein. Histamine augments leukocyte cyclic AMP and blocks antigenic histamine release. Science. 173:743-745. 1971.

17. Bremm, K.D., H.J. Brom, J.E. Alouf, W. Konig, B. Spur, A. Crea, W. Peters. Generation of leukotrienes from human granulocytes by alveolysin from *Bacillus alvei*. Infect. Immun. 44:188-193. 1984.

18. Brown, J.H., J.L. Taylor, I.W. Walters. Effect of pH on erythrocyte stabilization by anti-inflammatory drugs. Proc. Soc. Exper. Biol. Med. 136:137-140. 1971.

19. Clancy, R.M., and T.E. Hugli. Role of complement anaphylatoxins in neutrophil arachidonic acid metabolism. In: Progress in clinical and biological research. Leukotrienes in cardiovascular and pulmonary function. Lefer, A.M. and M.H. Gee, eds. Alan R. Liss. New York. pp.173-184. 1985.

20. Clark, R.A.F., J.A. Sandler, J.I. Gallin, A.P. Kaplan. Histamine modulation of eosinophil migration. J. Immunol. 118:137-145. 1977.

21. Cline, M.J., and K.L. Melmon. Plasma kinins and cortisol: a possible explanation of the anti-inflammatory action of cortisol. Science. 153:1135-1138. 1966.

22. Cox, J.S.G., J.E. Beach, A.M.J.N. Blair, A.J. Clarke, J. King, T.B. Lee, D.E.E. Loveday, G.F. Moss, T.S.C. Orr, J.T. Ritchie, P. Sheard. Disodium cromoglycate (Intal). In: Advances in drug research. Vol. 5. Harper, N.J., and A.B. Simmonds, eds. Academic Press. New York. pp.115-196. 1970.

23. Dahlen, S.E. Pulmonary effects of leukotrienes. Acta Physiol. Scand. 512(supp):1-51. 1983.

24. Dale, H.H., and P.P. Laidlaw. The physiological action of ß-imidazolylethylamine. J. Physiol., Lond. 41:318-344. 1910.

25. Dale, H.H., and P.P Laidlaw. Further observations on the action of ß-imidazolylethylamine. J. Physiol., Lond. 43:182-195. 1911.

26. Dale, H.H., and P.P. Laidlaw. Histamine shock. J. Physiol., Lond. 52:355-390. 1919.

27. Davis, L.E., Fever. J. Amer. Vet. Med. Assoc. 175:1210-1211.1979.

28. Dirosa, M., R.J. Flower, F. Hirata, L. Parente, F. Russo-Marie. Antiphospholipase proteins. Prostaglandins. 28:441-442. 1984.

29. Domermuth, C.H., and W.B. Gross. Hemorrhagic enteritis and related infections. In: Diseases of poultry. Calnek B.W., H.J. Barnes, C.W. Beard, W.M. Reid and H.W. Yoder, Jr., eds. 9th ed. Iowa State University Press. Ames, IA. pp. 567-572. 1991.

30. Domermuth, C.H., W.B. Gross, R.T. DuBose. Microimmunodiffusion test for hemorrhagic enteritis of turkeys. Avian Dis. 17:439-444. 1973.

31. Domermuth, C.H., W.B. Gross, C.S. Douglass, R.T. DuBose, J.R. Harris, R.B. Davis. Vaccination for hemorrhagic enteritis of turkeys. Avian Dis. 21:557-565. 1977.

32. Domschke, W., S. Domschke, M. Classen, L. Demling. Histamine and cyclic 3',5'-AMP in gastric acid secretion. Nature. 241:454-455. 1973.

33. Dougherty, T.F., M.L. Berliner, G.L. Schneebeli, and D.L. Berliner. Hormonal control of lymphatic structure and function. Ann. N.Y. Acad. Sci. 113:825-829. 1964.

34. Dousa, T.P., and C.F. Code. Effect of histamine and its methyl derivatives on cyclic AMP metabolism in gastric mucosa and its blockade by an H_2-receptor antagonist. J. Clin. Invest. 53:334-337. 1974.

35. Douwes, F.R. The effect of anti-inflammatory drugs on phagocytosis and lysosomal enzymes in granulocytic leukocytes. Int. J. Clin Pharm. Therap. Tox. 9:243-248. 1974.

36. Dukor, D., and F.M. Dietrich. Characteristic features of immunosuppression by steroids and cytotoxic drugs. Int. Arch. Allergy Appl. Immunol. 34:32-48. 1968.

37. Duncan R.J., and K.W. Prasse. Veterinary laboratory medicine. Iowa State University Press. Ames, IA. pp. 121-144. 1986.

38. Flower, R.J. Drugs which inhibit prostaglandin biosynthesis. Pharmacologic. Rev. 26:33-67. 1974.

39. Flower, R.J., and G.J. Blackwell. Anti-inflammatory steroids induce biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation. Nature. 278:456-459. 1979.

40. Ford-Hutchinson, A.W., M.A. Bray, M.V. Doig, M.E. Shipley, M.J.H. Smith. Leukotriene B, a potent chemokinetic and aggregating substance from polymorphonuclear leukocytes. Nature. 286:264265. 1980.

41. Foreman, J.C., and L.G. Garland. Cromoglycate and other anti-allergic drugs: a possible mechanism of action. Br. Med. J. 1:820-822. 1976.

42. Foreman, J.C., M.B. Hallett, J.L. Mongar. Site of action of the anti-allergic drugs cromoglycate and doxantrozole. Br. J. Pharmacol. 59:473P-474P. 1977.

43. Foreman, J.C., J.L. Mongar; B.D. Gomperts, L.G. Garland. A possible role for cyclic AMP in the regulation of histamine secretion and the action of cromoglycate. Biochem. Pharmacol. 24:538-540. 1975.

44. Freeman, B.A., and J.D. Crapo. Free radicals and tissue injury. Lab. Invest. 47:412-426. 1982.

45. Fruham, G.J. Extravascular mobilization of neutrophils. Ann. N.Y. Acad. Sci. 113:968-982. 1964.

46. Gabrielson, A.E. and R.A. Good. Chemical suppression of adoptive immunity. Advan. Immunol. 6:91-230. 1967.

47. Gale, H., and J.W. Wyne. Preliminary observations on hemmorhagic enteritis of turkeys. Poult. Sci. 36:1267-1270. 1957.

48. Gerber, D.A., R. Giustra, N. Cohen. The ability of anti-inflammatory compounds to accelerate a disulfide interchange reaction in serum. Arthritis and Rheumatism. 9:507. 1966.

49. Germuth, F.G. The role of adrenocortical steroids in infection, immunity and hypersensitivity. Pharmacol. Rev. 8:1-24. 1956.

50. Goldberg, R.N., C. Suguihara, T. Ahmed, B. Deseda de Cudemus, P. Barrios, E. Setzer, E. Bancalari. Influence of an antagonist of slow-reacting substance of anaphylaxis on the cardiovascular manifestations of hypoxia in piglets. Pediatric Res. 19:1201-1205. 1985.

51. Gross, W.B., and G. Colmano. Corticosterone and ACTH as treatments for *Escherichia coli* infection in chickens. Poult. Sci. 49:1256-1258. 1970.

52. Gross, W.B., and W.E.C. Moore. Hemorrhagic enteritis of turkeys. Avian Dis. 11:296-307. 1967.

53. Gross, W.B., and H.S. Siegel. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27:972-979. 1983.

54. Gross, W.B., P.B. Siegel, and R.T. DuBose. Some effects of feeding corticosterone to chickens. Poult. Sci. 59:516-522. 1980.

55. Haggie, S.J., D.C. Fermont, J.H. Wyllie. Treatment of duodenal ulcer with cimetidine. Lancet. 1:983-984. 1976.

56. Hammarstrom, S., X.Y. Hua, S.E. Dahlen, J.M. Lundberg, P. Hedqvist. Microcirculatory effects of leukotrienes C_4, D_4 and E_4 in the guinea pig. In: Progress in clinical and biological research. Leukotrienes in cardiovascular and pulmonary function. Lefer, A.M., and M.H. Gee, eds. Alan R. Liss. New York. pp.35-46. 1985.

57. Hansen, B. Leukotrienes. J. Vet. Intern. Med. 3:59-72. 1989.

58. Hedqvist, P., S.E. Dahlen, L. Gustafsson, S.A. Hammarstrom, B. Samuelsson. Biological profiles of leukotrienes C_4 and D_4. Acta Physiol. Scand. 110:331-333. 1980.

59. Heinzerling, R.H., C.F. Nockels, C.L. Quarels, R.P. Tengerdy. Protection of chicks against *E. coli* infection by dietary supplementation with vitamin E. Proc. Soc. Exp. Biol. Med. 146:279-283. 1974.

60. Henn, R.M., J.I. Isenberg, V. Maxwell, R.A.L. Sturdevant. Inhibition of gastric acid secretion by cimetidine in patients with duodenal ulcer. N. Eng. J. Med. 293:371-376. 1975.

61. Hetzel, D.J., J. Hansky, D.J.C. Shearman. Cimetidine treatment of duodenal ulcer: Short term clinical trial and maintenance study. Gastroenterol. 74(supp):389-392. 1978.

62. Johnson, C.L., and H. Mizoguchi. The interaction of histamine and guanylnucleotides with cardiac adenylate cyclase and its relationship to cardiac contractility. J. Pharmacol Exp. Ther. 200:174-186. 1977.

63. Johnson, H.G., and C.A. Van Hout. Inhibition of allergic reactions by cromoglycate and by a new anti-allergic drug (10-chloro-1,4,6,9,-tetrahydro-4, 6- dioxopyrido [3,2-g] quinoline-2, 8-dicarboxylic acid). Int. Arch. Allergy Appl. Immunol. 50:446-453. 1976.

64. Johnson, H.G., C.A. Van Hout, J.B. Wright. Inhibition of allergic reactions by cromoglycate and by a new anti-allergy drug U42,585E. Int. Arch. Allergy Appl. Immunol. 56:416-423. 1978.

65. Jubiz, W. A reevaluation of the chemotactic potency of leukotriene B_4 (LTB_4). Biochem. Biophys. Res. Commun. 110:842-850. 1983.

66. Kass E.H., Hormones and host resistance to infection. Bacteriol. Rev. 24:177-185. 1960.

67. Kindahl, H., Prostaglandin biosynthesis and metabolism. J. Amer. Vet. Med. Assoc. 176:1173-1177. 1980.

68. Konig, W., J. Scheffer, K.D. Bremm. Role of bacterial adherence and toxin production from *Escherichia coli* on leukotriene generation from human polymorphonuclear granulocytes. Int. Arch. Allergy Appl. Immunol. 77:118-120. 1985.

69. Kusner, E.J., B. Dubnick, D.J. Herzig. The inhibition by disodium cromoglycate *in vitro* of anaphylactically induced histamine release from rat peritoneal mast cells. J. Pharmacol. Exp. Ther. 184:41-46. 1973.

70. Lavin, N., G.S. Rachelefsky, S.A. Kaplan. An action of disodium cromoglycate: inhibition of cyclic 3',5'-AMP phosphodiesterase. J. Allergy Clin. Immunol. 57:80-88. 1976.

71. Lee, T.H., E. Israel, J. Drazen, A.G. Leitch, J. Ravalese, E.J. Corey, D.R. Robinson, R.A. Lewis, K.F. Austen. Enhancement of plasma levels of biologically active leukotriene B compounds during anaphylaxis in guinea pigs pretreated by indomethacin or by a fish oil-enriched diet. J. Immunol. 136:2575-2582. 1986.

72. Lett-Brown, M.A., and E.J. Leonard. Histamine induced inhibition of normal human basophil chemotaxis to C5a. J. Immunol. 118:815818. 1977.

73. Levi, R., G. Allen, J.H. Zavecz. Prostaglandins and cardiac anaphylaxis. Life Sci. 18:1255-1264. 1976.

74. Lickoff, R.O., D.R. Guptill, L.M. Lawrence, C.C., McKay, M.M. Mathias, C.F. Nockels, R.P. Tengerdy. Vitamin E and aspirin depress prostaglandins in protection of chickens against *Escherichia coli* infection. Am. J. Clin. Nut. 34:245-251. 1981.

75. Lichtenstein, L.M., and E. Gillespie. Inhibition of histamine release by histamine is controlled by an H_2 receptor. Nature. 244:287-288. 1973.

76. Lichtenstein, L.M., and E. Gillespie. The effects of H_1- and H_2-antihistamines on 'allergic' histamine release and its inhibition by histamine. J. Pharmacol. Exp. Ther. 192:441-450. 1975.

77. Lichtenstein, L.M., and S. Margolis. Histamine release *in vitro*: Inhibition by catecholamines and methylxanthines. Science. 161:902-903. 1968.

78. Machlin, J.E., Vitamin E and prostaglandins. In: Tocopherol, oxygen, and biomembranes. de Duve, C., and O. Hayaishi, eds. Elsevier. N.Y. pp. 179-189. 1978.

79. Majno, G., S.M. Shea, M. Leventhal. Endothelial contraction induced by histamine-type mediators. An electron microscopic study. J. Cell. Biol. 42:647-672. 1969.

80. McDonald, L.E. Hormones influencing metabolism. In: Veterinary pharmacology and therapeutics. 6th ed. Booth, N.E. and L.E. McDonald, eds. Iowa State University Press, Ames, IA. pp. 616-655. 1988.

81. McNeill, J.H., and S.C. Verma. Stimulation of rat gastric adenylate cyclase by histamine and histamine analogues and blocked by burimamide. Br. J. Pharmacol. 52:104-106. 1974.

82. Mizushima, Y., S. Sakai, M. Yamaura. Mode of stabilizing action of non-steroid anti-inflammatory drugs on erythrocyte membranes. Biochem. Pharmacol. 19:227-234. 1970.

83. Moqbel, R., S.P. Sass-Kuhn, E.J. Goetzl. Enhancement of neutrophil- and eosinophil-mediated complement-dependent killing of schistosomula of *Schistosoma mansoni in vitro* by leukotriene B_4. Clin. Exp. Immunol. 52:519-527. 1983.

84. Nakanishi, M., and K. Goto. Inhibitory effects by anti-inflammatory drugs on enzyme release from rabbit polymorphonuclear leukocyte lysosomes. Biochem. Pharmacol. 24:421-424. 1975.

85. National Research Council. Nutrient Requirements of Poultry. National Acadamy Press. Washington, D.C. pp. 16-18. 1984.

86. Nockels, C.F. Protective effects of supplemental vitamin E against infection. Fed. Proc. 38:2134-2148. 1979.

87. Palmer, R.M.J., R.J. Stepney, G.A. Higgs, K.E. Eakins. Chemokinetic activity of arachidonic acid lipoxygenase products on leukocytes of different species. Prostaglandins. 20:411-418. 1980.

88. Panganamala, R.V., and D.G. Cornwell. The effects of vitamin E on arachidonic acid metabolism. Ann. N.Y. Acad. Sci. 393:376-390. 1982.

89. Parwaresch, M.R. The human blood basophil. Springer Verlag. New York. pp. 69-76. 1976.

90. Phelps, P., and D.J. McCarty. Suppressive effects of indomethacin on crystal-induced inflammation in canine joints and on neutrophilic motility in vivo. J. Pharmacol. Exp. Ther. 158:546-553. 1967.

91. Piper, P.J. Pharmacology of leukotrienes. Br. Med. Bull. 39:255-259. 1983.

92. Plaut, M., L.M. Lichtenstein, E. Gillespie, C.S. Henney. Studies on the mechanism of lymphocyte-mediated cytolysis. IV. Specificity of the histamine receptor on effector T cells. J. Immunol. 111:389-394. 1973.

93. Plaut, M., L.M. Lichtenstein, and C.S. Henney. Properties of a subpopulation of T-cells bearing histamine receptors. J. Clin. Invest. 55:856-874. 1974.

94. Pomeroy, B.S., and R. Fenstermacher. Hemorrhagic enteritis in turkeys. Poult. Sci. 16:378-382. 1937.

95. Ramesha, C., and W.C. Pickett. Platelet-activating factor and leukotriene biosynthesis in inhibited in polymorphonuclear leukocytes depleted of arachidonic acid. J. Biol. Chem. 261:7592-7595. 1986.

96. Richardson, C.T., J.H. Walsh, M.I. Hicks. The effect of cimetidine, a new histamine H_2-receptor antagonist, on meal-stimulated acid secretion, serum gastrin, and gastric emptying in patients with duodenal ulcer. Gastroenterol. 71:19-23. 1976.

97. Riley, J.R. The mast cells. Livingstone. Edinburgh. 1959.

98. Robert, A., An intestinal disease produced experimentally by a prostaglandin deficiency. Gastroenterology 69:1045-1047. 1975.

99. Roubal, Z., and O. Nemecek. Anti-inflammatory compounds exhibiting fibrinolytic activity. J. Med. Chem. 9:840-842. 1966.

100. Sato, K., and B. Glick. Antibody and cell mediated immunity in corticosteroid-treated chicks. Poult. Sci. 49:982-986. 1970.

101. Schayer, R.W. Induced synthesis of histamine, microcirculatory regulation and the mechanism of action of the adrenal glucocorticoid hormones. In: Progress in allergy. Vol. 7. New York. Karger. pp. 187-212. 1963.

102. Schayer, R.W. Enzymatic formation of histamine from histidine. In: Handbook of experimental pharmacology. Vol. 18. Rocha E. Silva, ed. Springer. Berlin. pp. 688-725. 1966.

103. Scholes, P., A. Cooper, D. Jones, J. Major, M. Walters, C. Wilde. Characterization of an adenylate cyclase system sensitive to histamine H_2-receptor excitation in cells from dog gastric mucosa. Agents Actions. 6:677-682. 1976.

104. Serhan, C.N., A. Radin, J.E. Smolen. Leukotriene B_4 is a complete secretagogue in human neutrophils: A kinetic analysis. Biochem. Biophys. Res. Commun. 107:1006-1012. 1982.

105. Sheard P., M.C. Holroyde, A.M. Ghelani, J.R. Bantick, T.B. Lee. Antagonist of SRS-A and leukotrienes. In: Advances in prostaglandin, thromboxane and leukotriene research. vol. 9. Samuelsson, B., and R. Paoletti, eds. Raven Press. New York. pp. 229-235. 1982.

106. Shen Y.T., and C.A. Winter. Chemical and biological studies on indomethacin, sulindac, and their analogs. In: Advances in drug research. Harper, N.J., and A.B. Simmonds, eds. Academic Press. New York. pp. 89-245. 1977.

107. Snyder, F. Ether-linked choline-phospholipids. Med. Res. Rev. 5:107-140. 1985.

108. Soback, S.E., E. Bogin, and Y. Weisman. Biochemical changes in the blood, spleen, and duodenum of turkeys experimentally and naturally exposed to haemorrhagic enteritis virus. Avian Pathol. 14:189-197. 1985.

109. Tappel, A.L., Vitamin E as the biological lipid antioxidant. Vit. Hor. 20:493-510. 1962.

110. Tateson, J., and D.G. Trist. Inhibition of adenosine-3',5'-cyclic monophosphate phosphodiesterase by potential antiallergic compounds. Life Sci. 18:153-161. 1976.

111. Tauber, A.I., M. Kaliner, D.J. Stechschulte, K.F. Austen. Immunologic release of histamine and slow reacting substance of anaphylaxis from human lung. V. Effects of prostaglandins on release of histamine. J. Immunol. 111:27-37. 1973.

112. Tengerdy, R.P., R.H. Heinzerling, M.M. Mathias. Effect of vitamin E on disease resistance and immune responses. In: Tocopherol, oxygen, and biomembreanes. deDuve, C., and O. Hayaishi, eds. Elsevier. N.Y. pp. 191-200. 1978.

113. Tengerdy, R.P. and J.C. Brown. Effect of vitamin E and A on humoral immunity and phagocytosis in *E. coli* infected chickens. Poult. Sci. 56:957-963. 1977.

114. Tengerdy, R.P. and C.F. Nockels. Vitamin E and vitamin A protect chickens against *E. coli* infection. Poult. Sci. 54:1292-1296. 1975.

115. Theoharides, T.C., W. Sieghart, P. Greengard, W.W. Douglas. Antiallergic drug cromolyn may inhibit histamine secretion by regulating phosphorylation of a mast cell protein. Science. 207:80-82. 1980.

116. Vane, J.R., Inhibition of prostaglandin synthesis as a mechanism of action of aspirin like drugs. Nature. 231:232-235 1971.

117. Wilson, A.F., and J.J. McPhillips. Pharmacological control of asthma. Ann. Rev. Pharmacol. Toxicol. 18:541-561. 1978.

Table 1a. The effect of corticosterone on hematocrit, lesion score, spleen weight, SW/BW ratio, and serum lipid, protein, and albumin concentration and heterophil lymphocyte ratio.[1]

Treatment	Hematocrit	Lesion Score	Spleen Wt. (g)	SW/BW Ratio	(Lipid) (mg/dl)	(Protein) (g/dl)	(Albumin) (g/dl)	H/L Ratio
Control (CS 25 mg/kg, no virus)	30.3 ± 1.1^{a}	0.00^{a}	$1.42 \pm .26^{a}$	$0.76 \pm .11^{a}$	25.6 ± 3.2^{b}	$4.81 \pm .31^{d}$	$1.86 \pm .12^{bc}$	$1.73 \pm .34^{a}$
CS 15 mg/kg	36.6 ± 1.4^{b}	0.50^{a}	$1.84 \pm .37^{a}$	$1.62 \pm .16^{bc}$	14.5 ± 3.9^{a}	$5.14 \pm .37^{d}$	$1.99 \pm .16^{d}$	$1.14 \pm .45^{a}$
CS 25 mg/kg	33.6 ± 1.4^{b}	0.40^{a}	$1.48 \pm .37^{a}$	$1.31 \pm .16^{b}$	14.9 ± 3.9^{a}	$4.81 \pm .37^{d}$	$1.98 \pm .16^{d}$	$0.94 \pm .45^{a}$
CS 35 mg/kg	35.1 ± 1.4^{b}	0.30^{a}	$1.24 \pm .37^{a}$	$1.07 \pm .16^{ab}$	17.7 ± 3.9^{ab}	$4.75 \pm .37^{d}$	$1.88 \pm .15^{bcd}$	$1.18 \pm .45^{a}$
CS 45 mg/kg	31.5 ± 1.4^{a}	3.00^{b}	$3.66 \pm .39^{b}$	$1.43 \pm .16^{b}$	31.6 ± 6.2^{b}	$1.42 \pm .53^{a}$	$0.46 \pm .21^{a}$	$3.60 \pm .51^{b}$
(-) Control (no virus, no drug)	31.2 ± 1.0^{a}	0.00^{a}	$1.74 \pm .26^{a}$	$0.92 \pm .11^{a}$	25.3 ± 3.2^{b}	$3.41 \pm .30^{c}$	$1.58 \pm .12^{c}$	$0.88 \pm .33^{a}$
(+) Control (virus only, no drug)	28.5 ± 0.8^{a}	2.21^{b}	$4.11 \pm .22^{b}$	$1.91 \pm .09^{c}$	15.0 ± 2.8^{a}	$2.77 \pm .26^{b}$	$1.27 \pm .40^{b}$	$3.01 \pm .29^{b}$

[1]Means within parameter with different superscripts are significantly different from each other ($P \leq .05$).

Table 1b. The effect of corticosterone on serum alkaline phosphatase (ALKPHOS), creatine phosphokinase (CPK), sodium, potassium, chloride, and bicarbonate.[1]

Treatment	ALKPHOS (U/L)	CPK (U/L)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)	% Infected[2]
Control (CS 25 mg/kg, no virus)	2294 ± 257^{c}	1234 ± 838^{a}	152.7 ± 2.0^{ab}	$7.0 \pm .5^{c}$	110.5 ± 1.6^{a}	20.1 ± 1.0^{b}	0.00
CS 15 mg/kg	2064 ± 257^{bc}	4940 ± 1185^{b}	153.6 ± 2.6^{abc}	$3.1 \pm .7^{ab}$	112.6 ± 2.1^{a}	17.0 ± 1.4^{a}	40.00
CS 25 mg/kg	2286 ± 257^{c}	1810 ± 1185^{a}	161.0 ± 2.6^{c}	$3.2 \pm .7^{ab}$	119.0 ± 2.1^{bc}	18.7 ± 1.4^{ab}	40.00
CS 35 mg/kg	1732 ± 257^{abc}	1462 ± 1185^{a}	154.6 ± 2.6^{abc}	$2.8 \pm .7^{a}$	114.2 ± 2.1^{ab}	18.6 ± 1.4^{ab}	30.00
CS 45 mg/kg	1494 ± 257^{ab}	890 ± 1185^{a}	147.7 ± 2.6^{a}	$7.6 \pm .7^{c}$	112.9 ± 2.1^{ab}	26.1 ± 1.4^{c}	60.00
(-) Control (no virus, no drug)	1653 ± 182^{b}	3061 ± 838^{ab}	159.0 ± 1.9^{c}	$8.3 \pm .5^{c}$	120.7 ± 1.5^{c}	22.4 ± 1.0^{bc}	0.00
(+) Control (virus only, no drug)	1360 ± 148^{a}	993 ± 684^{a}	155.2 ± 1.5^{b}	$4.5 \pm .4^{b}$	116.5 ± 1.2^{b}	20.3 ± 0.8^{b}	83.33

[1]Means within parameter with different superscripts are significantly different from each other ($P \leq .05$).
[2]Percent of total birds per treatment which tested (+) for HEV antigen with AGID.

Table 2a. The effect of vitamin E on hematocrit, lesion score, spleen weight, SW/BW ratio, and serum lipid, protein, and albumin concentration and heterophil lymphocyte ratio.[1]

Treatment	Hematocrit	Lesion Score	Spleen Wt. (g)	SW/BW Ratio	(Lipid) (mg/dl)	(Protein) (g/dl)	(Albumin) (g/dl)	H/L Ratio
Control (VE 50 mg/kg, no virus)	31.6 ± 1.0^{b}	0.00^{a}	$1.77 \pm .27^{a}$	$0.93 \pm .11^{a}$	24.8 ± 3.5^{c}	$3.07 \pm .31^{bc}$	$1.67 \pm .17^{b}$	$0.93 \pm .32^{a}$
VE 25 mg/kg	29.8 ± 1.5^{ab}	2.10^{b}	$3.08 \pm .37^{b}$	$2.26 \pm .16^{c}$	6.4 ± 5.1^{a}	$2.83 \pm .44^{bc}$	$1.71 \pm .16^{b}$	$1.47 \pm .45^{a}$
VE 50 mg/kg	32.3 ± 1.4^{b}	1.00^{a}	$3.13 \pm .37^{b}$	$2.42 \pm .16^{c}$	10.1 ± 4.1^{a}	$4.00 \pm .39^{c}$	$1.44 \pm .13^{b}$	$1.72 \pm .45^{a}$
VE 100 mg/kg	31.0 ± 1.5^{ab}	0.60^{a}	$2.63 \pm .37^{ab}$	$2.11 \pm .16^{bc}$	12.1 ± 3.9^{a}	$3.28 \pm .37^{bc}$	$1.56 \pm .16^{b}$	$1.10 \pm .45^{a}$
VE 200 mg/kg	28.3 ± 1.6^{ab}	4.40^{c}	$4.54 \pm .37^{c}$	$1.93 \pm .16^{b}$	8.1 ± 6.2^{a}	$1.04 \pm .52^{a}$	$0.26 \pm .21^{a}$	$3.90 \pm .48^{b}$
(-) Control (no virus, no drug)	31.2 ± 1.0^{ab}	0.00^{a}	$1.74 \pm .26^{a}$	$0.92 \pm .11^{a}$	25.3 ± 3.2^{c}	$3.41 \pm .30^{bc}$	$1.58 \pm .12^{b}$	$0.88 \pm .33^{a}$
(+) Control (virus only, no drug)	28.5 ± 0.8^{a}	2.21^{b}	$4.11 \pm .22^{c}$	$1.91 \pm .09^{b}$	15.0 ± 2.8^{b}	$2.77 \pm .26^{b}$	$1.27 \pm .40^{b}$	$3.01 \pm .29^{b}$

[1]Means within parameter with different superscripts are significantly different from each other ($P \leq .05$).

Table 2b. The effect of vitamin E on serum alkaline phosphataste (ALKPHOS), creatine phosphokinase (CPK), sodium, potassium, chloride, and bicarbonate.[1]

Treatment	ALKPHOS (U/L)	CPK (U/L)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)	% Infected[2]
Control (VE 50 mg/kg, no virus)	1643 ± 182^{b}	1099 ± 838^{a}	159.7 ± 2.9^{b}	$7.9 \pm .5^{b}$	119.5 ± 1.5^{a}	21.3 ± 1.6^{b}	0.00
VE 25 mg/kg	1255 ± 287^{b}	1460 ± 1325^{a}	154.4 ± 2.9^{ab}	$7.4 \pm .8^{b}$	121.4 ± 2.4^{a}	20.4 ± 1.6^{b}	90.00
VE 50 mg/kg	1262 ± 257^{b}	2118 ± 1185^{a}	157.7 ± 1.9^{ab}	$7.6 \pm .8^{b}$	116.8 ± 2.4^{a}	20.4 ± 1.0^{b}	80.00
VE 100 mg/kg	1098 ± 257^{b}	2628 ± 1185^{a}	157.8 ± 2.6^{ab}	$3.4 \pm .7^{a}$	121.3 ± 2.1^{a}	16.4 ± 1.4^{a}	90.00
VE 200 mg/kg	632 ± 257^{a}	538 ± 1185^{a}	152.2 ± 2.6^{a}	$6.8 \pm .7^{b}$	118.3 ± 2.1^{a}	20.5 ± 1.4^{b}	70.00
(-) Control (no virus, no drug)	1653 ± 182^{b}	3061 ± 838^{a}	159.0 ± 1.9^{b}	$8.3 \pm .5^{b}$	120.7 ± 1.5^{a}	22.4 ± 1.0^{b}	0.00
(+) Control (virus only, no drug)	1360 ± 148^{b}	993 ± 684^{a}	155.2 ± 1.5^{ab}	$4.5 \pm .4^{a}$	116.5 ± 1.2^{a}	20.3 ± 0.8^{b}	83.33

[1]Means within parameter with different superscripts are significantly different form each other ($P \leq .05$).
[2]Percent of total birds per treatment which tested (+) for HEV antigen with AGID.

Table 3a. The effect of indomethacin on hematocrit, lesion score, spleen weight, SW/BW ratio, and serum lipid, protein, and albumin concentration and heterophil lymphocyte ratio.[1]

Treatment	Hematocrit	Lesion Score	Spleen Wt. (g)	SW/BW Ratio	(Lipid) (mg/dl)	(Protein) (g/dl)	(Albumin) (g/dl)	H/L Ratio
Control (I 1.56 mg/kg, no virus)	31.1 ± 1.0^{b}	0.00^{a}	$1.70 \pm .26^{a}$	$0.91 \pm .11^{a}$	32.5 ± 3.2^{b}	$3.34 \pm .30^{bc}$	$1.59 \pm .12^{bc}$	$0.86 \pm .34^{a}$
I 0.78 mg/kg	34.4 ± 1.4^{c}	1.70^{b}	$3.07 \pm .37^{b}$	$2.28 \pm .16^{d}$	13.9 ± 4.1^{a}	$4.11 \pm .41^{c}$	$1.70 \pm .15^{c}$	$1.22 \pm .45^{a}$
I 1.56 mg/kg	32.2 ± 1.4^{bc}	1.50^{b}	$2.13 \pm .37^{ab}$	$1.73 \pm .16^{bc}$	26.3 ± 4.1^{b}	$3.28 \pm .37^{bc}$	$1.51 \pm .15^{bc}$	$1.57 \pm .45^{a}$
I 3.12 mg/kg	30.7 ± 1.4^{ab}	1.30^{b}	$2.09 \pm .37^{ab}$	$1.47 \pm .16^{b}$	27.1 ± 3.9^{b}	$3.28 \pm .39^{bc}$	$1.69 \pm .15^{c}$	$1.42 \pm .48^{a}$
I 6.24 mg/kg	29.6 ± 1.5^{ab}	3.00^{c}	$4.59 \pm .39^{c}$	$1.88 \pm .16^{bc}$	26.8 ± 7.2^{b}	$0.65 \pm .58^{a}$	$0.23 \pm .24^{a}$	$2.81 \pm .54^{b}$
(-) Control (no virus, no drug)	31.2 ± 1.0^{b}	0.00^{a}	$1.74 \pm .26^{a}$	$0.92 \pm .11^{a}$	25.3 ± 3.2^{b}	$3.41 \pm .30^{bc}$	$1.58 \pm .12^{bc}$	$0.88 \pm .33^{a}$
(+) Control (virus only, no drug)	28.5 ± 0.8^{a}	2.21^{bc}	$4.11 \pm .22^{c}$	$1.91 \pm .09^{c}$	15.0 ± 2.8^{a}	$2.77 \pm .26^{b}$	$1.27 \pm .40^{b}$	$3.01 \pm .29^{b}$

[1]Means within parameter with different superscripts are significantly different from each other ($P \leq .05$).

Table 3b. The effect of indomethacin on serum alkaline phosphatase (ALKPHOS), creatine phosphokinase (CPK), sodium, potassium, chloride, and bicarbonate.[1]

Treatment	ALKPHOS (U/L)	CPK (U/L)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)	% Infected[2]
Control (I 1.56 mg/kg, no virus)	1530 ± 192^{b}	6113 ± 937^{c}	152.2 ± 2.0^{a}	$5.5 \pm .5^{ab}$	118.5 ± 1.6^{a}	23.3 ± 1.0^{a}	0.00
I 0.78 mg/kg	1724 ± 257^{b}	4264 ± 1185^{bc}	168.5 ± 2.6^{b}	$6.3 \pm .7^{b}$	124.0 ± 2.1^{b}	21.6 ± 1.4^{a}	90.00
I 1.56 mg/kg	1284 ± 257^{b}	7388 ± 1185^{c}	156.3 ± 2.6^{a}	$4.3 \pm .7^{a}$	114.8 ± 2.1^{a}	19.9 ± 1.4^{a}	70.00
I 3.12 mg/kg	1286 ± 257^{b}	2748 ± 1185^{b}	154.4 ± 2.6^{a}	$4.9 \pm .7^{ab}$	124.7 ± 2.1^{b}	22.5 ± 1.4^{a}	70.00
I 6.24 mg/kg	572 ± 287^{a}	1012 ± 1325^{a}	165.3 ± 2.9^{ab}	$8.0 \pm .8^{c}$	116.4 ± 2.4^{a}	22.3 ± 1.6^{a}	66.67
(-) Control (no virus, no drug)	1653 ± 182^{b}	3061 ± 838^{b}	159.0 ± 1.9^{a}	$8.3 \pm .5^{c}$	120.7 ± 1.5^{ab}	22.4 ± 1.0^{a}	0.00
(+) Control (virus only, no drug)	1360 ± 148^{b}	993 ± 684^{a}	155.2 ± 1.5^{a}	$4.5 \pm .4^{a}$	116.5 ± 1.2^{a}	20.3 ± 0.8^{a}	83.33

[1]Means within parameter with different superscripts are significantly different form each other ($P \leq .05$).
[2]Percent of total birds per treatment which tested (+) for HEV antigen with AGID.

Table 4a. The effect of chlorpheniramine on hematocrit, lesion score, spleen weight, SW/BW ratio, and serum lipid, protein, and albumin concentration and heterophil lymphocyte ratio.[1]

Treatment	Hematocrit	Lesion Score	Spleen Wt. (g)	SW/BW Ratio	(Lipid) (mg/dl)	(Protein) (g/dl)	(Albumin) (g/dl)	H/L Ratio
Control (AH1 2 mg/kg, no virus)	26.9 ± 1.5^{a}	0.00^{a}	$2.51 \pm .37^{a}$	$1.16 \pm .16^{a}$	28.3 ± 5.7^{b}	$3.58 \pm .53^{a}$	$1.44 \pm .21^{ab}$	$2.13 \pm .48^{b}$
AH1 1 mg/kg	28.3 ± 1.4^{ab}	2.60^{c}	$4.60 \pm .37^{b}$	$1.97 \pm .16^{b}$	21.2 ± 5.7^{ab}	$2.64 \pm .53^{a}$	$0.88 \pm .21^{a}$	$3.48 \pm .51^{c}$
AH1 2 mg/kg	28.8 ± 1.5^{ab}	0.80^{a}	$5.16 \pm .37^{c}$	$2.13 \pm .16^{b}$	23.7 ± 6.2^{ab}	$3.08 \pm .59^{a}$	$1.00 \pm .24^{a}$	$4.47 \pm .45^{d}$
AH1 4 mg/kg	29.3 ± 1.4^{ab}	1.50^{b}	$5.65 \pm .37^{c}$	$2.20 \pm .16^{b}$	37.2 ± 5.7^{b}	$3.22 \pm .53^{a}$	$1.06 \pm .21^{a}$	$3.90 \pm .45^{cd}$
(-) Control (no virus, no drug)	31.2 ± 1.0^{b}	0.00^{a}	$1.74 \pm .26^{a}$	$0.92 \pm .11^{a}$	25.3 ± 3.2^{ab}	$3.41 \pm .30^{a}$	$1.58 \pm .12^{b}$	$0.88 \pm .33^{a}$
(+) Control (virus only, no drug)	28.5 ± 0.8^{ab}	2.21^{bc}	$4.11 \pm .22^{b}$	$1.91 \pm .09^{b}$	15.0 ± 2.8^{a}	$2.77 \pm .26^{a}$	$1.27 \pm .40^{a}$	$3.01 \pm .29^{c}$

[1]Means within parameter with different superscripts are significantly different from each other ($P \leq .05$).

Table 4b. The effect of chlorpheniramine on serum alkaline phosphatase (ALKPHOS), creatine phosphokinase (CPK), sodium, potassium, chloride, and bicarbonate.[1]

Treatment	ALKPHOS (U/L)	CPK (U/L)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)	% Infected[2]
Control (AH1 2 mg/kg, no virus)	1969 ± 257^{b}	1624 ± 1185^{a}	148.2 ± 2.6^{a}	$13.6 \pm .7^{c}$	114.0 ± 2.1^{a}	19.5 ± 1.4^{a}	0.00
AH1 1 mg/kg	1516 ± 257^{ab}	928 ± 1185^{a}	156.6 ± 2.6^{b}	$4.6 \pm .7^{a}$	117.6 ± 2.1^{ab}	22.4 ± 1.4^{a}	80.00
AH1 2 mg/kg	1786 ± 257^{ab}	930 ± 1185^{a}	157.1 ± 2.6^{b}	$3.6 \pm .7^{a}$	117.2 ± 2.1^{ab}	23.1 ± 1.4^{a}	77.78
AH1 4 mg/kg	1774 ± 257^{ab}	1320 ± 1185^{a}	159.0 ± 1.9^{b}	$3.3 \pm .7^{a}$	116.2 ± 2.1^{ab}	22.0 ± 1.4^{a}	75.00
(-) Control (no virus, no drug)	1653 ± 182^{ab}	3061 ± 838^{a}	159.0 ± 1.9^{b}	$8.3 \pm .5^{b}$	120.7 ± 1.5^{b}	22.4 ± 1.0^{a}	0.00
(+) Control (virus only, no drug)	1360 ± 148^{a}	993 ± 684^{a}	155.2 ± 1.5^{b}	$4.5 \pm .4^{a}$	116.5 ± 1.2^{ab}	20.3 ± 0.8^{a}	83.33

[1]Means within parameter with different superscripts are significantly different form each other ($P \leq .05$).
[2]Percent of total birds per treatment which tested (+) for HEV antigen with AGID.

Table 5a. The effect of cimetidine on hematocrit, lesion score, spleen weight, SW/BW ratio, and serum lipid, protein, and albumin concentration and heterophil lymphocyte ratio.[1]

Treatment	Hematocrit	Lesion Score	Spleen Wt. (g)	SW/BW Ratio	(Lipid) (mg/dl)	(Protein) (g/dl)	(Albumin) (g/dl)	H/L Ratio
Control (AH2 50 mg/kg, no virus)	31.9 ± 1.5^{b}	0.00^{a}	$2.12 \pm .39^{a}$	$0.98 \pm .16^{a}$	25.2 ± 5.6^{a}	$3.06 \pm .53^{bc}$	$1.28 \pm .21^{ab}$	$2.26 \pm .48^{b}$
AH2 25 mg/kg	27.4 ± 1.6^{a}	3.00^{bc}	$4.74 \pm .48^{b}$	$2.29 \pm .20^{b}$	20.9 ± 5.6^{a}	$1.98 \pm .53^{ab}$	$0.72 \pm .21^{ab}$	$4.23 \pm .64^{c}$
AH2 50 mg/kg	29.2 ± 1.5^{ab}	3.10^{c*}	$3.48 \pm .39^{b}$	$1.87 \pm .16^{b}$	21.0 ± 5.6^{a}	$0.98 \pm .53^{a}$	$0.24 \pm .21^{a}$	$2.65 \pm .59^{b}$
AH2 100 mg/kg	28.8 ± 1.5^{ab}	3.10^{c*}	$4.05 \pm .45^{b}$	$2.07 \pm .19^{b}$	24.9 ± 5.6^{a}	$1.66 \pm .53^{ab}$	$0.56 \pm .21^{a}$	$3.64 \pm .54^{bc}$
(-) Control (no virus, no drug)	31.2 ± 1.0^{b}	0.00^{a}	$1.74 \pm .26^{a}$	$0.92 \pm .11^{a}$	25.3 ± 3.2^{a}	$3.41 \pm .30^{c}$	$1.58 \pm .12^{b}$	$0.88 \pm .33^{a}$
(+) Control (virus only, no drug)	28.5 ± 0.8^{ab}	2.21^{b}	$4.11 \pm .22^{b}$	$1.91 \pm .09^{b}$	15.0 ± 2.8^{a}	$2.77 \pm .26^{b}$	$1.27 \pm .40^{ab}$	$3.01 \pm .29^{bc}$

[1]Means within parameter with different superscripts are significantly different from each other ($P \leq .05$).
[*]Significant at $P \leq .10$

Table 5b. The effect of cimetidine on serum alkaline phosphatase (ALKPHOS), creatine phosphokinase (CPK), sodium, potassium, chloride, and bicarbonate.[1]

Treatment	ALKPHOS (U/L)	CPK (U/L)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)	% Infected[2]
Control (AH2 50 mg/kg, no virus)	1850 ± 257^{b}	1812 ± 1185^{a}	148.0 ± 2.6^{a}	$13.6 \pm .7^{c}$	117.4 ± 2.1^{a}	18.9 ± 1.4^{a}	0.00
AH2 25 mg/kg	1075 ± 257^{a}	1184 ± 1185^{a}	146.4 ± 2.6^{a}	$13.1 \pm .7^{c}$	119.8 ± 2.1^{a}	18.9 ± 1.4^{a}	50.00
AH2 50 mg/kg	676 ± 257^{a}	1778 ± 1185^{a}	147.5 ± 2.6^{a}	$13.3 \pm .7^{c}$	119.7 ± 2.1^{a}	19.0 ± 1.4^{a}	40.00
AH2 100 mg/kg	934 ± 257^{a}	890 ± 1185^{a}	147.8 ± 2.6^{a}	$15.1 \pm .7^{c}$	119.3 ± 2.1^{a}	18.8 ± 1.4^{a}	33.33
(-) Control (no virus, no drug)	1653 ± 182^{b}	3061 ± 838^{a}	159.0 ± 1.9^{b}	$8.3 \pm .5^{b}$	120.7 ± 1.5^{a}	22.4 ± 1.0^{b}	0.00
(+) Control (virus only, no drug)	1360 ± 148^{ab}	993 ± 684^{a}	155.2 ± 1.5^{b}	$4.5 \pm .4^{a}$	116.5 ± 1.2^{a}	20.3 ± 0.8^{a}	83.33

[1]Means within parameter with different superscripts are significantly different form each other ($P \leq .05$).
[2]Percent of total birds per treatment which tested (+) for HEV antigen with AGID.

Table 6a. The effect of aminophylline on hematocrit, lesion score, spleen weight, SW/BW ratio, and serum lipid, protein, and albumin concentration and heterophil lymphocyte ratio.[1]

Treatment	Hematocrit	Lesion Score	Spleen Wt. (g)	SW/BW Ratio	(Lipid) (mg/dl)	(Protein) (g/dl)	(Albumin) (g/dl)	H/L Ratio
Control (AP 10 mg/kg, no virus)	30.4 ± 1.5^{bc}	0.00^{a}	$2.22 \pm .37^{a}$	$0.97 \pm .16^{a}$	34.0 ± 5.6^{c}	$3.16 \pm .53^{a}$	$1.22 \pm .21^{ab}$	$1.65 \pm .54^{a}$
AP 5 mg/kg	27.1 ± 1.5^{b}	2.70^{b}	$5.11 \pm .39^{c}$	$2.15 \pm .16^{b}$	18.4 ± 7.2^{ab}	$3.00 \pm .68^{a}$	$1.03 \pm .28^{ab}$	$4.93 \pm .51^{c}$
AP 10 mg/kg	27.7 ± 1.4^{b}	2.70^{b}	$5.95 \pm .37^{d}$	$2.49 \pm .16^{c}$	24.7 ± 6.2^{b}	$2.74 \pm .59^{a}$	$0.90 \pm .24^{a}$	$3.59 \pm .51^{b}$
AP 20 mg/kg	22.8 ± 1.5^{a}	2.80^{b}	$4.91 \pm .37^{bc}$	$2.04 \pm .16^{b}$	24.1 ± 5.6^{b}	$2.86 \pm .53^{a}$	$0.96 \pm .21^{a}$	$2.11 \pm .54^{ab}$
(-) Control (no virus, no drug)	31.2 ± 1.0^{c}	0.00^{a}	$1.74 \pm .26^{a}$	$0.92 \pm .11^{a}$	25.3 ± 3.2^{b}	$3.41 \pm .30^{a}$	$1.58 \pm .12^{b}$	$0.88 \pm .33^{a}$
(+) Control (virus only, no drug)	28.5 ± 0.8^{bc}	2.21^{b}	$4.11 \pm .22^{b}$	$1.91 \pm .09^{b}$	15.0 ± 2.8^{a}	$2.77 \pm .26^{a}$	$1.27 \pm .40^{ab}$	$3.01 \pm .29^{b}$

[1]Means within parameter with different superscripts are significantly different from each other ($P \leq .05$).

Table 6b. The effect of aminophylline on serum alkaline phosphatase (ALKPHOS), creatine phosphokinase (CPK), sodium, potassium, chloride, and bicarbonate.[1]

Treatment	ALKPHOS (U/L)	CPK (U/L)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)	% Infected[2]
Control (AP 10 mg/kg, no virus)	1854 ± 257^{b}	1634 ± 1185^{a}	155.5 ± 2.6^{a}	$7.0 \pm .7^{c}$	114.8 ± 2.1^{a}	27.4 ± 1.4^{b}	0.00
AP 5 mg/kg	976 ± 257^{a}	944 ± 1185^{a}	154.8 ± 2.6^{a}	$3.3 \pm .7^{ab}$	114.0 ± 2.1^{a}	23.3 ± 1.4^{a}	100.00
AP 10 mg/kg	1436 ± 257^{ab}	986 ± 1185^{a}	153.6 ± 2.6^{a}	$2.9 \pm .7^{a}$	112.9 ± 2.1^{a}	24.1 ± 1.4^{a}	80.00
AP 20 mg/kg	1692 ± 257^{b}	850 ± 1185^{a}	153.6 ± 2.6^{a}	$4.2 \pm .7^{b}$	115.5 ± 2.1^{a}	21.4 ± 1.4^{a}	40.00
(-) Control (no virus, no drug)	1653 ± 182^{b}	3061 ± 838^{a}	159.0 ± 1.9^{a}	$8.3 \pm .5^{c}$	120.7 ± 1.5^{b}	22.4 ± 1.0^{a}	0.00
(+) Control (virus only, no drug)	1360 ± 148^{ab}	993 ± 684^{a}	155.2 ± 1.5^{a}	$4.5 \pm .4^{b}$	116.5 ± 1.2^{a}	20.3 ± 0.8^{a}	83.33

[1]Means within parameter with different superscripts are significantly different form each other ($P \leq .05$).
[2]Percent of total birds per treatment which tested (+) for HEV antigen with AGID.

Table 7a. The effect of disodium cromoglycate on hematocrit, lesion score, spleen weight, SW/BW ratio, and serum lipid, protein, and albumin concentration and heterophil lymphocyte ratio.[1]

Treatment	Hematocrit	Lesion Score	Spleen Wt. (g)	SW/BW Ratio	(Lipid) (mg/dl)	(Protein) (g/dl)	(Albumin) (g/dl)	H/L Ratio
Control (DSCG 10 mg/kg, no virus)	28.5 ± 1.4^{ab}	0.00^{a}	$2.55 \pm .37^{a}$	$0.96 \pm .16^{a}$	36.3 ± 5.6^{c}	$3.30 \pm .53^{a}$	$1.20 \pm .21^{ab}$	$2.16 \pm .48^{b}$
DSCG 5 mg/kg	26.8 ± 1.5^{ab}	2.10^{b}	$5.60 \pm .39^{c}$	$2.12 \pm .16^{bc}$	12.8 ± 8.8^{a}	$2.48 \pm .59^{a}$	$0.75 \pm .24^{a}$	$3.23 \pm .48^{c}$
DSCG 10 mg/kg	24.9 ± 1.6^{a}	2.60^{b}	$5.94 \pm .41^{c}$	$2.38 \pm .17^{c}$	16.4 ± 5.6^{a}	$2.26 \pm .53^{a}$	$0.70 \pm .21^{a}$	$4.57 \pm .48^{d}$
DSCG 20 mg/kg	31.3 ± 1.6^{b}	2.40^{b}	$5.15 \pm .39^{c}$	$2.43 \pm .16^{c}$	26.7 ± 8.8^{b}	$2.50 \pm .53^{a}$	$0.94 \pm .21^{a}$	$3.88 \pm .83^{cd}$
(-) Control (no virus, no drug)	31.2 ± 1.0^{b}	0.00^{a}	$1.74 \pm .26^{a}$	$0.92 \pm .11^{a}$	25.3 ± 3.2^{b}	$3.41 \pm .30^{a}$	$1.58 \pm .12^{b}$	$0.88 \pm .33^{a}$
(+) Control (virus only, no drug)	28.5 ± 0.8^{ab}	2.21^{b}	$4.11 \pm .22^{b}$	$1.91 \pm .09^{b}$	15.0 ± 2.8^{a}	$2.77 \pm .26^{a}$	$1.27 \pm .40^{ab}$	$3.01 \pm .29^{c}$

[1]Means within parameter with different superscripts are significantly different from each other ($P \leq .05$).

Table 7b. The effect of disodium cromoglycate on serum alkaline phosphatase (ALKPHOS), creatine phosphokinase (CPK), sodium, potassium, chloride, and bicarbonate.[1]

Treatment	ALKPHOS (U/L)	CPK (U/L)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)	% Infected[2]
Control (DSCG 10 mg/kg, no virus)	1838 ± 257^{a}	692 ± 1185^{a}	155.6 ± 2.7^{ab}	2.9 ± 0.7^{a}	112.8 ± 2.1^{a}	25.6 ± 1.4^{b}	0.00
DSCG 5 mg/kg	1412 ± 257^{a}	972 ± 1185^{a}	156.0 ± 2.7^{ab}	3.6 ± 0.7^{ab}	116.2 ± 2.1^{ab}	23.3 ± 1.4^{ab}	100.00
DSCG 10 mg/kg	1002 ± 257^{a}	730 ± 1185^{a}	155.0 ± 2.6^{ab}	3.3 ± 0.7^{ab}	116.1 ± 2.1^{ab}	22.0 ± 1.4^{ab}	80.00
DSCG 20 mg/kg	1272 ± 257^{a}	1547 ± 1325^{a}	148.2 ± 4.2^{a}	14.6 ± 1.1^{d}	116.6 ± 3.4^{ab}	19.6 ± 2.2^{a}	50.00
(-) Control (no virus, no drug)	1653 ± 182^{a}	3061 ± 838^{a}	159.0 ± 1.9^{b}	8.3 ± 0.5^{c}	120.7 ± 1.5^{b}	22.4 ± 1.0^{ab}	0.00
(+) Control (virus only, no drug)	1360 ± 148^{a}	993 ± 684^{a}	155.2 ± 1.5^{ab}	4.5 ± 0.4^{b}	116.5 ± 1.2^{ab}	20.3 ± 0.8^{a}	83.33

[1]Means within parameter with different superscripts are significantly different form each other ($P \leq .05$).
[2]Percent of total birds per treatment which tested (+) for HEV antigen with AGID.

Table 8a. The effect of specific leukotriene receptor antagonists (FPL 57231 and FPL 55712) on hematocrit, lesion score, spleen weight, SW/BW ratio, and serum lipid, protein, and albumin concentration and heterophil lymphocyte ratio.[1]

Treatment	Hematocrit	Lesion Score	Spleen Wt. (g)	SW/BW Ratio	(Lipid) (mg/dl)	(Protein) (g/dl)	(Albumin) (g/dl)	H/L Ratio
Control (231 1.0 mg/kg, no virus)	32.4±1.5[b]	0.00[a]	2.06±.39[a]	0.98±.16[a]	26.9±5.7[a]	2.86±.53[b]	1.12±.21[b]	1.99±.51[b]
231 0.5 mg/kg	31.6±1.5[ab]	3.20[c*]	5.09±.48[b]	2.53±.20[c]	25.9±5.7[a]	2.18±.53[ab]	0.80±.21[ab]	4.14±.64[d]
231 1.0 mg/kg	26.1±1.5[a]	3.80[c]	4.64±.39[b]	2.17±.16[bc]	20.9±5.7[a]	1.60±.53[a]	0.50±.21[a]	6.01±.51[e]
231 2.0 mg/kg	30.8±1.5[ab]	3.10[c*]	4.33±.39[b]	2.12±.16[bc]	20.1±5.7[a]	1.26±.53[a]	0.38±.21[a]	1.56±1.40[ab#]
712 20 mg/kg[#]	35.0±1.9[c]	4.00[c]	3.69±.53[b]	1.95±.22[b]	24.6±6.2[a]	1.87±.59[a]	0.50±.24[a]	5.70±1.4[de]
(-) Control (no virus, no drug)	31.2±1.0[ab]	0.00[a]	1.74±.26[a]	0.92±.11[a]	25.3±3.2[a]	3.41±.30[c]	1.58±.12[c]	0.88±.33[a]
(+) Control (virus only, no drug)	28.5±0.8[a]	2.21[b]	4.11±.22[b]	1.91±.09[b]	15.0±2.8[a]	2.77±.26[b]	1.27±.40[bc]	3.01±.29[c]

[1]Means within parameter with different superscripts are significantly different from each other ($P \leq .05$).
[*]Significant at $P \leq .10$.
[#]Limited sample size, n=5.

Table 8b. The effect of specific leukotriene receptor antagonists (FPL 55712 and FPL 57231) on serum alkaline phosphatase (ALKPHOS), creatine phosphokinase (CPK), sodium, potassium, chloride, and bicarbonate.[1]

Treatment	ALKPHOS (U/L)	CPK (U/L)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)	% Infected[2]
Control (231 1.0 mg/kg, no virus)	1572 ± 257^{ab}	1544 ± 1185^{a}	149.0 ± 2.6^{a}	$13.9 \pm .7^{d}$	116.4 ± 2.1^{a}	19.5 ± 1.4^{ab}	0.00
231 0.5 mg/kg	1596 ± 257^{ab}	1258 ± 1185^{a}	148.4 ± 2.6^{a}	$12.9 \pm .7^{cd}$	117.0 ± 2.1^{a}	18.3 ± 1.4^{a}	100.00
231 1.0 mg/kg	1325 ± 257^{ab}	1605 ± 1325^{a}	147.8 ± 2.6^{a}	$11.7 \pm .7^{c}$	119.2 ± 2.1^{a}	19.3 ± 1.4^{ab}	30.00
231 2.0 mg/kg	966 ± 257^{a}	868 ± 1185^{a}	147.5 ± 2.6^{a}	$12.0 \pm .7^{c}$	118.7 ± 2.1^{a}	20.6 ± 1.4^{ab}	44.44
712 20 mg/kg[#]	1015 ± 287^{ab}	873 ± 1325^{a}	147.6 ± 2.9^{a}	$14.5 \pm .7^{d}$	118.6 ± 2.4^{a}	18.9 ± 1.4^{a}	60.00
(-) Control (no virus, no drug)	1653 ± 182^{b}	3061 ± 838^{a}	159.0 ± 1.9^{b}	$8.3 \pm .5^{b}$	120.7 ± 1.5^{a}	22.4 ± 1.0^{b}	0.00
(+) Control (virus only, no drug)	1360 ± 148^{ab}	993 ± 684^{a}	155.2 ± 1.5^{b}	$4.5 \pm .4^{a}$	116.5 ± 1.2^{a}	20.3 ± 0.8^{ab}	83.33

[1]Means within parameter with different superscripts are significantly different form each other ($P \leq .05$).
[2]Percent of total birds per treatment which tested (+) for HEV antigen with AGID.
[#]Limited sample size, n=5.

Chapter IV

Involvement of Mast Cells in the Formation of Intestinal Lesions Associated with Hemorrhagic Enteritis Virus Infection in Turkeys. Alteration in Total Duodenal Mast Cell Number, Mast Cell Subpopulations, Basophil Numbers, and Duodenal Vascular Permeability.

Abstract

The involvement of mast cells in the formation of lesions characteristic of hemorrhagic enteritis virus (HEV) infection was studied in seven-week-old turkeys by quantifying duodenal mast cells using the histochemical stain astra blue. Changes in vascular permeability within the duodenum in birds infected with HEV was examined using colloidal carbon and ferritin as vascular markers. Turkeys infected with hemorrhagic enteritis virus (HEV) had significantly higher total duodenal mast cell counts than uninfected controls (227.2 ± 143.7 vs. 124.4 ± 69.2). An increase in vascular permeability was demonstrated in birds infected with HEV and was not present in birds which received saline inoculation. This study suggests that mast cells and the vasoactive mediators contained within mast cells may be important in the manifestation of HEV infection and lesion production. It also provides a possible mechanism through which biochemical and physiologic changes characteristic of HEV infection occur.

Introduction

Hemorrhagic enteritis (HE) of turkeys, a disease of young poults caused by a type II avian adenovirus, is characterized by splenic enlargement and a distended, congested small intestine (13). In the most severe cases birds hemorrhage intra-luminally, losing both red blood cells and serum protein as the intestinal barrier becomes increasingly compromised. Gross and histopathologic changes seen in the small intestine have been reviewed (13) but to date no studies have addressed the sequence of events that leads to the formation of intestinal lesions associated with HEV infection.

It has been suggested that histamine may play a significant role in the immunopathogenesis of HE (37). The mutually opposing actions of H_1 and H_2 type histamine receptors in the inflammatory process has been well documented (3). Pharmacologic antagonism of H_1 receptors by chlorpheniramine maleate and H_2 receptor antagonism by cimetidine has led to differential effects on the formation of intestinal lesions characteristic of HEV infection. H_1 receptor antagonism significantly decreased lesion scores while the administration of an H_2 receptor antagonist increased lesion scores (37).

Histamine is primarily found in mast cells (3). Within the duodenum these mast cells are located in the lamina propria of the villus, usually adjacent to blood vessels (38). An increase in mast cell numbers within the intestine of animals with intestinal diseases has been reported for many diseases in many species (20,24,29-32,41,49,51). The involvement of the mast cell within the intestine of turkeys infected with HEV in the development of characteristic lesions has not yet been investigated.

The inflammatory response to histamine release is manifested as a decrease in blood flow as a result of vasodilation, an increase in microvascular permeability due to endothelial

contraction, and edema formation from subsequent plasma leakage (38). Congestion and hemorrhage in the intestinal villi has been described in turkeys infected with HEV (13), indicating the probable involvement of a vasoactive mediator in lesion formation. Additionally, others (37,43) have reported a decrease of serum albumin in infected birds and have suggested that this may be due to villus leakage of plasma protein. Permeability studies, however, have not yet been used to study the potential effects of vasoactive mediators in the formation of intestinal lesions associated with HEV. The present studies were designed to examine the possible role duodenal mast cells may have in the development of intestinal lesions associated with HEV infection and whether or not these effects are accompanied by an alteration in intestinal vascular permeability (experiment 1), and to examine the quantitative changes in circulating basophils and duodenal mast cell subpopulations (mucosal and connective tissue) in turkeys challenged with HEV (experiment 2).

Materials and Methods

Experimental turkeys - One-day-old, commercial, broad-breasted white, female turkey poults were reared until six weeks of age in confinement in identical battery units. The birds were then transferred to an enclosed house where they were housed on wire floors until eight weeks of age.

Virus - The inoculum was a splenic suspension reconstituted from a master seed of lyophilized spleens infected with a pathogenic HEV isolate propagated in susceptible turkeys ($PID_{50}=10^{4.10}$).

Stress - To maximize the clinical effects of viral infection in poults, birds were subjected to heat stress (30°C) from the time of viral challenge until the experiment ended (experiment 1). Administration of stress in experiment 2 entailed of lowering the environmental temperature to 7°C for 2 hr, 24 hr prior to inoculation. Efficacy of these procedures in stressing birds was determined by heterophil to lymphocyte ratio determination 24 hr post-stress.

Inoculum - Each bird challenged with virus received 0.5 ml of a 10^{-2} PBS dilution of the previously described splenic suspension per os, while controls received 0.5 ml sterile PBS *per os*.

Experimental design -

Experiment 1 - Seventy-five, eight-week-old hen poults were randomly assigned to four groups: 1) unchallenged, unstressed negative controls (15 birds), 2) unchallenged, heat stressed (20 birds), 3) HEV challenged, unstressed (20 birds), and 4) HEV challenged, heat stressed (20 birds). At the time of virus inoculation, each group of birds was moved into a separate room for the duration of the experiment.

Experiment 2 - Forty, six-week-old poults were randomly assigned to four groups: 1) unchallenged, unstressed negative controls, 2) unchallenged, cold stressed, 3) HEV challenged, unstressed, and 4) HEV challenged, cold stressed. At the time of virus inoculation, each group of birds was moved into a separate room for the duration of the experiment.

Basophil and heterophil to lymphocyte ratio (HLR) determination - Three ml of blood was drawn via brachial venipuncture 24 hr post cold stress, just prior to viral challenge. Additionally, 3 ml of blood was obtained from each bird just prior to euthanasia. Blood

smears were made from each sample and stained with May-Grunwald/Giesma stain (35) for determination of heterophil to lymphocyte ratios (23). A total of 100 heterophils, lymphocytes, and basophils were counted from each slide and HLRs were determined by dividing the total number of heterophils by lymphocytes.

Total absolute basophil counts were obtained by first quantitating differential leukocyte counts using the hemocytometer method (21). The percentage of basophils found when counting the blood smear was then multiplied by the ratio of absolute heterophils (hemocytometer) to percent heterophils (smear), i.e. [absolute heterophils number (10^3) / % heterophils] x (% basophils) = absolute basophils number (10^3).

Necropsy - Birds were euthanatized 4.5 days post-inoculation and the spleens removed and frozen. The spleens were later assayed for HEV antigen using the agar gel precipitin (AGP) test (14). The intestinal tract was incised from the pancreatico-duodenal junction posteriorly for approximately 20 cm. Gross intestinal lesion scores were graded on a scale of 1 to 5; 1) marked congestion < 1 cm in length, 2) marked congestion 1-10 cm in length, 3) marked congestion > 10 cm in length, 4) sanguineous material present within the entire length of the intestinal tract, and 5) death caused by intra-luminal blood loss.

Duodenal mast cell determination - At necropsy, duodenal cross sections were taken from each bird and placed in Carnoy's fixative to facilitate mast cell preservation and staining (11,16-18,45). At 24 hr post-fixation the tissues were routinely processed for paraffin embedding beginning in absolute ethanol.

Total mast cells per duodenal cross section were determined in sections which had been stained with astra blue (pH=0.2-0.3, 30 min) and counterstained with eosin (5,17). For each bird, three cross sections were counted and an average of the three sections was

considered to be the total duodenal mast cell count for that poult. Similarly, in sections stained with 0.5% Bismark brown Y (26, 44, 47) in 80% ethanol and 1% HCl (1.5 hr), duodenal connective tissue mast cells were counted. Mucosal mast cell (MMC) counts were determined indirectly by subtracting average connective tissue mast cells (CTMC) for each bird from average total duodenal mast cells for each bird.

Microscopic and ultrastructural permeability studies - To determine if alterations in vascular permeability had occurred, each bird was injected intravenously with either 0.1 ml/100 g bw (1) colloidal carbon (Pelikan, West Germany) or 100 mg ferritin (Sigma Chemical Co., St. Louis, MO) 10 min prior to euthanasia. Those sections containing carbon were stained with Periodic-Acid-Schiff (PAS) stain while those containing ferritin were first stained with ferrocyanide to demonstrate iron presence (47) and then with PAS.

One mm^3 duodenal sections were immediately placed in 0.05 M Na cacodylate buffer containing 5% glutaraldehyde and 3% formaldehyde. The tissues were washed with two changes of 0.10 M Na cacodylate buffer and post-fixed in Na cacodylate-buffered 1% osmium tetroxide for 1 hr at 4°C. The specimens were rapidly dehydrated in graded series of ethanol, passed through propylene oxide and embedded in resin. Ultra-thin sections were cut and mounted on copper grids and double stained with aqueous uranyl acetate and lead citrate and examined using a Joel 100CXII transmission electron microscope.

Statistical methods - Lesion scores, total duodenal mast cells, CTMC, MMC, basophil, and heterophil to lymphocyte ratios (HLR) were analyzed for significant differences ($p \leq .05$) using analysis of variance and the Duncan's multiple range test.

Results

Effect of HEV infection and thermal stress on duodenal mast cell counts -

Experiment 1. Poults which were inoculated with HEV had significantly higher numbers of total mast cells than those poults receiving only a saline inoculation (227.3 ± 143.7 vs. 124.4 ± 69.2, respectively) (Table 1). The presence of heat stress did not significantly alter total duodenal mast cell number in either HEV-infected or saline inoculated groups (Table 2). There were, however, significant differences between the viral-challenged groups and the unchallenged groups. Mast cells became more prominent within the lamina propria of the duodenal villi in HEV-infected birds compared to the unchallenged birds.

Experiment 2. Inoculated birds had significantly higher numbers of MMC (Figures 1a and 1b) than uninoculated birds (Table 3). Both of the inoculated groups (stressed and unstressed) had MMC counts which were significantly greater than the uninoculated, unstressed group (Table 4). Although not statistically significant, the inoculated groups had numerically higher MMC counts than the uninoculated, stressed group. Cold stressing the birds appeared to cause a slight increase in MMC counts in both inoculated and uninoculated groups. Connective tissue mast cell counts were not significantly different when any groups were compared (Figures 2a and 2b).

Effect of HEV infection and cold stress on circulating basophil numbers - Both stressed and unstressed inoculated birds had significantly lower numbers of basophils than birds which were neither stressed nor inoculated (Table 4). Because, prior to inoculation, this group had more basophils than the other groups and because of great variability in individual basophil counts, the importance of this results is questionable. Considering that

at this point in the study there was no difference among groups, this difference may possibly be due to chance. It was concluded, therefore, that neither cold stress nor HEV inoculation significantly altered circulating basophil numbers.

Effect of HEV inoculation and thermal stress on lesion score and presence of intestinal lesions -

Experiment 1 - Turkey poults receiving HEV inoculation (Table 1) had significantly higher lesion scores (1.68±1.95) than saline inoculated controls (0.00±0.00). The addition of heat stress to the environment of HEV-infected turkeys significantly increased lesion scores compared to the unstressed, HEV-infected turkeys (2.80±1.83 vs. 0.55±1.32). It is important to note that the addition of heat stress did not affect the severity of lesions formed but did affect the number of birds which had intestinal lesions (17/20 vs 3/20). The average lesion score for birds which had lesions in the two HEV-infected groups was not significantly different (not shown). The difference in lesion scores, therefore, is a reflection of the number of birds with lesions within a group.

Experiment 2 - Although not statistically significant, poults which were cold stressed prior to inoculation had lesion scores greater than poults which were inoculated and unstressed (1.70 vs. 0.80, respectively) (Table 4). The inoculated, stressed group had lesion scores significantly greater than either of the uninoculated groups (lesion scores of 0.00) while the group which was inoculated and unstressed had lesion scores which approached being significantly different from the stressed and unstressed uninoculated groups. Lesion scores were significantly different when inoculated groups were compared with uninoculated groups (Table 3).

Effect of HEV infection on changes in duodenal vascular permeability - The presence of carbon or ferritin particles within vessels of the duodenum was common in all birds (Figure 3). Only those birds which were infected with HEV and had intestinal lesions had vessels which were "labelled" indicating an increase in vascular permeability (Figure 4). The majority of the vascular labelling occurred in the lamina propria of the duodenal villus. Additional evidence of vascular compromise was diapedesis of red blood cells through intact vessel walls (Figure 5).

Effect of HEV infection and cold stress on HLR - (Table 4) Heterophil to lymphocyte ratios significantly increased in the birds which were inoculated 24 hr post-cold stress (1.44 vs 0.93). In the group which was stressed but not inoculated, only a slight increase in HLR was observed when compared to the unstressed groups (1.17 vs. 0.93, respectively). Inoculated, unstressed birds had significantly greater HLRs than inoculated, cold stressed birds five days post-inoculation (3.85 vs. 2.39). Inoculated groups had significantly higher HLRs than uninoculated groups five days post-infections (Table 3).

Discussion

The sequence of events leading to the development of intestinal lesions associated with HEV infection remains unclear. An earlier study suggested that histamine may play a role in lesion formation (37). Chlorpheniramine, an H_1 antagonist, caused a reduction in lesion scores, while cimetidine, an H_2 antagonist, exacerbated lesion formation. The majority of histamine in the body is stored within mast cells in the tissue and in circulating basophils

(3). Examination of the effects of HEV infection on these cells may provide evidence for their involvement in lesion formation.

There have been numerous studies examining intestinal mast cell numbers in diseased animals. An exponential increase in the total number of mast cells in rats beginning approximately 10 days post-infection with *Nippostrongylus brasiliensis* has been reported (31,51). Similar results were reported in sheep infected with *Ostertagia circumcincta* (51) and chickens infected with *Raillietina cesticillus* (20). Others, however, have reported an initial decrease in intestinal mast cells of chickens following inoculation with coccidial oocysts, followed by an increase in mast cell numbers (41). The increase in cell numbers has been attributed to differentiation and division of the mast cell population into globule leukocytes--mast cells which are in the process of discharging their granule content (33). The globule leukocyte has been described in the fowl intestine. At the time of its description it was thought to be derived from the small lymphocyte (48). Two histochemically and possibly functionally distinct populations of mast cells are said to exist within rat and human intestine (4,16,17). Befus *et al.* (4) reported that mucosal and connective tissue mast cell populations within the intestine were more accurately characterized histochemically than by location. The distribution and ultrastructure of mast cells in the chicken (8,52,53), duck (50), pheasant and quail (8) have been studied, but mast cell heterogeneity has not been reported. Attempts to demonstrate histochemical differences in the mast cells of chickens using techniques which were originally described in the rat have been unsuccessful (41). In our study, two different histochemical stains (astra blue to determine total intestinal mast cells and Bismark brown Y to determine connective tissue mast cells) were employed to enable quantification and localization of the two populations of mast cells in the duodenum. The

drawback of this method, however, is that MMC were then determined indirectly by subtracting CTMC from total mast cells, since there is no histochemical stain which is selective for MMC. It was assumed that this method would result in increased variability of mast cells counts. However, because the method was consistent throughout the study, the resulting variability would be minimized.

Mucosal mast cells were significantly increased in the duodenal sections of birds which were inoculated, regardless of whether or not the birds also had intestinal lesions. This suggests that the migration of MMC into the lamina propria of the duodenum may actually precede lesion formation and subsequently be associated with lesion development. Mucosal mast cells were most commonly located within the lamina propria of the duodenal villus (Figures 1a and 1b). Connective tissue mast cells, however, were not significantly altered by viral challenge. Those mast cells which were histochemically identified as CTMC were actually located deep within the lamina propria (mucosa) adjacent to the glandular crypts (Figures 2a and 2b). This indicates, as previously described (4), that the terminology 'mucosal' and 'connective tissue' must be used cautiously and may not accurately describe these subpopulations.

Galli (19) suggested that in disease, mast cell number and phenotype may be altered by factors secreted by other cells which normally control mast cell number and phenotype in health. It has been postulated that one of these factors might be interleukin-3, a product of lymphocytes (39). Others have also postulated that proliferation and differentiation of MMC progenitors in the small intestine may be a secondary event dependent upon a 'growth-stimulating factor' produced by activated T-lymphocytes (25). The spleen, therefore, may not only be important for viral replication in HEV infection, but as a source of activated

T-lymphocytes which migrate to the small intestine and produce interleukin-3.

The importance of circulating basophils in the inflammatory reaction in the fowl is not understood. Chand and Eyre (9) suggested that basophils of chickens might fulfill a similar function in inflammatory and hypersensitivity reactions as do mast cells in mammals. Previously, basophils had been reported to be involved in acute inflammatory reactions (6,7,34) and in passive cutaneous anaphylaxis (10). Others have not observed changes in circulating basophils in chickens infected with *Eimeria spp.* (40) or in other birds affected by bacteria, parasites, toxins, or environmental conditions (21).

The results of this study indicate that basophils were unaffected by either stress or viral challenge. The high variability among individuals in the same treatment group was indicated by high standard deviations. Variability of this magnitude has been reported by others (27,36,42) and has been attributed to age, sex, breed, and strain differences. This study, however, used birds of identical age, sex, breed, and strain. Variability must, therefore, have been largely attributable to individual variation.

Increased permeability of the intestinal mucosa (25,31) as well as mucosal hyperemia and an increase in mucus production in the small intestine (49) have been demonstrated following disease-induced intestinal mast cell proliferation. These changes have been attributed to the release of vasoactive agents by mast cells and their subsequent action on the endothelial cells of small veins which induce the formation of intercellular gaps. Plasma protein and red blood cells may pass through these gaps and subsequently filter through the basement membrane (28). Jarrett *et al.* (25) suggested that additional leakage of macromolecules into the lumen may also occur between intestinal epithelial cells. Although vascular markers such as colloidal carbon and ferritin are able to pass between endothelial

cells of "leaky" vessels, they become trapped by the basement membrane and are unable to pass through the vessel wall. The appearance of "labelled" vessels within the lamina propria of the duodenal villi of birds which have characteristic intestinal lesions indicates a possible mechanism through which serum albumin is initially lost in HEV infection. The loss of serum albumin early in HEV infection has previously been reported (37,43). The loss of vessel wall integrity as a result of vasoactive mediator release may also be an early change which results in villus congestion, subsequent villus tip necrosis, and intraluminal hemorrhage characteristic of HEV-induced lesions.

Heterophil to lymphocyte ratios (HLRs) provide an accurate and relatively quick measurement of stress in birds (21). Cold stress significantly increased HLRs pre-inoculation. This is in agreement with what has been reported (21-23). Five days post-inoculation, there was no difference between stressed and unstressed groups. This may be due to the readaptation of the birds. Viral challenge, however, significantly increased HLRs five days post-inoculation. The increase in HLRs was the direct result of a significant decrease in the number of lymphocytes in inoculated vs. uninoculated birds (17,400 vs 33,000, respectively). The decline in lymphocyte number is thought to occur in HEV infection for two reasons. First, a transient sequestration of lymphocytes by lymphoid tissue as a result of the stress of viral infection and subsequent endogenous corticosteroid release (15). Second, induction of a primary lymphocytopenia due to the lymphocytic nature of HEV infection. Unstressed, inoculated birds had significantly higher HLRs than stressed, inoculated birds. One possible explanation for this is that cold stressed birds were less able to respond to the subsequent stress of viral challenge. Deaton *et al.* (12) and Teeter *et al.* (46) showed that pre-stressing birds led to adaptation enabling them to respond less severely

to subsequent stress.

An additional effect of the application of stress to an animal is suppression of the immune system (2). The results from our study suggest that thermal stress, both cold and heat, lowered defense against HEV challenge resulting in more severe lesion formation (Tables 2 and 4).

These studies suggest that mast cells, more specifically MMC, may be important in the manifestation of HEV infection and lesion production. It also provides a possible mechanism through which biochemical (decreased serum lipid and albumin) and physiologic (increased vascular permeability) changes characteristic of HEV infection may occur.

References

1. Awadhiya, R. P., J. L. Vegad, and G. N. Kolte. A topographical study of increased vascular permeability in acute inflammatory reaction in chicken skin. Res. Vet. Sci. 29:203-210. 1980.

2. Baxter, J.D., and A.W. Harris. Mechanism of glucocorticoid action: General features, with reference to steroid-mediated immunosuppression. Transplant. Proc. 7:55-65. 1975.

3. Beavan, M. A. Histamine: Its role in physiological and pathological processes. Monographs in allergy. vol. 13. Dukor, P., P. Kallos, Z. Trnka, B. H. Waksman, A. L. deWeck, eds. Karger. New York. 1978.

4. Befus, D., R. Goodacre, N. Dyck, and J. Bienenstock. Mast cell heterogeneity in man. I. Histologic studies of the intestine. Int. Arch. Allergy Appl. Immunol. 76:232-236. - 1985.

5. Blaies, D. M., and J. F. Williams. A simplified method for staining mast cells with astra blue. Stain Technol. 56:91-94. 1981.

6. Carlson, H. C. The acute inflammatory reaction in chicken breast muscle. Avian Dis. 16:553-558. 1972.

7. Carlson, H. C., and J. R. Allen. The acute inflammatory reaction in chicken skin. Blood cellular response. Avian Dis. 14:817-833. 1970.

8. Carlson, H. C., and M. A. Hacking. Distribution of mast cells in chicken, turkey, pheasant and quail and their differentiation from basophils. Avian Dis. 16:574-577. 1972.

9. Chand, N., and P. Eyre. Rapid method for basophil count in domestic fowl. Avian Dis. 22:639-645. 1978.

10. Chand, N., H. C. Carlson, and P. Eyre. Passive cutaneous anaphylaxis in domestic fowl. Int. Arch. Allergy. 51:508-517. 1975.

11. Crowle, P. K. Differentiation between connective tissue and mucosal mast cells in tissue sections. J. Histotechnol. 8:195-196. 1985.

12. Deaton, J. W., F. N. Reece, S. L. Branton, and J. D. May. High environmental temperature and broiler livability. Poul. Sci. 65:1268-1269. 1986.

13. Domermuth, C. H., and W. B. Gross. Hemorrhagic enteritis and related infections. In: Diseases of poultry. 9th edition. B.W. Calnek, H.J. Barnes, C.W. Beard, W. M. Reid, and H. W. Yoder, Jr., eds. Iowa State University Press. Ames, IA. pp. 567-572. 1991.

14. Domermuth, C. H., W. B. Gross, and R. T. DuBose. Microimmuno-diffusion test for hemorrhagic enteritis of turkeys. Avian Dis. 17:439-444. 1973.

15. Duncan, R. J. and K. W. Prasse. Leukocytes. In: Veterinary laboratory medicine. 2nd ed. Iowa State University Press. Ames, IA. pp. 31-60. 1986.

16. Enerback, L. Mast cells in rat gastrointestinal mucosa. 1. Effects of fixation. Acta Pathol. Microbiol. Scand. 66:289-302. 1966.

17. Enerback, L. Mast cells in rat gastrointestinal mucosa. 2. Dye binding and metachromatic properties. Acta Pathol. Microbiol. Scand. 66:303-312. 1966.

18. Fei, A. C. Y., and Y. C. Lee. The fixation effects of mast cells in chicken intestinal mucosa. Bull. Inst. Zool. Acad. Sinica. 22:119-122. 1983.

19. Galli, S. J. New insights into "the riddle of the mast cells": Microenvironmental regulation of mast cell development and phenotypic heterogeneity. Lab. Invest. 62:5-33. 1990.

20. Gray, J. S. The cellular response of the fowl small intestine to primary and secondary infections of the cestode *Raillietina cesticillus* (Molin). Parasitol. 73:189-204. 1976.

21. Gross, W. B. Differential and total avian blood cell counts by the hemacytometer method. Avian/Exotic Practice. 1:31-36. 1984.

22. Gross, W. B. Factors affecting chicken thrombocyte morphology and the relationship with heterophil:lymphocyte ratios. Br. Poul. Sci. 30:919-925. 1989.

23. Gross, W. B., and H. S. Seigel. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27:972-979. 1983.

24. Hiatt, R. B., and L. Katz. Mast cells in inflammatory conditions of the gastrointestinal tract. Amer. J. Gastroenterol. 37:541-545. 1962.

25. Jarrett, E. E. E., and D. M. Haig. Mucosal mast cells in vivo and in vitro. Immunol. Today. 5:115-118. 1984.

26. Leach, E. H. Bismark brown as a stain for mucoproteins. Stain Technol. 22:73-76. 1947.

27. Lucas, A. M., and C. Jamroz. Atlas of avian hematolgy. U. S. Dept. of Agric. Washington, D. C. 1961.

28. Majno, G., G. E. Palade, and G. I. Schoefl. Studies on inflammation. II. The site of action of histamine and serotonin along the vascular tree: A topographic study. J. Biophys. Biochem. Cytol. 11:607-626. 1961.

29. McAuley, R. L., and S. C. Sommers. Mast cells in nonspecific ulcerative colitis. Amer. J. Dig. Dis. 6:233-236. 1961.

30. McGovern, V. J., and G. T. Archer. The pathogenesis of ulcerative colitis. Aust. Annals of Med. 6:68-74. 1957.

31. Miller, H. R. P., and W. F. H. Jarrett. Immune reactions in mucous membranes. 1. Intestinal mast cell response during helminth expulsion in the rat. Immunol. 20:277-288. 1971.

32. Murray, M., W. F. H. Jarrett, and F. W. Jennings. Mast cells and macromolecular leak in intestinal immunological reactions. The influence of sex of rats infected with *Nippostrongylus brasiliensis*. Immunol. 21:17-31. 1971.

33. Murray, M., H. R. P. Miller, and W. F. H. Jarrett. The globule leukocyte and its derivation from the subepithelial mast cell. Lab. Invest. 19:222-234. 1968.

34. Nair, M. K. The early inflammatory reaction in the fowl. A light microscopical, ultrastructural and autoradiographic study. Acta Veterinaria Scand. 42:1-13. 1973.

35. Natt, M. P., and C. A. Herrick. A new blood diluent for counting the erythrocytes and leukocytes of chickens. Science. 31:735-738. 1952.

36. Olson, C. Variation in the cells and hemoglobin content in the blood of the normal domestic chicken. Cornell Vet. 27:235-263. 1937.

37. Opengart, K. N., P. Eyre, and C. H. Domermuth. The effects of steroidal and non-steroidal anti-inflammatory agents on the manifestations of hemorrhagic enteritis in turkeys. Proc. 127th A.V.M.A. Conv. San Antonio, Texas. July 21-25, 1990. pp. 113.

38. Owen, D. A. A. Inflammation - histamine and 5-hydroxtryptamine. Brit. Med. Bull. 43:356-369. 1987.

39. Razin, E., J. N. Ihle, D. Seldin, J. M. Mencia-Huerta, H. R. Katz, P. A. Lablanc, A. Hein, J. P. Caulfield, K. F. Austen, and R. L. Stevens. Interleukin 3: A differentiation and growth factor for the mouse mast cell that contains chondroitin sulfate E proteoglycan. J. Immunol. 132:1479-1486. 1984.

40. Rose, M. E., P. Hesketh, and B. M. Ogilvie. Peripheral blood leukocyte response to coccidial infection. A comparison of the response in rats and chickens and its correlation with resistance to reinfection. Immunol. 36:71-79. 1979.

41. Rose, M. E., B. M. Ogilvie, and J. W. A. Bradley. Intestinal mast cell response in rats and chickens to coccidiosis, with some properties of chicken mast cells. Int. Archs. Allergy Appl. Immunol. 63:21-29. 1980.

42. Schermer, S. The chicken. In: The blood morphology of laboratory animals. 3rd ed. F. A. Davis Co. Philadelphia, PA. pp. 137-158. 1967.

43. Soback, S. E., E. Bogin, and Y. Weisman. Biochemical changes in the blood, spleen, and duodenum of turkeys experimentally and naturally exposed to hemorrhagic enteritis virus. Avian Pathol. 14:189-197. 1985.

44. Spatz, M. Bismark brown as a selective stain for mast cells. Tech. Bull. Regis. Med. Tech. 30:141-143. 1960.

45. Strobel, S., H. R. P. Miller, and A. Ferguson. Human intestinal mucosal mast cells: Evaluation of fixation and staining techniques. J. Clin. Pathol. 34:851-858. 1981.

46. Teeter, R. G., M. O. Smith, S. Saugiah, and F. B. Mather. Effects of feed intake and fasting duration upon body temperature and survival of thermostressed broilers. Nutr. Rep. Int. 35:531-537. 1987.

47. Thompson, S. W. Histochemical methods for the demonstration of carbohydrates. In: Selected histochemical and histopathological methods. C. C. Thomas. Springfield, Ill. pp. 370-575. 1966.

48. Toner, P. G. The fine structure of the globule leukocyte in the fowl intestine. Acta Anat. 61:321-330. 1965.

49. Urquhart, G. M., W. Mulligan, R. M. Eadie, and F. W. Jennings. Immunologic studies on *Nippostrongylus brasiliensis* infection in the rat: The role of local anaphylaxis. Exp. Parasitol. 17:210-217. 1965.

50. Vasala, K. V., B. Jarplid, and H. J. Hansen. Distribution and ultrastructure of mast cells in the duck. Avian Dis. 30:653-657. 1986.

51. Whur, P. Relationship of globule leukocytes to gastrointestinal nematodes in the sheep, and *Nippostrongylus brasiliensis* and *Hymenolepis nana* infections in rats. J. Comp. Path. 76:57-65. 1966.

52. Wight, P. A. I. The mast cells of *Gallus domesticus*. 1. Distribution and ultrastructure. Acta Anat. 75:100-113. 1970.

53. Wight, P. A. I., and G. M. Mackenzie. The mast cells of *Gallus domesticus*. 2. Histochemistry. Acta Anat. 75:263-275. 1970.

Table 1. The effect of HEV inoculation on total duodenal mast cell counts and lesion scores.[1]

Treatment	n	Total mast cell counts	Lesion scores	# of birds with lesions
HEV inoculation	40	227.3 ± 143.7^{a}	1.68 ± 1.95^{a}	17/40
Saline inoculation	35	124.4 ± 69.2^{b}	0.00 ± 0.00^{b}	0/35

[1]Means with different superscripts are significantly different from each other ($p \leq .05$).

Table 2. The effect of heat stress and HEV inoculation on total duodenal mast cell counts and lesion scores in HEV infected turkeys.[1]

Treatment	n	Total mast cell counts	Lesion scores	# of birds with lesions
Heat stressed, HEV inoculation	20	247.0 ± 171.4^{b}	2.88 ± 1.83^{b}	14/20
Unstressed, HEV inoculated	20	207.3 ± 105.6^{b}	0.55 ± 1.32^{a}	3/20
Heat stressed, uninoculated	20	122.8 ± 75.3^{a}	0.00 ± 0.00^{a}	0/20
Unstressed, uninoculated	15	126.4 ± 60.5^{a}	0.00 ± 0.00^{a}	0/20

[1]Means with different superscripts are significantly different from each other ($p \leq .05$).

Table 3. The effect of HEV infection and cold stress on lesion score, duodenal mucosal mast cell number (MMC), duodenal connective tissue mast cell number (CTMC), basophil number, and heterophil to lymphocyte ratio (HLR).[1]

				Basophil		HLR	
Treatment	Lesion Score	MMC	CTMC	pre-inoc	5 days post-inoc	pre-inoc	5 days post-inoc
Inoculated[2]	1.25±1.67[b]	120±64[b]	82±52[a]	4758±3130[a]	4426±3209[a]	1.18±.65[a]	3.08±1.51[b]
Uninoculated	0.00±0.00[a]	55±39[a]	102±52[a]	7343±4805[b]	7037±5067[a]	1.05±.36[a]	1.01±0.45[a]
Cold Stress[3]	0.85±1.42[a]	100±54[a]	99±54[a]	5562±3720[a]	4954±3199[a]	1.31±.61[b]	1.76±1.20[a]
Unstressed	0.40±1.20[a]	83±51[a]	83±51[a]	6539±4681[a]	6482±5295[a]	0.93±.35[a]	2.29±1.74[a]

[1]Means with different superscripts are significantly different ($P \leq .05$) among inoculated and uninoculated or stressed and unstressed groups.
[2]Inoculated with HEV.
[3]7°C for 2 hr, 24 hr prior to HEV inoculation.

Table 4. Effect of HEV infection and cold stress on lesion score, duodenal mucosal mast cell number (MMC), duodenal connective tissue mast cell number (CTMC), basophil number, and heterophil to lymphocyte ratio (HLR).[1]

					Basophil		HLR	
Treatment	n	Lesion Score	MMC	CTMC	pre-inoc	5 days post-inoc	pre-inoc	5 days post-inoc
Inoculated[2], cold stressed[3]	10	1.70 ± 1.60^{b}	123 ± 57^{b}	97 ± 63^{a}	5020 ± 3730^{a}	4510 ± 3572^{a}	$1.44 \pm .74^{b}$	2.39 ± 1.42^{b}
Inoculated, unstressed	10	0.80 ± 1.60^{ab}	117 ± 70^{b}	67 ± 32^{a}	4490 ± 2352^{a}	4342 ± 2797^{a}	$0.93 \pm .41^{a}$	3.85 ± 1.21^{c}
Uninoculated, cold stressed	10	0.00 ± 0.00^{a}	66 ± 48^{ab}	101 ± 44^{a}	6104 ± 3629^{a}	5566 ± 3448^{ab}	$1.17 \pm .40^{ab}$	1.14 ± 0.29^{a}
Uninoculated, unstressed	10	0.00 ± 0.00^{a}	44 ± 24^{a}	103 ± 62^{a}	8582 ± 5472^{a}	8673 ± 5992^{b}	$0.93 \pm .26^{a}$	0.89 ± 0.53^{a}

[1]Means within a column with different superscripts are significantly different ($P \leq .05$).
[2]Inoculated with HEV.
[3]7°C for 2 hr, 24 hr prior to HEV inoculation.

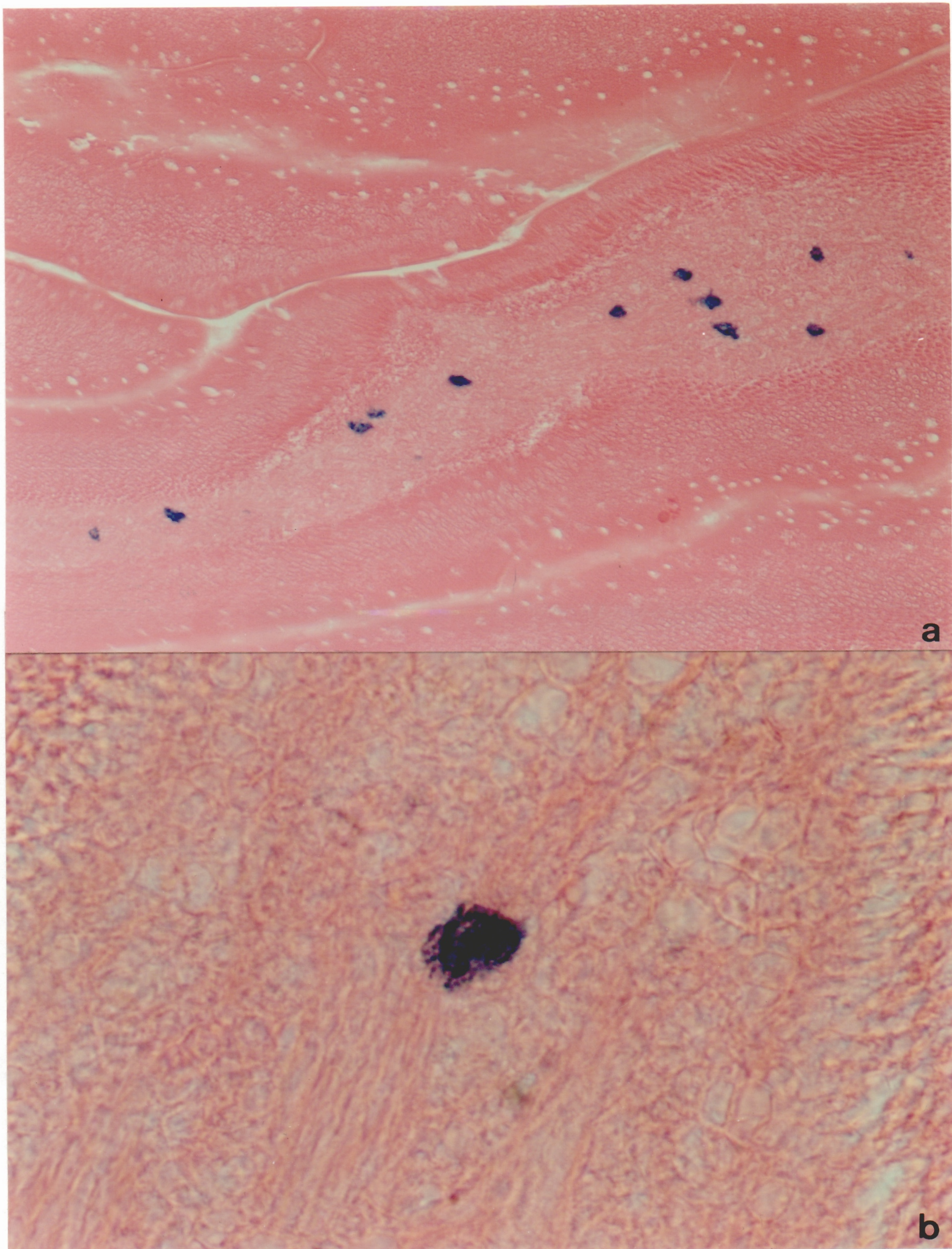


Figure 1. a) Mucosal mast cells stained with astra blue in the lamina propria of a duodenal villus in an HEV infected turkey. b) Higher power magnification of a mucosal mast cell in the lamina propria.

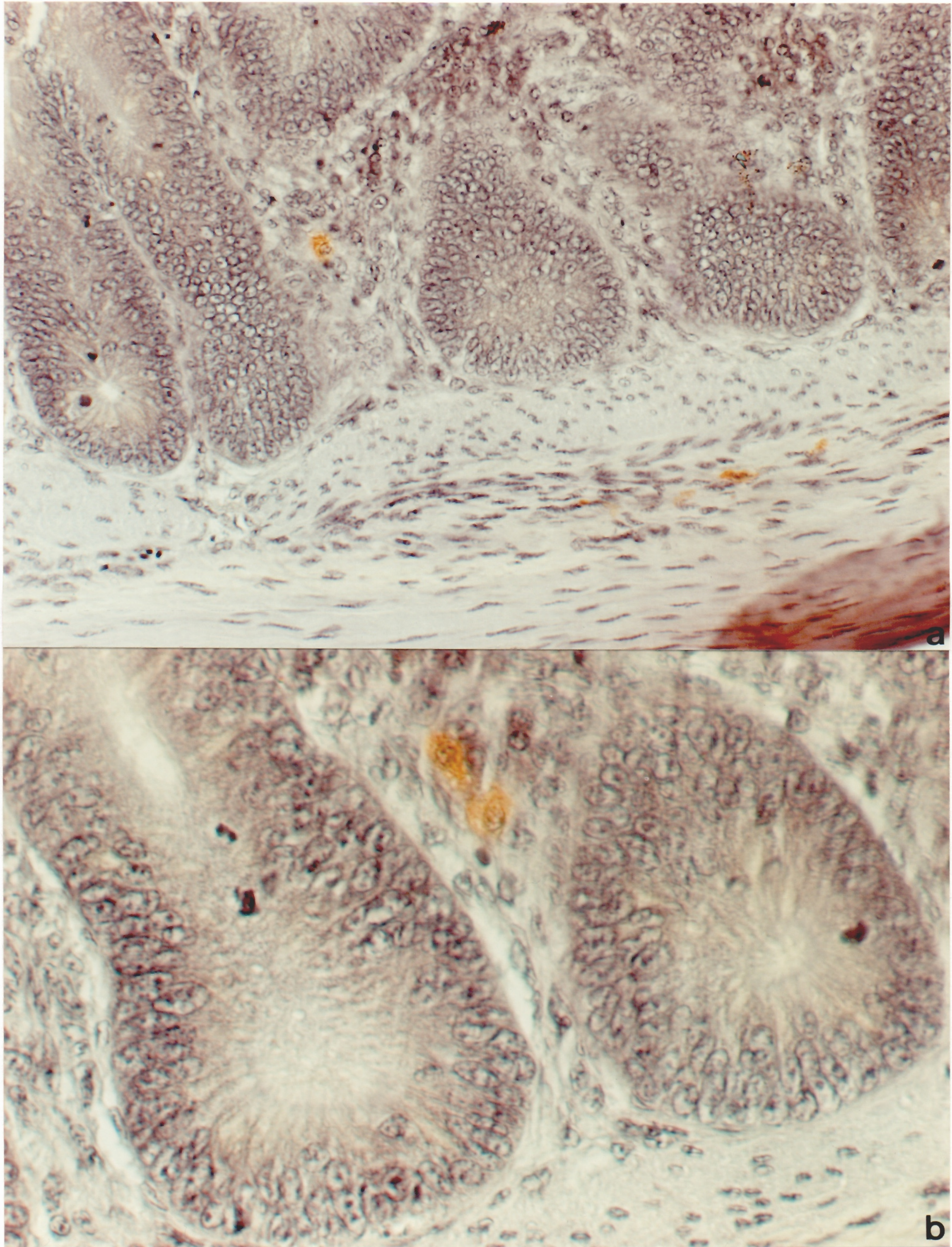


Figure 2. a) Connective tissue mast cells stained with Bismark brown Y in the deep mucosa of the duodenum of an HEV infected turkey. b) High power magnification of a connective tissue mast cell in the glandular crypts of the duodenal villi.

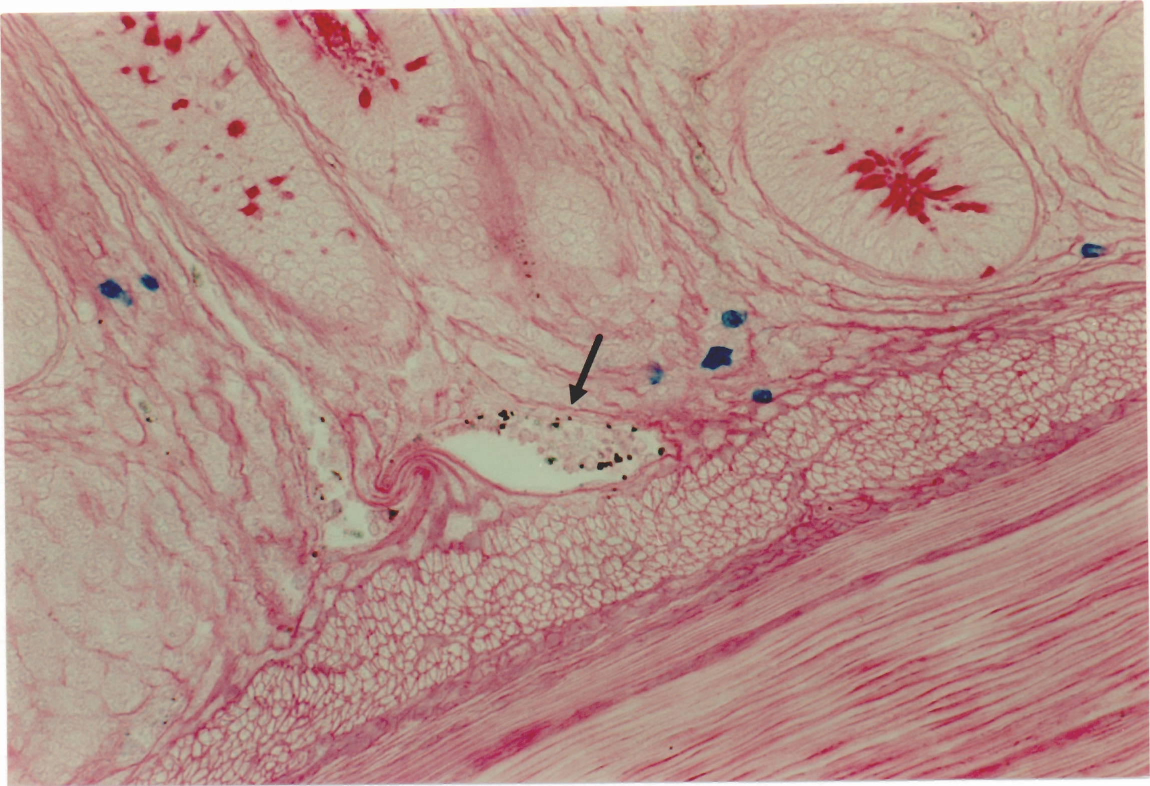


Figure 3. The presence of carbon particles within a vessel (arrow) near the base of the glandular crypts of the duodenal villus. Note the presence of mast cells stained with astra blue in close proximity to the vessel.

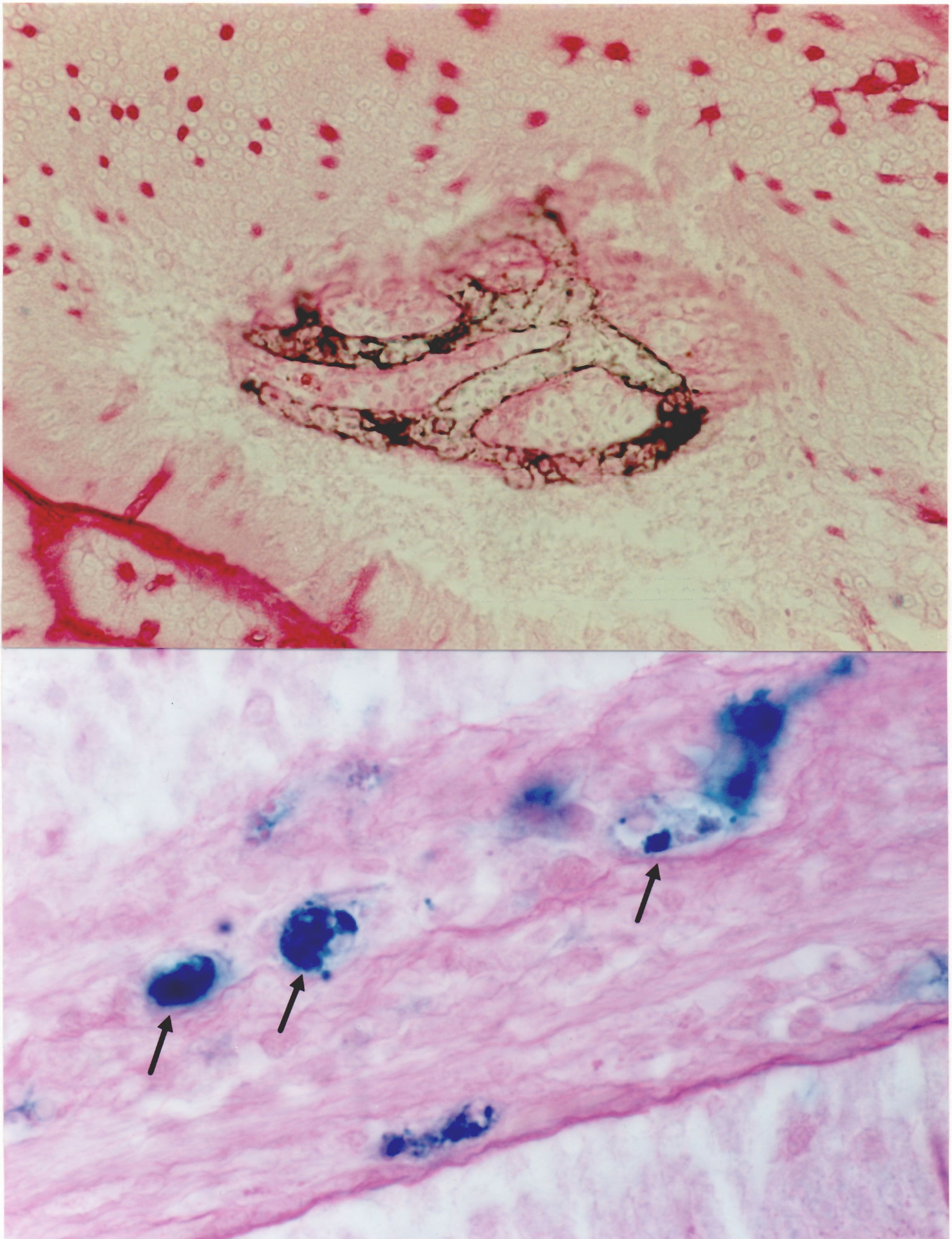


Figure 4. a) A network of vessels within the lamina propria showing the characteristic "labelling" by carbon indicative of a vessel with increased permeability. b) Vessels within the lamina propria labelled with ferritin and stained with ferrocyanide (arrows) to demonstrate the presence of iron.

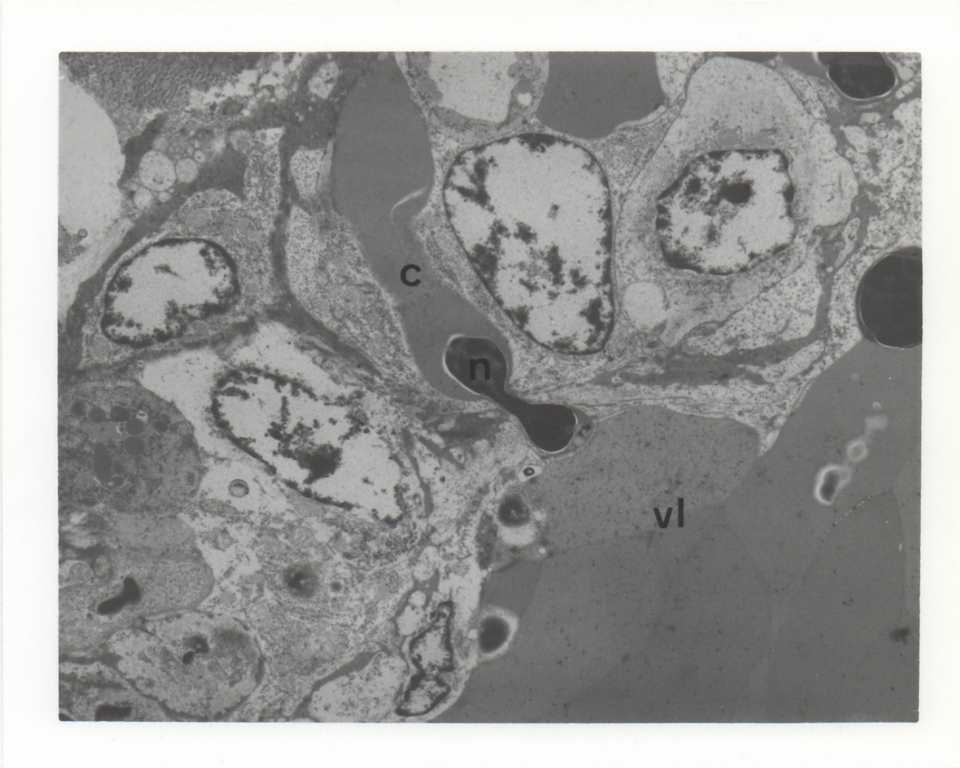


Figure 5. A red blood cell moves through a compromised vells wall in the lamina propria of the duodenal villus. VL = vessel lumen; N = RBC nucleus; C = RBC cytoplasm. (Courtesy of Dr. G. Saunders).

Summary

With the exception of gross and histologic descriptions, little is known about the intestinal lesions associated with hemorrhagic enteritis virus (HEV) infection of turkeys. The immunopharmacologic mechanisms by which these intestinal lesions are produced is unclear. Although in most viral diseases the site of primary viral replication is usually the site of the most severe clinical lesion, HEV is an exception. In HEV infection the primary site of viral replication appears to be the spleen. The site of the most severe clinical lesion, however, is the intestine. The objective of these studies was to elucidate more clearly specific immunoactive cells and inflammatory mediators which might play a role in lesion formation, and hopefully relate these to the relationship between the spleen and the intestine.

These studies have shown that histamine and the eicosanoids (prostaglandins and leukotrienes) are important in the formation of intestinal lesions associated with HEV infection. A source of vasoactive mediators appears to be the mucosal mast cells within the lamina propria of the intestinal villus. The relationship of the mucosal mast cells to the inflammatory mediators and their specific "anti-inflammatory" compounds is illustrated in Figure 1. The close relationship between those mediators which were found to be important, HEV-induced lesion formation, and mast cells is very apparent.

Mucosal mast cells increased in HEV infected birds whether or not an intestinal lesion was present. This suggests that these cells may actually be involved in the early stages of lesion formation. What then is the signal for these cells to proliferate? Others (1, 3) have postulated that activated T-lymphocytes (perhaps those infected with HEV) may secrete

lymphokines, such as interleukin-3, which induce mast cell proliferation and development. This may provide at least a partial link between the spleen and intestine. The spleen may be important for both HEV replication and as a source of activated lymphocytes and their products.

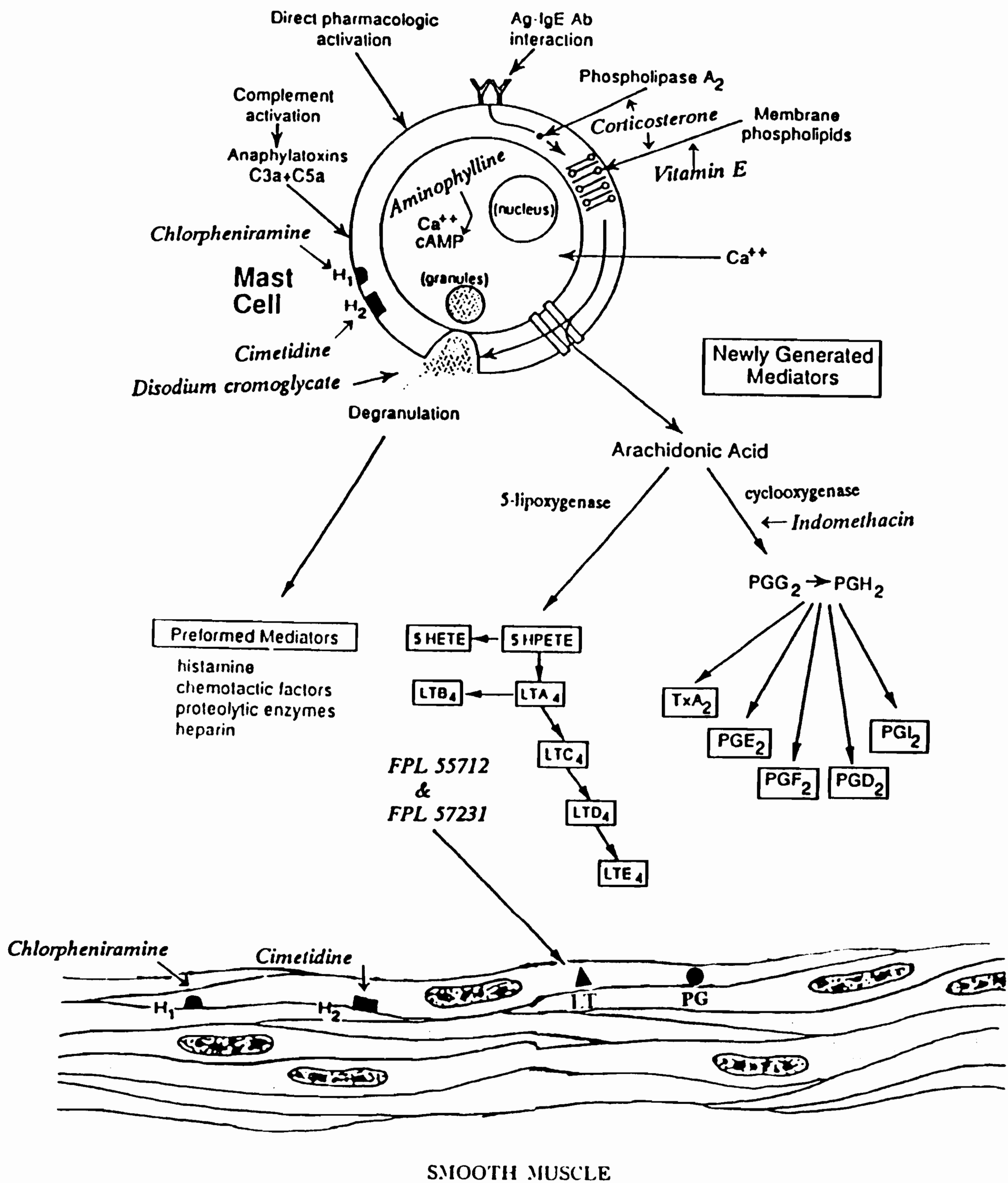
Although this work provides some interesting clues as to the formation of intestinal lesions associated with HEV infection there are many questions which still remain unanswered. This information hopefully provides future researchers with a solid first step to stand on.

References

1. Jarrett, E. E. E., and D. M. Haig. Mucosal mast cells *in vivo* and *in vitro*. Immunol. Today. 5:115-118. 1984.

2. Mueller, D. L., and J. O. Noxon. Anaphylaxis: pathophysiology and treatment. Comp. Cont. Educ. 12:157-170. 1990.

3. Razin, E. J., N. Ihle, D. Seldin, J. M. Mencia-Huerta, H. R. Katz, P. A. LaBlanc, A. Hein, J. P. Caulfield, K. F. Austen, and R. L. Stevens. Interleukin-3: a differentiation and growth factor for the mouse mast cell that contains chondroitan sulfate E proteoglycan. J. Immunol. 132:1479-1486. 1984.



SMOOTH MUSCLE

Figure 1. The interrelationship between mast cells and some of the inflammatory mediators studied. Note the site of action of the anti-inflammatory drugs (italics) used in these studies. Adapted from Mueller and Noxon, 1990 (2).

Vita

Kenneth Neil Opengart, son of Alfred and Arlene Opengart, was born on September 14, 1962 in New York City. He graduated from Douglas S. Freeman High School in Richmond, Virginia in June of 1980. After receiving his B.S. in Biology from the University of Richmond in 1983, he began a M.S. program in Poultry Science at Virginia Polytechnic Institute and State University. He completed this degree in May of 1985. In the fall of 1985, he began to pursue a professional degree in Veterinary Medicine from the Virginia-Maryland Regional College of Veterinary Medicine. Following his first year of professional studies, he was enrolled in the graduate program as the first student in the dual degree program. He received his Doctor of Veterinary Medicine in 1989.

He is a member of the Poultry Science Association, the World Poultry Science Association, the American Association of Avian Pathologists, the Association of Avian Veterinarians, the Virginia Veterinary Medical Association, and the American Veterinary Medical Association. He also is a member of Beta Beta Beta Biological Honorary Society, Gamma Sigma Delta Agricultural Honorary Society, Sigma Xi, and was a recipient of the E.L. Stubbs Award in Avian Medicine in 1989, and the Reed Rumsey Award for the Advancement of Avian Medicine in 1991.