THE EFFECT OF HISTAMINE ON CERTAIN HEMATOLOGICAL FACTORS IN THE CANINE

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INTRODUCTION

The significance of allergic and anaphylactic manifestations in many disease conditions has become recognized with advances in medical knowledge. However, the principle mediator of allergic and anaphylactic reactions has not been adequately documented. Biologically active substances which have been implicated as mediators of allergic reactions include histamine, acetylcholine, serotonin, bradykinin, heparin, leucotaxin, slow reacting substance and complement.

It has long been assumed that histamine plays a role in allergic reactions and that it may in part be responsible for many of the clinical signs of allergy. Unfortunately, histamine has become established in some literature as the primary cause of allergic and anaphylactic reactions without due regard for its pharmacologic limitations and without adequate supporting evidence.

Advances in the study of histamine have been slow in spite of the voluminous literature available from scientific investigation. Species variation in response to exogenous histamine is well documented in the literature and has been responsible for widely varying results. However, consideration of the dosage and method of administration of histamine seems to be lacking in the literature.

The successful treatment of allergies involving the respiratory and integumentary systems of man with histaglobin (human gamma globulin, histamine and sodium thiosulfate) has been reported in Europe in the past few years (Gelfand et. al. 1963). A similar product, Hapamine[1], containing histamine

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[1]Parke Davis and Company. Detroit, Michigan.

and protein, derived from equine serum, is available for treatment of various allergic diseases of man in the United States. Histamine has been recommended for therapy of allergic conditions in dogs (Schirmer, 1965); however, its therapeutic value is questionable on clinical trial and has been nearly discontinued. A survey of the literature failed to reveal studies on the possible antigenicity of histamine in the human or animal subject. Consequently, a determination of the tolerance and antigenic response appeared to be of value and was incorporated into this study.

Alterations produced in the blood during allergic and anaphylactic reactions have been demonstrated by several workers, (Webb, 1924; Dean and Webb, 1924b; Campbell et. al., 1935; Biggart, 1932; Litt, 1964). Investigations of hematologic changes induced by exogenous histamine have centered primarily on the eosinophil response. Changes in other fractions of the blood have not been adequately established.

The purpose of this study was to investigate the clinical and hematologic response induced by sublethal doses of histamine over an extended period of time in the dog. The parameters which were investigated with regard to the effect of exogenous histamine injection were the cellular elements of the blood, the coagulation mechanism, clinical signs and the possible existence of a tolerance phenomenon.

LITERATURE REVIEW

Histamine was first synthesized in 1907 as a chemical curiosity (Windaus and Vogt, 1907). Abel and Kubota (1919) first identified histamine as the essential depressor constituent of a number of animal organs and tissues and suggested there was a similarity between the effects of injected histamine and anaphylactic symptoms.

Dale (1929) stated that sufficient evidence existed for regarding histamine as the physiological agent responsible for the relaxation of muscle tone of the small blood vessels in response to irritation or injury of the tissue and for the initial phase of the response to an injury more severe in degree. Histamine appeared to increase the rate of healing in its early phases following linear surgical wounds in the rat (Boyd and Smith, 1959).

The first limitations of the histamine theory were suggested by Dale (1929) who proposed that a fully developed, local inflammatory reaction involved much that histamine could not reproduce. He specified the wandering of leucocytes, the leakage of erythrocytes into the injured tissue, and thrombosis with resulting arrest of the circulation as examples of changes not attributable to histamine. He noted that certain features of the anaphylactic reaction, such as the loss of coagulability of the blood, thrombosis, hemorrhage and necrosis could not be produced by direct injection of histamine. He further pointed out that there was no justification to a supposition that histamine was the only cell constituent liberated by injury. He did suggest, however, that only histamine might be liberated, or only its effects were visible, in a mild injury. Nielsen and Feigen (1962) confirmed this supposition by demonstrating that in the late stages of anaphylactic release of active materials, after the degradation of histamine became dominant, there was a sudden

upsurge in the bio-assay which was due to the elaboration of a new material.

Critics of the histamine theory continued to question its validity. Best and McHenry (1930) stated that histamine had not been established as the causative agent of any pathological condition. Katz and Cohen (1941) reported that although the fact that histamine was responsible for many of the signs observed in allergic responses seemed to be well documented in the literature, actual experimental evidence for this hypothesis was very meager and circumstantial.

The hypothesis that the liver is the primary shock organ in the dog has become well established in the literature (Dean and Webb, 1924; Dale, 1929; Ojers, Holmes, and Dragstedt, 1941 and Melli, 1963). Even so its relationship with histamine is still somewhat obscure. The liver tissue of the canine has been found to contain 33 mg histamine per kg (33 mcg/g) by Best and McHenry (1930) and 8-110 mcg/g by Feldberg (1956). The average weight of the liver was 450 gm in 91 dogs with an average body weight of 13.3 kg. (Miller, Christensen, and Evans, 1964). By calculation the liver from a dog weighing 13.3 kg contains 3.6 to 49.5 mg of histamine. It would, therefore, be possible for sufficient histamine to be released to produce histamine shock. In the dog the specific anaphylactic reaction produced by antigen-antibody complex chiefly affects the liver cells. When liver cells are injured by immune reactions occurring in the protoplasm, histamine is released in such quantities as to produce effects not only on the vessels but on the histamine sensitive cells of the body (Dale, 1929). Ojers, Holmes, and Dragstedt (1941) obtained biopsy specimens of liver before and after an injection of equine serum in equine-serum-sensitized dogs and found that the histamine lost by the liver was adequate to account for the degree of shock displayed. Release of the maximum concentration of histamine following antigen injection was found

to occur in 3-10 minutes by Code (1939) and in 60 seconds by Spink, Chartrand, and Davis (1963). Other tissues may participate to a lesser degree. Katz and Cohen (1941) found that when blood from a patient sensitive to an allergen was incubated with an extract of that allergen, the plasma histamine rose considerably.

The destruction of histamine was thought to be quite rapid in the body since blood pressure returned to normal within 15-20 minutes following injection (Spink, Chartrand, and Davis, 1963). A study of the distribution of the histamine destroying enzyme, histaminase, in canine tissues revealed that the kidney and intestine were the richest sources; lung and blood contained moderate amounts; skeletal muscle, liver, spleen and stomach contained very little and the heart contained none (Best and McHenry, 1930).

Present views concerning the manner in which antigens release histamine from sensitized tissues fall into two principal categories embodied either by the enzymatic hypothesis or the displacement hypothesis (Nielsen and Feigen, 1962). The displacement theory postulates that organic bases penetrate the mast cell and enters the intracellular granules to release histamine, thought to be loosely linked by ionic affinity to acid groups. (Uvnas, 1958)

The enzymatic hypothesis postulates that enzymatic disruption of the anatomical structure or permeability barrier of the mast cell apparently suffices to cause a release of intracellular histamine (Uvnas, 1958). Histamine release in guinea pig tissues as a result of antigen-antibody reactions has been ascribed to an energy-requiring enzymatic process, since the histamine release is reduced by oxygen lack, iodo-acetic acid and other metabolic inhibitors (Mongar and Schild, 1955).

Eosinophilia has long been associated with allergic responses in animals. A heterogeneous group of pathologic conditions are known to be associated with

a local or systemic eosinophilia, e.g. allergic conditions such as asthma and hay fever, metazoal parasitic infestations, certain nonbacterial skin diseases, Hodgkin's disease, malignant tumors and convalescence following infectious diseases (Campbell et. al., 1935). Litt (1964) maintains that there is a degree of eosinophilia at some stage of virtually all pathologic conditions and that the relationship of allergy and eosinophilia should be reviewed. He suggests that no specificity is attributed to the presence of eosinophils in tissue where along with plasma cells, they are regarded as being indicative of the chronicity of a lesion, while eosinophilia is considered as indicative of an allergic response and of a process which has the high order of specificity typical of any immune response. As early as 1932, Biggart gave repeated injections of protein intramuscularly as well as subcutaneously and produced a general eosinophilia in guinea pigs. The eosinophil reaction became more marked with each successive injection. Later investigation showed that local eosinophil accumulation was not produced by the injection of foreign proteins if an allergic reaction did not occur. Eosinophilia was produced with succeeding injections under conditions compatible with the occurrence of an allergic reaction, i.e. a time interval following the first injection which would correspond to that necessary for sensitization to develop (Campbell, Diennan, and Rettie, 1935). More recent studies with the electron microscope have shown the presence of immune complexes within the cytoplasm of eosinophils thus providing evidence at a molecular level that at least one of the functions of the eosinophil in allergic reactions is the phagocytosis of antigen-antibody complexes (Campbell, Diennan, and Rettie, 1935; Sabesin, 1963; and Litt, 1964). Litt (1964) demonstrated that lymph nodes exhibit substantial eosinophil accumulation following a single stimulus in response to antibody accumulation during the primary immune reaction.

It has long been theorized that histamine release associated with cellular injury, was responsible for the eosinophilia following foreign protein injection or allergic responses. If true, then it would appear that histamine injection should elicit eosinophilia similar to foreign body injection or contact with natural allergens. Vaughn (1953) produced eosinophilia in the guinea pig by injecting 0.25 mg histamine. However, when a single dose of histamine of sufficient amount to produce obvious clinical signs (sweating, gasping for breath, etc.) was given either intravenously or subcutaneously to the horse, there was a marked diminution in the number of circulating eosinophils (Archer, 1963). Archer further observed that some of the eosinophils disappeared from the circulation within five minutes with a decrease to the lowest level in about two hours. Thereafter, the number of eosinophils returned to pre-injection levels at about the 8th or 10th hour. In contrast to this, if an intravenous infusion of histamine was given continously over a two hour period, an immediate eosinopenia follows as before within five minutes, but at approximately one hour the eosinophils return to the starting level. Thereafter, they increase so that a peripheral blood eosinophilia is found. Suyuki (1963) concluded that intravenous injection of histamine in doses of 0.05-1.0 mg per kg body weight produced a marked acceleration of 17-hydroxycorticoid secretion in the dog. Code and Mitchell (1954) found a high concentration of histamine occurring in association with large numbers of eosinophils in the blood of the dog. Based on these reports there would seem to be some confusion in the literature in regard to the response of eosinophils to injections of histamine in the various species.

A most characteristic sign of anaphylaxis in the dog is the increased clotting time of blood (Dale, 1929, Jaques and Waters, 1941, and Rocha e Silva, 1955). The anticoagulant substance responsible for this characteristic

prolongation of the clotting time in anaphylactic shock is heparin (Jaques and Waters, 1941 and Rocha e Silva, 1955). Liberation of histamine during anaphylaxis in the dog appears to be correlated with the release of heparin, and although there was no strict parallelism, the release of both substances probably depends upon an analogous mechanism (Rocha e Silva, 1955).

Current concepts of canine shock are inconsistent with earlier reports. Brewer (1965) reported that in the early stages of shock, the blood has a reduced coagulation time and intravascular clotting may occur, especially in the stagnated microcirculation of the small intestine, thus interferring with metabolism of the tissues and causing their breakdown with extensive ecchymotic hemorrhages. Also, stagnation of the splanchnic pool as a result of hepatic vein stricture, leads to contamination of the blood stream by coliform organisms and their toxins. Robb (1963) demonstrated platelet emboli in small diameter vessels in various types of experimental shock. Microscopic observations of living blood vessels in the gut, mesentery, liver and lung showed that micro-emboli formed after severe hemorrhage, trauma to the vessels, anaphylaxis, norepinephrine injection and injections of coagulase positive Staphylocci and Escherichia coli. He further concluded that the large number of platelet emboli formed could account for the hypocoagulability of blood and for the reported drop in platelet count following prolonged shock.

The direct effects of histamine altering the coagulation mechanism are not adequately documented in the literature. Morgan et. al. (1959) found no changes in clotting time of blood in dogs following intravenous histamine injection. Dale (1929) believed that the loss of the coagulability of the blood was not a feature of the histamine reaction. However, Bierman et. al. (1951) reported an immediate decrease in clotting time in humans following intravenous administration of histamine phosphate. Therefore, it appears

that the phenomenon is subject to some controversy.

With the exception of a reference by Wintrobe (1961) relating to an increase in platelet count, detailed study of the coagulation factors following histamine administration seems to be lacking.

Histamine produces a generalized dilation of the capillaries and contraction of small arteries and veins (Rocha e Silva, 1955). It has been thought to be the only substance known capable of dilating capillaries and increasing their permeability. Evidence supporting the supposition that it is the physiological agent responsible for the relaxation of the tone of the small blood vessels in response to irritation or injury of the tissues appeared to be available as early as 1929 (Dale).

In the dog, the injection of 10 mg of histamine diphosphate per kg of body weight elicited a fall in arterial blood pressure with a return to the initial level after two hours. Afterwards, a slow drop in blood pressure was observed leading to the death of the animal. During the secondary reaction, hemoconcentration up to 130 per cent of the initial value occurred (Smith, 1928). Small doses of histamine administered intravenously to the dog and cat do not produce alterations in the packed cell volume (Morgan et. al. 1959).

In both sensitized and normal dogs, the intravenous injection of 20 cc of equine serum is followed by an abrupt rise of red cells per cubic millimeter as well as a rise in the level of hemoglobin in the peripheral blood (Dean and Webb, 1924b). They recorded an intense leucopenia followed by a well marked leucocytosis. Sections taken from lung tissue of dogs injected with equine serum revealed enormous numbers of polymorphonuclear cells (PMN) adhering to the capillaries (Webb, 1924). The first and most important change following equine serum injection in the sensitized dog was extreme congestion of the liver and gall bladder (Dean and Webb, 1924).

Local eosinophil concentration may be found in the intestinal mucosa during digestion in the guinea pig, rabbit and dog (Biggart, 1932). No local eosinophil accumulation was observed in animals which had been starved for two days. Selye (1938) observed an increased absorption of histamine in the intestinal tract in stressed animals. Oral doses of histamine that were completely harmless in the normal guinea pig produced severe signs of intoxication and death in animals subjected to prolonged and exhaustive work (Rocha e Silva, 1955). Ivy and Javois (1924) reported that 500 to 700 mg of histamine administered orally to dogs would not produce signs of acute intoxication.

MATERIALS AND METHODS

Experimental Animals

Eight mongrel dogs ranging from one to three years of age were employed in this investigation. The experimental animals were maintained in the small animal ward in the Department of Physiology. A preliminary period consisting of a minimum of fourteen days was allowed for conditioning the dogs to their environment and ration.[1] During this period, the dogs were examined for parasitism, treated if necessary, and blood urea nitrogen[2] determinations made to verify relatively normal kidney function. (See Table 1)

The animals were randomly divided into two groups of four animals each. Group I (animals I-IV) received subcutaneous injections and group II (animals V-VIII) received intravenous injections. Animals I and V were retained as controls and received only saline on comparable volume basis. The remaining animals in group I (II-IV) received histamine base at a dosage of 0.5 mg per kg subcutaneously while the remaining animals in group II (VI-VIII) received 0.2 mg per kg histamine base intravenously.

The study was conducted over a period of three months. Twenty-five blood sample determinations were performed on each experimental animal in group I and 22 blood sample determinations were performed on each animal in group II for a total of 188 blood sample determinations.

The experimental animals in group I were on continuous study for a period of 36 days. Histamine injections were initiated on the fourth day and continued twice daily until the 20th day at a level of 0.5 mg per kg body weight. An additional histamine injection was administered on the 32nd

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[1]Gaines Meal: General Foods Corporation, White Plains, New York
[2]Bun-O-Graph: Haver-Lockhart Laboratories, Kansas City, Missouri

day of the experiment for a total of 35 injections.

The experimental animals in group II were maintained on experiment for 28 days. Histamine injections were initiated on the fourth day and continued twice daily until the 20th day at a dosage level of 0.2 mg per kg body weight for a total of 34 injections.

Table 1: Initial Examination

Breed	Dog	Sex	Weight (kg)	BUN (mg/100ml)	Comments
Sheltie Mix	I	F	12.4	12-15	Toxocara canis
Beagle Mix	II	F	14.0	15-17	No ova demonstrated
Cocker Mix	III	M	10.9	15-17	Dipylidium caninum
German Shepherd	IV	M	21.6	12-14	Toxocara leonina
Terrier Mix	V	F	11.8	15-17	Dipylidium caninum and Toxocara canis
Collie Mix	VI	M	22.5	12-15	No ova demonstrated
Wirehair Terrier	VII	M	12.0	15-17	Ancylostoma caninum
Terrier Mix	VIII	M	10.9	12-15	No ova demonstrated

Sampling Procedure

Normal values for each animal were established by taking blood samples on alternate days (-4, -2 and 0 days) before histamine or saline injections were initiated. This method was employed so that each animal would represent an individual study, with a control and experimental phase.

Histamine injections were initiated on day 0 and continued twice daily until day 16. One additional injection of histamine was made on day 28 to all animals on subcutaneous injection studies. Saline injections were given to

control animals on the same schedule.

Serial blood sample determinations were made 15 minutes pre-histamine injection and 15, 45, 90, 150, and 240 minutes post-histamine injection on day 0 and day 16. Serial blood sample determinations made on days 4, 8, 12 and 28 consisted of only two samples taken 15 minutes pre-histamine injection and 45 minutes post-histamine injection.

Single blood sample determinations were made on days 20, 24 and 32. These were determined on days in which no histamine was administered.

The plan consisted of establishing normal values for each animal on days -4, -2 and 0. A period of day 0 to day 16 in which histamine was administered twice daily and samplings made on days 0, 4, 8, 12 and 16. All animals were maintained from day 17 to day 24 without histamine administration and samplings made on days 20 and 24. The study was continued on the animals receiving histamine subcutaneously and an additional histamine injection was made on day 28. A final determination was made on day 32 and the study terminated.

Preparation of Histamine

Histamine base was suspended in physiological saline at a concentration of 25 mg per ml and divided into multiple dose vials. To prevent decomposition these were stored at -10°C. Additional solutions were compounded as needed. Difficulties were encountered in weighing histamine base due to its hygroscopic properties and therefore absolute weights were exceedingly difficult to obtain.

Dosage was determined on other available animals and limited to the minimum level evoking clinical signs. The levels utilized in this investigation were 0.5 mg per kg subcutaneously and 0.2 mg per kg intravenously. Physiological saline was administered to control animals on comparable volume basis.

Procedure for Obtaining Blood Samples

Three ml of blood was withdrawn from the cephalic or lateral saphenous vein into a siliconized syringe and distributed as follows: 1 ml into each of two 13 x 100 mm test tubes for coagulation time and clot retraction determinations and 1 ml into a 13 x 100 mm test tube containing 0.1 ml 3.8% sodium oxalate solution, stoppered with parafilm and stored under refrigeration for prothrombin time determination. One ml blood was then collected directly from the needle and allowed to flow down the side of a siliconized test tube containing one drop of a 10% disodium ethylenediaminetetraacetate (EDTA) solution. This sample was utilized for platelet, eosinophil, total leucocyte, differential leucocyte, hemoglobin and packed cell volume (PCV) determinations.

Platelet Determination

The red cell pipette stem was rinsed in a solution of 3.6 per cent sodium citrate and blood from the siliconized test tube containing EDTA drawn to 0.5 mark and diluted with 3.6 per cent sodium citrate to 101 mark. The mixture was shaken for five minutes and placed in a clean Neubauer counting chamber. The chamber was then placed in a covered Petri dish containing a piece of wet filter paper and allowed to settle for approximately five minutes. The platelets in the center square millimeter were counted under high dry lens (430x) and multiplied by 2000.

Eosinophil Determination

EDTA anticoagulant treated blood was drawn to the 0.1 mark on a white cell pipette and diluted to the 1.0 mark with a diluting solution which consisted of 5 ml of 1 per cent eosin, 5.25 ml of acetone, and q.s. 100 ml with distilled water. The diluting solution was compounded every three days, and kept under refrigeration. The diluted blood in the pipette was thoroughly mixed for approximately three minutes on a pipette rotor. The first few drops

was discarded and then both chambers of a hemacytometer were filled. The hemacytometer was then placed within a covered Petri dish containing a wet filter paper and set aside for 5 minutes. Duplicate counts were made under low power (100x). Calculations were completed by multiplying the total count in the entire ruled area times the dilution factor of 11.

Prothrombin Time Determination

The 13 x 100 mm test tube containing 1 ml of blood and 0.1 ml 3.8% sodium oxalate was centrifuged, and the plasma decanted and stored in a test tube under refrigeration immediately following collection. The one-stage plasma prothrombin time of Quick was used for the determination. More specifically, 0.1 ml of plasma was measured and added to a 12 x 75 mm Pyrex tube and placed in a waterbath at 37°C, along with a reconstituted vial of Simplastin.[1] 0.2 ml Simplastin was added quickly to the plasma with a blowout pipette and a stopwatch started simultaneously. The tube was agitated immediately for good mixing and then tilted from vertical to almost horizontal position until a gel appeared which was the end point. Paired samples were determined and an average reading recorded.

Coagulation Time Determination

Coagulation time was determined on blood withdrawn into a siliconized syringe and distributed into two 13 x 100 mm test tubes at room temperature. A stopwatch was started simultaneously as the blood was withdrawn. Starting at two minutes after withdrawal, tube number one was tilted 45 degrees at approximately 30 second intervals until it could be inverted 180 degrees without the blood flowing. The same procedure was then repeated with tube two and the time recorded.

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[1]Warner-Chilcott, Morris Plains, New Jersey

Hemoglobin Determination

The Cyan-methemoglobin method was utilized to determine hemoglobin values. 6.0 ml Hycel Cyan-methemoglobin Reagent[1] and 0.02 ml of EDTA-treated blood were measured into a test tube with an Auto Dilutor.[2] The contents of the test tube were mixed by inverting several times. This solution was transferred to a cuvette and read on a Coleman Junior Spectrophotometer[3] at 540 mu against a reagent blank. The optical density reading was converted to gms hemoglobin per 100 ml blood by means of a calibrated standard curve.

The standard curve was determined by employing increasing dilutions of the Hycel Cyan-methemoglobin Standard.[4] The optical density readings were then plotted on straight graph paper to establish the standard curve.

Packed Cell Volume

The microhematocrit method of packed cell volume (PCV) determination was performed with an International Micro-capillary Centrifuge.[5] Blu-Tip Capillary Tubes[6] were filled from one-half to three-fourths of capacity from the siliconized test tube containing three ml of blood and one drop of EDTA. One end of the capillary tube was sealed with clay and the tube then placed in the centrifuge for a period of three minutes at approximately 12,000 revolutions per minute. The PCV was then read on a standard chart in per cent total volume. Duplicate determinations were made and an average of the two readings recorded.

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[1]Hycel, Inc., P. O. Box 36329, Houston, Texas 77036
[2]Model 1034; Scientific Products, Evanston, Illinois
[3]Model 6C; Coleman Instruments, Inc., Maywood, Illinois
[4]Hycel, Inc., P. O. Box 36329, Houston, Texas 77036
[5]Model MB, International Equipment Company, Boston, Massachusetts
[6]Scientific Products, Evanston, Illinois

Total Leucocyte Count

All total leucocyte counts were determined on a Coulter Counter[1] adjusted to a threshold of 20 and an aperture current setting of 5. A standard dilution of 0.02 ml of EDTA-treated blood and 10 ml of saline[2] was made on an Auto Dilutor.[3] Duplicate dilutions were prepared from each blood sample. Additional dilutions were made to verify results when wide variations occurred.

Clot Retraction

Clot retraction studies were made on blood samples previously used in coagulation time determinations. The two coagulated blood samples were immediately placed in a waterbath at 37°C following coagulation. Visual observations were made at one-half, one, two, four and twenty-four hours to determine the degree of clot retraction as evidenced by the amount of serum expelled and the firmness and rigidity of the clot.

Clinical Signs

Clinical signs were observed following histamine injection throughout the investigation. The respiratory, digestive, urinary and integumentary systems, the eye and associated structures and the general response were noted. Clinical impressions were relied upon to determine the possibility of a tolerance developing in relation to histamine administration.

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[1]Model A, Serial 1056; Coulter Electronics, Chicago, Illinois
[2]Sodium Chloride 0.85%; Scientific Products, Evanston, Illinois
[3]Model 2680; Scientific Products, Evanston, Illinois

RESULTS AND DISCUSSION

Eosinophil Response (Table 2 - 7)

Eosinopenia occurred within fifteen minutes following histamine administration in all subjects in eight trials. The per cent decrease in eosinophils averaged 36.75 in the subcutaneous study and 29.94 in the intravenous study 45 minutes following histamine injections. An absolute eosinopenia occurred within 45 minutes in 32 out of 33 trials. Absolute eosinopenia, 45 minutes following histamine administration, ranged from 26.5 to 50.7 per cent of 15 minute pre-injection values in group I and 14.9 to 49.5 per cent in group II. In the control animals, the eosinophil count increase in five of six trials in the subcutaneous subject and two of five trials in the intravenous subject 45 minutes following saline administration.

Eosinophil increases based on average control values established on days -4, -2 and 0 (Table 2) became apparent prior to histamine administration on day 16 in group I and on day 8 in group II. An absolute progressive eosinophilia occurred in the subjects receiving histamine subcutaneously and reached a maximum average of 1074 or 233 per cent of the control values (Table 1) on day 32. The absolute eosinophilia occurring in the subjects receiving histamine intravenously was erratic. A peak of 774 eosinophils or 146.0 per cent of average control values occurred on day 12, followed by a decline to 107.1 per cent of average control values on day 20. A maximum of 148.1 per cent of average control values or 785 eosinophils per cmm blood occurred on day 24.

Eosinophil increases based on average values for all experimental animals receiving histamine were markedly decreased by animals IV and VII. These animals had a relatively high eosinophil count initially and did not respond with an eosinophil increase. Excluding these animals in computation, the eosinophil

response would be 202 per cent of control values for group II on day 24 and 113 and 290 per cent of control values for group I on day 24 and 28, respectively.

The control subject on the subcutaneous study showed an eosinophil variation of 57.5 to 305.2 per cent of control values (Table 2). The eosinophilia was correlated with a mucoid enteritis and an infected laceration of the right dorsal metacarpal region which was clinically evident on day 24. The eosinophil count remained within 129.8 per cent of control values through day 24. The eosinophilia was maximal on day 28 at a level of 583 eosinophils per cmm of blood and declined to 324 eosinophils per cmm of blood on day 32.

The control subject on intravenous study showed an initial high eosinophil count as recorded in Table 1. The eosinophil count showed a declining trend throughout the investigation, except for a slight increase on day 8. An eosinophil variation of 57.1 to 116.6 per cent of control values occurred during the period of study.

Total Leucocyte Response (Table 8)

Total leucocyte values were highly variable throughout the investigation. The total leucocyte count decreased in 14 of 18 trials in the group I and in 12 of 15 trials in group II within 45 minutes following histamine administration. The intravenous control subject responded with a leucocyte decrease in four of five trials while a leucocyte decrease occurred in five of six trials in the SC subject 45 minutes following injection of 0.9 per cent saline solution.

A slight increase in cell count occurred on day 0 and 16 at 150 and 240 minutes post-injection. The increase was more predominant in group II. The differential leucocyte count indicated that an increase in neutrophils occurred simultaneously.

High leucocyte values were obtained on both control and histamine injected animals during the investigation. These values showed no correlation with histamine activity or eosinophil response and appear to be normal for the environment and condition of the experimental animals.

Differential Leucocyte Count (Table 9)

Trends in eosinophil response are very closely correlated in both the differential and eosinophil counts. The average differential count for both the intravenous and subcutaneous subjects showed an absolute eosinopenia in ten of eleven trials 45 minutes post-histamine injection. An absolute eosinophil increase on day 32 in group I was in close agreement with the eosinophil count per cmm blood. The per cent eosinophils occurring in group II on day 24 differed from the results in Table 6 and was not increased over previous responses.

No variation in per cent neutrophils can be correlated with the direct action of histamine. A slight neutrophil increase occurred on day 0 and 16 at 150 and 240 minutes post-injection in both the subcutaneous and intravenous animals receiving histamine. The per cent average neutrophils in group I varied from 60 to 77 while those in group II varied from 62 to 81. The per cent average neutrophils in the subcutaneous control subject ranged from 58 to 77 and 58 to 79 in the intravenous control subject.

Packed Cell Volume (Table 10)

Packed Cell Volume (PCV) studies indicate the absence of significant alterations occurring following histamine administration. A decreasing trend in PCV occurred in consecutive samples on the same day in both control and experimental animals. This decrease was attributed to the removal of 5 ml of blood for each determination.

A slight hemoconcentration occurred during the 15 minute post-injection

trial on day 0 and 16. Hemoconcentration occurred in 4 of 6 trials in the subcutaneous experimental animals and in 6 of 6 trials in the intravenous animals. The average PCV increase was 2.56 for group I and 4.23 for group II.

The trend in hemoconcentration was less pronounced at the 45 minute post-injection trial. The subcutaneous subjects showed an average hemoconcentration of 1.97 ml of RBC in 12 of 18 trials and the intravenous subjects 1.03 ml RBC in 9 of 15 trials.

Hemoglobin Values (Table 11)

Hemoglobin values in initial samples were miscalculated due to a faulty spectrophotometer. An attempt was made to superimpose the results on a corrected standard curve without success. The results were discarded and a different spectrophotometer employed.

The hemoglobin values correlate very closely with PCV results. Hemoconcentration occurred in 6 of 6 trials in the intravenous group 15 minutes following histamine administration and in 3 of 4 trials in the subcutaneous group. An increased hemoglobin value occurred in 11 of 14 trials in the subcutaneous subjects and in 12 of 15 trials in the intravenous subjects 45 minutes post-histamine injection. The increase ranged from 0.3 to 2.5 grams hemoglobin per 100 ml of blood.

Control subjects showed a definite trend toward hemoglobin decrease at 15 and 45 minutes post-saline injection. The subcutaneous control subject showed a decrease in hemoglobin in 1 of 2 trials at 15 minutes post-injection of saline. A decrease in hemoglobin values occurred in 2 of 2 trials 15 minutes post-saline injection and 5 of 5 trials 45 minutes post-saline injection in the intravenous control subject.

Prothrombin Time (Table 12)

Prothrombin time for all subjects averaged 10.5 seconds with a range of

7.6 to 15.6 seconds. Normal values usually given, range from 7 to 9 seconds. the extreme range was due to the method employed. Values obtained compared to normal values established prior to experimental injections, support a conclusion that no significant changes can be attributed to histamine administration.

Thrombocyte Values (Table 13)

Thrombocyte values remained within normal limits throughout the study. The mean thrombocyte value for all animals was 287,000 per cmm blood while the high and low values ranged from 153,000 to 440,000 thrombocytes per cmm of blood.

The results of this study are in agreement with the results of the clot retraction and coagulation time studies in which no changes were demonstrated following histamine administration. The critical level of thrombocytes necessary for clot retraction is approximately 70,000 or less per cmm of blood. Based on the results of this study it is concluded that histamine has no demonstrable effect on the thrombocyte value in the blood.

Coagulation Time (Table 14)

The mean coagulation time for all animals in the study was 7.8 minutes with a range of 4.5 to 14 minutes. No abnormal values or consistent variations occurred following histamine administration. An upper limit of 14 minutes is considered normal for this study. Prolongation of coagulation time can be attributed to variable room temperatures throughout the study. Based on these results, coagulation time is not altered by histamine administration.

Clot Retraction

Clot retraction studies were conducted on all blood samples previously used in coagulation time determinations. Visual observation of the degree of clot retraction and the firmness and rigidity of the clot showed normal progression in all samples.

Clot retraction was complete at 4 hours, while clot rigidity and firmness was normal at 24 hours. From the results of this study, there is no alterations produced in clot retraction or more specifically, platelet activity in the coagulation process.

Clinical Signs

Clinical signs were variable. Tolerance to exogenous histamine was not apparent as signs were as severe at the termination of histamine injections as they were initially. Clinical signs noted in group I included scleral injection, salivation, lacrimation, defecation, vomition, micturition, thirst and hyperpnea with abdominal breathing. Dog number II developed a cough and dyspnea within two minutes following injection and exhibited only mild signs as enumerated above. Individual variation in the target organs affected by histamine was prevalent in this study.

Dogs number III and IV exhibited edema at the injection site on the 6th and 7th day on experiment. Dogs numbered II and III developed a swelling and focal induration at the injection site on the 16th day on experiment. This reaction appeared to meet the requirements of an Arthus phenomenon.

A focal dermatitis appeared on dog number IV on the 32nd day of the experiment. Histamine base injection resulted in a slight puritis, edema, and erythema of the lesion. Skin scrapings for cutaneous acariasis were inconclusive, however, nineteen days following appearance of the lesion, skin scrapings were positive for Demodex canis.

Clinical signs observed in group II consisted of scleral injection, prostration, abdominal breathing, salivation, lacrimation, vomition, defecation, and micturition. Dog number VII exhibited severe respiratory embarrassment with occasional mild signs as enumerated above.

The method of injection appeared to have very little influence on the

induction period before appearance of clinical signs. The severity of response was correlated with the method of histamine administration.

The health of the animals in general was not affected by histamine injections. All animals maintained or increased their body weight throughout the investigation.

Dosage was based upon response in prior studies and adjusted to give approximately the same response by different routes of injection. The dosage administered in this study was 0.5 mg histamine base per kg body weight to group I and 0.2 mg per kg body weight to group II. In general, the intravenous subjects showed a greater severity based upon clinical signs.

GENERAL DISCUSSION

Response of Cellular Components

Variations noted in the total leucocyte count following histamine injection as shown in Table 3 were minor. The slight increase in cell count occurring on day 0 and 16 at 150 and 240 minutes post-injection suggested that a leucocytosis may have occurred. This response was most predominant in group I. The differential leucocyte count indicated that an increase in neutrophils occurred simultaneously and may therefore be responsible for the leucocytosis. This phenomenon may have been due to labored respiration and resulting physiological leucocytosis.

The differential leucocyte count reflected the eosinophil response, but with less accuracy than an actual eosinophil count per cmm of blood. An absolute eosinopenia occurred within 45 minutes following subcutaneous and intravenous histamine injections in 32 of 33 trials. The lowest levels were reached within two and one-half hours in eight of twelve trials. These findings are in agreement with the eosinopenia found by Archer (1963) in the horse, but not in agreement with the eosinophilia produced by Vaugh (1954) in the guinea pig. This difference may be due to species variation. The explanation for the eosinopenia remains a mystery, however, several possibilities exist. Litt (1964) found that the lymph nodes exhibit substantial eosinophilia following a single foreign protein stimulus. Histologic studies would verify or refute the possibility of eosinophil migration due to a histamine stimulus. Suzuki (1963) concluded that intravenous injection of histamine produced a marked acceleration of 17-hydroxycorticoid secretion which could result in an eosinopenia. Marked increases in 17-hydroxycorticoid were noted within five minutes following intravenous administration of histamine hydrochloride in the dog.

The rapid occurrence of an eosinopenia suggests that an alteration in the staining qualities of the eosin staining cellular components may have occurred with a resulting change in appearance of the cell. Uvnas (1958) describes profound morphological changes in the tissue mast cell when histamine is released as a result either of an antigen-antibody reaction or the action of a histamine liberator. The cells become degranulated and more or less lose their staining characteristics. It would, therefore, appear that a similar phenomenon may occur in the eosinophil in the presence of exogenous histamine.

Eosinophilia was well established on the sixteenth day following histamine base injections twice daily. Additional increases in absolute eosinophils per cmm occurred in all experimental animals except dogs number IV and VII during a period of eight days following histamine injections twice daily for 19 days. Apparently the initial high eosinophilia in dogs IV and VII, established on days -4, -2 and 0, indicated a physiopathological condition existing which interfered with the eosinophilic response to histamine injections. An additional histamine injection on the 28th day following the initial injection elicited an even higher eosinophilia in group I except dog number IV. The increase in absolute eosinophils per cmm of blood was approximately 233 per cent of the control levels established prior to the initial experimental injection. The response of animals II and III indicated that the eosinophilia may be due to an immune response. The first definite eosinophilia developed 16 days following the first injection of histamine and continued to develop further upon discontinuing injections for 12 days. An additional histamine injection at that time appeared to stimulate a greater eosinophilic response as determined four days later. Litt (1964) and Sabesin (1963) found evidence that one of the eosinophil in allergic reactions was the phagocytosis of antigen-antibody complexes and assumed that eosinophilia was a phenomenon which occurred after

formation of antigen-antibody complexes.

The eosinophil increase, even at the level of highest response, was less than ten per cent of the total leucocyte count. Based on dosage level and method of administration employed in this study, it would appear unlikely that eosinophil levels up to 30% exhibited in animals showing allergic clinical signs such as dermatitis, colitis, allergic rhinitis and parasitism could be produced by histamine injections.

Hemoglobin and PCV studies indicate no changes occuring following histamine injection. Hemoconcentration did not appear to be a phenomenon of consequence in this study. This is in agreement with Dale (1928), and Morgan et. al. (1959), however, Smith (1928) found hemoconcentration occuring following 10 mg of histamine diphosphate per kg body weight. The dosage employed was sufficient to produce death. Hemoconcentration occurred two hours following histamine administration during the secondary reaction. Hemoconcentration, as noted in anaphylactic shock, does not appear to be a physiopathological response to histamine at the dosage administered in this investigation.

Coagulation Mechanism

Clotting time, clot retraction, platelet count and prothrombin time values were within normal ranges following histamine injection. This finding is supported by Morgan et. al. (1959) and Dale (1929) in which normal values for clotting time were found following histamine administration. Bierman et. al. (1951) reported an immediate decrease in clotting time in humans following intravenous administration of histamine phosphate at a level equivalent to 0.1-0.3 mg histamine base. While Wintrobe (1961) reported an increase in platelet count following histamine administration in humans. Species variation may be responsible for the differences in the above described investigations.

Changes in the coagulation mechanism have been verified repeatedly in

anaphylactic studies. Brewer (1965) found a reduced coagulation time and intravascular clotting in the early stages of canine shock. While Robb (1963) demonstrated hypocoagulability of blood and a decreased platelet count following prolonged shock. The increased clotting time of blood during anaphylaxis found by earlier investigators (Dale 1929, Jaques and Waters 1941 and Rocha e Silva 1955) is compatible with current concepts of anaphylaxis. However, the absence of significant changes in the coagulation mechanism in this investigation does not support the histamine theory of anaphylaxis. The hypothesis advanced by Robb (1963) that large numbers of platelet emboli formed could account for the hypocoagulability of blood following prolonged shock and for the reported drop in platelet count would appear to be highly acceptable in light of present concepts.

Clinical Signs

Clinical signs appear in less than one minute following either subcutaneous or intravenous injection of histamine. The signs become progressively more severe for approximately five minutes, then tend to subside during the subsequent 15 minutes. The signs most commonly exhibited were scleral injection, salivation, lacrimation, severe abdominal pain, micturition, and thirst. Intravenous administration of histamine base at a dosage level of 0.2 mg per kg, occasionally produced prostration. Two of the six animals exhibited respiratory distress. Dog number III on subcutaneous study showed dyspnea and coughing suggestive of laryngeal spasm or edema. Dog number VII responded to intravenous histamine administration with a severe dyspnea and developed alveolar emphysema during the investigation.

These results appear to be significant in that one-third of the experimental subjects showed respiratory distress. The liver is assumed to be the primary shock organ in anaphylaxis in the dog, while asphyxiating contraction

of the bronchioles is the primary sign of anaphylaxis in the guinea pig. It would appear that either the respiratory system or the liver may be the primary shock organ in histamine shock in the canine.

The development of a clinical case of demodectic mange in dog number IV following histamine injections may be of significance. Histamine injection resulted in a slight puritis, edema and erythema of the lesion suggesting a definite aggravation of the process. Therefore, further study of histamine and demodectic mange is indicated.

Clinical signs did not appear to decrease in severity during the 16 days in which histamine administration was performed twice daily. When the final injection of histamine was administered on the 28th day to the dogs on subcutaneous study, the clinical response was nearly identical to those noted during the initial injection.

Clinical signs were not indicative of those occurring during anaphylactic shock. The response elicted by histamine injection differed from anaphylactic shock in several aspects. Abdominal pain was the outstanding sign in four of six subjects. Histamine shock was rapid and severe in onset and resulted in a vocal response and biting at the abdominal area. There was no change in appearance of the mucous membranes, no secondary response and no deaths from histamine shock. Anaphylactic shock, as severe in nature as the histamine shock, would undoubtably result in a grave prognosis. Based on the impressions and findings of this study, it would seem that histamine is notthe primary substance responsible for death in anaphylactic shock.

Clinical response was suggestive of direct activation of the parasympathetic nervous system reactor sites, thereby causing salivation, urination, lacrimation, defecation and pain due to stimulation of smooth musculature of the gastro-intestinal and urinary systems. Respiratory embarrassment could be

due to activation of the parasympathetic nervous system reactor sites resulting in contraction of the smooth musculature of the alveolar ducts and bronchioles.

Tolerance and Antigenicity

Evidence of tolerance development was lacking following histamine injections. The severity of clinical signs were consistent following each injection of histamine throughout the experimental period.

Histamine is suspected of being responsible for an immunological response. The occurrence of the Arthus Phenomenon in all experimental subjects on subcutaneous histamine administration supports a conclusion that a hypersensitivity had developed. Further circumstantial evidence of an immunological response is the occurrence of a prominent eosinophil increase 16 days following the initial injection of histamine. Based on the findings of Campbell, Drennan and Rettie (1938), Sabesin (1963) and Litt (1964), eosinophilia occurs following formation of antigen-antibody complex.

SUMMARY

An absolute eosinopenia occurs within 15 minutes following subcutaneous and intravenous injection of histamine base. Eosinophilia becomes well established on the 16th day following initiation of histamine base injections twice daily. The highest level of eosinophilia attained did not exceed ten per cent of the total white cell count.

Hemoglobin and hematocrit values were within normal range throughout histamine injection studies. Hemoconcentration did not appear to occur as a result of histamine administration.

Clotting time, clot retraction, platelet count and prothrombin time studies were not altered following histamine injection. Histamine does not appear to be a factor in producing changes in the coagulation mechanism.

Clinical signs appeared in less than one minute following either subcutaneous or intravenous histamine injections. The signs most commonly exhibited were scleral injection, salivation, lacrimation, severe abdominal pain, micturition, and thirst.

Histamine may be sufficiently antigenic to produce antibody formation twelve days following initial injections of histamine. This supposition is based on the occurrence of eosinophilia and Arthus Reactions at sufficient intervals for antibody formation to occur.

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APPENDIX

Table 2: Pre-injection Eosinophil Values

Dog No.	Status	Eosinophils per cmm Control Values			
		Day -4	Day -2	Day 0	Ave.
I	Control	253	198	123	191
II	SC	430	420	390	413
III	SC	540	418	240	399
IV	SC	853	973	730	852
Ave.	SC	608	604	453	555
V	Control	1122	742	880	915
VI	IV	468	605	286	453
VII	IV	1045	990	797	944
VIII	IV	264	170	231	222
Ave.	IV	592	588	438	530

Table 3: Post-injection Eosinophil Values, Day 0

Dog No.	Status	Eosinophils per cmm Day 0					
		Pre-injection 15 min.	Post-injection 15 min.	45 min.	90 min.	150 min.	240 min.
I	Control	123	122	132	143	138	122
II	SC	390	240	210	170	120	*
III	SC	240	120	90	71	75	95
IV	SC	730	215	390	400	137	132
Ave.	SC	453	192	230	214	111	114
V	Control	880	863	847	907	869	808
VI	IV	286	208	178	228	72	94
VII	IV	797	402	440	532	503	374
VIII	IV	231	38	44	81	77	102
Ave.	IV	438	216	221	280	217	190

*Sample lost through technical difficulties.

Table 4: Post-injection Eosinophil Values, Days 4 - 8 - 12

Dog No.	Status	Eosinophils per cmm Day 4 Pre-inj. 15 min.	Day 4 Post-inj. 45 min.	Day 8 Pre-inj. 15 min.	Day 8 Post-inj. 45 min.	Day 12 Pre-inj. 15 min.	Day 12 Post-inj. 45 min.
I	Control	116	127	231	236	110	143
II	SC	350	210	358	163	242	149
III	SC	247	160	340	220	390	187
IV	SC	720	462	726	363	880	660
Ave.	SC	439	277	475	349	504	248
V	Control	742	698	1067	935	764	767
VI	IV	99	72	385	365	649	550
VII	IV	891	704	770	731	1155	786
VIII	IV	396	291	577	644	517	330
Ave.	IV	462	356	577	380	774	555

Table 5: Post-injection Eosinophil Values, Day 16

Dog No.	Status	Eosinophils per cmm Day 16					
		Pre-injection 15 min.	Post-injection 15 min.	45 min.	90 min.	150 min.	240 min.
I	Control	192	187	187	231	176	143
II	SC	500	429	341	247	324	341
III	SC	412	297	291	330	413	396
IV	SC	1034	407	550	561	603	754
Ave.	SC	649	378	394	379	447	411
V	Control	566	544	561	539	523	495
VI	IV	561	539	490	429	314	297
VII	IV	1072	803	968	671	841	863
VIII	IV	561	368	407	451	363	242
Ave.	IV	731	570	622	517	506	534

Table 6: Post-injection Eosinophil Values, Days 20 - 24

Dog No.	Status	Eosinophils per cmm			
		Day 20	Day 24	Ave.	Per cent control values (Table 2)
I	Control	247	202	225	118
II	SC	742	729	736	178
III	SC	484	528	506	127
IV	SC	736	902	819	96
Ave.	SC	654	719	687	134
V	Control	523	633	578	63
VI	IV	528	715	621	137
VII	IV	572	990	781	83
VIII	IV	605	649	627	282
Ave.	IV	568	785	677	167

Table 7: Post-injection Eosinophil Values, Days 28 - 32

Dog No.	Status	Eosinophils per cmm			
		Day 28		Day 32	Per cent Control (Table 2)
		Pre-inj. 15 min.	Post-inj. 45 min.		
I	Control	583	665	324	170
II	SC	660	451	1177	285
III	SC	*	814	1177	295
IV	SC	990	743	869	120
Ave.	SC	825	669	1074	233

*Sample lost through technical difficulties.

Table 8: Total Leucocyte Counts[1]

Dog No.	Status	Control Values		Day 0		
		Day -4	Day -2	Pre-inj. 15 min.	Post-inj. 15 min.	45 min.
I	Control	13,100	14,500	13,700	13,200	11,800
II	SC	9,800	10,700	9,200	8,500	7,900
III	SC	10,900	12,300	16,800	12,600	12,400
IV	SC	12,000	17,200	17,100	16,300	14,900
Ave.	SC	10,900	13,400	14,400	12,500	11,700
V	Control	15,400	11,900	11,000	11,300	12,300
VI	IV	22,500	24,600	13,500	12,500	12,500
VII	IV	10,100	8,700	15,100	14,000	12,000
VIII	IV	8,800	7,700	11,700	8,400	9,100
Ave.	IV	13,800	13,700	13,400	11,600	11,200

[1]Total leucocytes per cmm blood

Table 8 continued - Total Leucocyte Counts

Dog No.	Day 0 Post-injection 90 min.	150 min.	240 min.	Day 4 Pre-inj. 15 min.	Post-inj. 45 min.
I	11,900	12,200	11,600	19,700	14,800
II	7,800	10,500	*	9,600	11,400
III	10,600	16,400	20,700	11,600	12,700
IV	21,500	27,900	22,600	19,900	17,700
Ave.	13,300	18,300	21,600	13,700	13,900
V	10,000	10,000	9,600	10,700	9,800
VI	13,800	14,200	14,400	11,800	11,900
VII	16,500	18,900	19,600	15,000	14,500
VIII	10,500	11,600	12,900	9,000	8,700
Ave.	13,600	14,900	15,600	11,900	11,700

*Sample lost through technical difficulties.

Table 8 continued - Total Leucocyte Counts

Dog No.	Day 8 Pre-inj. 15 min.	Day 8 Post-inj. 45 min.	Day 12 Pre-inj. 15 min.	Day 12 Post-inj. 45 min.
I	16,500	16,100	17,900	16,100
II	13,400	11,600	13,600	19,500
III	18,600	13,900	19,900	17,900
IV	21,300	20,000	19,400	14,900
Ave.	17,800	15,200	17,600	17,400
V	11,300	10,900	10,900	9,100
VI	19,700	18,700	22,400	19,900
VII	14,500	14,600	14,400	14,900
VIII	14,200	11,600	12,200	10,700
Ave.	16,100	15,000	16,300	15,200

Table 8 continued - Total Leucocyte Counts

Dog No.	Pre-inj. 15 min.	Day 16 Post-injection 15 min.	45 min.	90 min.	150 min.	240 min.
I	17,800	19,000	20,200	17,500	16,400	19,400
II	12,900	13,200	14,000	15,000	18,000	22,000
III	16,600	14,200	15,500	15,700	17,500	15,700
IV	11,900	9,800	10,200	12,000	13,500	14,000
Ave.	13,800	12,400	13,200	14,200	16,300	17,200
V	11,900	10,900	10,400	10,300	8,600	8,400
VI	15,200	16,700	11,700	15,600	16,400	16,900
VII	12,100	10,100	10,600	10,100	10,100	9,000
VIII	13,100	11,300	11,400	12,200	13,400	12,300
Ave.	13,500	12,700	11,200	12,600	13,300	12,700

Table 8 continued - Total Leucocyte Counts

Dog No.	Day 20	Day 24	Day 28		Day 32
			Pre-inj. 15 min.	Post-inj. 45 min.	
I	17,000	19,500	15,600	17,500	15,900
II	13,800	16,800	15,600	13,100	13,400
III	15,500	15,300	19,700	14,000	16,000
IV	14,800	11,600	11,900	11,000	13,000
Ave.	14,700	14,600	15,700	12,700	14,100
V	14,500	9,500			
VI	14,500	12,100			
VII	12,600	10,200			
VIII	12,000	10,500			
Ave.	13,000	10,900			

Table 9: Differential Leucocyte Count

Dog No.	Status	Control Values Day -4					Day -2					Day 0 Pre-injection 15 min.				
		N	E	B	L	M	N	E	B	L	M	N	E	B	L	M
I	Control	64	1	0	29	6	66	2	0	26	6	74	0	0	21	5
II	SC	76	2	0	19	3	75	2	0	20	3	61	2	0	29	7
III	SC	53	1	0	43	3	49	6	1	36	8	72	3	0	20	5
IV	SC	68	5	2	19	6	56	7	0	30	8	62	8	0	21	9
Ave.	SC	66	3	1	28	4	60	5	0	29	6	65	4	0	23	7
V	Control	65	7	2	23	3	69	10	0	14	7	69	5	0	24	2
VI	IV	73	4	0	16	7	67	7	0	21	5	69	3	0	20	8
VII	IV	68	8	0	18	8	71	8	0	17	4	83	3	0	11	3
VIII	IV	68	4	0	24	4	50	3	0	36	11	65	3	0	24	8
Ave.	IV	70	5	0	19	6	62	6	0	25	7	73	3	0	18	6

N - Neutrophils
E - Eosinophils
B - Basophils
L - Lymphocyte
M - Monocyte

Table 9 continued - Differential Leucocyte Count

Day 0 Post-injection														
15 min.					45 min.					90 min.				
N	E	B	L	M	N	E	B	L	M	N	E	B	L	M
66	3	0	22	9	73	2	0	19	6	68	1	0	26	6
51	2	0	42	5	49	2	0	48	2	54	2	0	37	7
72	0	0	21	7	69	0	1	25	5	66	0	0	27	7
63	0	0	30	7	72	2	0	21	5	72	3	0	21	4
63	1	0	31	6	64	1	0	31	4	64	2	0	28	6
71	4	0	17	8	67	3	0	22	8	61	9	0	19	11
67	2	0	22	9	71	1	0	21	7	71	1	0	21	7
83	1	0	11	5	77	2	0	17	4	81	4	0	11	4
61	1	0	35	3	64	0	0	27	9	76	1	0	15	8
70	1	0	23	6	70	1	0	22	7	76	2	0	16	6

Table 9 continued - Differential Leucocyte Count

Day 0 (cont.) Post-injection 150 min.					240 min.					Day 4 Pre-injection 15 min.				
N	E	B	L	M	N	E	B	L	M	N	E	B	L	M
72	0	0	18	10	66	1	0	28	5	64	2	0	28	6
66	2	0	25	7	--	-	-	--	-	55	5	1	36	3
70	1	1	24	4	72	2	0	20	6	63	3	0	28	6
77	4	1	11	7	82	1	0	12	5	63	5	0	27	5
71	2	1	20	6	77	2	0	16	5	60	4	0	31	5
74	6	0	16	4	70	10	1	14	5	70	5	0	21	5
79	0	0	16	5	80	0	0	14	6	70	0	0	24	6
82	3	0	12	3	81	3	0	10	6	72	5	0	19	4
81	0	0	15	5	79	0	0	16	4	63	4	0	26	7
81	1	0	14	4	80	1	0	14	5	68	3	0	23	6

Table 9 continued - Differential Leucocyte Count

Day 4 (cont.) Post-injection 45 min.					Pre-injection 15 min.					Day 8 Post-injection 45 min.				
N	E	B	L	M	N	E	B	L	M	N	E	B	L	M
70	2	0	25	3	64	1	0	27	8	69	2	0	25	4
50	3	0	42	5	65	3	0	28	4	54	2	0	38	6
60	1	0	33	6	67	4	0	22	7	71	1	0	22	6
76	2	0	18	4	68	6	0	20	6	75	1	0	19	5
62	2	0	31	5	67	4	0	23	6	67	1	0	26	6
79	5	0	14	2	69	7	0	18	6	65	7	1	23	4
62	0	0	29	9	70	3	0	25	4	72	2	0	23	3
77	4	0	17	2	71	5	0	21	3	79	7	0	13	1
61	5	0	30	4	71	2	0	24	3	67	3	0	26	4
67	3	0	25	5	71	3	0	23	3	73	4	0	31	3

Table 9 continued - Differential Leucocyte Count

Day 12											Day 16				
Pre-injection 15 min.					Post-injection 45 min.					Pre-injection 15 min.				
N	E	B	L	M	N	E	B	L	M	N	E	B	L	M
70	2	0	22	6	67	2	0	23	8	67	1	0	23	9
60	4	0	30	6	59	1	1	34	5	67	5	1	25	2
66	3	0	25	6	65	1	0	29	5	62	5	0	28	5
66	6	0	19	9	69	4	0	20	7	63	10	0	25	2
64	4	0	25	7	64	2	0	28	6	64	7	0	26	3
62	6	1	27	5	59	6	0	34	3	63	3	0	30	4
71	5	0	18	6	70	4	0	23	3	64	6	0	26	4
73	5	1	19	2	74	7	0	12	8	83	2	0	17	0
58	8	0	28	6	66	2	0	27	5	65	4	0	26	5
67	6	0	22	5	70	4	0	21	5	70	4	0	23	3

Table 9 continued - Differential Leucocyte Count

Day 16 continued

Post-injection 15 min.					45 min.					90 min.				
N	E	B	L	M	N	E	B	L	M	N	E	B	L	M
72	1	0	21	6	69	1	0	25	4	60	1	0	35	4
58	2	0	37	3	57	4	0	35	4	72	2	0	27	4
65	2	0	28	5	66	2	0	26	6	73	1	1	16	9
46	2	0	50	2	56	4	1	34	5	70	5	0	19	6
57	2	0	38	3	60	3	0	32	5	72	3	0	21	6
58	7	0	30	5	61	5	0	31	4	67	5	0	25	3
59	6	0	31	4	61	2	0	30	7	67	3	0	24	6
72	6	1	19	2	75	4	0	19	2	72	8	0	16	4
69	2	0	24	5	75	5	0	16	4	75	2	0	14	9
66	5	0	25	4	70	4	0	22	4	71	4	0	18	6

Table 9 continued - Differential Leucocyte Count

Day 16 continued											Day 20				
150 min.					Post-injection 240 min.										
N	E	B	L	M	N	E	B	L	M		N	E	B	L	M
59	0	0	35	6	58	2	0	34	5		67	0	0	27	6
76	2	0	19	3	80	2	0	11	7		50	7	0	38	5
75	1	1	18	5	66	1	0	29	4		64	2	0	28	6
73	5	0	18	4	64	4	1	27	4		72	3	0	22	3
75	3	0	18	4	70	2	0	23	5		62	4	0	29	5
63	4	0	30	4	59	6	0	28	7		62	3	0	33	2
73	5	0	16	6	75	0	0	21	4		66	4	0	24	6
71	8	0	15	6	71	8	1	15	5		76	5	0	14	5
78	2	0	14	6	76	1	0	19	4		70	3	2	23	4
74	5	0	15	6	74	3	0	18	4		71	4	1	20	5

Table 9 continued - Differential Leucocyte Count

Day 24					Day 28									
					Pre-injection					Post-injection				
N	E	B	L	M	N	E	B	L	M	N	E	B	L	M
77	2	0	18	3	62	3	0	27	8	65	4	0	25	6
67	5	1	22	3	66	3	0	27	4	64	2	0	30	4
56	4	0	34	6	68	5	0	20	9	57	5	0	33	5
66	2	1	24	7	59	6	0	32	2	64	5	1	22	8
63	4	1	27	5	64	5	0	26	5	62	4	0	28	6
60	5	1	31	3										
66	8	0	20	6										
72	3	0	23	3										
67	3	0	26	5										
68	5	0	23	5										
Day 32														
72	0	0	22	6										
56	6	0	33	5										
55	7	0	32	6										
69	8	0	19	4										
60	7	0	28	5										

Table 10: Packed Cell Volume[1]

Dog No.	Status	Normal Values Day -4	Day -2	Day 0 Pre-inj. 15 min.	Post-inj. 15 min.	45 min.	90 min.	150 min.	240 min.
I	Control	43	43.5	40	37.5	37.5	35.0	36.0	34.5
II	SC	47.5	47.5	49.5	49.5	50.0	48.5	45.5	*
III	SC	49.0	52.0	45.5	48.0	45.5	43.0	42.0	45.5
IV	SC	47.5	45.0	46.0	44.5	47.0	44.0	37.5	37.0
Ave.	SC	48.0	48.0	47.0	47.5	47.5	45.0	41.5	41.0
V	Control	41.5	41.0	41.0	39.5	40.0	39.0	36.0	37.5
VI	IV	45.0	47.0	42.5	47.5	48.0	48.0	43.5	44.0
VII	IV	48.0	46.5	46.5	52.5	49.0	47.0	42.5	41.5
VIII	IV	43.5	42.5	43.5	46.5	45.0	43.5	42.0	41.0
Ave.	IV	45.5	45.5	44.0	49.0	47.5	46.0	42.5	42.0

[1]ML of red blood cells per 100 ml of blood.

*Values lost due to technical difficulties.

Table 10 continued - Packed Cell Volume

Day 4		Day 8		Day 12	
Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.	Post-inj. 45 min.
40.5	38.0	38.5	37.0	37.5	36.0
43.5	43.5	41.5	41.0	39.0	42.0
41.5	39.0	42.0	43.0	42.0	44.0
42.5	45.0	42.0	41.0	41.0	46.0
42.5	42.5	42.0	41.5	40.5	44.0
39.5	35.0	37.0	35.0	39.0	38.0
44.5	47.0	42.5	46.5	44.5	43.0
46.0	48.0	49.5	50.5	49.0	49.0
38.5	40.5	45.0	44.5	43.5	42.5
43.0	45.0	45.5	47.0	45.5	45.0

Table 10 continued - Packed Cell Volume

Dog No.	Day 16 Pre-inj. 15 min.	Post-inj. 15 min.	45 min.	90 min.	150 min.	240 min.	Day 20
I	35.5	37.0	36.0	36.0	40.0	37.0	39.0
II	41.5	43.0	45.5	41.5	41.0	40.0	43.0
III	43.5	50.5	46.5	41.5	44.0	40.0	41.0
IV	44.5	50.5	48.0	41.5	39.5	39.0	40.0
Ave.	43.0	48.0	46.5	41.5	41.5	39.5	41.5
V	41.0	41.5	43.0	39.5	34.5	36.5	42.0
VI	47.5	51.0	49.0	46.5	46.0	47.5	43.0
VII	48.0	51.5	47.5	46.0	43.0	44.0	44.0
VIII	43.0	47.5	39.5	47.0	41.0	39.5	45.5
Ave.	46.0	50.0	45.5	46.5	43.5	43.5	44.0

Table 10 continued - Packed Cell Volume

Day 24	Day 28		Day 32
	Pre-injection 15 min.	Post-injection 45 min.	
40.0	39.5	40.0	41.0
41.0	41.0	46.5	40.5
48.0	44.0	44.5	44.5
42.0	41.5	51.0	*
43.5	42.0	47.5	42.5
44.0			
42.0			
44.5			
47.5			
44.5			

*Values lost due to technical difficulties.

Table 11: Hemoglobin Values[1]

Dog No.	Status	Normal Values Day -4	Day -2	Day 0 Pre-inj. 15 min.	Post-inj. 15 min.	45 min.	90 min.	150 min.
I	Control	13.9	14.7	12.6	12.3	12.2	11.8	11.4
II	SC	*	*	*	*	*	*	*
III	SC	*	*	*	*	*	*	*
IV	SC	*	15.4	16.0	15.2	16.0	15.0	13.0
Ave.	SC	*	15.4	16.0	15.2	16.0	15.0	13.0
V	Control	14.1	15.8	13.9	13.2	15.5	14.3	12.5
VI	IV	14.4	15.4	14.1	15.8	15.8	15.6	14.5
VII	IV	16.1	15.3	15.4	17.2	16.6	15.8	14.1
VIII	IV	14.6	15.4	15.4	16.2	15.8	16.0	15.4
Ave.	IV	15.0	15.4	15.0	16.4	16.1	15.8	14.7

[1]Gm per 100 ml of blood

*Value discarded because of faulty spectrophotometer.

Table 11 continued - Hemoglobin Values

Day 0 (cont.)	Day 4		Day 8		Day 12
Post-inj. cont. 240 min.	Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.
11.1	13.6	12.2	12.6	12.2	12.6
*	*	*	*	*	13.5
*	14.8	13.0	14.6	14.7	13.6
13.6	13.5	15.1	13.9	13.4	13.1
13.6	14.2	14.1	14.3	14.1	13.4
13.0	14.7	12.1	12.6	12.4	14.3
14.6	14.3	14.9	13.9	14.7	15.0
13.6	13.6	16.3	16.8	17.0	17.2
15.0	14.4	14.7	15.3	15.4	14.3
14.4	14.1	15.3	15.3	15.7	15.5

Table 11 continued - Hemoglobin Values

Day 12 cont. Post-inj. 45 min.	Day 16 Pre-inj. 15 min.	Post-inj. 15 min.	45 min.	90 min.	150 min.	240 min.
12.2	12.4	12.8	12.7	12.0	13.1	12.8
14.1	13.9	15.1	15.6	14.4	14.1	13.7
14.8	14.7	17.2	15.6	13.7	14.5	12.8
15.0	14.5	16.3	15.8	13.4	12.8	12.6
14.6	14.4	16.2	15.7	13.8	13.8	13.0
13.7	16.2	16.1	16.2	16.1	13.3	14.7
14.4	15.1	17.0	16.4	14.8	14.6	15.4
17.3	18.6	17.0	16.2	16.6	15.4	15.3
15.6	14.5	17.1	13.5	16.3	14.0	15.2
15.8	16.1	17.0	15.4	15.9	14.7	15.3

Table 11 continued - Hemoglobin Values

Day 20	Day 24	Day 28		Day 32
		Pre-inj. 15 min.	Post-inj. 45 min.	
12.6	14.5	13.3	14.0	13.1
14.1	12.3	13.7	15.0	13.8
13.8	17.2	15.8	15.0	14.9
13.1	14.2	14.0	17.0	*
13.7	14.6	14.5	15.7	14.4
15.4	15.6			
15.1	14.0			
14.8	14.4			
16.6	15.5			
15.5	14.6			

Table 12: Prothrombin Time Determinations[1]

Dog No.	Status	Normal Values Day -4	Day -2	Day 0 Pre-inj. 15 min.	Post-inj. 15 min.	45 min.	90 min.	150 min.	240 min.
I	Control	10.3	12.0	9.9	11.2	10.9	11.0	10.6	10.3
II	SC	9.6	11.0	11.8	12.0	13.0	12.3	12.3	*
III	SC	12.9	11.0	12.3	11.8	13.4	11.7	12.3	11.8
IV	SC	10.2	11.0	11.6	12.5	11.9	12.6	13.4	12.5
Ave.		10.9	11.0	11.9	12.1	12.8	12.2	12.7	12.2
V	Control	8.0	10.1	10.2	10.3	10.1	*	8.4	8.1
VI	IV	9.2	11.8	11.9	12.0	11.6	11.6	12.0	11.5
VII	IV	10.3	10.2	11.6	11.2	12.4	11.7	11.8	11.2
VIII	IV	11.5	11.2	14.1	14.4	15.6	13.7	13.1	13.3
Ave.		10.3	11.1	12.5	12.5	13.2	12.3	12.3	12.0

[1]Plasma prothrombin time of Quick

*Values lost due to technical difficulties

Table 12 continued - Prothrombin Time Determinations

Day 4		Day 8		Day 12		Day 16
Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.
10.6	9.7	10.9	10.7	10.2	9.9	11.9
13.3	11.2	10.3	11.6	11.1	11.3	10.3
10.8	10.5	10.7	11.3	12.2	11.8	10.4
11.3	11.8	*	9.7	11.7	11.5	12.1
11.8	11.2	10.5	10.9	11.7	11.6	10.9
8.1	7.6	9.3	9.1	8.7	8.8	9.7
10.3	10.4	10.1	10.2	9.2	9.3	11.3
11.5	11.3	10.2	9.8	12.8	12.6	9.1
12.0	11.5	9.5	9.4	9.9	10.1	11.3
11.3	11.1	9.9	9.8	10.6	10.7	10.6

Table 12 continued - Prothrombin Time Determinations

Day 16 cont. Post-inj. 15 min.	45 min.	90 min.	150 min.	240 min.	Day 20	Day 24
10.3	10.5	10.5	10.6	10.6	10.6	9.3
10.1	11.5	11.3	9.8	9.7	12.2	10.4
10.7	10.8	11.3	11.1	11.1	12.2	10.8
11.7	11.6	11.9	11.5	11.7	10.7	10.7
10.8	11.3	11.5	10.8	10.8	11.7	10.6
9.6	10.3	9.4	9.3	9.3	10.2	9.2
11.3	*	10.0	12.1	9.6	11.6	8.4
9.2	8.7	8.7	8.6	8.8	8.5	8.3
11.5	11.5	11.7	11.4	11.3	10.4	10.5
10.7	10.1	10.1	10.7	9.9	10.2	9.1

Day 28		Day 32
Pre-injection 15 min.	Post-injection 45 min.	
8.6	9.1	10.4
11.2	10.2	10.4
10.0	10.0	12.0
11.3	11.0	13.0
10.8	10.4	11.8

Table 13: Thrombocyte Values[1]

Dog No.	Status	Normal Values Day -4	Day -2	Day 0 Pre-inj. 15 min.	Post-inj. 15 min.	45 min.	90 min.	150 min.	240 min.
I	Control	352	212	220	232	232	230	239	253
II	SC	230	200	227	240	213	197	200	*
III	SC	440	306	288	262	278	202	243	301
IV	SC	298	263	210	270	279	216	346	268
Ave.	SC	323	256	241	257	257	205	263	285
V	Control	395	423	245	266	290	378	364	358
VI	IV	231	250	231	234	212	193	194	216
VII	IV	248	276	251	189	140	218	223	200
VIII	IV	300	308	272	334	350	384	359	416
Ave.	IV	260	278	251	252	234	265	259	277

[1]Platelets in thousand per ml of blood

*Values lost due to technical difficulties

Table 13 continued - Thrombocyte Values

Day 4		Day 8		Day 12		Day 16
Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.
290	302	254	224	320	210	248
268	270	400	247	380	362	258
222	274	314	344	430	370	303
348	432	340	355	243	319	340
279	325	351	315	351	350	300
285	239	195	184	252	257	312
163	176	174	153	176	185	230
352	357	272	360	342	262	269
222	232	450	441	324	267	346
246	255	299	318	287	241	248

Table 13 continued - Thrombocyte Values

Day 16 continued					Day 20	Day 24
Post-inj. 15 min.	45 min.	90 min.	150 min.	240 min.		
266	287	322	324	252	339	358
235	260	278	382	382	417	396
398	370	376	370	368	356	246
394	438	399	342	271	300	334
342	356	351	365	340	358	325
271	241	268	240	239	235	291
264	220	220	267	274	274	208
247	239	244	230	244	309	310
341	303	349	337	374	376	215
284	254	271	278	297	320	244

Pre-injection	Day 28	Post-injection	Day 32
416		411	368
223		380	440
268		279	340
310		370	275
267		346	352

Table 14: Coagulation Time

Dog No.	Status	Normal Values Day -4	Day -2	Day 0 Pre-inj. -15 min.	Post-inj. 15 min.	45 min.	90 min.	150 min.	240 min.
I	Control	5.5	4.5	8.5	9.0	6.5	7.0	7.0	8.0
II	SC	7.0	5.0	5.0	5.5	5.0	5.0	5.5	*
III	SC	5.0	6.0	5.0	*	6.5	6.0	5.5	8.0
IV	SC	6.5	7.0	6.5	7.5	9.5	7.0	7.5	8.0
Ave.	SC	6.2	6.0	5.5	6.5	7.0	6.0	6.2	8.0
V	Control	7.0	7.0	7.5	7.5	6.5	7.5	6.0	6.0
VI	IV	8.5	12.5	13.0	13.5	13.5	13.0	13.0	14.0
VII	IV	10.5	13.5	9.0	7.5	6.5	5.5	7.0	6.0
VIII	IV	9.0	9.0	8.5	*	10.5	9.5	7.0	6.0
Ave.	IV	9.3	8.3	10.2	10.5	10.2	9.3	9.0	8.7

[1]Method of Lee and White

*Values lost due to technical difficulties

Table 14 continued - Coagulation Time

Day 4		Day 8		Day 12		Day 16	
Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.	Post-inj. 45 min.	Pre-inj. 15 min.	Post-inj. 15 min.
11.0	10.5	10.0	9.0	8.5	8.5	12.0	10.5
5.0	6.5	7.5	5.5	5.0	5.5	8.0	7.0
6.5	7.0	7.5	7.5	8.5	12.0	7.5	10.0
5.5	8.5	5.5	7.5	11.5	13.5	10.5	13.0
5.7	7.2	6.8	6.8	8.3	10.3	8.7	10.0
7.0	6.5	13.0	8.0	7.0	10.0	8.5	9.5
8.5	10.0	7.0	6.0	8.0	12.0	*	9.0
12.0	13.0	8.0	7.0	12.5	11.5	10.5	9.5
6.0	5.0	8.5	9.0	8.5	9.5	10.0	9.0
8.8	9.3	7.8	6.7	9.7	11.0	12.5	9.2

Table 14 continued - Coagulation Time

Day 16 continued				Day 20	Day 24
Post-inj. 45 min.	90 min.	150 min.	240 min.		
9.0	8.5	8.0	11.5	9.0	7.5
6.5	8.0	6.0	6.0	11.0	10.5
9.5	9.0	*	12.0	8.5	8.0
14.0	13.5	12.0	11.0	8.0	10.5
10.0	10.2	9.0	9.7	9.2	9.7
9.5	10.0	10.0	9.5	6.0	10.5
10.5	8.5	9.0	7.0	9.5	7.0
8.0	10.5	9.0	7.5	13.5	10.0
9.5	9.5	9.5	8.2	11.2	9.2
9.3	9.5	9.2	7.7	11.4	8.7

Day 28		Day 32
Pre-injection 15 min.	Post-injection 45 min.	
11.0	12.0	7.5
4.5	7.0	7.0
*	5.5	9.5
9.0	9.0	8.0
8.2	7.2	8.2

THE EFFECT OF HISTAMINE ON CERTAIN HEMATOLOGICAL FACTORS IN THE CANINE

by

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AN ABSTRACT OF A MASTER'S THESIS

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requirements for the degree

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Allergic and anaphylactic mechanisms in many disease syndromes have become more commonly recognized. The principle mediator has not been fully documented nor has the role of histamine been adequately defined in relation to its basic pharmacological activity. Therefore, a study encompassing some of the hemal factors and clinical signs was conducted in an effort to determine changes induced by sublethal doses of histamine base administered over an extended period of time to the dog.

Eight mongrel dogs were selected and randomly divided into two groups of four animals each for subcutaneous (SC) and intravenous (IV) studies. One animal in each group was retained as a control.

Observations included total leucocyte, eosinophil, and differential leucocyte counts, hematocrit, hemoglobin, prothrombin time, clotting time, clot retraction and platelet count determinations. These observations were made over a period of 36 days for the animals on subcutaneous study and 28 days for the animals on intravenous study. Normal values for each animal were established from the results of sampling on alternate days for three consecutive determinations before histamine or saline injections were initiated. A total of 9 different serial hematological determinations were conducted on the 4 SC study animals and 7 different serial hematological determinations were conducted on the 4 IV study animals.

Clinical signs appeared in less than one minute following either subcutaneous or intravenous injections of histamine. Histamine injections resulted in signs of scleral injection, salivation, lacrimation, severe abdominal pain, micturition, defecation, and prostration. However, other changes associated with systemic allergic syndromes, such as increased clotting time, hemoconcentration, leucopenia, thrombosis, and hemorrhage were not observed. The absence of these signs would tend to confirm suggestions that

substances other than histamine are responsible for several of the signs of allergic responses.

Histamine appears to elicit an antigenic response 12 days following initial administration. This assumption is based on the occurrence of eosinophilia and the Arthus Reaction. There was no indication that a tolerance developed for the pharmacologic action of histamine.

Hemoglobin and hematocrit values remained within the normal range throughout the study. Hemoconcentration did not appear to be a part of the histamine reaction. Clotting time, clot retraction, platelet count and prothrombin studies showed no alterations in the coagulation mechanism following histamine administration.

In this study, the effects of histamine on observed hematologic factors was confined to an eosinophil response. An eosinopenia occurred in all subjects 15 minutes following histamine administration and reached the lowest levels with 150 minutes in eight of twelve trials. Eosinophil increases became well established on the 16th day following initiation of histamine injections twice daily. Progressive increases were noted following additional histamine injections. The highest level of eosinophils did not exceed ten per cent of the total white cell count. Further studies are indicated to clarify the eosinophil phenomenon.

Finally, there appears to be a noted absence of systemic effects following histamine administration which can be compared with permanent changes commonly associated with anaphylactic shock. However, the effects of histamine on smooth muscle, lacrimation, scleral injection and eosinophil increases combined with apparent absence of changes in other hemotologic factors are consistent with findings in local allergic syndromes.