HAEMATOLOGICAL STUDY OF INFECTED PYE DOGS (Canis familiaris) IN KARACHI

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M. Sc

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IN KARACHI

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INTRODUCTION

The blood dyscrasias produced by adverse reactions to hemoparasitic infections and the drugs are of diverse groups. They are cytopenias that are undesired and have unexpected side-effects, developing in only a very small proportion of dog population exposed to the hemoparasitic infection and drugs, and occurring, for the most part, unpredictably. The cytopenias include agranulocytosis, thrombocytopenia, hemolytic anemia, megaloblastic anemia, and aplastic anemia. As unrelated to these conditions, consideration of them as a group is justified not only because of their common etiology, but also because they require awareness on the part of the veterinarians to assist, diagnosis and treatment.

One of the most important aspects of these reactions to hemoparasitic infection and drugs is their pathogenesis. If we can learn the factor or combination of factors which make an occasional dog react to hemoparasitic infection and drug in a way in which the great majority of dog population do not, we might be able to identify susceptible dogs and potentially toxic drugs and hemoparasites before disaster. This goal has been attained for one type of reaction; the hemolytic action of hemoparasites and oxidant drugs in dogs with glucose-6-phosphate dehydrogenase deficiency (Beutler, 1966). This purpose of this project is to provide the current knowledge of hematologic reactions to hemoparasites and drugs, emphasizing pathogenetic mechanisms. Dr. Haider has observed the veterinary manifestations and has documented the hemoparasites and drugs responsible (Haider, 1992). I brief my
discussion of these aspects. Knowledge of the pathogenesis of most of the syndrome remains obscure. Often, particularly in aplastic anemia, some veterinarians cannot even be sure of that a particular case is the result of exposure to any hemoparasites drug and have no way to prove such a casual relationship. The mechanism by which a given type of dyscrasia is produced by one blood protozoan and produced by a one drug may be different from that by which another blood protozoan and another drug produces the same reaction. A drug and blood protozoan may, in different dogs produce the same cytopenia in more than one way.

Several general mechanisms have been devised by which a blood protozoan and drug might lead to a cytopenia in a small proportion of the dog population.

a) The blood protozoan and drug might act as a heptene which stimulates the production of an antibody capable of attacking the cell in the presence of the blood protozoan and drug. Such a mechanism has been demonstrated in some cases of agranulocytosis and of hemolytic anemia and appears to be a frequent mode of production of blood protozoan intensity and drug thrombocytopenia (Shulman, 1964).

b) The effect on the blood cell might result from intensity of the blood protozoan and concentrations of drug greater than those ordinarily obtained through therapeutic use. A cell deficient in a mechanism capable of protecting it against blood protozoan and therapeutic concentrations of the drug would be highly susceptible. An example of this is the heritable deficiency of erythrocytes glu-
c) The susceptible dog might have a metabolic abnormality such as that toxic product is formed from the blood protozoan, and then the drug, to a much greater extent than in most dogs.

d) The blood protozoan and drug might antagonize a metabolic reaction that is not vital in most dogs, but may become vital in the susceptible dog because of failure of an alternative pathway fails due to enzyme deficiency.
PRESENT WORK

A search of the literature reveals scanty information as to average, normal or abnormal values or standards for the dog under controlled and experimental conditions or for various lengths of time, although this animal has been very commonly used in the study of changes involving the hematopoietic system. The data here presented are adequate in this respect, but they are submitted in the hope that workers in other laboratories in which dogs are extensively used will be stimulated to add their findings, so that ultimately the number of values will justify the establishment of normal and abnormal levels. This project includes 4000 determinations of normal animals (50% males and 50% females), in which 3000 hemoparasitized non-splenectomised naturally infected animals (50% males and 50% females) and 500 splenectomised, hemoparasitized, naturally infected animals (250 males and 250 females) and 500 experimentally infected animals (50% males and 50% females)(250 splenectomised and 250 non-splenectomised) pye dogs (Canis familiaris) of various breeds and ages, obtained from the pond and used in various investigations.

Routine examinations were usually made in the morning. Blood samples were taken from the ear, the first few drops discarded. Larger quantities, when necessary, were drawn from the femoral vein or artery.

This project was made during July 1990 to June 1996 in Karachi (Pakistan). A record of collection was kept regularly to
see if dogs' hemoparasites are common in certain areas, and the blood dyscrasias produced by adverse reactions to blood protozoan and drugs are a diverse group. Similarly a record of all the hemoparasites and hematologic values are kept and numbers were assigned to each of them for ready reference. A total of 3 different genera of hemoparasites including Babesia, Trypanosoma and Leishmania are described and illustrated. This include 2 species of each Babesia (B. canis and B. gibsoni), Trypanosoma (T. brucei and T. evansi) and one species of Leishmania (L. donovani). Prevalence of hemoparasites (blood protozoa) in dog at Karachi (Pakistan) are listed in Table 1.

Since no previous attempts have been made to study the response to dog in hemoparasites, and hematologic examination in Pakistan, the present project was therefore designed for study
OBJECTIVES

This project has been focused for the studies of hematology during hemoparasitic infections and effect of drugs which are influenced on the hematology of *Canis familiaris* (common or pye dog), therefore other infections such as intestinal parasitic infections (helminthic infections) and other types of ectoparasites and endoparasites have not been considered for this project.

1) I have attempted to report the current information of the blood protozoan of dogs in Karachi (Pakistan).

2) I have attempted to record current knowledge of the hemoparasitized and the drug-induced hematologic reactions in dogs.

Details are given in the section of result.
REVIEW
OF
LITERATURE
Review of Literature

Study of available literature depicts that normal and adverse hematologic reactions to blood protozoa and drugs in dog have been reported and discussed by a quite large number of workers belonging to almost every region of the world. Most of particular regions are U.S.A, Mexico, Canada, France, Germany, Britain, Australia, Africa, Middle East, Central Asia, China, Malaysia, Bangladesh, Srilanka, India and Pakistan.

A description of the hematology of dog and review of the literature on this subject are found in papers by various authors. A review of available literature in chronological order is as under:

Dawson, 1900; Busch and Van Bergen, 1902; Von Dungern and Hirschfield, 1910; Ottenberg et al., 1913; Musser and Krimbhaar, 1914; Wells and Sutton, 1916; McEnery et al., 1924; Krueger and Schultz, 1925; Joseph, 1927; Emmons, 1927; Stebbins and Leake, 1927; Pierce and Plant, 1928; Archley and Benedict, 1927; Mayerson, 1930; Searborough, 1930; Powers et al., 1930; Dayton, 1931; Leich Senling et al., 1932; Ashley and Guest, 1934; Holman et al., 1934; Madison and Squier, 1934; McNaught, 1935; Melnick et al., 1935; Wintrobe et al., 1936; Wright, 1936; Dameshek and Colmes, 1936; Bruner and Wakerlin, 1937; Stasney and Higgins, 1937; Melnick and Cowgill, 1937; Gibson et al., 1938; Hunter, 1939; Mallory et al., 1939; Landsberg, 1939; Morris et al., 1940; Olson, 1940; Rawson et al., 1941; Bornford and Rhoads, 1941; Mulligan, 1941; Hahn and Bale, 1942; Meyer and Bloom, 1943; Vontoon and Clark, 1943; Mulligan et
al., 1943; Miller et al., 1945; Mulligan, 1945; Moore, 1946; Ederstrom and Deboer, 1946; Medawar, 1946; Mettler et al., 1948; Bartels, 1948; Ackroyd, 1948; Rekers and Coulter, 1948; Eyquem, 1948; Ackroyd, 1949; Eyquem, 1949; Hamilton, 1949; Young et al., 1949; Yule et al., 1949; Young et al., 1950; Ackroyd, 1951; Lowe and Davey, 1951; Christian et al., 1951 (a) and 1951 (b); Young et al., 1951 (a) and 1951 (b) and 1952; Discombe, 1952; Moeschlin and Wagner, 1952; Saint Paul et al., 1952; Friesen et al., 1952; Mannheimer et al., 1952; Lewis et al., 1952; Giack and Mclean, 1953; Snapper et al., 1953; Cohen and Fuller, 1953; Swisher, et al., 1953 (a) and 1953 (b) Stohlman, 1953; Dela Riviere and Eyquem, 1953; Dern et al., 1954; Thompson et al., 1954; McGavock and Chevally, 1954; H'Doubler and H'Doubler, 1954; Welch et al., 1954; Mourant, 1954; Swisher, 1954; Moeschlin, 1955; Feichtmeier et al., 1955; Steinkamp et al., 1955; Berlyne et al., 1955; Beutler et al., 1955; Dern et al., 1955; Smith, 1955; Girdwood and Lenman, 1956; Savilanchi, 1956; Freedman et al., 1956; Koszowski and Hubbard, 1956; Freedman et al., 1956 (a) and 1956 (b); Bolton and Dameshek, 1956; Carson et al., 1956; Jandl et al. 1956; Stohlman and Schneiderman, 1956; Stohlman 1956; Swisher, 1956; Newman and Sumner, 1957; Pavne, 1957; Butler, 1957; Beulter, 1957; Szeinberg et al., 1958; Flanagan et al., 1958; Hawkins and Meynell, 1958; Pisciotta et al., 1958; Mohler and Leavell, 1958; 'Muirhead et al., 1958; Anastason, 1958; Ley et al., 1958; Andersen and Gee, 1958; Szeinberg et al., 1959; Fiore and Noonan, 1959; Scott et al., 1959; Muirhead et al., 1959; Ley et al., 1959; Garriga and Crosby, 1959; Desorges et al., 1959; Beutler, 1959; Marks and Gross, 1959; Mollison, 1959; Troup et al., 1960; Watson et al., 1960; Fudenberg and German, 1960; Shinton and
Wilson, 1960; Gangarosa, et al., 1960; Weissman, 1960; Deavers et al., 1960; Tullis, 1961; Dausset and Bergerot - Blondel, 1961; Engelfriet and Vanlooghem, 1961; Benjamin, 1961; Carson et al., 1961; Brewer et al., 1961; Allen and Jandl, 1961; Anderson et al., 1961; Oort et al., 1961; Lyon, 1961; Wintrobe, 1961; Swisher and Young, 1961; Shulman et al., 1962; Strumia and Raymond, 1962; Beutler, et al., 1962; Fairbank and Beutler, 1962; Eritlev and Wintrobe, 1962; Robins, 1962; Huguley, 1963; Shulman, 1963; Walzer and Einbinder, 1963; Hoffman et al., 1963; Jandl, 1963; Penny, 1963; Bettman, 1963; Weisberger et al., 1963; Jiji et al., 1963; DeGowin, 1963; Huggins et al., 1963; Maloney et al., 1963; Shulman, 1964; Shulman et al., 1964; Stein et al., 1964; Beutler and Baluda, 1964; Rodriguez et al., 1964; Huguley, 1964; Pisciotta and Santos, 1964; Yunus and Bloomberg, 1964; Vigliani and Saita, 1964; Quagliani et al., 1964; Ross and Rosenbourn, 1964; Beal et al., 1964; Lawrence, 1964; Pollini and Colombi, 1964; Raab et al., 1964; Boggs et al., 1964; Desforges, 1965; Beutler, 1965; Schmid et al., 1965; Yunus et al., 1965; Pisciotta and Santos, 1965; Pisciotta, 1965; Daugan and Woodif, 1965; Sjoberg and Perers, 1965; Wijnja et al., 1965; Beutler, 1966; Swanson et al., 1966; Carstair et al., 1966; Tarlov et al., 1967; Jandl et al., 1967; de Leeuw et al., 1967; Frick et al., 1967; Robinson and Ziegler, 1968; Bover et al., 1968; Pannacciulli et al., 1968; Griggs, 1968; Motulsky 1969; Shively et al., 1969; Dellenback et al., 1969; Schmid et al., 1970; Soulsby, 1970; Bertanga, 1970; Sonoda and Kobayashi, 1970; Scott et al., 1970; Sherman et al., 1970; Deavers et al., 1971; Adam et al., 1971; Huggins et al., 1971; Hung, 1972; Ingall et al., 1973; Bean, 1974; Minter and Ingram, 1974; Deubelbeiss et al., 1975 (a) and 1975 (b); Manwell, 1976; Cohen, 1977; Walliker, 1978; Grab,
1979; Sidaiqui, 1980; Cullen, 1980; Brander ano Rugh, 1980; Mackey, 1981; Gregor, 1981. Lambert, 1982; Levine, 1983; Zavala, 1983; Gwadz, 1984; Snedecor, 1984; Zaugg, 1984; Sinha, 1984; Schalm, 1985; Collins, 1985; Nussenzweig, 1985; Ramsey, 1985; Haider and Mallik, 1985; Beaudoin, 1986; Kuttler and Kreier, 1986; Haider and Mallik, 1986; Haider and Gondal 1987; Baden, 1987; Espinol, 1988; Haider and Iqbal, 1988 (a, b, c, and d); Davidson, 1989; Motulsky, 1989; Haider and Iqbal, 1989 (a, b, c and d); Brodey, 1989; Robertson, 1990; Jensen, 1990; Steel and Torrie, 1990; Wintrobe, 1990; Haider and Iqbal, 1990; (a, b, c and d); Maegher and Bowie, 1991; Haider and Iqbal, 1991 (a, b, c and d). Fandeur, 1992; Bailey, 1992; Haider and Iqbal, 1992; (a, b, c and d); Haider, 1992; Merck, 1993; Gysin, 1993; Molineaux, 1953; CDAMA, 1993, Haider and Iqbal, 1993; (a, b, c and d) Gramiccia, 1994; Russel, 1994; Haider, 1994; Haider and Iqbal, 1994 (a and b); Frei, 1994; Bloom et al., 1994; Erslev, 1994; Beeson et al., 1994; American Medical Association, 1994; Best, 1994.

The earliest systematic observations of blood group of dogs were made in by Von Dungern and Hirschfield (1910) who recognized four different blood groups in this aspects. Ottenberg, et al. (1913) described transfusion reactions in dogs following isoimmunization and infusion of incompatible cells. McEnery et al. (1924), and later Melnick et al. (1935) and Melnick & Cowgill (1937) recognized the Phenomenon of isoimmunization of dogs during the course of experiments in which recipients were infused with the red cells of other animals. Similar experiences were recorded on several occasions by Holman, et al. (1934), wright (1936),
Hahn and Bale (1942), Miller, et al. (1945), from the department of pathology, University of Rochester, and it was these observation that stimulated much of the work to be summarized here. A number of other workers, including Olson (1940), Hamilton (1949), and Eyquem (1948, 1949) have also investigated various aspects of dog blood groups. Dela Riviere and Eyquem (1953) and Mourant (1954) have published brief reviews of canine blood grouping systems. A variety of blood grouping systems in dogs have been under investigation in the department of pathology, University of Rochester since 1947 (Christian, et al., 1951 (a), 1951 (b); Swisher, et al., 1953 (a), 1953 (b); Young, et al., 1949, 1950, 1951 (a), 1951 (b); & 1952). These systems had been developed, identified, and characterized in the course of studies on the mechanisms of destruction of incompatible red cells by isoantibodies.

The several blood grouping systems in the dog provide models analogous to a number of human blood grouping systems. Since even massive hemolytic transfusion reactions are rarely fatal in dogs, it is possible to observe repeatedly the phenomena which occur when incomparable red cells are infused. The blood grouping systems which have been identified in the department of pathology, University of Rochester have been encountered largely fortuitously by investigating the isoantibodies produced by immunizing transfusions in a series of randomly cross-transfused dogs. No effort has been made to define all the possible blood grouping systems which may exist in this species, since the first seven systems encountered provided adequate models of the general types of
systems they desired to study. There is adequate evidence to suggest that dog blood grouping systems other than those described in department of pathology, University of Rochester exist and could be identified by further systematic study. This review will discuss those systems which are best characterized in department of pathology, University of Rochester with full recognition of the fact that additional systems will be identified if they are sought for. As in human transfusion practice compatibility between donor and recipient dogs for all known blood grouping systems is no assurance of biological compatibility for all possible significant antigenic differences. The blood volume (ml/kg body wt.) of a number of species of animals is significantly higher at birth than in the adults phase whether this is true in the human is less clear because of the variability of the data for the newly born.

In a number of species including that of human being there is a period during the early part of growth in which there is a continuous decrease in venous hematocrit, hemoglobin content, total hemoglobin and in the red cell volume per unit body weight.

In the dog, the change in the relationship of these variables in body weight and with each other in growth has received little attention. Therefore, the changes in these parameters were measured in beagles from the day of birth to one year of age (Deavers, et al., 1971). Since Wintrobe, et al. (1936) have pointed out the relative paucity of information in the literature concerning normal blood values for the dog, I am briefly recording additional data obtained during certain experimental studies. Various authors have reported
the total blood volume of normal dog is in terms of percentage of body weight. Thus Haider and Mallik (1985) using the carbon monoxide method, found the blood volume to average 6.65 percent of body weight. Haider and Iqbal (1988 b) using the acacia method and the pulfrich refractometer, obtained an average value of 5.55 percent of body weight. Using various modification of the original dye method of Baden (1987), Haider and Gondal (1987) reported 6-11 percent; Haider and Iqbal (1988 a, c, d) 6 percent; Davidson (1989) 7.6 percent; and Jensen (1990) 3 percent as the average percentage of body weight for normal and abnormal total blood volume. In terms of cubic centimeters per kilogram of body weight average values hereby reported very widely. From the data presented by Haider and Mallik (1986) an average value of 40 cc per k. gm. is obtained. Haider and Iqbal (1989 a, b, c, d) found an average of 35cc per k. gm., while the average value reported by Maegher and Bowie (1991) 60 cc per k. gm. Further analysis of the findings of these two groups, workers show a disagreement as to the relationship of unit volume in terms of body weight to variations in body weight in the 300 dogs studied by Haider and Iqbai (1990 a, b, c, d) unit volume ranges from 30 cc - 40 cc per k. gm. and tended toward a constant value with decrease in body weight. In the series of 50 dogs reported by Fandeur (1992) unit volume varied from 35 cc per k. gm. to 45 cc per k. gm. and tended to decrease with decreasing weight.

In the series of Gysin (1993) unit volume varied from 40 cc to 50 cc per k. gm. and showed a marked decrease in weight.
In a study of spontaneous lymphoma in dogs, it was necessary to develop a technique for reported bone marrow studies in the living animal. A review of the literature disclosed no consistent methods or results which could be used as standard. Davidson (1989) employed Mackey (1981) sternal aspiration method in the study of the bone marrow of 12 normal dogs, and calculated the percentage distribution of marrow cells. Haider and Iqbal (1991) investigated the marrow of 35 apparently normal dogs immediately after death of the animals by making imprints and sections of the 7th right ribs and the proximal and middle portions of the right femur. They found only slight variations in the cellular organization from the different sites selected, although their quantitative results differed from those of Davidson (1989). This was attributed to the fact that their animals were anemic as reflected by lowered peripheral red cell counts. Mulligan (1945) examined paraaffin sections and smears of the bone marrow in 35 normal living adult dogs and 4 puppies by resecting portions of the 10th, 22nd and 12th ribs under nembutal anesthesia. The smears were made by expressing the marrow from the rib with bone forceps, emulsifying it with plasma from the same animals, and then spreading this mixture on glass slides. His results were similar to those of Davidson (1989) and this procedure was suggested for repeated bone marrow studies, as 12 biopsies may be done on an animal if the 7th, 8th and 9th ribs (which are easily accessible) are also used.

In evaluating these techniques for obtaining marrow, Mackey (1981) aspiration method is the simplest and an ultimate number
of successive biopsies can be made upon the same animal. I have applied this procedure successfully in young dogs of medium and large sizes, but difficulty was experienced in small dogs due to the marrow medullary cavity, and in other dogs due to an increase in density and thickness of the outer layer of compact bone. Gwadz (1984) described a rapid method for the diagnosis of canine leishmaniasis by aspiration of bone marrow from the crest of the ilium. I have used this site successfully in obtaining marrow from animals of all ages and sizes. In addition to greater accessibility, it has all the advantages of Mackey (1981) method, as the sternal marrow in the dog can only be aspirated from the upper 4-5 segments.

The frequency with which Grab (1979) thesis has been consulted during the past fourteen years indicated the desirability of rendering it accessible to investigators who are engaged in blood studies upon animals. The normal blood picture of the commonly used animals has been determined at great length by many investigators, but almost always as incidental to and for the control of experimental work in which alterations in the picture were expected. In consequence the data relating to the normal findings are hidden under titles which refer only to the object of the experimental work. The purpose of this compilation is to make these data relating to normal blood pictures more easily available.

No attempt has been made to include the literature since 1976. The mass of material up to that time was so extensive that the recent additions would not significantly alter the averages.
New and more accurate methods may be expected to change these normal values.

The study has brought to light many mistakes of authors who were not aware of similar work already done by others, as well as several important deficiencies in our knowledge of the blood picture of laboratory animals (Haider and Iqbal, 1993 d).

Evidence was presented from this laboratory in 1982 that the physiologic normal hemoglobin concentration for the adult dog is 18 gm. or more gm/100 ml of blood, with a corresponding red cell count of 7,590,000 mm$^3$ and hematocrit of 54-1 Vol. % (Haider and Iqbal, 1991 a). This was in sharp contrast to the then currently accepted value of 14 gm (Haider and Iqbal, 1991 b). Experience in canine medicine is well stated by Haider and Iqbal (1991 c) as follows: "Most of the reports during the past 20 years give the number of red cells in the adult dogs as 6.16 to 7.24 million per cmm. of blood, with hemoglobin values of 12.5 to 16.6 Gm. per 100 ml of blood. The values we have obtained are somewhat higher than those generally reported, ranging from 15 to 18 Gm. of hemoglobin and red cell counts from 6.5 to 8 million". In a recent communication they state that "In private canine practice I see a fair number of adult dogs with hemoglobin values in the 17-19 gram percent range".

Haider and Iqbal (1991 d) have observed even higher values in adult dogs considered to be perfectly normal, namely, red blood count 7-9 million, hemoglobin 19-20 gm/100 ml of blood and
hematocrit 50-55 vol. %. In spite of this, we find a concentration of 14-8 gm% listed as the hemoglobin level of the normal adult dog in a recent handbook of blood values (Haider, 1994).

Since it has been observed that the hemoglobin level in a healthy animal varies directly with the quality of the nutriment up to the point where the physiologic norm is reached, we are presenting data on six dogs subsisting on diets adequate for this purpose. In addition, since charge with age 6 (Haider, 1994) and traumatic situations 6' (Haider and Iqbal 1992 a) have been indicated, one dog has been followed on such a diet over a period of 10½ years or the approximate life span of this species.

A satisfactory quantitative determination of erythropoiesis can be obtained from the measurement of the total circulating red cell volume (TRCV) with labeled red cells, the rate of production of red cells from the rate of disappearance of Fe⁵⁹ from the plasma and subsequent uptake by red cells, and the red cell life span with glycine-2-C¹⁴ (Haider and Iqbal, 1992 b).

The total red cell volume of the dog has been measured with labelled red cells (Haider and Iqbal, 1992 c, d) and the red cell life span determined various protoporphyrin and globin precursors containing C¹⁴ and Fe⁵⁹ (Haider and Iqbal, 1993, a, b & c) and by the Ashby differential agglutination technique (Haider and Iqbal, 1993 a, b & c) There are no reported measurements of the red cell iron turnover rate as measured with Fe³⁷.
The studies reported here are part of a series dealing with the distribution of phosphorus in the blood in a number of pathologic conditions which are known to effect the phosphorus metabolism of the body. In earlier papers attention has been directed especially to the organic acid soluble fraction of the blood phosphorus, designated "ester P", and to changes of its concentration in relation to other chemical constituents of the blood. Following experimental high intestinal obstruction in dogs there were found marked increase of ester P in the blood cells, as well as of inorganic P in the plasma (Ashley and Guest, 1934).

In another study, it was found that following the administration of large doses of irradiated ergosterol to rabbits the increase of inorganic P in the plasma -- changes already made familiar through the work of previous investigators -- were accompanied by increase of ester P in the cell which were of greater magnitude than the increase of inorganic-P. (Joseph, 1927; Atchley and Benedict, 1927).

While the mechanism of such increase of ester P in the cells appeared obscure, it was recognized that in these conditions the partial suppression of renal function might be a factor influencing the changes observed. In further study of this phase of the problem, several experimental procedures known to bring about acute suppression of renal function in animals have been employed.

Many studies, both clinical and experimental, have furnished a fairly complete picture of the chemical changes which occur in
the blood plasma as a result of \( \text{HgCl}_2 \) poisoning. Acute nephrites with suppression of renal function is generally held to be responsible for the increase of nonprotein nitrogen and other metabolites in the blood, while vomiting and diarrhea apparently account for the depletion of the blood chlorides commonly observed (Haider, 1992).

Although a few papers concerning the ultrastructure of the dog eosinophil leukocyte have been published (Shively et al. 1969; Sonoda and Kobayashi, 1970; Hung, 1972), the presence of lamellated crystalloid in dog eosinophil granules have not been shown until brief report (Hung 1972).

The disappearance from the circulation of neutrophils labeled with \( ^{32}\text{P} \) disopropylphosphorofluoridate (DF \( ^{32}\text{P} \)) has served as a basis for calculating the turnover of circulating neutrophils in the both dogs (Raab et al., 1964) and man (Haider, 1994). These studies provide estimates of effective neutrophil production in other studies using tritiated thymidine attempts to determine total marrow neutrophil production have been made (Deubelbeiss et al., 1975 a). However, these latter studies have been of limited usefulness because of the inadequate methods available for measuring marrow cellularity. In an accompanying paper, a method for determining the number of erythroid and neutrophilic cells in dog marrow has been described (Boggs et al., 1964).

In a previous report Mulligan (1941 and 1945) the quantitative data on the bone marrow of thirty five adult mongrel dogs
and of four puppies were presented and a method of serial
biopsy of the bone marrow of the living dog by resection of
segments of the seventh through the twelfth ribs was described.
Since then two other papers dealing with the bone marrow of
adult dogs have been published. Van Loon and Clark (1943)
performed differential counts on smears of extruded rib marrow
examined immediately following the sacrifice of eight normal
mongrel adult dogs. Meyer and Bloom (1943) studied the cellular
composition of bone marrow obtained by needle puncture from
the crest of the ilium in ten normal living dogs (eight through bred,
two mongrel), 1 to 11 years old.

The blood volume of the newborn of a number of species of
animals, expressed in milliliters per kilogram body weight (blood
volume ratio), is significantly larger than that of the adult. (Andersen
and Gee, 1958) Although at least one animal, the pig, appears to
be an exception to this generalization (Deavers et al., 1960), it is
impossible to decide, because of the contradictory data in the
literature, whether or not there is a similar decrease in blood

Even if blood volume in milliliters per kilogram volume (milliliters
per kilogram, hereafter called plasma volume) does not necessarily
decrease, or, if a decrease occurs, it may not be parallel to the change
in blood volume. Mott's data of rabbit (Mott, 1965) illustrate the later
possibility, although the mean plasma volume was stable from
birth until day 31 of age, blood volume ratio decreased signifi-
cantly. In the adult rabbit, however, the plasma volume was less
than that of the newborn.

Despite a considerable quantity of data on the plasma volume of humans at different ages, it is difficult to decide whether the plasma volume of the adult is significantly less than that of the newborn and young (Robinson and Ziegler, 1968).

Thus, from the data available in literature it was impossible to make firm conclusions on the relation between age, body weight, plasma volume, and hematocrit from birth to maturity for any single species.

**Agranulocytosis**

As early as 1934, Madison and Squier suggested an immune mechanism for aminopyrine-induced agranulocytosis based on the observations that only a few test animals receiving aminopyrine developed agranulocytosis, and the granulocytes disappeared and returned rapidly after a change. Moeschlin and Wagner first demonstrated the presence of leukocyte agglutinins in aminopyrine-agranulocytosis in 1952. They found that serum or plasma from an aminopyrine-sensitive test animals caused agglutination of leukocytes from normal or aminopyrine-sensitive test animals only if the aminopyrine-sensitive donor had ingested the drug shortly before his serum was obtained. They also found that fresh blood from an aminopyrine-sensitive donor who had ingested the drug shortly before donation, when transfused into a normal recipient, produced striking leukopenia within 40 minutes, followed by recovery in four
hours. By Moeschlin 1955 had demonstrated a similar immune mechanism for sulfapyridine agranulocytosis. Although he was able to enhance in vitro leukocyte agglutination by addition of a small amount of sulfapyridine, he again pointed out that he was unable to demonstrate this drug dependence in vitro with aminopyrine. He presented a complete scheme for immunoagranulocytosis with histologic evidence suggesting that agglutinated leukocytes are destroyed and removed from the circulation in pulmonary capillaries.

**Thrombocytopenia**

In 1948 - 1951, Ackroyd demonstrated a drug-dependent antibody against platelets in purpura due to allylisopropylacetylene (Sedormid). Four years later, he summarized his work on the mechanism of allergic purpura, particularly that due to Sedormid. In vitro platelet agglutination by Sedormid required platelets from normal or from Sedormid-sensitive test animals and serum from a Sedormid-sensitive test animals. Only upon the addition of complement was platelet lysis observed. An attack of thrombocytopenic purpura was produced by as little as 1.4 x 10^6 Gm. of Sedormid to a highly sensitized test animals. He also observed a positive Sedormid patch test in the absence of thrombocytopenia, suggesting that a second mechanism of allergic purpura with Sedormid was a drug-dependent immune reaction against capillary endothelium. Steinkamp et al (1955) reported a patient who had habitually used a proprietary quinine preparation and who, several hours after ingestion of quinine, developed massive generalized hemorrhage with a platelet count of
The presence of quinine-dependent antibodies was proven by drug challenge, by in vitro quinine-dependent agglutination studies, and by passive transfer to a normal animal: the quinine-sensitive test animal's plasma caused thrombocytopenia only when the recipient ingested quinine shortly before the infusion.

In 1956, Bolton and Dameshek described the clinical features of 23 cases of quinidine purpura, of which 19 were proven by drug challenge or agglutination tests. They reported 5 cases of their own, systematically demonstrating quinidine-dependent platelet agglutination in vitro in each case.

**Hemolytic Anemia**

In 1953 Snapper and coworker studied a test animal with Coombs'-positive hemolytic anemia and pancytopenia secondary to Mesantoin ingestion who recovered completely after administration of the drug was stopped. Haider and Iqbal (1994) reported dog male who had been treated with diminazene at age of one year. At age 2 years he was retreated with diminazene. Eleven days after the first injection and three hours after the fifth injection, he suddenly developed symptoms and signs of hemolytic anemia. Subsequent in vitro tests revealed a diminazene dependent Coombs'-positive red cell antibody. In vivo evidence for the dependence of red cell agglutination on the presence of diminazene was provided by the passive transfer of the agglutination to a normal recipient who did not develop Coombs-positivity or red cell agglutination until he was given an injection of diminazene.
Mechanism of Action

Shulman et al. (1962) has emphasized the greater specificity, sensitivity, reliability, and versatility of the complement-fixation techniques over those utilizing agglutination. Complement fixation techniques allow a much fuller demonstration of the principles of the antigen-antibody reaction.

Moeschlin (1955) suggested that a common mechanism existed for the various immune drug cytopenias. He and many others had proposed that the affected cell, altered by the offending drug or complex with it as a heptane, became antigenic, inducing a cell-specific antibody response. In 1963, Shulman, by measuring association constants, described the progressive steps in a quinidine antibody-platelet reaction; he graphed the effect of quinidine concentration on the adsorption of antibody, on complement fixation, and on agglutination, quantitated the equilibrium concentrations of the reactants in each step, and then related the platelet count to the number of free platelet sites for binding. He pointed out that thrombocytopenia may develop rapidly in the sensitized individual with quinidine concentration levels which in vitro are not sufficient to allow measurable platelet agglutination or even complement fixation.

Shulman (1963) has suggested that the haptenic drug must couple firmly with a macromolecule of the serum (usually a protein) to become effectively antigenic. The drug adsorbed on blood cells is easily eluted by washing in saline. Concentrations of
drug a million times greater than needed to fill all available antibody binding sites on a cell will not interfere with the adsorption of antibody by the cell. Drug antibodies have a high affinity for their respective drugs, binding them in the absence of blood cells and competing successfully against the nonspecific but efficient binding capacity of other plasma proteins. Shulman (1964) believes that the drug-induced immune mechanism consists of the formation of an antigenic complex of drug and a noncellular soluble plasma macromolecule which then leads to the production of antibodies that bind the drug with high affinity. The antibody-drug complex is then adsorbed non-specifically by one of the types of blood cell. He has emphasized that many examples of nonspecific blood cell adsorption of antigen or antigen-antibody complexes are known. Further evidence suggested that the type of blood cell involved may be more dependent on physical characteristics (size, configuration, charge) of the antibody than on any property of the drug. In six cases of quinidine or quinine thrombocytopenia the antibodies were 7S gamma globulins, but in two cases of stibophen hemolytic anemia the antibodies were 19S gamma globulins. In one test animal, two forms of quinidine antibodies a 7S gamma globulin reacting only with platelets and a 19S gamma globulin reacting only with red cells were identified. It is important to note that although the adsorption of the drug antibody complex on the cell is nonspecific, complement is bound to the cell (Shulman 1964). Cells to which sublytic amounts of complement are attached through the action of drug antibodies can be washed or dialyzed to elute drug or antibody completely; but complement remains fixed to the cell (Shulman 1964). Such a situation might also occur in
vivo after complete excretion of the drug. In the case of hemolytic anemia, the adsorption of the drug-antibody complex on the red cell can be detected by an anti-gamma globulin Coombs' serum. If the Coombs' serum is of a broad spectrum and contains antibodies against the complement components involved, the Coombs' test might continue to be positive after complete excretion of the drug. A "non-gamma" Coombs' test serum might be necessary to detect the adsorbed complement. (Shulman 1964). The phenomenon might possibly explain the persistence of a positive Coombs' test for 50 days after discontinuation of diminazene in dog (Haider and Iqbal, 1994).

The basic immune mechanisms involved seems to apply to all the immunocytopenias so far studied. Shulman's work with isoantibodies provides an insight into later stages of the isoimmune reaction. (Shulman et al., 1964). Although these antibodies are specific for the cell and unrelated to a drug, it is likely that their later effects on blood cells leading to cell destruction are common to both types of reaction.

The mechanism of the hemolytic anemia caused by antibodies induced by penicillin differs in some respects from the above generalization. Circulating antibodies directed against penicillin may be demonstrable by the agglutination of erythrocytes sensitized to penicillin. (Ley et. al., 1958; Watson et al., 1960; Fudenberg and German, 1960). The cells bind penicillin in the absence of the antibody, and the drug is not easily washed off. Cell thus sensitized will be agglutinated by the antibody. Whether or not
hemolytic anemia can be caused by these hemagglutinating antibodies is not clear. Coombs' positive hemolytic anemia has occurred in several test animals after the prolonged administration of penicillin in high dosage (20 million units or more daily for matter of weeks). (Ley et al., 1959 and Swanson et al., 1966). Other manifestations of penicillin allergy are not necessarily present. The usual hemagglutinating antibodies demonstrable in test animals sensitized to penicillin are IgM (19S) type (Fudenberg and German, 1960, and Swanson et al., 1966). The antibodies associated with a positive Coombs' test, which presumably are responsible for the hemolytic anemia, are IgG (7S) type, and differ from the typical penicillin antibodies in other ways. (Fudenberg and German, 1960, and Swanson et al., 1966).

In drug agranulocytosis, the rapid disappearance of circulating granulocytes after a test dose of the offering drug demonstrates attack on the mature granulocytes themselves. The marrow early in the reaction may show a complete lack of granulocytes or only very immature granulocytes. Marrow precursors, therefore, are also susceptible to destruction. In thrombocytopenia produced by this mechanism, it is again clear, both from the rapid fall in the platelet count and from the in vitro destruction of platelets, that the immune reactions attacks the circulating platelets. The appearance of the megakaryocytes in the marrow suggests that they also are damaged and that platelet production is held up for a time.

We know of no evidence that the marrow red cell precursors are destroyed by the drug-dependent antibodies which attack circulating red cells.
Regardless of the cell type involved, the clinical pattern of an immune hematologic drug reaction is similar. Usually the test animals have previously ingested the drug, although occasionally no such history can be documented. The test animals may have been taking the offending drug regularly or intermittently for a period varying from two weeks to many years. When suddenly, after taking as little as one tablet, symptoms appear.

The clinical manifestations of acute agranulocytosis, acute thrombocytopenic purpura and acute hemolytic anemia are well known and need not be discussed here. In a test animal with any of these conditions the possibility of a drug or chemical immune mechanism must be considered. All drugs should be stopped until the diagnosis is clarified. Rapid improvement of the test animal will support the impression of an immune response related to a drug. Often the only way to prove the relationship to the drug is to challenge the test animal cautiously with the drug after recovery.

Platelet Antibodies

Platelet antibodies may be demonstrated by a number of technics: (1) microscopic observation of platelet agglutination and lysis in a mixture of platelets, serum from the test animal and varying concentrations of the drug (Shulman, 1963), (2) demonstration of complement fixation during the above reaction; (Shulman,
1963). (3) demonstration of alteration of platelet function by the test animal’s serum in the presence of the drug by inhibition of clot retraction of normal blood (Bolton and Dameshek, 1956) (4) the transfer of sensitivity to a normal animal by transfusion of the test animal’s blood or plasma, followed by administration of the offending drug.

The passive transfer experiment appears unwarranted. Agglutination and complement fixation technics have not always been easy to perform. A simple method of detecting serum antibodies against platelets by inhibition of clot retraction has been described and appears to be not only easy, but quite reliable (Freedman et al., 1956). Saturated solutions of the suspected drugs in saline are prepared and serial dilutions made. Two ml. of fresh compatible normal blood are added to a series of tubes containing 0.2 ml. of a drug dilution or 0.2 ml. of a saline control, mixed with 0.2 ml of the test animal’s serum or 0.2 ml of control serum. After inverting to mix, the tubes are incubated at 37°C and observed for clot retraction after two hours. The test is positive if clot retraction occurs in the control tubes but does not occur in tubes containing the drug and the test animal’s serum. By this means, the diagnosis is quickly established and the offending drug identified.

Antibodies may disappear rather promptly after administration of the drug is discontinued (Freedman et al., 1956). A negative test, therefore, may not necessarily rule out an immune mechanism and certainly does not eliminate other mechanisms, as yet not understood, through which thrombocytopenia may be pro-
duced by a drug. In such cases, a cautious challenge of the test animal with small doses of the drug may be justified in order to establish the relationship.

Ackroyd (1948 - 1949) has reviewed reports of anti-platelet antibodies demonstrated in association with allylisopropylacetyleurea, ethylallylacetyurea, quinidine, quinine, chlorpheniramine maleate, sulfamezathine, p-amino-salicylic acid, antazoline, methyl paraphenol, and bromoallylmethylisopropylbarbituric acid. Tullis (1961) demonstrated drug-dependent platelet antibodies related to tolbutamide, phenacetin, biphetamine, ethchlorovynol, digitoxin, and vitamin K.

Other examples, involving mephenytoin (Snapper et al., 1953), diphenylhydantoin (Freedman et al., 1956) and chlorpropamide (Stein et al., 1964) have been reported. The AMA registry on adverse reactions includes reports of antibodies against promethazine, sorbitan trioleate and hydrochlorothiazide (CDAMA, 1993). Although the demonstration of a drug-dependent antibody attacking platelets appears to be clear evidence for the causal relationship of the drug to thrombocytopenia in the cases described, there is only a single report for many of the drugs in the list above. As can be seen from the infrequency of reports to the registry of adverse reactions, some of these drugs cannot be regarded as particularly likely causes of thrombocytopenia.

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<th>Red Cell Drug-dependent Antibodies</th>
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Red cell drug-dependent antibodies have not been difficult
to demonstrate. The indirect Coombs' test is positive in the presence of the drug and many remain so for weeks. Shulman (1964) has pointed out that the drug-antibody complex can be washed off easily, but that complement will remain fixed to the red cells. The Coombs' test would then become negative if the Coombs' serum; specific for gamma globulin and, without anti-complement activity, the Coombs' test using serum specific for complement (non-gamma Coombs' test) would remain positive. Antibodies dependent on the drug have been demonstrated in hemolytic anemia due to mephenytoin (Snapper et al., 1953) dimenazene (Haider and Iqbal, 1994) quinidine (Freedman et al., 1956) quinine, phenacetin insecticides (Muirhead et al., 1958) p-aminosalicylate (Ley et al., 1959 Strumia and Raymond 1962; Swanson et al., 1966) penicillin (Carstair et al., 1966; Dausset and Bergerot - Blondel, 1961) Salicylazosulfapyridine (Shinton and Wilson 1960) and methyldopa (Carstair et al., 1966). For many of these drugs, there has been only a single case report. This mechanism of producing hemolytic anemia cannot be regarded as being very common. However, since they are not specific for red cells and may have a weak action against them, these antibodies may occur more frequently, but without producing hemolysis. Since sublytic amounts of complement attached to red cells through the action of the antibody may produce a positive Coombs' test, particularly the "non-gamma" Coombs' test, a drug must be suspected as the cause of an unexplained positive Coombs' test in the absence of anemia. Carstair et al. (1966) have found a positive anti-gamma Coombs' test in 5 of 57 test animals tested while receiving methyldopa. None of the positive test animals was
anemic. Their study was initiated by the finding of a positive Coombs' test in 3 test animals on methyldopa. Two of these 3 test animals were actively hemolyzing their erythrocytes. One recovered after cessation of methyldopa. The other recovered after reduction of the dose. The third test animal like those found in the survey, was not anemic.

Leukocyte Drug-dependent Antibodies

Leukocyte drug-dependent antibodies have been more difficult to demonstrate and less is known about them. In their original report Moeschlin and Wagner (1952) were able to demonstrate leukoagglutinins in the test animals' serum only if aminopyrine was present and only when the drug was administered to the sensitive test animal before drawing blood. Tullis (1961) has reviewed the case for leukocyte agglutinins related to drugs. When the agglutination is truly drug-dependent, there seems no reason to doubt that the drug is exerting its effect through an immune mechanism. Certainly the production of agranulocytosis by passive transfer seems clear proof of such a mechanism.

Drug-dependent leukocyte agglutinins have been described in agranulocytosis due to aminopyrine (Moeschlin and Wagner, 1952), dipyrone (CDAMA, 1993) Sulfapyridine (Moeschlin, 1955), Sulfathiazole (Tullis 1961), Chlorpropamide (Stein et al., 1964), Propylthiouracil (Walzer and Einbinder, 1963), methylthiouracil (Anastason, 1958), p-aminosalicylic acid (Dausset and Bergerot -

**OXIDANT DRUG REACTIONS**

**Mechanism of Action**

A century ago, it was known that chemical workers in Germany sometimes experienced a syndrome characterized by: (1) an acute, self-limited hemolytic anemia, (2) a variable degree of cyanosis due to methemoglobin and sulftemoglobin and (3) the presence of intra-erythrocytic inclusion bodies called Heinz bodies, demonstrable by supravital stains. Various chemicals and drugs were found to produce such hemolysis, namely, aniline, nitrobenzene, phenylhydrazine, trinitrotoluene, and later, the sulphonamides and p-amaquine.

Dern and coworkers discovered in 1954 that the susceptibility
to hemolysis from ordinary doses of pramaquine depended upon an abnormality of the erythrocyte. This abnormality was proven to be a deficiency of the enzyme, glucose-6-phosphate dehydrogenase (G-6-PD), through a series of studies Dern, Beutler, Alving, Carson, Flanagan and others (1954 and 1956) at the University of Chicago. These studies have been reviewed by Tarlov et al., (1967) and by Beutler (1966). The current knowledge of erythrocyte metabolism involved in hemolysis has been reviewed by Desforges (1965) Only a summary will be attempted here.

The red cell is completely dependent upon carbohydrate for its metabolism. The availability of energy depends upon the production of ATP by anaerobic glycolysis through the Embden-Meyerhof pathway. About 10 percent of glucose is metabolized through the aerobic pentose phosphate pathway. The erythrocyte, even though it transports molecular oxygen, is predominantly anaerobic. This mechanism may serve to "insulate" the cell from the dangerous charge of oxygen (Jandl, 1963). The pentose phosphate pathway, however, has important functions for the red cell, particularly under stress. The only source of NADAH (reduced from the nicotinamide-adenine-dinucleotide phosphate, TPNH) is from the first two steps of the pentose phosphate pathway: the conversion of glucose-6-phosphate to 6-phosphogluconate and the subsequent conversion of the latter to ribulose-5-phosphate. The first reaction is catalyzed by the enzyme glucose-6-phosphate dehydrogenase (G-6-PD), it is the enzyme which is deficient in the "primaquine-sensitive" individual. Lack of the enzyme blocks the pentose phosphate pathway, thereby, blocking the formation of NADPH in the red cell. NADPH is neces-
sary for the generation of reduced glutathione. Lack of reduced glutathione, in turn, interferes with still other reactions (Desforges, 1965). Normally, even the relatively severe G-6-PD deficiency of the primaquine-sensitive Negro male leads to only a moderate reduction of red cell life span (Brewer et al., 1961). Jandl has pointed out, however, that the drugs and chemicals active in producing hemolysis in the G-6-PD deficient person have the property of reacting with molecular oxygen to form active or potential redox intermediates between oxygen and hemoglobin or other cellular components (Jandl et al., 1967). If the cell reducing mechanism cannot overcome the oxidative potential of the drug, oxidative destruction of cellular component will take place, reduced glutathione (GSH) levels will fall, methemoglobin may be formed. Jandl has suggested that the Heinz body, the hallmark of oxidant drug hemolytic anemia, may be a product of oxidative breakdown of hemoglobin by the drug (Allen and Jandl., 1961). Evidence also suggests that hemolysis may be brought about by oxidation of sulfhydryl groups in the membranes (Desforges, 1965).

Whatever mechanism finally leads to hemolysis of the cell, it is clear that the degree of susceptibility to hemolysis by an oxidant drug is relative. If the concentration of the drug within the red cell exceeds the capacity of the available mechanisms to reduce it, irreversible changes will take place and hemolysis will result. The individual with G-6-PD deficiency will be more sensitive and may develop hemolysis at drug concentration within the range used therapeutically. On the other hand, larger doses of the same drug may induce milder hemolysis in normal animals (Dern
et al., 1955). With some drugs, such as phenylhydrazine, the difference in susceptibility between the normal and G-6-PD deficient animals is not as striking. A mild hemolytic anemia is almost the rule in test animals treated with sulfones (Lowe and Davey, 1951; Desforges et al., 1959). Still other drugs, such as chloramphenicol, may induce hemolysis only in test animals with a very severe degree of G-6-PD deficiency (Beutler, 1959).

It must not be forgotten that instances of hemolytic anemia following administration of these drugs are not limited to test animals with G-6-PD deficiency. In one series, only 2 of 16 test animals with drug-induced hemolytic anemia could be shown to have evidence of G-6-PD deficiency (de Leeuw, 1967). In many of the others, the presence of diminished renal function suggested that the cause of the hemolysis was an excessively high blood level of the drug. The reason for hemolysis in the other was not clear. The implication is obvious: caution in using these drugs must not be limited to population groups with a high incidence of G-6-PD deficiency, but must also be extended to include at least cases with renal insufficiency.

Three other specific hematologic abnormalities associated with sensitive to the same group of drugs have been discovered: erythrocyte GSH reductase deficiency (Carson et al., 1961) erythrocyte GSH deficiency (Cort et al., 1961; Frick et al., 1967) and the hemoglobinopathy associated with hemoglobin Zurich.

Beutler has reviewed the genetics of the G-6-PD deficiency (Beutler, 1965 and 1966). It is a sex-linked heritable defect but
there are several variants. Approximately 13 percent of American Negro males are hemizygotes (Tarlov et al., 1967). They have a G-6-PD activity of 4 to 37 percent (Marks and Gross, 1959). About 20 percent of American Negro women are heterozygotes, but only 3 percent are fully susceptible to hemolysis (Tarlov et al., 1967). Some non-deficient Negroes possess an electrophoretically rapid enzyme, designated A (Bover et al., 1968). Enzyme-deficient hemizygotes always possess an enzyme with electrophoretic mobility A (Beutler, 1965). The "inactive X" (Lyon) hypothesis (Lyon, 1961) has been invoked to explain the striking variability in enzyme activity of female heterozygotes. Suppression of one or the other of the X-chromosomes of the female might lead to a mosaic with variable proportions of two populations of cells, one deficient and one normal (Beutler et al., 1962). There is evidence to support this concept (Beutler and Baluda, 1964). Several different types of G-6-PD have been found in Caucasians that can be distinguished by clinical and biochemical characteristics. The deficiency is rare among north European people, but is common among certain Mediterranean people and Asiatic (Sephardic) Jews (Szeinberg, 1958 and 1959) with an incidence as high as 48 percent in some areas of Sardinia. (Motulsky, 1969).

The clinical picture of drug-induced hemolytic anemia varies with the type of deficiency present. In the G-6-PD deficient Negro, only the older cells are deficient: once these have been destroyed, hemolysis decreases sharply and, eventually, the patient will compensate, even though the drug is continued. In the G-6-PD deficient Caucasian, however, the deficiency is more
striking and even immature cells are susceptible, so that hemolysis may continue until death supervenes (Pannacciulli et al., 1968)

**Diagnosis and Treatment**

The history of ingestion of a drug known to be capable of producing this type of hemolysis, the demonstration of Heinz bodies early in the course, and the short duration of the episode are important. The general findings are those of acute hemolysis. Other mechanisms, particularly immune mechanisms of hemolysis, must be ruled out. The activity of G-6-PD in the red cell may be assayed by a spectrophotometric measurement of NADPH (TPNH) produced by a mixture of glucose-6-phosphate, NADP (TPN), buffer and hemolysate (Glock and McLean, 1953). A number of screening tests have been devised in which NADP (TPN) reduction is measure indirectly through the reduction of a dye and color development (Beutler, 1966; Tarlov et al., 1967). One of these has been adopted to a spot-test technic commercially available (Fairbanks and Beutler, 1962). The G-6-PD activity of red cells may be higher in G-6-PD deficient subjects immediately following an episode of hemolysis (Flanagan et al., 1958). If a deficiency of the enzyme cannot be demonstrated, then one must consider the possibility of erythrocyte GSH deficiency (Oort et al., 1961), erythrocyte GSH reductase deficiency, (Carson et al., 1961) or the presence of hemoglobin Zurich, (Frick et al., 1967). Finally, it must be remembered that none of these deficiencies may be present, and that hemolysis may have been induced in a non-deficient person for some other reason, such as an excessively high blood level of the drug.
The self-limited nature of the disease in the American Negro usually necessitates very little in the way of therapy. In the affected Caucasian, however, it is imperative that the drug be stopped immediately and it is often necessary to transfuse with non-deficient red cells. The routine screening of Negroes for G-6-PD deficiency by means of a simple spot test must be considered as a means of determining those in whom the administration of this group of drugs must be avoided.

**MEGALOBLASTIC ANEMIA**

**Mechanism of Action**

In 1952, Mannheimer described a case of megaloblastic anemia in a patient with epilepsy under treatment with diphenylhydantoin (Mannheimer et al., 1952). Recovery on treatment with vitamin B₁₂ was very slow. Since then, a number of cases have been described, mostly from the British Isles, in patients and test animals under treatment with a single drug: diphenylhydantoin, primidone (Girdwood and Lenman, 1956) methphenobarbital, (Haider and Iqbal, 1991 b) and tuinal (a mixture of amobarbital and secobarbital) (Haider and Iqbal 1991 a). Most instances have occurred in patients and test animals who were under treatment with more than one anticonvulsant. Recently, a case of megaloblastic anemia has been attributed to nitrofurantoin (a hydantoin derivative). Haider and Iqbal 1991 d. Although this case was clearly not due to deficiency of cyanocobalamin, it is not clear whether folic acid deficiency was
the cause. The test animals had three courses of nitrofurantoin for two to three weeks each over a period of nine months, the last one immediately preceding diagnosis. Symptoms of anemia had been developing for sometime and improvement, not progression, would have been expected during the four months between the second and third courses if the anemia was indeed due to the drug.

**Penny** has reviewed 78 cases from the literature (Penny 1963). Eighty percent were under 50 years of age. Seventy-eight percent were women. In addition to the usual symptoms and signs of a megaloblastic anemia lesions of the mouth and gums have been common, and neurologic lesions have been described (Hawkins and Meynell, 1958). When measured, the serum levels of cyanocobalamin and the absorption of cyanocobalamin and folic acid have usually been normal. Twenty patients of this collection responded to cyanocobalamin alone (Penny, 1963). Nineteen failed to respond to cyanocobalamin but subsequently responded to folic acid; 26 patients responded to folic acid alone; and 8 responded to combinations of folic acid and cyanocobalamin. No patient treated with folic acid failed to respond, even when the anticonvulsant drug was continued. The remaining patients improved after withdrawal of the drug. It is possible that the improved folic acid content of the hospital diet hastened their improvement. Withdrawal of the drug and the hospital diet may possibly have accounted for the improvement of those patients treated with cyanocobalamin alone.

**Hawkins and Meynell** (1958), studied 159 patients with epilepsy who were receiving phenobarbital, diphenylhydantoin or
primidone. A modest degree of macrocytosis was observed in about one-third of the patients, but none of them was anemic. Discontinued administration of the anticonvulsant drug, or treatment with folic acid, corrected the macrocytosis. The administration of cyanocobalamin did not. These authors found a strong correlation between the dose of the anti-convulsants and the incidence of macrocytosis. There was no macrocytosis in 30 controls. Despite the frequency with which large doses of these drugs produce macrocytosis, the incidence of frank megaloblastic anemia is quite low. In a total of 30 test animals and 534 patients under treatment with these drugs, only 12 test animals and 4 cases of megaloblastic anemia definitely related to their administration were found. (Hawkins and Meynell 1958; Berlyne et al., 1955; Haider and Iqbal 1990c; Newman and Sumner, 1957). In view of the very common use of anticonvulsant drugs in large doses, frequently in combination, over a period of many years and the scarcity of reports of megaloblastic anemia related to them, the overall incidence may be even less than reports indicate.

These various findings support the opinion that the drug is interfering in some way with folic acid metabolism. The administration of excess folic acid can overcome the effect of the drug, even though the administration of the drug is continued. Discontinuance of folic acid while the drug was continued has been followed by relapse in 4 to 18 months. (Haider and Iqbal, 1990 b, c and 1991 c). Although a superficial structural resemblance of primidone, phenobarbital and diphenylhydantoin to the pteroyl portion of the folic acid molecule has been pointed out (Girdwood
and Lenman, 1956), no evidence exists for metabolic antagonism of folic acid in microbiological systems. (Girdwood and Lenman, 1956; Haider and Iqbal 1989 d and 1990 a).

The precise mechanism which leads to the development of megaloblastic anemia in an occasional test animals after many months of therapy with anticonvulsant drugs remains unknown. It is clear, however, that dietary folic acid deficiency is a factor in many (Haider and Iqbal 1989 d). The dietary history is not mentioned in many of the reports, and in others the diet is simply described as adequate. In a large proportion of cases, however, the diet was described as inadequate, (Haider and Iqbal, 1989 d). Whatever the mechanism by which these drugs antagonize the action of the folic acid, the antagonism may not be strong enough to overcome the amounts of folic acid present in a good diet.

### Diagnosis and Treatment

The diagnosis depends upon ruling out other causes of megaloblastic anemia. The administration of folic acid is clearly effective, even though the anticonvulsant drugs are continued. There seems little reason to give cyanocobalamin as well.

Because of the severity of the anemia in some cases, and the tendency of some patients with epilepsy to eat a poor diet, the prophylactic administration of a small daily dose of folic acid to patients on long-term anticonvulsant therapy should be considered.
SINGLE CYTOPENIAS OF UNKNOWN PATHOGENESIS

Types

The drug-related hematologic reactions discussed and produced through mechanism which we understand to some extent. The reactions remaining to be discussed are, for the most part, unexplained.

There is a tendency for a given drug to be associated with a single type of cytopenia, e.g., the phenothiazines and antithyroid drugs with acute agranulocytosis. Yet drugs which have a high association with aplastic anemia are also frequently reported in association with single cytopenias as well, e.g. chloramphenicol. In such cases, the cytopenias may be of an acute variety, with rapid recovery following withdrawal of the drug, or of a chronic type, with a course like that of aplastic anemia itself. Whether these types of reactions are interrelated is not clear. This problem will be taken up later in this chapter.

Hemolytic Anemia

Most of the reported cases of hemolytic anemia (CDAMA, 1993) have been related to the drugs capable of producing oxidant drug hemolytic anemia, or the drugs which have been associated with an immune type of reaction. An exception is lead (Pb.). The various mechanisms by which lead affects the red cell
have been thoroughly discussed by Griggs in the preceding volume of this series. (Griggs, 1968).

**Thrombocytopenia**

An acute thrombocytopenic purpura, in which recovery rapidly follows withdrawal of the drug, is not necessarily associated with a demonstrable immunologic mechanism. For example, drug-dependent inhibition of clot retraction has been demonstrated in one case of thrombocytopenia associated with hydrochlorothiazide (Haider and Iqbal, 1989 c) but not in others (Bettman, 1963; Rodriguez et al., 1964). Bettman was unable to demonstrate inhibition of clot retraction or of platelet lysis, but did demonstrate drug-dependent platelet agglutination in thrombocytopenia related to hydrochlorothiazide. He suggested that an antibody might have been present which was weaker than those described previously (Bettman, 1963). Ristocetin has been demonstrated to produce thrombocytopenia by a dose-related direct effect upon the platelets (Gangarosa et al., 1960). Thrombocytopenia following some drugs may pursue a more chronic course, possibly depending upon the rate at which the drug is excreted (Mettier et al., 1948; Thompson et al., 1954).

**Pure Red Cell Aplasia**

The condition is characterized by anemia and a virtual lack of red cell precursors in an otherwise normal marrow. Drugs such as benzene and chloramphenicol, which are associated with
aplastic anemia are usually also associated with pure red cell aplasia, though less frequently, (CDAMA, 1993). The AMA registry of adverse reactions contains 91 cases in which red cells alone were depressed. Of the 43 reported to have received only one drug, 19 had received chloramphenicol (CDAMA, 1993).

Schmid et al. have reviewed 39 cases of acquired pure red cell aplasia (Schmid et al., 1970). Fourteen had been exposed to drugs; six to benzene for long periods; 2 to sulfonamides; and the other 6 to different drugs of chemicals. The same syndrome is sometimes associated with thymoma. In a series of 169 thymomas, there were 4 instances of pure red cell aplasia (Schmid et al., 1965). Of 37 cases associated with thymoma from the literature, 7 progressed to aplastic anemia. Three of these had received chloramphenicol and 3 had been irradiated. Nine additional cases of aplastic anemia with thymoma were found - 4 had been irradiated and one had received sulfonamides. These observations suggest that drugs may play a part in inducing the anemia or pancytopenia associated with thymoma.

Yunis has demonstrated the mechanism of a pure red cell aplasia related to diphenylhydantoin (Yunis et al., 1965). His patient differed from those referred above in that the aplasia quickly recovered following withdrawal of the drug. The administration of riboflavin prevented the drug effect, whereas cyanocobalamin or folic acid did not. The in vitro uptake of C14 formate into marrow DNA was reduced by the drug when erythroblasts were present in the marrow, but not when they were
absent. The uptake of deoxyuridine or thymidine was not inhibited. Radioautography confirmed that this inhibitory effect of diphenylhydantoin was exerted on red cells but not on granulocytes. Yunis concluded that the toxic effect of diphenylhydantoin in this unusual patient was exerted through a specific defect in DNA synthesis, probably at the step of formation of deoxyribotides. The clinical pattern of this particular reaction is similar to that of the non-immune agranulocytosis, but is quite different from the chronic pure red cell aplasia referred to previously.

Granulocytopenia

Several types of granulocytopenia are induced by drugs: a slowly developing mild neutropenic, with little or no leukopenia; an acute "agranulocytosis" with severe leukopenia and virtually no granulocytes; and a chronic granulocytopenia. Most interest has centered around the second of these reactions and very little is known about the other two. Further discussion is given in the later section on aplastic anemia.

The clinical and hematological manifestation of acute agranulocytosis, in which an immune mechanism has been demonstrated, differs very little from that in which no evidence of an immune mechanism has been found (Huguley, 1963). Antibodies against leukocytes have been more difficult to demonstrate than those active against platelets or erythrocytes. They have usually been demonstrable when looked for in agranulocytosis due to aminopyrine or dipyrone (Huguley, 1964). On the other hand,
agranulocytosis induced by the phenothiazines, the most common cause of agranulocytosis, today. (CDAMA, 1993) is seldom accompanied by demonstrable antibodies, (Pisciotta et al., 1958), although they have been found occasionally, (Tullis, 1961; Hoffman et al., 1963). The immune and non-immune type of agranulocytosis may differ in their mode of onset. On re-administration of the causative drug after recovery, the type due to aminopyrine will exhibit an explosive fall after as little as 10 mg. of drug (Dameshek & Colmes, 1936). The type due to thiouracils and phenothiazines may not recur until after standard doses have been given for a number of days (Pisciotta et al., 1958; McGavock & Chevally, 1954), and may not always recur (Pisciotta et al., 1958). The granulocyte count may not reach its nadir for 24 hours or longer.

Pisciotta has investigated the pathologic histochemical changes of agranulocytosis induced by chlorpromazine (Pisciotta & Santos, 1964). Using tritiated thymidine and tritiated uridine uptake to correlate DNA synthesis with morphological changes during marrow recovery, he found a four-stage pattern: (1) an aplastic or hypoplastic marrow with peripheral lymphopenia; (2) the appearance of small mononuclear cells in the marrow showing little DNA synthesis, rare granulocyte precursors showing intense DNA synthesis, and peripheral lymphocytosis; (3) repopulation of the marrow with granulocyte precursors vigorously synthesizing DNA and occurring one to two days after maximum peripheral lymphocytosis; and (4) qualitative and quantitative restoration of marrow and blood cell types.
In 13 randomly selected hematologically normal test animals, Pisciotta found that incubation of marrow cells with chlorpromazine led to approximately 50 percent reduction in the number of labeled granulocyte precursors in 8 test animals, and no reduction in the remaining 5 test animals (Pisciotta & Santos, 1965). This entire group was then treated with chlorpromazine. None developed agranulocytosis. After treatment, the marrow of all test animals were again studied with in vitro tritiated thymidine labelling. The 5 original non-sensitive individuals were unchanged; the 8 who had pretreatment chlorpromazine suppression now showed a 50 percent reduction in the number of granulocytic precursors incorporating tritiated thymidine, whether or not chlorpromazine was added to the incubation mixture. This effect might have explained the temporary early leukopenia often seen in chlorpromazine-treated individuals. The compensatory mechanism which allowed escape from this suppressive effect in 2 of the group who were resulted after one year of continuous therapy with chlorpromazine in unexplained.

Pisciotta next investigated the difference between the individuals who did develop chlorpromazine agranulocytosis and those who did not but, instead, exhibited suppression of DNA synthesis in vitro when their marrow cells were incubated with chlorpromazine (Pisciotta & Santos, 1965). In test animals who had recovered from chlorpromazine agranulocytosis, granulocyte precursor labelling with tritiated thymidine was significantly lower after three hours than that a normal controls of comparable age whose labeling index was similar to that of non-sensitive younger test
animals. When chlorpromazine was added to the marrow incubation, non-sensitive individuals had indices of cell division similar to, but usually slightly lower than, controls, whereas chlorpromazine-sensitive individuals failed to show significant cell division at all. Since DNA synthesis is under enzymatic control, Pisciotta suggests that the limited proliferative potential of these chlorpromazine-sensitive individuals may be due to a diminished enzyme activity, unrelated to aging.

The implications of these various findings are interesting; but the precise pathogenesis of chlorpromazine-induced agranulocytosis remains far from clear. There is no real information about possible mechanisms of non-immune agranulocytosis from other drugs.

Agranulocytosis is the most common of the hematologic adverse reactions, accounting for 39 percent of those reported to the registry (CDAMA, 1993). The drugs which most commonly produce it do so with much greater frequency than is seen with other hematologic reactions. The incidence in test animals receiving steady doses appears to be in the neighborhood of 0.13 to 0.7 percent with chlorpromazine and mepazine (Pisciotta et al., 1958; Fiore and Noonan, 1959), 0.86 percent with aminopyrine (Discombe, 1952), 0.45 to 1.75 percent with thiouracils, (Moore, 1946; Bartles, 1948), and 1.5 percent with methimazone (H'Doubler and H'Doubler, 1954).
As has been indicated, the mechanism of these reactions is not known and no specific tests are available to identify a drug which might be responsible. Since some of these same reactions may occur in test animals and patients who, insofar as can be determined, have not been exposed to any drugs or chemicals, the etiologic agent may not have been a drug even in those test animals/patients who were taking drugs. The diagnosis of a drug etiology for the reaction must therefore be tentative.

The treatment for these reactions is the discontinuance of any drug at all likely to have caused it. Therapy is supportive.

APLASTIC ANEMIA

Mechanism of Action

Aplastic anemia is the most disabling, most frequently fatal of these reactions, and next to agranulocytosis, the most frequently reported (CDAMÁ, 1993). The term "aplastic anemia" usually describes a functional or morphological failure of the marrow manifested by pancytopenia and may include cases in which the marrow is of normal or even increased cellularity, as well as those with hypocellular marrow (Scott et al., 1959; Mohler and Leavell, 1958). Cases are usually excluded in which there is extramedullary myelopoiesis with or without myelofibrosis, reduction in erythroid elements only ("pure red cell aplasia"), or marrow hypoplasia
induced by radiation or by the chemotherapeutic agents used in the treatment of malignancy. The relationship to reactions associated with the same drugs in which depression of one or two blood elements occurs without pancytopenia is controversial (Yunis and Bloomberg, 1964; Best, 1994).

Unlike most of the drug-induced hematologic syndromes, the pathologic changes of aplastic anemia progress long after the drug has been discontinued. Recovery, if it occurs, may require years. Perhaps half of the cases in reported series have been considered to be idiopathic. (Scott et al., 1959; Mohler and Leavell, 1958; Erslev and Wintrobe 1962). Yet many may have resulted from undisclosed drugs or other unsuspected agents to which the test animals and patient had been exposed. For a discussion of the clinical manifestations, pathology, treatment and drugs which may produce it, see the review of Scott Cartwright and Wintrobe. (Scott et al., 1959).

In any discussion of drug-induced aplastic anemia, benzene and chloramphenicol must be considered-benzene because of the frequency with which hematologic disorders occur among those exposed. (Hunter, 1939; Savilahti, 1956; Haider and Iqbal, 1989 a), and chloramphenicol because it is the agent most commonly associated with aplastic anemia in recent years (CDAMA, 1993; Best, 1994).

The injection of a benzene-olive oil mixture into test animals produces an initial rise in leukocytes in the blood and granulocytic
hyperplasia of the marrow followed by a leukopenia (Haider and Mallik, 1986). Larger doses and prolonged treatment lead eventually to an aplastic marrow.

Chronic exposure of workers in certain industries to benzene fumes has led to a very high incidence of hematologic change. Among 89 workers in a shoe factory exposed to atmospheric concentrations averaging about 100 ppm for 7 weeks to 22 years, only 11 had normal blood counts, 21 had a depression of one type of cell, and 57 of more than one, including all possible combinations (Hunter, 1939). One had acute myeloblastic leukemia. Among 332 test animals exposed in the benzene fumes, 14.5 percent had leukopenia (Haider and Iqbal 1989). Other changes were less regularly looked for, but among those examined, 47.8 percent were anemic and 32.7 percent were thrombocytopenic. The incidence of combinations was not reported. Among another group of 147 workers exposed to concentrations above 300 ppm from a few months to 20 years, 73 percent had abnormal blood findings: 20 percent pancytopenia, 7 percent anemia, 7 percent leukopenia, and 38 percent thrombocytopenia (Savilahti, 1956). Among shoe workers in Italy (an estimated 3,000 with possible benzene exposure), there were 47 cases of hemopathy: 40 of hypoplastic anemia and six of acute leukemia (Vigliani and Saita, 1964). These various authors conclude that the concentration of benzene vapor and duration of exposure are important, but that individual susceptibility is very variable. Follow-up data are sketchy. Apparently, most of the less severely affected test animals and patients recover within two months to one year after
cessation of exposure (Haider and Iqbal, 1989 a and b), although severe reactions may appear for the first time after exposure has ceased (Hunter, 1939). Obviously, benzene can produce mild disease, reversible even after long periods of exposure, a severe and progressive aplastic anemia, or myelofibrosis (Rawson, et al., 1941; Mallory et al., 1939; Haider and Iqbal, 1988 d and 1989 b).

Despite this propensity of benzene for producing reactions it is not the cause of very many reactions at the present. The registry has received reports of only 10 cases of aplastic anemia, 2 of erythroid-hypoplasia without pancytopenia, and 1 each of thrombocytopenia and leukopenia (CDAMA, 1993). Apparently, exposure is minimal in the U.S.A.; yet we must always be on guard against the presence of benzene, not only in materials used in industry, but also in household materials such as solvents and paint removers.

The pathogenesis of the hematologic toxicity of benzene is not understood. We can only speculate on the possible interrelationship of these various reactions. The milder single cytopenias may simply be early and more readily reversible manifestations of the same process which, with continued exposure, may lead to aplasia or fibrosis. The evidence suggests a continuous spectrum from single cytopenias and double cytopenias to mild pancytopenias with a cellular marrow to severe pancytopenias with an aplastic marrow or fibrosis (Hunter 1939; Mallory et al., 1939; Haider and Iqbal, 1939 b). The severity of the reaction may be correlated with the intensity of the exposure (Haider and Iqbal 1989b). The
frequency and rapidity with which recovery takes place appear to decrease with increasing severity (Hunter 1939; Haider and Iqbal, 1989 b).

These speculations may be extended to aplastic anemia in general. In the extensive reviews of Bomford and Rhoads (1941) and of Scott, Cartwright and Wintrobe (1959), there is little correlation between the clinical findings and the cellularity of the marrow or the presence or absence of exposure to possibly toxic agents. Patients and test animals with a mild pancytopenia and a cellular marrow are more likely to recover. Scott, Cartwright and Wintrobe found no correlation between tendency to recover and exposure to noxious agents (Scott et al., 1959); however, Bomford and Rhoads felt that recovery was more common in patients who developed the aplastic anemia after exposure to benzene. (Bomford and Rhoads 1941).

The information about chloramphenicol is considerably more extensive than that about benzene. The entire subject of chloramphenicol toxicity has been thoroughly reviewed by Yunis and Bloomberg in the preceding volume of this series (Yunis and Bloomberg, 1964). I will limit myself to comments. There is no question of the potential of chloramphenicol to produce aplastic anemia. There are 724 cases of aplastic anemia in the registry on adverse reactions, and 321 of these (44.3 percent) had received chloramphenicol (CDAMA, 1993); It was the only drug reported in 143 cases (19.7 percent). In two years (July 1, 1963, through June 30, 1965), there were 86 reports of aplastic anemia; 30 had
received chloramphenicol and 15 of these had taken no other
drug. In addition, 68 reports of leukopenia, 29 of thrombocytope-
nia, 28 of erythroid hypoplasia, 5 of hemolytic anemia, and 7 of
combinations without pancytopenia have been made for patients
who had received chloramphenicol. Of 2,492 reports of hematologic
reactions in the registry, 471 listed chloramphenicol. Only 29 of these
cases were among those included by Yunis and Bloomberg in their
94 cases (Best, 1994). The incidence of aplastic anemia among re-
cipients of chloramphenicol is not known. A common estimate is about
1 in 200,000 (Haider and Gondal, 1987). A higher frequency has
been estimated in California for the period of 1957-60 (Haider
and Iqbal, 1988 c). Assuming that all deaths during that period
from aplastic anemia due to chloramphenicol were recognized
and reported, that all chloramphenicol dispensed was given to
people (veterinary use was included), and that the average course
was only 4 Gm., and incidence of 1 fatal case of aplastic
anemia in 60,000 recipients was indicated (Haider and Iqbal,
1988 c). This interpretation of the data seems conservative.

Yunis and Bloomberg (1964) have reviewed the several pro-
spective studies of the reversible dose-related marrow inhibition
occurring with chloramphenicol, which is characterized by in-
creased plasma iron, increased saturation of plasma iron-binding
globin, delayed plasma iron clearance, failure of the radioactive
iron to appear in circulating red cells, and reticulocytopenia.
Some test animals and patients subsequently developed mild
anemia, leukopenia or thrombocytopenia, and some developed
vacuoles in the marrow erythroblasts and early granulocytes. This
reaction has received further documentation by Scott et al., in a controlled double-blind study of the hematologic toxicity of chloramphenicol (Scott et al., 1970). Hematologic changes in a group of 20 patients receiving 2 Gm. of chloramphenicol a day for serious infections were similar to those in 12 comparable patients not receiving chloramphenicol, except that in 2 of the chloramphenicol-treated patients the constellation of changes, described above, developed. Of 20 patients receiving 6 Gm. of chloramphenicol daily, evidence of erythropoietic depression occurred in 18, with thrombocytopenia in 14, leukopenia in 3, and vacuolization of marrow erythroblasts in 15. There was a striking correlation of erythropoietic damage with the six-hour plasma total chloramphenicol level. No patient developed damage at a level of less than 20 μg/ml, and all patients developed damage at a level of more than 35 μg/ml. All recovered promptly on discontinuing the drug.

The observation that bone marrow vacuoles appeared in children with phenylketonuria, while on treatment with a diet low in phenylalanine and that these disappeared after the ingestion of phenylalanine (Sherman et al., 1970) led to a trial of phenylalanine in patients who exhibited vacuolization of marrow erythroblasts while taking chloramphenicol (Ingall et al., 1973). In 3 patients, a striking decrease occurred in the number of vacuoles within two to four days after beginning 100mg. of phenylalanine per Kg. per day, although chloramphenicol was continued. These patients did not have cytopenias. These data suggest some interference with the metabolism of phenylalanine as a possible mechanism of the acute toxic effect of chloramphenicol, but do not
justify more than speculation at this time. They also suggest the possibility that the vacuoles observed in erythroblasts of patients with alcoholism (Best, 1994) might be the result of dietary deficiency of phenylalanine.

The pathogenesis of the acute dose-related (or plasma drug level-related), readily reversible changes described above is not well understood. It may be related to an interaction of chloramphenicol with messenger RNA and a resulting inhibition of protein synthesis (Weisberger et al., 1963). Yunis and Bloomberg do not believe any relationship exists between these two group of effects and the rare progressive pancytopenia, associated with an aplastic marrow, usually appearing much later not necessarily dose-related, and frequently leading to a fatal outcome. (Yunis and Bloomberg, 1964). They believe the two types of reaction have a different pathogenesis. There is no explanation for the late type of reaction.

Best has reviewed the reports previously alluded to form the registry on adverse reactions (Best, 1994). The majority of reports fell into the late onset aplastic type with another cluster suggesting an acute, reversible, normocellular type. However, a number of cases fell between or overlapped. Nine of 60 patients who had at least two week’s delay between the last dose and onset of reaction showed a normocellular or only isolated cellular depression. All combinations of blood cell depression were seen in association with non-aplastic marrows, with half of the series showing pancytopenia. Cases with hypoplastic marrows tended to
have received a smaller dose of the drug than those with cellular marrows, but there was an appreciable overlapping. Duration of therapy for the two marrow groupings was similar, with a median of 11 to 12 days. There appears to be little question that the acute changes described in prospective studies are readily reversible. No case detected has been observed to progress to aplastic anemia (Jiji et al., 1963). One subject was kept on the drug for 41 days after erythroblast vacuolization developed without any serious hematologic changes. (Jiji et al., 1963). There are no reports of the drug having been continued after the development of cytopenia (Best, 1994). There is no way of knowing how many of the other patients who subsequently developed aplastic anemia may have developed such changes or how many might not have progressed if the drug had been stopped as soon as acute changes were noted. Furthermore, even if the two types of reaction have a different pathogenesis, some of the cases with only a single cytopenia and a cellular marrow may actually represent instances of early and still reversible changes of aplastic anemia, rather than manifestations of the acute type of changes. Until more is known about the mechanism involved, caution should be used concerning the continuation of chloramphenicol in the presence of hematologic changes. I would at least reduce the dose so as to lower the blood level. The author heartily agree, however, with the statement that "the most important step in preventing bone marrow aplasia from chloramphenicol is not the monitoring of its administration, but the initial judgment of the physician as to whether the drug should or should not be given (Yunis and Bloomberg, 1964).
Even less is known about the pathogenesis of aplastic anemia due to other drugs. The information available has been reviewed for quinacrine (Haider and Iqbal 1988 a), trimethoprim (Haider and Mallik, 1985), and anticonvulsant drugs (Robins, 1962).

| Relationship between aplastic anemia and Paroxysmal nocturnal hemoglobinuria |

The interesting possibility of a relationship between aplastic anemia and paroxysmal nocturnal hemoglobinuria has been proposed by Quagliani, Cartwright and Wintrobe, who have described six cases of their own and five from the literature in which patients initially diagnosed as aplastic anemia were later found to have paroxysmal nocturnal hemoglobinuria (Quagliani et al., 1964). Nine of 11 cases had been exposed to drugs, 4 of them to chloramphenicol repeatedly. Of their own 6 cases, 4 had negative studies for paroxysmal nocturnal hemoglobinuria early in the course of the disease, but developed them later. In 2 of these, the findings were transient. Ross and Rosenbaum have added a twelfth case of their own, in which hemoglobinuria appeared over a year after the diagnosis of aplastic anemia attributed to chloramphenicol (Ross and Rosenbaum, 1964). These authors also report another in the literature in which paroxysmal nocturnal hemoglobinuria apparently developed ten years after the diagnosis of hypoplastic anemia (Feichtmeier et al., 1955). A fourteenth case has been described in which a pancytopenia with hypoplastic marrow developed after repeated courses of chloramphenicol and was
followed some months later by paroxysmal nocturnal hemoglobinuria (Beal et al., 1964). Of these 14 patients, 6 had received chloramphenicol prior to the discovery of anemia.

Such clinical description lead to a strong inference that paroxysmal nocturnal hemoglobinuria may, in some instances, be an acquired abnormality resulting from a drug-induced aplastic anemia, and suggest the wisdom of repeated acid hemolysin and thrombin tests in patients during the course of aplastic anemia (Quagliani et al., 1964).

**Diagnosis and Treatment**

The diagnosis and treatment of aplastic anemia are complex subject. Nothing new can be added to previous reviews (Huguley, 1963; Scott et al., 1959).

**LEUKEMIA**

In nearly every reported series of patients with benzene toxicity, there are cases of leukemia (Hunter, 1939; Rawson et al., 1941; Haider and Iqbal, 1989 b). Vigliani and Saita have reviewed the data and present a compelling case for an etiologic relationship (Vigliani and Saita, 1964). In the Italian provinces of Milan and Pavia, no more than 5000 workers are exposed to benzene vapors, and probably not less than 3000 are exposed to concentration considered dangerous. Sixty-eight hematologic reactions were recognized among this group in the years 1960-63, of which
11 were leukemic. This incidence of leukemia was about twenty
times higher than expected. The authors review other reports with
similar findings. The cases of leukemia which have appeared most
likely to be related to benzene have been predominantly acute
myeloblastic leukemia, have usually followed prolonged exposure
to benzene, and have often been preceded by a state of
pancytopenia or chronic granulocytopenia (Vigliani and Saita,
1964; Bomford and Rhoads, 1941; DeGowin, 1963).

Few reports have attributed leukemia to other drugs. A
possible association between phenylbutazone and acute leukem-
bia was described in 1960 (Bean, 1974). The literature has been
reviewed by Dougan and Woodliff (1965). From among the pub-
lished cases and their own experience, they have accepted nine
cases of acute leukemia as possibly induced by phenylbutazone.
Six others were excluded because of exposure to a known car-
cinogen (radiation), or because only a short time had elapsed
between the initial administration of the drug and the onset. In
order to establish some sort of control, they compared the inci-
dence of prior phenylbutazone administration in other hemat-
logic malignancies with that in acute leukemia. The 'leukemia and
allied disorders registry' of the cancer council of Western Australia
for 1959 through 1963 contained reports of 55 patients with acute
leukemia, of whom 8 had received phenylbutazone. Three of
these were excluded because of prior radiation therapy or short
time interval after phenylbutazone administration. Among 417 pa-
tients with other hematologic malignancies reported in the same
period, there were 2 with Hodgkin's disease, 1 with chronic lym-
phocytic leukemia, and with polycythemia vera who were recorded as having received phenylbutazone. The association in 5 to 55 cases of acute leukemia and in only 5 of 417 other hematologic malignancies suggests a possible causal relationship between phenylbutazone and some cases of acute leukemia.

Although I cannot regard the etiologic relationship or either benzene or phenylbutazone to leukemia as being established, the data do indicate the need for a continued search for such relationships. As Dougan and Woodliff have pointed out, some of the repeated cases of leukemia following administration of phenylbutazone have occurred at such short interval after the initial administration of the drug as to eliminate the possibility of a casual relationship (Dougan and Woodliff, 1965). The same reasoning probably applies to one of the two cases of acute leukemia recently reported as following oxyphenylbutazone, a metabolic product of phenylbutazone (Sjoberg and Perers, 1965). From my current concept of the kinetics of cell growth in acute leukemia, I may infer that at least 40 doubling at intervals of about four days would be required to produce a sufficient mass of leukemic cells to cause symptoms and to permit diagnosis (Frei, 1994). Hence, 160 days would be required from induction to diagnosis. Even if the same malignant change took place over a period of time in 1 million different cells, there would still be a requirement of 20 doubling or 80 days to clinical onset. It appears naive to consider the possibility of a casual relationship between the administration of a drug and the development of leukemia, unless a period of many months had transpired since initial exposure.
If drugs sometimes do lead to the induction of leukemia, they might do so as a primary effect or as a secondary effect resulting from a severe drug-induced hematologic reaction. Although leukemia has been the first evidence of hematologic abnormality in some patients after long exposure to benzene (Hunter, 1939), there is more commonly a preceding aplastic anemia or chronic granulocytopenia (Vigliani and Saita, 1964; Bomford and Rhoads, 1941; DeGowin, 1963). A lapse of as much as 15 years has occurred between the onset of the initial benzene hematologic depression and leukemia (DeGowin, 1963). A parallel exists with other cause of aplastic anemia. Radiation can produce aplasia and can also induce leukemia (Lawrence, 1964). A suggestive high incidence of leukemia occurs in patients with the hereditable Fanconi’s syndrome, of which aplastic anemia is a major manifestation in them and in their families (Garriga and Crosby, 1959). Chromosomal abnormalities are common after radiation (Lawrence, 1964), and are thought to play a part in radiation leukemogenesis. Similar chromosomal changes are also found in Fanconi’s syndrome (Bloom et al., 1994), and in aplastic anemia secondary to benzene (Pollini and Colombi, 1964).
PARASITOLOGICAL FINDINGS
MATERIALS AND METHODS

During the period from June 1990 to July 1992, a group of 250 adult dogs (n=400) was captured in Karachi (Pakistan). Individual blood samples from each of them were examined for the presence of blood parasites (i.e., Babesia canis, B. gibsoni, Trypanosoma evansi, Leishmania donovani). Blood was taken from the ear, the first few drops being discarded. Larger quantities, when necessary, were drawn from the femoral vein or artery. A record of collection was maintained regularly to see in which of the dog hemoparasites were common in a certain area. Similarly a record of all hemoparasites were maintained and specific numbers were assigned to each of them for ready reference.

SAMPLE OF BLOODS

When the blood sample were collected, the following points were observed. (1) Inserted the sample number so that the sample could be identified. (2) Most of the blood samples have been taken in the morning between 8.00 A.M. to 9.00 A.M. In some cases at 4.00 P.M. also. (3) Sterilized the skin with a dust free swab moistened with 70% ethanol.

VENOUS BLOOD

Materials and reagents used for collection of venous blood sample were as follows:
1) Swab made from dust-free material or absorbed cotton wool.
2) 70% ethanol v/v.
3) Sterile disposable syringe, preferably with wide bore needle.
4) EDTA test tubes.

Blood samples were taken directly from an uncongested vein into the sample tube. The venous blood was mixed with EDTA (Ethylene Diamine Tetra Acetic Acid) to prevent clotting. The EDTA was prepared by allowing 0.02 ml of a 10% Titriplex III GR solution (MERCK cat. No. 8418) to dry in small tubes (2ml blood was drawn and sealed with plastic stoppers with a tube labelled the dog number with its locality). The blood sample collected and the tube, was sealed immediately after the operation was over and the tube was carefully inverted several times in order to avoid clot formation. The tube was not shaken to avoid hemolysis. Blood sample obtained in the field were collected in evacuated glass tubes via jugular vein puncture.

**SPECIMEN COLLECTION AND PREPARATION OF SERUM**

Five ml of blood was collected from the jugular vein of dog. Serum preparation blood was allowed to clot. Centrifuge for 10 min at 900 rcf. Removed serum from clot. Hemolyzed, lipemic or icteric sera was not used.

No additive or preservatives were necessary to maintain the integrity of the sample. Samples were stored at room temperature for 4 hours and refrigerated (2-8 °C) for 24 hours. Some samples frozen and packed in dry ice.
DIAGNOSIS

Diagnosis of hemoparasites depends on epidemiology clinical signs, identification of the parasites in blood smears, stained either Giemsa-stained or Wright-stained.

In case of chronic infection, diagnosis is usually made using a variety of serologic tests for the detection of specific antibodies since the organism disappears or is presented in extremely low numbers soon after the acute infection. All sera were evaluated for antibody reactions to babesiosis, leishmaniasis and trypanosomiasis, by the rapid card agglutination (RCA) test, complement fixation (CF) test and the indirect fluorescent antibody (IFA) test. Reactions from tests were read as either positive (agglutination) or negative (clear). A batch of dog was considered positive if at least one animal in the batch was identified as positive for hemoparasites.
RESULTS AND DISCUSSION

Table 1 shows the species of protozoans recorded, locations from the peripheral blood/bone marrow and prevalences and intensities of infections of each hemoparasites. There were two Babesia, one Trypanosoma and one Leishmania species (Figs 37-41). The two most prevalent hemoparasites were Babesia canis (44%), Trypanosoma evansi (20%). Not only the Babesia canis found the most prevalent parasite, but had also the greatest mean intensity (X=50). Other blood protozoa were prevailed as Babesia gibsoni (28%), and Leishmania donovani (8%). Of 4000 positive hemoparasitic samples were collected from 250 groups. All dogs were found to be infected with at least one species of hemoparasites, with an average of two species per dog (range 1-3). The percentage of infected dogs having one, two or three hemoparasitic species were respectively as 100, 25 and 15.

Table 2 shows the ranges and means for the number of hemoparasites species, and intensity of infection for sex and age. There were no significant differences in the mean number of hemoparasites species per dog at sex, but it was present at age categories. Based upon the 250 groups for which total counts were obtained, intensities ranged from one to 60/100 RBCs in case of T. evansi / cmm blood and in case of Leishmania donovani percent parasitized macrophage cells (X=16.7). Although there was no significant difference in the mean intensity among sexes, a significant difference was found between ages, with young dogs exhibiting higher means.
intensities of babesiosis than adult dogs with regard to ages, there was a significant higher prevalence of babesiosis in younger dogs when all dogs were considered, but no significant difference was found nor other blood protozoans.

The intensities of trypanosomiasis and Leishmaniosis were significantly greater in dogs of all ages. These findings in addition to the greater intensity of infection found in dogs of all ages when all hemoparasites were considered except babesiosis. In general these studies on 250 groups (n=9000) in Pakistan, most of which were from those areas, agreed with data reported by Benjamin, 1961; Adam et al., 1971; Siddiqui, 1980; Sinha, 1984 and Haider, 1992, from larger sample sizes in variety of districts of Pakistan.

As in their study, intensity of infections were not emphasized. Notable difference was much greater prevalence of babesiosis and trypanosomiasis and the greater mean intensity of Babesia canis in the present studies.
HEMATOLOGICAL FINDINGS
MATERIALS AND METHODS

This project (July 1992 To June 1994) includes 4000 determinations of 4000 normal and naturally infected animals (50% Males and 50% Females), in which 3000 hemoparasitized non-splenectomised naturally infected dog (50% Males and 50% Females) and 500 splenectomised, hemoparasitized, naturally infected dogs (250 Males and 250 Females) and 500 experimentally infected (50 % Males and 50 % Females)(250 splenectomised and 250 non-splenectomised) pye-dogs (a vagrant mongrel), splenectomised males and females and non-splenectomised males and females of various breeds and ages, obtained from the pond and used in various investigations. When received, each dog (Canis familiaris) was bathed and placed in a large, roomy, outside cage and fed a standard diet for at least a week before any determinations were begun. At this time test animals (Pye dogs) that refused to eat or appeared unwell were discarded. The indiscriminate use of pound animals for work of this sort is open to criticism, but by careful selection and allowance for proper control periods it is believed that truly representative results are obtained. Routine examination were usually made in the morning. Blood was taken from the ear, the first few drops being discarded. Larger quantities, when necessary, were drawn from the femoral vein or artery. A record of collection was maintained regularly to see in which of the dog hemoparasites were common in a certain area. Similarly a record of all hemoparasites were maintained and specific numbers were assigned to each of them for ready reference.

SAMPLE OF BLOODS

When the blood sample were collected, the following points were
observed. (1) Inserted the sample number so that the sample could be identified. (2) Most of the blood samples have been taken in the morning between 8.00 A.M. to 9.00 A.M. In some cases at 4.00 P.M. also. (3) Sterilized the skin with a dust free swab moistened with 70% ethanol.

**VENOUS BLOOD**

Materials and reagents used for collection of venous blood sample were as follows:

1. Swab made from dust-free material
2. 70% ethanol v/v.
3. Sterile disposable syringe, preferably with wide bore needle.
4. EDTA test tubes.

Blood samples were taken directly from an un congested vein into the sample tube. The venous blood was mixed with EDTA (Ethylene Diamine Tetra Acetic Acid) to prevent clotting. The EDTA was prepared by allowing 0.02 ml of a 10% Titriplex III GR solution (MERCK cat. No. 8418) to dry in small tubes (2 ml blood was drawn and sealed with plastic stoppers with a tube labelled the dog number with its locality). The blood sample collected and the tube, was sealed immediately after the operation was over and the tube was carefully inverted several times in order to avoid clot formation. The tube was not shaken to avoid hemolysis. Blood sample obtained in the field were collected in evacuated glass tubes via jugular vein puncture.
SPECIMEN COLLECTION AND PREPARATION OF SERUM

Five ml of blood was collected from the jugular vein of dog. Serum preparation blood was allowed to clot. Centrifuge for 10 min at 900 rcf. Removed serum from clot. Hemolyzed, lipemic or icteric sera was not used.

No additive or preservatives were necessary to maintain the integrity of the sample. Samples were stored at room temperature for 4 hours and refrigerated (2-8 °C) for 24 hours. Some samples frozen and packed in dry ice.

HEMATOCRIT (Hct)

Graduated centrifuge tube filled up to 100 marks with blood rendered incoagulable by the addition of EDTA. The hematocrit value was recorded directly from the graduated scale of the tube after centrifuging for 10 min at 3,000 rpm in a standard centrifuge.

HEMOGLOBIN (Hb) DETERMINATION

The following reagents and apparatus were used:

REAGENTS:

Merck cat. No. 3298

Reagent solution (1.0 mmol/l potassium cyanide; 0.6 mmol/l po-
tassium hexacyanoferrate (III): 2.5 mmol/l phosphate buffer pH 7.2; 1.5 mmol/l sodium chloride; 0.05% detergent).

**APPARATUS:**
Spectrophotometer, pipettes 20 μl and 5 ml.

**PROCEDURE:**
Spectrophotometer adjusted at: Absorbance maximum: 540 nm

Filter: Hg 546
Light Path: 1 cm.

Reagent solution 5ml + Blood 0.02 ml in test tube. Measured the absorbance of the sample (As) against the reagent solution after at least 3 min.

**CALCULATION:**
Hemoglobin concentration = As. 36.8 (g/dl)
Hemoglobin concentration = As. 22.8 (mmol/l)

**ERYTHROCYTE COUNTING**

The following reagents and apparatus were used in the common laboratory manual counting method.

**REAGENTS:**
Hayem's reagent (Merck cat. No. 9260)

Mercury (11) chloride solution 2.5g/l isotonic with blood, ready for use.

APPARATUS:
NEUBAUER counting chamber, erythrocyte pipette, microscope, pipetting-tube with mouthpiece, electric shaker.

CALCULATION

\[
\text{Erythrocyte count} = \frac{X \times D \times 4,000}{80}
\]

= X. 10,000 (Cells/μl)

when (D=200)

= X 5,000 (Cells/μl)

when (D=100)

= X 100 (Cells/μl)

When (D=2)

D = Dilution factor (200 or 100)

X = Number of erythrocytes counted in 80 of the smallest squares.

Calculation parameters (MCH, MCV, MCHC)

Important erythrocyte constant were calculated from the hemoglobin, erythrocyte and hematocrit values.

1. \( \text{Hb}_e = \text{MCH} \)

   = mean hemoglobin content of a single erythrocyte
   = mean corpuscular hemoglobin
\[ \text{Hb}_{e} = \frac{\text{Hb (g/dl)} \times 0.1 \text{ log}}{\text{Ery}} \]

Ery = erythrocyte count in millions/\(\mu\)l

Pg = Picogram = \(10^{-12}\) g

2. **MCV**
   
   = mean cell volume
   
   = mean corpuscular volume
   
   = mean volume of a single erythrocyte

   \[ \text{MCV} = \frac{\text{hematocrit} \times 10^{12}}{\text{Ery}} \]

   Ery = erythrocyte count in millions/\(\mu\)l
   
   fl = femtoliter = \(10^{-15}\) l

3. **MCHC**
   
   = mean corpuscular hemoglobin concentration.

   \[ \text{MCHC} = \frac{\text{Hb (g/dl)} \times 100}{\text{Hematocrit (Vol %)}} \]

**COLOR INDEX (C.I.)**

\[ \text{C. I.} = \frac{\text{HB, expressed as a percentage of normal}}{\text{RBC, expressed as a percentage of normal}} \]


**RETICULOCYTE COUNTING**

The following reagents and apparatus were used in common laboratory method.

**REAGENTS:**

*Merck* cat. No. 13742

0.5% physiological brilliant crystal blue solution.

**APPARATUS:**

Hemoglobin pipette, Petri dish, microscope, grid for counting reticulocytes.

**CALCULATION:**

\[
\text{Reticulocyte count} = \frac{\text{Ery/µl} \times (\%)}{1000} \times \frac{\text{cells/µl}}{
\]

\[Ery = \text{erythrocyte count}\]

\[R = \text{reticulocytes count}\]

**THROMBOCYTE COUNTING**

The following reagents and apparatus were used in common laboratory method.

**REAGENTS:**

*Merck* cat. 13795
Ammonium oxalate solution with mercury (II) chloride, ready for use.

**APPARATUS:**

**NEUBAUER** counting chamber, leukocyte pipette (with a white mixing bead), Petridish and microscope.

**CALCULATION:**

\[
\text{Thrombocyte count} = \frac{X \cdot D \cdot 4000}{80} = X \cdot 1000 \text{ cells/μl}
\]

\(D = \text{Dilution factor (20 or 100)}\)

\(X = \text{Number of thrombocytes in the 5 group squares (80 small squares)}\)

**TOTAL LEUKOCYTE COUNTING**

The following reagents and apparatus were used by common laboratory method.

**REAGENTS :**

**Merck** cat. No. 13739.

Acetic acid-gentian violet solution or azure B solution.

**APPARATUS :**

**WBC** pipette and other same as used in thrombocytes counting.
CALCULATION:

\[
\text{Leukocyte count} = \frac{X \times 10}{0.10} \quad \text{(dilution 1:10)}
\]

\[
\text{Leukocyte count} = X \times 25 \quad \text{(cells/μl)}
\]

\[
X = \text{Total number of cells counted in the 4 corner squares.}
\]

DIFFERENTIAL LEUKOCYTIC PICTURE (DLC)

The following reagents and apparatus were used by common laboratory method.

REAGENTS:
Merck cat. No. 1577 and 1161.
Hemacolor (rapid staining), immersion oil.

APPARATUS:
Microscope, DLC counter, slides, spider.

Blood smears for DLC and reticulocyte counts were prepared with in 5h. at room temperature

TEMPERATURE AND PPE

Inoculated test animal were bled, rectal temperature were recorded and clinical observations were made daily. Percent parasitized erythrocytes (PPE) was estimated from Giemsa-Stained smears and PCV’s were determined.
**BLOOD pH**

Blood pH estimated by pH meter. The coagulation, bleeding and prothrombin time was carried out by common laboratory methods.

**OSMOTIC FRAGILITY**

Osmotic fragility of the RBC has been estimated by saline solution

**BONE MARROW SAMPLES**

The necessary bone marrow puncture was performed by the clinician using a strong needle after administration of a local anesthetic to the skin and underlying periosteum at easily accessible sites: sternum at the second intercostal spine, anterior and posterior crest of the ilium, the spinal process of the lumbar vertebrae. The bone marrow was aspirated by a short but strong such in order to avoid dilution by mixing with peripheral blood. This procedure was carried out quickly anticoagulants was not required. The aspirated bone marrow cell was smeared immediately on to microscopic slides.

**BONE MARROW SMEARS**

Immediately after the bone marrow samples were taken, prepared and dry the smear in air in the same manner as described for the blood smears.
MORPHOLOGY OF RBC & IMMATURE CELLS

Standard blood methods were used for the study of RBC morphology and immature cells.

SURGICAL REMOVAL
OF THE SPLEEN

750 dogs were splenectomised as suggested by Brodey (1989) and Zaugg (1984).
RESULTS

Various published reports are available on normal hematology of dogs but there are very scanty published informations on splenectomised and non-splenectomised dogs during different infections specially those caused by blood protozoa (i.e. babesiosis, leishmaniasis and trypanosomiasis). Only a few reports are available on the induced hemoparasitic infections in dogs and their observation for all periods (i.e. prepatent, early and acute and late phases). In this experiment 4000 normal and naturally infected (500 splenectomised and dogs 3000 non-splenectomised dogs, and 500 experimentally infected dogs (250 splenectomised and 250 non-splenectomised monitored dogs) respectively given trial doses of Babesia canis (1.5x10⁶); Babesia gibsoni (1.5 x 10⁶); Trypanosoma evansi (1 x 10⁶); Trypanosoma brucei (1 x 10⁶); and Leishmania donovani (1 x 10⁶) were used. It was very difficult to maintain the accuracy of different phases of infection in cases of naturally infected dogs. Therefore this study is based on monitored dogs. Following results are compiled according to the tables and figures.

EC = Erythrocytes (millions/µl) Figure 1 (A, B)

In prepatent period Babesia canis, Babesia gibsoni, Trypanosoma evansi, Trypanosoma brucei, and Leishmania donovani (BC, BG, TE, TB, LD). EC was near to normal than in other phases. But decreased significantly in late and early and acute phases (P<0.01). Between EC of splenectomised dogs (SPD), and non-splenectomised dogs (NSPD) shows slight difference (Tables 3, 5, 7, 9 and 11).
SEC = Size of erythrocytes (µm) Figure 2 (A, B)

In all phases (BC, BG, TB, TE, LD) of SPD and NSPD, shows
SEC was usually normal (P<0.05), but slight difference was noted
between prepatent period and other phases (Tables 3, 5, 7, 9 and
11).

RC = Reticulocytes (°/°o) Figure 3 (A, B)

SPD and NSPD in prepatent periods (BC, BG, TE, TB, LD) RC
was noted on normal range but increased significantly (P<0.01) in
eyearly and acute and late phases. These values were showed
significant difference at (P<0.01) between SPD and NSPD. (Tables
3, 5, 7, 9 and 11).

RE = Reduction of erythrocytes (%) Figure 4 (A, B)

SPD and NSPD in prepatent (BC, BG TE, TB, LD) RE was low,
but it was significantly higher (P<0.01) in early and acute and late
phases (Tables 3, 5, 7, 9 and 11).

HBC = Hemoglobin concentration (g/dl) Figure 5 (A, b)

SPD and NSPD in prepatent periods (BC, BG TE, TB, LD) HBC
was usually normal but it was decreased significantly (P<0.01)
during early and acute and late phases (Tables 3, 5, 7, 9 and 11).

C I = Color Index Figure 6 (A, B)

SPD and NSPD in all phases (BC, BG TE, TB, LD) CI was
usually normal. There was no significant difference (P<0.01) between
SPD and NSPD (Tables 3, 5, 7, 9 and 11).

Hct = Hematocrit (vol %) Figure 7 (A, B)

SPD and NSPD in prepatent period (BC, BG, TE, TB, LD)
HCT was noted in normal range but it was decreased significantly (P<0.01) during early and acute and late phases (Tables 3, 5, 7, 9 and 11).

MCV = Mean Corpuscular Volume (fl) Figure 8 (A, B)
SPD and NSPD in all phases (BC, BG, TE, TB, LD) MCV was usually in normal range with significant (P<0.01) and nonsignificant (P>0.05) levels between the SPD and NSPD. (Tables 3, 5, 7, 9 and 11).

MCH = Mean Corpuscular Hemoglobin (pg/Cell) Figure 9 (A, B)
NSPD in all phases (BC, BG, TE, TB LD) MCH was usually in normal range with significant (P<0.01) and nonsignificant (P<0.05) levels between SPD and NSPD but it was slightly increased in the case of SPD (Tables 3, 5, 7, 9 and 11).

MCHC = Mean Corpuscular Hemoglobin Concentration (g/dl RBCS) Figure 10 (A, B)
NSPD in all phases (BC, BG, TE, TB, LD) MCHC was noted in normal range with significant (P<0.01) and nonsignificant (P<0.05) levels between SPD and NSPD but it was slightly increased in the case of SPD. (Tables 3, 5, 7, 9 and 11).

TC = Thrombocytes (per µl) Figure 11 (A, B)
SPD and NSPD in prepatent periods (BC, BG TE, TB, LD) TC was usually in normal range but it was low significantly (P<0.01) in NSPD and much lower significantly (P<0.01) in case of SPD.
during early and acute and late phases (Tables 3, 5, 7, 9 and 11).

**OF = Osmotic Fragility (% NaCl)** Figure 12 (A, B)

SPD and NSPD in all phases (BC, BG, TE, TB, LD) OF was usually within normal range but it was non significantly increased (P<0.05) in early and acute and late phases (Tables 3, 5, 7, 9 and 11).

**BpH = Blood pH** Figure 13 (A, B)

SPD and NSPD in all phases (BC, BG, TE, TB, LD) were showed the BpH weak alkalinity. There was noted only nonsignificant (P<0.05) between SPD and NSPD. (Tables 3, 5, 7, 9 and 11).

**BT= Bleeding Time** Figure 14 (A, B)

BT was observed within normal range in SPD and NSPD during prepantent periods (BC, BG, TE, TB, LD) but it was increased significantly (P<0.01) in other phases. SPD had a BT slightly increased in NSPD but they were not at significant (P<0.05) levels (Tables 3, 5, 7, 9 and 11).

**CT = Coagulation Time (min)** Figure 15 (A, B).

CT was slightly increased nonsignificantly (P<0.05) in SPD and NSPD during all phases (BC, BG, TE, TB, LD) CT of SPD was increased nonsignificantly (P<0.05) than NSPD (Tables 3, 5, 7, 9 and 11).

**PT = Prothrombin time (sec)** Figure 16 (A, B)

PT was noted in normal range during prepantent periods (BC, BG, TE, TB, LD), but PT of SPD was increased nonsignificantly
(P<0.05) than NSPD in all phases (Tables 3, 5, 7, 9 and 11).

**LC = Leukocytes (10³/μl) Figure 17 (A, B)**

LC was decreased significantly (P<0.01) during all phases (BC, BG, TE, TB, LD) LC of SPD was further slightly decreased than NSPD (Tables 4, 6, 8, 10 and 12).

**MC = Myelocytes (%) Figure 18 (A, B)**

MC was noted in normal range during all phases (BC, BT, TE, TB, LD) there was no significant difference (P<0.05) between SPD and NSPD (Tables 4, 6, 8, 10 and 12).

**MMC = Metamyelocytes (%) Figure 19 (A, B)**

MMC was noted in normal range during all phases (BC, BT, TE, TB, LD), but there was no significant difference (P<0.05) between SPD and NSPD (Tables 4, 6, 8, 10 and 12).

**BN = Band Neutrophils (%) Figure 20 (A, B)**

All Phases (BC, BG, TE, TB, LD) in SPD and NSPD were showed in the normal range of BN and nonsignificant difference (P<0.05) was noted between two groups (Tables 4, 6, 8, 10 and 12).

**SN = Segmented neutrophils (%) Figure 21 (A, B)**

However SN range was normal during all phases (BC, BG, TE, TB, LD) but its values increased significantly (P<0.01) during early and acute and late phases. SN of SPD was higher significantly (P<0.01) than NSPD. (Tables 4, 6, 8, 10 and 12).
LYC = Lymphocytes (%) Figure 22 (A, B)

LYC of SPD and NSPD were decreased significantly (P<0.01) during early and acute and late phase (BC, BG, TE, TB, LD) but its values was normal during prepatent period. LYC of SPD was lower significantly (P<0.01) than NSPD (Tables 4, 6, 8, 10 & 12).

EP = Eosinophils (%) Figure 23 (A, B)

EP of SPD and NSPD were significantly normal (P<0.01) during all phases (BC, BG, TE, TB, LD), it was slightly increased in late phases (Tables 4, 6, 8, 10 and 12).

MOC = Monocytes (%) Figure 24 (A, B)

MOC was increased significantly (P<0.01) during all phases (BC, BG, TE, TB, TD) in SPD and NSPD (Tables 4, 6, 8, 10 and 12).

BP = Basophils (%) Figure 25 (A, B)

BP was noted in normal range during all phases (BC, BG, TE, TB, LD) in SPD and NSPD. There was no significant difference (P<0.05) observed. (Tables 4, 6, 8, 10 and 12).

RL = Reduction of leukocytes (%) Figure 26 (A, B)

Percentage of RL during prepatent periods was very low than other phases (BC, BG, TE, TB, LD) in SPD and NSPD. RL significantly (P<0.01) observed in other phases (Tables 4, 6, 8, 10 and 12).
AP = Average Parasitemia (%) Figure 28 (A, B)

AP was not seen during prepatent period, but it was nonsignificantly (P<0.05) noted during other phases (BC, BG, TE, TB, LD) in SPD and NSPD (Tables 3-12).

PPE = Percent Parasitized Erythrocytes (%) Figure 29 (A, B)

PPE was not observed during prepatent periods, but it was not significant (P<0.05) increased between the SPD and NSPD during early and acute and late phases of BC and BG (Tables 3 and 5).

P/cm³ (blood) = Parasites per cubic millimeter in blood Figure 30 (A, B)

P/cm³ (blood) was not seen during prepatent period, but it was not significant (P<0.05) increased between SPD and NSPD during other phases of TE and TB (Tables 7 and 9).

PPMC = Percent Parasitized Macrophage Cell (%) Figure 31

PPMC was not seen during prepatent period, but it was observed gradually increased with nonsignificantly difference (P<0.05) between SPD and NSPD during other phases phase of LD. (Tables 11 and 12).
DISCUSSION

The current study presents a retrospective analysis of results for induced hemoparasitic infection IHPI. My approach to this analysis has incorporated several unique features designed to improve to yield the valid information and the accuracy of interpretation. First, responses were evaluated at a standardized but non-fixed single time point. This avoided both the selection of an arbitrarily fixed time point and the complexity of analyzing multiple time points. The evaluation time point selected for my study allows a maximum period of time for measurement of response for each dog, limited by initiation secondary effect of hemoparasites or in dogs without such effect, by death or a 100 days' end point. Second responses were assessed by using quantitative measures of improvement or deterioration rather than changes in staging category. The criteria were clearly defined, objective, and uniformly applied. Third no attempt was made to evaluate changes in the blood if another complication was present, even though this meant excluding organs from a large number of dogs. This avoided any in consistent or subjective judgement as to whether improvement or deterioration reflected IHPI or other processes concerns can be raised about validity and limitations of methods used to evaluate response to IHPI. In any analysis of response, the time of assessment and criteria for improvement or progression may not reflect clinical practice or bedside judgment. Furthermore, the rates of improvement and progression are not easily described. The attempts to improve accuracy by excluding organs with complications other than IHPI assumes that other organs remain valuable in such dogs.
Moreover, this approach also assumes either the sensitivity for detecting such complications is high or the indicated complications have a negligible influence on the response to IHPI concerns about the validity of these assumptions are allayed by the close correlation between response categories and non relapse mortality.

Unlike the analysis of response, the analysis of time for inoculation (infection) failure as indicated by the institution of secondary onset of infection reflects actual bedside judgment. I also excluded death from causes other than relapse as an indication of infection success.

This assumes, perhaps correctly, that most deaths following an episode of IHPI are related directly or indirectly to either IHPI or its doses. A second assumption is that deaths due to unrelated causes are relatively infrequent and randomly distributed among dog groups.

The analyses from time-to-time infections success has the advantage that all dogs remain valuable irrespective of complications other than IHPI.

The toxic effect of IHPI on the blood of dogs, when inoculated is distinct in the earlier authors investigations (Gregor, 1981; Zavala, 1983; Gwadz, 1984; Haider, 1992; Molineaux, 1993).

It was found that in dogs receiving IHPI the erythrocytes count and hemoglobin level decreased and the percentage participation or reticulocytes and polychromatophilic erythrocytes
increased in peripheral blood. In the present experiments the dogs received IHPI all test dogs were inoculated intravenously with a stabilate made from hemoparasitic infected (Babesia canis, B. gibsoni, (1.5 x 10^9) and Trypanosoma brucei T. evansi (1 x 10^8) and Leishmania donovani (1 x 10^8) canine blood.

Apart from this, the changes in blood were similar, but there was only one lethal case. In the earlier tests about 20% of the animals died, probably with the greatest changes in blood.

The decrease in the erythrocyte count with simultaneous increase of the percentage of reticulocytes and polychromatophilic erythrocytes in peripheral blood suggests that IHPI may affect maturation of red blood cells.

The hemolytic action of IHPI in vitro (Molineaux, 1993) and in vivo (Haider, 1992) has been described in the literature. Worthy of note are the studies of Haider (1992), who demonstrated a decrease in the erythrocyte count in canine after inoculation of blood protozoans. On the basis of the performed test for hemolysis, the author explains it by the hemolytic action of blood protozoa.

In the present investigations no significant changes in osmotic resistance of erythrocytes were observed. The experiments of Haider, 1992 however, were made twenty days after inoculation of a stabilate, whereas my tests of osmotic resistance of erythrocytes were carried out after ten weeks of chronic blood protozoic intoxication. It is possible, therefore, that after this time the less
resistant blood cells were eliminated from the blood stream, and adaptation to the changed conditions occurred.

Maegher 1991, observed a decreased of deformability of canine erythrocytes after incubation with blood protozoa. Less deformable cells are more frequently retained in the reticuloendothelial system of the spleen and more rapidly eliminated from the blood stream (Grab, 1979). Thus, when interpreting the decreased number of erythrocytes in the blood of the blood protozoa intoxicated canine, the possibility of this element influencing the survival time of erythrocytes should be taken into consideration. The decrease of the hemoglobin level in canine receiving stabilate was largely due to the decrease in the erythrocyte count. Less important, probably, was the increase of the percentage participation of immature erythrocytes in peripheral blood. The hematocrit value, almost decrease despite the reduced erythrocyte number, probably results from the volume in decrease of these blood cells and their loss of deformability (Gregor, 1981; Zavala, 1983; Gwadz, 1984; Collins, 1985; Haider and Iqbal, 1988). The decrease in the thrombocytic count with simultaneous increase of the bleeding time, coagulation and prothrombin during early and acute and late phases of hemoparasitic infection was noted in the present study.

Previous studies in dogs have shown similar hematological values obtained from normal and some abnormal dogs (Cohen, 1977). The thrombocytic efficacy of blood protozoa, given as a bolus has been tested in a femoral artery thrombosis model (Lambert, 1982) with reperfusion rates comparable to present project. However,
reocclusion was not monitored in those studies, and blood protozoans have not been studied for venous thrombolysis so far.

In conclusion IHPI applied as an intravenous bolus appears to be equipotent to infusion for coronary and venous thrombolysis in the dog, spite of its short plasma half-life and its lack of fibrin-binding. The combined animal model for arterial and venous thrombosis used in the present study may be useful for the evaluation of new thrombocytic strategies.

In the examined animals, no significant changes were observed in size of erythrocytes, color index, MCV, MCH, MCHC, pH, myelocytes, metamyelocytes, band neutrophils, segmented neutrophils and basophils count.

Although cases of a decrease of polymorphs activity caused by IHPI have been described in canine (Collins, 1985), feline (Molineaux, 1993) and in bovine polymorphs (Haider, 1994).

In earlier studies the authors did not note significant changes in the leukocyte system (Walliker, 1978; Grab, 1979; Gramiccia, 1994), but in the present investigations a statistically significant decrease in the leukocyte count has been shown. It comprises neutrophilic granulocytes, lymphocytes, monocytes, basophils, eosinophils, myelocytes and metamyelocytes, etc. These changes together with the depressed value of the leukocytic test by granulocytes suggest that blood protozoans affects the resistance of the tested animals. Similar results are reported by other authors.
The decrease in the percentage of formazane-positive cells in the nitroblue tetrazolium (NBT) reduction test due to dog macrophages after blood protozoic intoxication was observed by Haider, 1992 and Grab, 1979. Therefore, the influence of blood protozoans on the metabolism of the studied phagocytes seems to be distinct.

The decrease in the erythrocytes count and hemoglobin level with simultaneous increase of the percentage of reticulocytes and polychromatophilic erythrocytes in peripheral blood confirms the influence of blood protozoa on maturation of erythrocytes. Moreover, the significant decrease in the erythrocyte count with hardly any changes in bone marrow may suggest the shortening of the erythrocyte survival under conditions of intoxication with blood protozoa. On the other hand, changes in the leukocytes system seem to confirm the influence of this element on the resistance of the tested animals.

In the present studies both naturally and experimentally infected dogs were used. This study based on monitored dogs (detail see in result section).

The mean values of erythrocytic changes in splenectomised and non-splenectomised dogs during prepatent periods of infections was reported as normal values in the present studies, whereas during early and acute phases of infections, scarce value were observed, on the other hand rise values for osmotic fragility, bleeding time, coagulation time and prothrombin time were found. Erythrocytic values slightly increased during late phases of infection, but these values were not found as normal values.
In the present studies, the leukocytic picture differed considerably in the severely reacting splenectomised dogs with these in the less severely reacting non-splenectomised dogs. Whether the differences were caused directly by the presence or absence of the spleen or indirectly because the absence of the spleen causes more severe reactions remain unclear. It is more important to say that the differences indicate that the kinds and degrees of the leukocytic reactions are dependent on the severity of the infections, and that there is, therefore, no typical picture for canine hemoparasitic infections. The difference also allows the following discussions on the interrelationship of leukocytic, clinical and immunological reactions to canine hemoparasitic infections with special regard to the splenic function. It is well known that following initial B. canis infections, splenectomised dogs are more prone to fatal reactions than the non-splenectomised dogs. Thus the spleen seems to play a very important part in combatting primary infection. The actual function has not been clearly defined.

The mean values of leukocytic changes varied in different hemoparasitic infection and between the splenectomised and non-splenectomised dogs. During prepatent periods of infections no remarkable changes in leukocytes were noticed in non-splenectomised dogs, but leukopenia was, however, observed in splenectomised dogs. During early and acute phases of Babesia canis and B. gibsoni, blood abnormalities and the resulting diseases such as leukopenia, lymphopenia, eosinopenia, neutrophilia and monocytosis were recorded in splenectomised dogs whereas in non-splenectomised dogs, the blood disorders were mild. These values slightly undergo
changes in late phases.

In the present studies both naturally and experimentally infected dogs were used. This studies based on monitored dogs (detail see in result, tables and figures sections).

The mean values of hematological parameters changes in splenectomised and non-splenectomised dogs during prepatent periods of infection was reported usually as normal values (except some cases excluded) in the present studies, whereas during early and acute phases of infections, scarce values were observed. In late phases blood values tended to be normal, probably as a result of an immune response against hemoparasitic infections.

It is evident that, wherever, comparisons can be made the data obtained under the routine conditions of a small animal hospital are in good agreement with data reported from experimental laboratories. Apparently there is no essential difference between studies made on finer breeds of dogs kept as pets and the full in establishing indices for blood cytology of normal and abnormal (hemoparasitized dogs) young and adult SP and NSP dogs and can be used as reference for interpreting abnormal and pathologic findings from the hospitals.
CLINICAL & PHARMACOLOGICAL FINDINGS
MATERIALS AND METHODS

Experimental design for hematologic activity of phenytoin sodium, mepacrine hydrochloride, diminazene, suramin and acriflavine hydrochloride was made according to Haider, 1992.

Three hundred splenectomised dogs (SPD) and 300 non-splenectomised dogs (NSPD), these were pye dogs 1± 0.3 year of age were examined for this project from July 1994 to June 1996. Three hundred normal pye dogs (150 SPD and 150 NSPD were selected for control (no disease and no treatment). One fifty SPD and 150 NSPD were equally distributed among the 5 experiments (Tables 13, 14, 15, 16 and 17). These dogs were experimentally infected by Babesia canis, B. gibsoni, Trypanosoma evansi, T. brucel and Leishmania donovani. Each experimental batch of test dogs were treated with phenytoin sodium, mepacrine hydrochloride, diminazene, suramin and acriflavine hydrochloride and later exposed to trypanosomiasis (mixed infection of Trypanosoma evansi and T. brucel), babesiosis (mixed infection of Babesia canis and B. gibsoni) respectively.

SAMPLE OF BLOODS

When the blood sample were collected, the following points were observed. (1) Inserted the sample number so that the sample could be identified. (2) Most of the blood samples have been taken in the morning between 8.00 A.M. to 9.00 A.M. In some cases at 4.00 P.M. also. (3) Sterilized the skin with a dust free swab
moistened with 70% ethanol.

### VENOUS BLOOD

Materials and reagents used for collection of venous blood sample were as follows:

1) Swab made from dust-free material
2) 70% ethanol v/v.
3) Sterile disposable syringe, preferably with wide bore needle.
4) EDTA test tubes.

Blood samples were taken directly from an un congested vein into the sample tube. The venous blood was mixed with EDTA (Ethylene Diamine Tetra Acetic Acid) to prevent clotting. The EDTA was prepared by allowing 0.02 ml of a 10% Titriplex III GR solution (MERCK cat. No. 8418) to dry in small tubes (2ml blood was drawn and sealed with plastic stoppers with a tube labelled the dog number with its locality). The blood sample collected and the tube, was sealed immediately after the operation was over and the tube was carefully inverted several times in order to avoid clot formation. The tube was not shaken to avoid hemolysis. Blood sample obtained in the field were collected in evacuated glass tubes via jugular vein puncture.

### HEMATOCRIT (Hct)

Graduated centrifuge tube filled up to 100 marks with blood
rendered incoagulable by the addition of EDTA. The hematocrit value was recorded directly from the graduated scale of the tube after centrifuging for 10 min at 3000 rpm in a standard centrifuge.

**HEMOGLOBIN (Hb) DETERMINATION**

The following reagents and apparatus were used:

**REAGENTS:**

**Merck** cat. No. 3298
Reagent solution (1.0 mmol/l potassium cyanide; 0.6 mmol/l potassium hexacyanoferate (III); 2.5 mmol/l phosphate buffer pH 7.2; 1.5 mmol/l sodium chloride; 0.05% detergent).

**APPARATUS:**
Spectrophotometer, pipettes 20 μl and 5 ml.

**PROCEDURE:**
Spectrophotometer adjusted at: Absorbance maximum: 540 nm
Filter: Hg 546
Light Path: 1 cm.

Reagent solution 5 ml + Blood 0.02 ml in test tube. Measured the absorbance of the sample (As) against the reagent solution after at least 3 min.
CALCULATION:
Hemoglobin concentration = As. 36.8 (g/dl)
Hemoglobin concentration = As. 22.8 (mmol/l)

ERYTHROCYTE COUNTING

The following reagents and apparatus were used in the common laboratory manual counting method.

REAGENTS:

Hayem's reagent (Merck cat. No. 9260)

Mercury (II) chloride solution 2.5g/l isotonic with blood, ready for use.

APPARATUS:

NEUBAUER counting chamber, erythrocyte pipette, microscope, pipetting-tube with mouthpiece, electric shaker.

CALCULATION

\[
\text{Erythrocyte count} = \frac{X \times D \times 4000}{80}
\]

= \( X \times 10,000 \text{ (Cells/μl)} \) when (D=200)

= \( X \times 5,000 \text{ (Cells/μl)} \) when (D=100)

= \( X \times 100 \text{ (Cells/μl)} \) when (D=2)

\]

98
D = Dilution factor (200 or 100)
X = Number of erythrocytes counted in 80 of the smallest squares.

**Calculation parameters (MCH, MCV, MCHC)**

Important erythrocyte constant were calculated from the hemoglobin, erythrocyte and hematocrit values.

1. \( \text{Hb}_e = \text{MCH} \)
   
   = mean hemoglobin content of a single erythrocyte
   
   = mean corpuscular hemoglobin
   
   \( \text{Hb}_e = \frac{\text{Hb (g/dl)} \times 10^7 \text{pg}}{\text{Ery}} \)

   Ery = erythrocyte count in millions/µl

   Pg = Picogram = 10^{-12} g

2. \( \text{MCV} \)

   = mean cell volume
   
   = mean corpuscular volume
   
   = mean volume of a single erythrocyte

   \( \text{MCV} = \frac{\text{hematocrit} \times 10^6 \text{fl}}{\text{Ery}} \)

   Ery = erythrocyte count in millions/µl

   fl = femtoliter = 10^{-16} l
3. MCHC

\[ \text{MCHC} = \frac{\text{Hb (g/dl) \times 100}}{\text{Hematocrit (Vol %)}} \] (g Hb / dl erythrocytes)

**RETCULOCYTE COUNTING**

The following reagents and apparatus were used in common laboratory method.

**REAGENTS:**

*Merck* cat. No. 13742

0.5% physiological brilliant crystal blue solution.

**APPARATUS:**

Hemoglobin pipette, Petridish, microscope, grid for counting reticulocytes.

**CALCULATION:**

\[ \text{Reticulocyte count} = \frac{\text{Ery/\mu l} \times R (\% \text{Hb})}{1000} \]

\( \text{Ery} = \text{erythrocyte count} \)
\( R = \text{reticulocytes count} \)

**THROMBOCYTE COUNTING**

The following reagents and apparatus were used in common
laboratory method.

**REAGENTS:**
*Merck* cat. 13795

Ammonium oxalate solution with mercury (II) chloride, ready for use.

**APPARATUS:**
*NEUBAUER* counting chamber, leukocyte pipette. (with a white mixing bead), Petri dish and microscope.

**CALCULATION:**

\[
\text{Thrombocyte count} = \frac{X \cdot D \cdot 4000}{80} = X \cdot 1000 \text{ (cells/μl)}
\]

\(D = \text{Dilution factor (20 or 100)}\)

\(X = \text{Number of thrombocytes in the 5 group squares (80 small squares)}\)

**TOTAL LEUKOCYTE COUNTING**

The following reagents and apparatus were used by common laboratory method.

**REAGENTS:**
*Merck* cat. No. 13739.
Acetic acid-gentian violet solution or azure B solution.

**APPARATUS:**

WBC pipette and other same as used in thrombocytes counting.

**CALCULATION:**

\[ \frac{X \times 10}{10} = \frac{5}{(\text{dilution } 1:10)} \]

Leukocyte count = \( X \times 25 \) (cells/\( \mu l \))

\( X = \) Total number of cells counted in the 4 corner squares.

**DIFFERENTIAL LEUKOCYTIC PICTURE (DLC)**

The following reagents and apparatus were used by common laboratory method.

**REAGENTS:**

**Merck** cat. No. 1577 and 1161.

Hemacolor (rapid staining), immersion oil.

**APPARATUS:**

Microscope, DLC counter, slides, spider.

Blood smears for DLC and reticulocyte counts were prepared with in 5h. at room temperature.
BONE MARROW SAMPLES

The necessary bone marrow puncture was performed by the clinician using a strong needle after administration of a local anesthetic to the skin and underlying periosteum at easily accessible sites: sternum at the second intercostal spine, anterior and posterior crest of the ilium, the spinal process of the lumber vertebrae. The bone marrow was aspirated by a short but strong such in ordered to avoid dilution by mixing with peripheral blood. This procedure was carried out quickly anticoagulants was not required. The aspirated bone marrow cell was smeared immediately on to microscopic slides.

BONE MARROW SMEARS

Immediately after the bone marrow samples were taken, prepared and dry the smear in air in the same manner as described for the blood smears.

MORPHOLOGY OF RBC & IMMATURE CELLS

Standard blood methods were used for the study of RBC morphology and immature cells.

SURGICAL REMOVAL OF THE SPLEEN

750 dogs were splenectomised as suggested by Brodey (1989)

**PARASITES**

Parasitemia Babesia canis; B. gibboni, Trypanosoma evansi, T. brucei and Leishmania donovani were obtained from donor dogs and were inoculated intravenously 1.5 ml and 1 ml of a 1:10 dilution into each of 100 intact (test and control) dogs. Theoretically, the trial dose was $1.5 \times 10^5$ and $1 \times 10^6$ organisms. Stabiliates (Babesia canis B. gibboni Trypanosoma evansi, T. brucei and Leishmania donovani) were obtained from infected dogs and stored in liquid nitrogen (Love, 1972).

**DRUGS USED**

The following drugs were used in experiments; Monosodium derivative of 5:5'-diphenylhydantoin (Phenytoin sodium; Epanutin; Imperial Chemical Industries Ltd., Macclesfield, England), at the level of 0.040g kg$^{-1}$ (administered intramuscularly), Acridine derivative (3-Chloro-9-(4-diethylamino-1-methylbutylamino) 7-methoxyacridine dihydrochloride dihydrate), Mepacrine hydrochloride; Atebrin; Bayer 205. Bayer Co. Leverkusen, Germany) at the level of 0.040 g kg$^{-1}$ (administered intramuscularly), Diminazene (4,4-diamidino diazoaminobenzene diaceturate; Berenil; Farbwerke-Hoechst AG, Frankfurt, Germany), at the level of 3mg kg$^{-1}$ (administered intramuscularly), Suramin (Sulfonated naphthylamine Bayer 205, Bayer Co. Leverkusen, Germany) at the level of 7 - 10 mg Kg$^{-1}$ (administered intravenously), Acridine derivatives (Acriflavine
hydrochloride; Gonadrotropin; May and Baker Ltd., Dagenham, England), at the level of 2.2 mg Kg⁻¹ (administered intravenously).
RESULTS

The object of this study is to test the adverse hematologic reactions of drugs (which are usually used against hemoparasitic infection or against their pathogenesis). Therefore therapeutic results were not judged in relation to the parasitic infection.

Present, assessment is one of the most important aspects of reactions of drugs and their pathogenesis. If I can learn the factor or combination of factors which makes an occasional animal react to a drug in a way in which the great majority of animals do not, I might be able to identify susceptible animals and potentially toxic drugs before disasters.

**Effect of Phenytoin Sodium (0.035 Kg·IV twice a week) on hematology (Therapy for convulsions of Trypanosomiasis) of SP and NSP test dogs.**

**Fig. 32** shows the peripheral blood of dogs and their some parameters i.e., hemoglobin concentration, MCV, morphology of red blood cells, multi-segmented neutrophils and thrombocytes of trypanosomiatized dogs after 2400-4000 hours of inoculation of phenytoin sodium.

As shown in **Fig. 32A**, dogs had decreased (P < 0.01) hemoglobin concentration at hours 2400-4000. There was significant difference noted between SPD and NSPD (table 13).
Fig. 32B shows the MCV had gradually increased (P< 0.01) at hours 2400-4000. Significant difference was noted between SPD and NSPD (Table 13).

As shown in fig. 32C, the morphology of red blood cells were noted anisocytosis at 2400-2800 hours, and poikilocytosis is at 3200-4000 hours. (Table 13).

Fig. 32D shows the multi-segmented neutrophils had gradually decreased (P< 0.01) at hours 2400-4000. These values had significant difference at P< 0.01 between SPD and NSPD (Table 13).

Fig. 32E shows the thrombocytes count had gradually decreased (P< 0.01) at hours 2400-4000. These values had significant difference at P< 0.01 between SPD and NSPD (Table 13).

Fig. 32F (bone marrow) shows the cells per cm^3 x 1000 in bone marrow had gradually increased at 2400-4000 hours. These values had the significant difference at P< 0.01 between SPD and NSPD (Table 13).

Fig. 32G (bone marrow) shows the percentage of megaloblasts had gradually increased (P< 0.01). These values were noted with for significant difference at P< 0.01 between SPD and NSPD (Table 13).
Effect of mepracrine hydrochloride (0.04 g kg⁻¹ IM on alternate day) on hematology (therapy for babesiosis) of SP and NSP test dogs.

Effect of mepracrine hydrochloride shown on hematology of SP and NSP test dogs in fig 33, dogs had decreased (P< 0.01) thrombocytes count. Mepracrine hydrochloride produced the thrombocytopenia at hours 24-400. These values were significantly different at P< 0.01 between SPD and NSPD (table 14).

Effect of diminazine (3mg kg⁻¹ IM on alternate day) on hematology (therapy for babesiosis) SP and NSP of test dogs

Effect of diminazine on hematology of SP and NSP dogs shown in fig 34, had decreased significant by (P< 0.01) total leukocyte count (TLC). Leukopenia was noted due to the effect of diminazene at hours 24-400. These values had significant difference (P< 0.01) at hours 24-100 and nonsignificant difference (P< 0.05) was noted at hours 200-400 between SPD and NSPD (table 15).

Effect of suramin (8mg kg⁻¹ IV on alternate day) on hematology (therapy of trypanosomiasis) of SP and NSP test dogs.

Effect of suramin on hematology of SP and NSP test dogs shown in fig. 35 (A, B, C, D, E, F, & G). Fig. 35 A, B the erythrocyte count and total leukocyte count (TLC) of dogs were decreased significantly (P< 0.01) after 24-400 hours of suramin treatment. These
values were significantly different at $P < 0.01$ between SPD and NSPD (table 16).

As shown in fig 35C, dogs had decreased significantly ($P < 0.01$) hemoglobin concentration at 24-400 hours. The values were nonsignificantly different ($P < 0.05$) between SPD and NSPD (table 16).

As shown in fig 35D, dogs had increased significantly ($P < 0.01$) MCV at hours 24-400. These values indicated the significant difference ($P < 0.01$) at hours 24-400 (except 100 hours) between SPD and NSPD (table 16).

As shown in fig 35E, dogs had increased significantly ($P < 0.01$) reticulocytes count at 24-400 hours. These values were noted significantly ($P < 0.01$) at hours 50, 200 and 400 and nonsignificant difference ($P < 0.05$) was noted at 24 & 100 hours between SPD and NSPD (table 16).

As shown in fig 35F, morphology of red blood cells (MRBC) were noted as spherocytes at hours 24-400. MRBC shows no difference between SPD and NSPD (table 16).

As shown in fig 35G (bone marrow) dogs had increased significantly ($P < 0.01$) myeloid erythroid count at 24-400 hours. These values were significantly difference ($P < 0.01$) at 50-400 hours (except 24 hours). (table 16). Suramin was noted as associated with hemolysis of glucose-6-phosphate dehydrogenase deficient red cells.
Effect of Acriflavine hydrochloride (2.2 mg kg⁻¹ IV on alternate day) on hematology (therapy of babesiosis) of SP and NSP test dogs.

Effect of acriflavine hydrochloride on hematology of SP and NSP test dogs shows in fig. 36 (A, B, C, D, E, F, G, H, I, J, K & L). Fig. 36A, shows the reticulocyte count decreased significantly (P< 0.01) at 24-400 hours. No reticulocyte was noted at hours 200-400, after treatment of acriflavine hydrochloride. These values were showed non-significant difference between SPD and NSPD (table 17).

Fig. 36B, shows the thrombocytic count decreased significantly (P< 0.01) at 24-400 hours. These values showed significant difference (P< 0.01) between SPD and NSPD (table 17).

Fig. 36C, shows the morphology of red blood cells were noted normocytic-hypochromic at hours 24-50, and macrocytic cells were seen at 100-400 hours. There was no difference noted between SPD and NSPD (table 17).

Fig. 36D, shows the monocyte count was decreased significantly (P< 0.01) at 24-50 hours in SPD and 24-100 hours in NSPD. No monocyte was noted in SPD at hours 100-400 and in NSPD at hours 200-400 after treatment. At hours 24 shows significant difference (P< 0.01), and at hours 50 shows nonsignificant difference (P< 0.05) at hours 100 shows difference, where as no difference was noted at hours 200-400 between SPD and NSPD (table 17).
Fig. 36E, shows the eosinophilic count was decreased significantly (P < 0.01) at 24-50 hours after treatment. At hours 100-400 shows absence of eosinophils. At 24 hours show significant difference (P < 0.01), and nonsignificant difference (P < 0.05) was noted at hours 50, however no difference was seen at hours 100-400 between SPD and NSPD (table 17).

Fig. 36F, shows the lymphocytic count was decreased significantly (P < 0.01) at hours 24-400. The significant difference (P < 0.01) was noted between SPD and NSPD (table 17).

Fig. 36G, (bone marrow) shows the basophilic myelocytic count (BMC) decreased significantly (P < 0.01) at hours 24, but no BMC was noted at hours 50-400. At hours 24 shows nonsignificant difference (P < 0.05), where as no difference was noted at hours 50-400 between SPD and NSPD (table 17).

Fig. 36H, (bone marrow) shows the basophilic metamyelocytic count (BMMC) was decreased significantly (P < 0.01) at hours 24-50. At hours 100-400 no BMMC was seen. Non significant difference (P < 0.05) was note at hours 24-50, and no difference was seen at hours 100-400 between SPD and NSPD (table 17).

Fig. 36 I (bone marrow), shows the neutrophili myelocytic count (NMC) decreased significantly (P < 0.01) at hours 24-50, and no NMC was seen at hours 100-400. There was significant difference (P < 0.01) noted at hours 24-50, but no difference was seen at hours 100-400 between SPD and NSPD (table 17).
Fig. 36J (bone marrow), shows the neutrophilic metamyelocytic count (NMMC) decreased significantly ($P < 0.01$) at hours 24-50, where as no NMMC was seen at 100-400 hours. At hours 24 shows significant difference ($P < 0.01$) , where as at hours 50 shows non significant difference ($P < 0.05$), however no difference was seen at hours 100-400 between SPD and NSPD (table 17).

Fig. 36K (bone marrow), shows the eosinophilic myelocytic count (EMC) was decreased significantly ($P < 0.01$) at hours 24-50, also nonsignificant difference ($P < 0.05$) was noted between SPD and NSPD. No EMC was seen at hours 100-400, and also no difference was noted between SPD and NSPD (table 17).

Fig. 36L (bone marrow), shows the eosinophilic metamyelocytic count (EMMC) decreased significantly ($P < 0.01$) at hours 24-50, however nonsignificant difference ($P < 0.05$) was noted between SPD and NSPD. No EMMC was seen at hours 100-400, and no difference was seen between SPD and NSPD (table 17).
DISCUSSION

I have attempted to summarize current knowledge of the drug-induced hematologic reactions, emphasizing the mechanisms of induction and pathogenesis. In types of reactions in which the mechanisms is to some extent understood, such as immune cytopenias, the oxidant drug hemolytic anemias, and megaloblastic anemias. I have attempted to experiments in the text every drug shown to have been associated with such a reaction. For the reactions of unknown mechanism, for which there are no specific tests for the responsible drug other than a challenge. I have not attempted to list all the drugs reported to have been associated with the various reactions, even when the relationship was proven by challenge. I believe that almost any drug has the potential of causing some sort of adverse reaction in occasional drugs. Even when there is strong evidence in a few cases of a blood dyscrasia for incriminating a drug taken by millions of test animals, I do not believe this type of incriminating a warrants inclusion of the drug among those particularly likely to cause hematologic reactions. An example is acetylsalicylic acid, which is consumed in large amounts by many test animals and sometimes by nearly everyone in this country. In the registry, there reports of aspirin having been administered to 60 test animals with aplastic anemia, 70 test animals with thrombocytopenia, 50 test animals with leukopenia, 25 test animals erythroid hypoplasia without pancytopenia, 10 test animals of double cytopenias, and 85 test animals with hemolytic anemia (Molineaux, 1993; Haider, 1994).

Although the evidence that chloramphenicol may cause aplastic
anemia is purely circumstantial, it is of a different order of validity. A very small percentage of the test animals treated with chloramphenicol develop aplastic anemia (1 in 30,000 Haider & Gondal (1987) to 1 in 70,000 Haider & Iqbal (1988), but those who do constitute a large proportion of the total cases of aplastic anemia. Benzene is seldom reported in association with hematologic reactions. Yet from the discussion elsewhere in this project, it is clear that a rather large proportion of exposed test animals develop a hematologic reaction.

The present work (fig. 32 A, B, C, D, E, F & G) confirms the previous findings of several workers (Girdwood & Lenman, 1956; Penny, 1963; Haider & Iqbal, 1989-1991) of increased bone marrow megaloblast count in test drugs after treatment (Phenytoin sodium). In the present study, the megaloblast counts reached megaloblastic anemia at hours 2400-3200, and these raised to abnormal count after 3600-4000 hours of Phenytoin sodium administration. The precise mechanism which leads to the development of megaloblastic anemia in an occasional test animal after many months of therapy with anticonvulsant drugs remains unknown. It is clear, however, that dietary folic acid deficiency is a factor in many test animals (Haider & Iqbal, 1989). The dietary history is not mentioned in many of the reports, and in others, the diet is simply described as inadequate (Haider & Iqbal, 1989). Whatever the mechanism by which these drugs antagonize the action of folic acid, the antagonism may not be strong enough to overcome the amounts of folic acid present in a good diet.

Previously, Minter & Ingram (1974) proposed that large heavy
platelets obtained from acutely bled dogs are "stress" platelet and that these platelets do not decrease in size as their ages. However, other workers (Gangarosa et al., 1960; Haider & Iqbal, 1989) believed that platelets produced in response to thrombocytopenia decrease in size as their age. In the present work, platelet sizes were larger in dogs after mecaprine hydrochloride treatment and 24-400 hours after initiation of treatment than in normal dogs. The data of the present study are also consistent with the hypothesis that large platelets produced in response to thrombocytopenia decrease in size as their ages (Table 14). Previous work (Thompson et al., 1954; Haider & Iqbal, 1993) has shown that about 35% of the total number of platelets are sequestered in the spleen and that large platelets are preferentially stored. Moreover, Haider & Iqbal (1993 a) have demonstrated a rapidly mobilizable non splenic platelet pool that is not enriched with large platelets and is responsive to exercise and/or epinephrine. In agreement with these findings, the present study indicate that splenic release of platelets does not account for the increase in the platelet counts of dogs after 24-400 hours of treatment.

Leukocytopenia is the most common of the hematologic adverse reactions, accounting for 39 percent of test animals (Haider & Iqbal, 1988b). The drugs which most commonly produce it do so with much greater frequency than is seen with other hematologic reactions. The incidence in test animal receiving steady dose appears to be in the neighborhood of 0.1 to 0.7 percent with chlorpromazine and mepazine (Pisciotta et al., 1958; Fiore & Noonan, 1959) 0.86 percent with aminopyrine (Discombe, 1952) 0.45 to 1.75 percent with thiouracils (Moore, 1946; Bartels, 1948) and 1.5 percent
with methimazole (H' Doubler and H' Doubler, 1954). Although Haider & Iqbal (1994b) found that splenectomised dogs reduced the more total leukocytes counts (TLC) than non-splenectomised dogs (NSPD), TLC from splenectomised dogs (SPD) in the present study were lower than NSPD after administration (diminazene) for 24-400 hours. Present studies have reviewed the several prospective studies of the reversible dose related marrow inhabitation occurring with diminazene, which is characterized by increased plasma iron, increased saturation of plasma iron - binding globulin, delayed plasma iron clearance, failure of the radioactive iron to appear in circulatory red cells, and reticulocytopenia. Some test animals subsequently developed mild anemia leukopenia or thrombocytopenia. In 1992d, Haider & Iqbal studied a canine with Coombs'-positive hemolytic anemia and pancytopenia secondary to ingestion who recovered completely after administration of the drug was stopped (Espinal, 1988). Haider, (1994) reported a monkey male (Macaca mulata) who had been treated with suramin. Eight days after the first injection and six hours after the fourth injection, he suddenly developed symptoms and signs of hemolytic anemia subsequent in vitro tests revealed a suramin-dependence of red cell agglutination on the presence of suramin was provided by the passive transfer of the agglutinin in to a normal recipient who did not develop Coombs' positivity or red cell agglutination until he was given an injection of suramin.

The hemolytic action of suramin in vitro (Dern et al, 1954) and in vivo (Beutler, 1957) has been described in the literature. Worth of note are at the studies of Collins (1985), who demonstrated a decrease in the erythrocytes count in canine after administration of
suramin. On the basis of the performed test for hemolysis, the author explains it by the hemolysis action of suramin. In the present investigations no significant changes in osmotic resistance of erythrocytes were observed. The experiments of Haider, 1994, however, were made four days after administration of a single suramin dose, where as present tests of osmotic resistance of erythrocytes were carried out after three weeks of chronic suramin intoxication. It is possible, therefore, that after this time the less resistant blood cells were eliminated from the blood stream, and adaptation to the changed conditions occurred.

Aplastic anemia is the most disabling most frequently fatal of these reactions and next to agranulocytosis, the most frequently reported (Haider & Mallik, 1985) the term aplastic anemia usually describes a functional or morphologic failure of the marrow manifested by pancytopenia and may include cases in which the marrow is of normal or even increased cellularity, as well as those with hypocellular marrow (Haider and Iqbal 1988a) cases are usually excluded in which there is extramedullary myelopoiesis with or without myelofibrosis, reduction in erythroid elements only (pure red cell aplasia, or marrow hypoplasia induced by radiation or by the chemotherapeutic agents used in the treatment of malignancy. The relationship to reactions associated with the same drugs in which depression of one or two blood elements occurs without pancytopenia is controversial (Gysin, 1993).

Unlike most of the drug-induced hematologic syndromes, the pathologic changes of aplastic anemia progress long after the drug
has been discontinued Recovery, if it occurs, may require years.

In any discussion of drug-induced aplastic anemia, benzene and chloramphenicol must be considered. Benzene because of the frequency with which hematological disorders occur among those exposed (Haider and Mallik, 1985; Haider and Gondal, 1987; Haider and Iqbal, 1988a; Molineaux, 1993 and Russel, 1994) and chloramphenicol because it is the agent most commonly associated with aplastic anemia in recent years (Russel, 1994). In the present work, aplastic anemia was noted in dogs after administration of acriflavine hydrochloride at 24-400 hours. The administration of a drug is a calculated risk. If the risk of harm is greater than the possibility of help in a particular test animals the drug should not be used. Even if the risk is slight, as with acriflavine hydrochloride, suramin, mepacrine hydrochloride, phenytoin sodium and diminazene, the severity of the possible reaction may counsel against the use of even an excellent drug. If a satisfactory, less risky, substitute is available, the best way to treat these reactions is to prevent them. The knowledgeable and considered use of drugs would prevent many adverse reactions (Huguley, 1964; Yunis and Bloomberg, 1964; Best, 1994; Haider and Mallik, 1985; and 1986; Haider and Gondal, 1987; Haider and Iqbal, 1988).

If use of the drug is indicated, the veterinarians/physicians should be instructed as to the possibility, as well as the signs and symptoms of a reaction. Monitoring the administration of the drug with blood counts may sometimes identify a reaction before complications have occurred and lead to a better than usual outcome (Pisciotta et
al, 1958; Haider and Iqbal, 1989), but the reaction may appear suddenly, shortly after the last blood count. The test animal who is receiving regular doses of a drug which can cause agranulocytosis, such as the thiouracils or phenothiazines, should be warned to stop the drug and report to the veterinarians/physician if fever or any other symptoms of infection occurs. Similarly, the test animals receiving a drug which can produce thrombocytopenia, such as quinidine, mepacrine hydrochloride, must be taught that petechiae, or easy bruising, is a signal to stop the drug and report to the veterinarian/physician.

The second consideration in the treatment of these reactions is to be aware that a drug may have been the cause, and to exercise appropriate caution in the use of any drugs, the veterinarians/physicians may have been taking prior to onset until the diagnosis is clear. Drugs not listed in text may cause reactions. For other listings, the reader may consult the review titles in the bibliography, the AMA registry on adverse reactions (AMA, 1990).

Wintrobe's clinical hematology (Wintrobe, 1990; Schalm, 1985) has depicted the veterinary hematology and the annual listing of literature on adverse hematologic reactions included in the year book of medicine (Beeson et al, 1994). It is the responsibility of the veterinarians/physicians to inform himself of the possible adverse reactions which may follow the use of any drug he prescribes.

It is the particular responsibility of the hematologist to investigate possible pathogenetic mechanisms in any blood disorder related to
drugs. Simple tests are available for establishing the drug relationship in the immune thrombocytopenias and hemolytic anemias and for diagnosing the deficiency of G-6-PD. A careful drug history should be made in all cases of blood dyscrasias of the types caused by drugs. These cases should be reported to the appropriate agency: the food and drug administration and section on adverse reactions of the AMA council on drugs, both have reporting systems which are cooperating. Only by knowing how often a given drug produces a reaction and the severity of the reaction, can the veterinarians/physicians make competent judgement of the calculated risk of using a drug.
TABLES
### TABLE 1

Prevalence and intensity of infection of hemoparasites of dog in Pakistan during 1990-1992

<table>
<thead>
<tr>
<th>Parasites</th>
<th>DZM* No.</th>
<th>Sample Size</th>
<th>Prevalence</th>
<th>Intensity+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. infected/No. examined</td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Babesiosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Babesia canis</td>
<td>001</td>
<td>1760/4000</td>
<td>44</td>
<td>50</td>
</tr>
<tr>
<td>Babesia gibsoni</td>
<td>u02</td>
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<td>28</td>
<td>11</td>
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<td>Trypanosomiasis**</td>
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<tr>
<td>Trypanosoma evansi</td>
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<td>800/4000</td>
<td>20</td>
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<tr>
<td>Leishmaniasis**</td>
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<td>004</td>
<td>320/4000</td>
<td>8</td>
<td>25</td>
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</tbody>
</table>

* = DZM No. = Department of Zoology museum number
+ = Number of parasites per 100 RBCs.
** = Parasites per cubic millimeter blood bone marrow (P/cmm, in case of Trypanosoma evansi/Leishmania donovani)
<table>
<thead>
<tr>
<th>Category</th>
<th>Number of parasites species</th>
<th>Intensity</th>
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<td>N'</td>
<td>Range**</td>
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<tr>
<td>Sex</td>
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<td>1-3</td>
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<tr>
<td>Bitch</td>
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<td>Age-Class</td>
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<td>3-Month-</td>
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<td>&gt; 6 Years</td>
<td>1000</td>
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* = Number of test animals  
** = Range of parasites species  
+ = Ranges of parasites per 100 RBCs  
N.B. = Parasites per cubic millimeter (P/cmm) blood bone marrow in case of Trypanosoma evansi and Leishmania donovani  
S = Significant difference at P < 0.01  
NS = Non-significant difference at P < 0.05
# I Erythrocytic Changes

Comparative hematological average values, average temperatures, parasitemia and PPE in splenectomised and non-splenectomised dogs during *Babesia canis* (1.5 x 10⁶) trial dose.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (7-14 days)</th>
<th>Early and acute phase (15-21 days)</th>
<th>Late phase (25-45 days)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n=30)</td>
<td>NSP. dogs (n=30)</td>
<td>SP. dogs (n=30)</td>
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<td>Erythrocytes (million/µl)</td>
<td>6.60</td>
<td>6.80</td>
<td>3.00</td>
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<td></td>
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<td>2.00</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2.68 S</td>
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<td>11.15 S</td>
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<td>5.50</td>
<td>6.50</td>
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<td></td>
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<td>0.80 NS</td>
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<td>6.40</td>
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<td>6.00</td>
<td>19.00</td>
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<td>3.00</td>
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<td>3.74 S</td>
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<td>4.00</td>
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<td></td>
<td></td>
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<td>2.11 S</td>
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<td>0.80</td>
<td>0.90</td>
<td>1.00</td>
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<td></td>
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<td></td>
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<td>0.80</td>
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<td></td>
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<td></td>
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<td>1.90 NS</td>
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<th>Early and acute phase (15-21 days)</th>
<th>Late phase (25-45 days)</th>
<th>t-values</th>
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<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
<td>SP. dogs (n-30)</td>
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<tr>
<td>Hematocrit (vol %)</td>
<td>26.00</td>
<td>27.00</td>
<td>1.80 NS</td>
<td>12.00</td>
</tr>
<tr>
<td>Mean Corpuscular Volume (fl)</td>
<td>40.00</td>
<td>40.10</td>
<td>0.61 NS</td>
<td>40.00</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin (Pg/cell)</td>
<td>12.00</td>
<td>13.00</td>
<td>0.84 NS</td>
<td>13.00</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin Concentration (g/dl RBCs)</td>
<td>31.00</td>
<td>33.00</td>
<td>3.45 S</td>
<td>33.00</td>
</tr>
<tr>
<td>Thrombocytes (per μl)</td>
<td>150,000</td>
<td>160,000</td>
<td>1.20 NS</td>
<td>25,000</td>
</tr>
<tr>
<td>Osmotic Fragility (% NaCl)</td>
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<td>0.38</td>
<td>0.45 NS</td>
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<td>Blood pH</td>
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<td>7.20</td>
<td>0.95 NS</td>
<td>7.70</td>
</tr>
<tr>
<td>Bleeding Time (min)</td>
<td>5.00</td>
<td>4.00</td>
<td>0.68 NS</td>
<td>9.00</td>
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Contd........P/3
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<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
</tr>
<tr>
<td>Coagulation Time (min)</td>
<td>7.00</td>
<td>6.00</td>
<td>0.64 NS</td>
</tr>
<tr>
<td>Prothrombin Time (sec)</td>
<td>14.00</td>
<td>13.00</td>
<td>0.76 NS</td>
</tr>
<tr>
<td>Average Temperature (°C)</td>
<td>39.00</td>
<td>38.99</td>
<td>0.45 NS</td>
</tr>
<tr>
<td>Average Parasitemia (%)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
</tr>
<tr>
<td>PPE</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
</tr>
</tbody>
</table>

NSP. dogs = Non-splenectomised dogs  
SP. dogs = Splenectomised dogs  
NS = Non-significant difference at P < 0.05  
S = Significant difference at P < 0.01  
NR = No response  
NA = Not applicable  
PPE = Percent Parasitized erythrocytes
### TABLE 4

**II LEUKOCYTIC CHANGES**

Comparative hematological average values, average temperatures and parasitemia in splenectomised and non-splenectomised dogs during *Babesia canis* (1.5 x 10⁶) trial dose.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (7-14 days)</th>
<th>Early and acute phase (15-21 days)</th>
<th>Late phase (25-45 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
</tr>
<tr>
<td>Leukocytes (10⁹/µl)</td>
<td>4.00</td>
<td>7.00</td>
<td>5.50 S</td>
</tr>
<tr>
<td>Myelocytes (%)</td>
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<td>0.00</td>
<td>NA</td>
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<tr>
<td>Metamyelocytes (%)</td>
<td>1.00</td>
<td>0.99</td>
<td>0.89 NS</td>
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<td>Band Neutrophils (%)</td>
<td>2.00</td>
<td>3.00</td>
<td>2.68 S</td>
</tr>
<tr>
<td>Segmented Neutrophils (%)</td>
<td>50.00</td>
<td>38.00</td>
<td>12.91 S</td>
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<tr>
<td>Lymphocytes (%)</td>
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<td>50.00</td>
<td>25.51 S</td>
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Contd... .....P/2
<table>
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<th>Parameters</th>
<th>Prepatent period (7-14 days)</th>
<th>Early and acute phase 15-21 days</th>
<th>Late phase 25-45 days</th>
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</thead>
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<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>3.00</td>
<td>4.00</td>
<td>3.74 S</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>18.00</td>
<td>3.00</td>
<td>23.21 S</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>1.00</td>
<td>1.10</td>
<td>0.89 NS</td>
</tr>
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<td>Reduction of Leukocytes (%)</td>
<td>0.51</td>
<td>0.50</td>
<td>0.40 NS</td>
</tr>
<tr>
<td>Average Temperature (°C)</td>
<td>39.00</td>
<td>38.99</td>
<td>0.45 NS</td>
</tr>
<tr>
<td>Average Parasitemia (%)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
</tr>
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NSP. dogs = Non-splenectomised dogs  
SP. dogs = Splenectomised dogs  
NS = Non-significant difference at P < 0.05  
S = Significant difference at P < 0.01  
NR = No response  
NA = Not applicable
## TABLE 5

**I ERYTHROCYTIC CHANGES**

Comparative hematological average values, average temperatures, parasitemia and PPE in splenectomised and non-splenectomised dog during *Babesia gibsoni* (1.5x10⁶) trial dose.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (5-8 days)</th>
<th>Early and acute phase (9-15 days)</th>
<th>Late phase (20-45 days)</th>
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<td>SP. dogs (n=30)</td>
<td>NSP. dogs (n=30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>t</em>-values</td>
<td><em>t</em>-values</td>
<td><em>t</em>-values</td>
</tr>
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<td>Erythrocytes (millions/µl)</td>
<td>6.60</td>
<td>6.80</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td>1.59 NS</td>
<td>2.50</td>
<td>1.83 NS</td>
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<td></td>
<td>4.10</td>
<td>3.00</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>1.60 NS</td>
</tr>
<tr>
<td>Sizc of Erythrocytes (µm)</td>
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<td>5.50</td>
<td>6.50</td>
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<td>0.80 NS</td>
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<td>6.50</td>
<td>6.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.80 NS</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>10.00</td>
<td>6.00</td>
<td>20.00</td>
</tr>
<tr>
<td></td>
<td>6.09 S</td>
<td>23.00</td>
<td>4.16 S</td>
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<td></td>
<td>2.60 S</td>
</tr>
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<td>Reduction of Erythrocytes (%)</td>
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<td>0.50</td>
<td>58.00</td>
</tr>
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<td>40.00</td>
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<td>3.99 S</td>
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<td>Hemoglobin Concentration (g/dl)</td>
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<td>9.00</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>2.10 S</td>
<td>4.00</td>
<td>2.11 S</td>
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<td>5.00</td>
<td>4.00</td>
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<td>2.11 S</td>
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<th>Late phase (20-45 days)</th>
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</thead>
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<tr>
<td></td>
<td>SP. dogs (n=30)</td>
<td>NSP. dogs (n=30)</td>
<td>t-values</td>
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<td>Color Index</td>
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<td>0.90</td>
<td>0.92 NS</td>
</tr>
<tr>
<td>Hematocrit (vol %)</td>
<td>26.00</td>
<td>27.00</td>
<td>1.80 NS</td>
</tr>
<tr>
<td>Mean Corpuscular Volume (fl)</td>
<td>40.00</td>
<td>40.10</td>
<td>0.61 NS</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin (pg/cell)</td>
<td>12.00</td>
<td>13.00</td>
<td>0.84 NS</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin Concentration (g/dl RBCs)</td>
<td>31.00</td>
<td>33.00</td>
<td>3.45 S</td>
</tr>
<tr>
<td>Thrombocytes (per ul)</td>
<td>150,000</td>
<td>160,000</td>
<td>1.20 NS</td>
</tr>
<tr>
<td>Osmotic Fragility (% NaCl)</td>
<td>0.39</td>
<td>0.38</td>
<td>0.45 NS</td>
</tr>
<tr>
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<td>Late phase (20-45 days)</td>
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<td>--------------------------------</td>
<td>-----------------------------</td>
<td>-----------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td></td>
<td>SP. dogs (n=30)</td>
<td>NSP. dogs (n=30)</td>
<td>SP. dogs (n=30)</td>
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<td>7.70</td>
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<tr>
<td></td>
<td>0.95 NS</td>
<td>0.61 NS</td>
<td>0.58 NS</td>
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<tr>
<td>Bleeding Time (min)</td>
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<td>4.00</td>
<td>9.00</td>
</tr>
<tr>
<td></td>
<td>0.68 NS</td>
<td>1.10 NS</td>
<td>1.58 NS</td>
</tr>
<tr>
<td>Coagulation Time (min)</td>
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<td>6.00</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td>0.64 NS</td>
<td>0.98 NS</td>
<td>0.64 NS</td>
</tr>
<tr>
<td>Prothrombin Time (sec)</td>
<td>14.00</td>
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<td>17.00</td>
</tr>
<tr>
<td></td>
<td>0.76 NS</td>
<td>0.21 NS</td>
<td>1.66 NS</td>
</tr>
<tr>
<td>Average Temperature (°C)</td>
<td>39.00</td>
<td>38.99</td>
<td>42.40</td>
</tr>
<tr>
<td></td>
<td>0.45 NS</td>
<td>1.17 NS</td>
<td>1.13 NS</td>
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<tr>
<td>Average Parasitemia (%)</td>
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<td>NA</td>
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<td>1.14 NS</td>
</tr>
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</table>

NSP. dogs = Non-splenectomised dogs  
SP. dogs = Splenectomised dogs  
NS = Non-significant difference at P < 0.05  
S = Significant difference at P < 0.01  
NR = No response  
NA = Not applicable  
PPE = Percent parasitized erythrocytes
### TABLE 6

**II LEUKOCYTIC CHANGES**

Comparative hematological average values, average temperatures and parasitemia in splenectomised and non-splenectomised dogs during *Babesia gibsoni* (1.5 x 10^9) trial dose.

<table>
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<tr>
<th>Parameters</th>
<th>Prepatent period (5-8 days)</th>
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<th>Late phase (20-45 days)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
</tr>
<tr>
<td>Leukocytes (10^3/μL)</td>
<td>3.50</td>
<td>6.00</td>
<td>6.04 S</td>
</tr>
<tr>
<td>Myelocytes (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>NA</td>
</tr>
<tr>
<td>Metamyelocytes (%)</td>
<td>1.00</td>
<td>0.90</td>
<td>0.89 NS</td>
</tr>
<tr>
<td>Band Neutrophils (%)</td>
<td>2.00</td>
<td>3.00</td>
<td>2.68 S</td>
</tr>
<tr>
<td>Segmented Neutrophils (%)</td>
<td>53.00</td>
<td>38.00</td>
<td>12.91 S</td>
</tr>
</tbody>
</table>

Contd..........P/2


<table>
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<th>Prepatent period (5-8 days)</th>
<th>Early and acute phase (9-15 days)</th>
<th>Late phase (20-45 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
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<tr>
<td>Lymphocytes (%)</td>
<td>28.00</td>
<td>50.00</td>
<td>25.51 S</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>3.00</td>
<td>4.00</td>
<td>3.74 S</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>18.00</td>
<td>3.00</td>
<td>23.21 S</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>1.00</td>
<td>1.10</td>
<td>0.89 NS</td>
</tr>
<tr>
<td>Reduction of Leukocytes (%)</td>
<td>0.51</td>
<td>0.50</td>
<td>0.40 NS</td>
</tr>
<tr>
<td>Average Temperature (°C)</td>
<td>39.00</td>
<td>38.99</td>
<td>0.45 NS</td>
</tr>
<tr>
<td>Average Parasitemia (%)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Legend:**
- NSP. dogs = Non-splenectomised dogs
- SP. dogs = Splenectomised dogs
- NR = No response
- NA = Not applicable
- S = Significant difference at P < 0.01
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (5-21 days)</th>
<th>Early and acute phase (22-28 days)</th>
<th>Late phase (90-1065 days)</th>
<th>t-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
</tr>
<tr>
<td>Erythrocytes (millions/µl)</td>
<td>6.80</td>
<td>6.81</td>
<td>3.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Size of Erythrocytes (µm)</td>
<td>5.81</td>
<td>5.83</td>
<td>6.50</td>
<td>6.20</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>6.00</td>
<td>6.10</td>
<td>20.00</td>
<td>17.00</td>
</tr>
<tr>
<td>Reduction of Erythrocytes (%)</td>
<td>0.50</td>
<td>0.52</td>
<td>15.00</td>
<td>11.00</td>
</tr>
<tr>
<td>Hemoglobin Concentration (g/dl)</td>
<td>9.00</td>
<td>9.11</td>
<td>4.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Contd........P/2
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (5-21 days)</th>
<th>Early and acute phase (22-28 days)</th>
<th>Late phase (90-1065 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
</tr>
<tr>
<td>Color Index</td>
<td>0.90</td>
<td>0.92</td>
<td>0.99 NS</td>
</tr>
<tr>
<td>Hematocrit (vol %)</td>
<td>27.00</td>
<td>27.13</td>
<td>0.44 NS</td>
</tr>
<tr>
<td>Mean Corpuscular Volume (fl)</td>
<td>40.00</td>
<td>40.12</td>
<td>0.61 NS</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin (Pg/cell)</td>
<td>13.00</td>
<td>13.10</td>
<td>0.77 NS</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin Concentration (g/dl RBCs)</td>
<td>33.00</td>
<td>33.14</td>
<td>0.73 NS</td>
</tr>
<tr>
<td>Thrombocytes (per μl)</td>
<td>161,000</td>
<td>162,000</td>
<td>0.37 NS</td>
</tr>
<tr>
<td>Osmotic Fragility (% NaCl)</td>
<td>0.39</td>
<td>0.38</td>
<td>0.45 NS</td>
</tr>
</tbody>
</table>

Contd.....P/3
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (5-21 days)</th>
<th>Early and acute phase (22-28 days)</th>
<th>Late phase (90-1065 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>SP. dogs (n-30)</td>
</tr>
<tr>
<td>Blood pH</td>
<td>7.40</td>
<td>7.42</td>
<td>7.80</td>
</tr>
<tr>
<td>Bleeding Time (min)</td>
<td>4.00</td>
<td>5.00</td>
<td>9.00</td>
</tr>
<tr>
<td>Coagulation Time (min)</td>
<td>6.00</td>
<td>7.00</td>
<td>7.00</td>
</tr>
<tr>
<td>Prothrombin Time (sec)</td>
<td>12.00</td>
<td>14.00</td>
<td>18.00</td>
</tr>
<tr>
<td>Average Temperature (°C)</td>
<td>39.00</td>
<td>38.99</td>
<td>41.50</td>
</tr>
<tr>
<td>Average Parasitemia (%)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>P/cmm (blood)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

NSP. dogs = Non-splenectomised dogs  
SP. dogs = Splenectomised dogs  
NS = Non-significant difference at P < 0.05  
S = Significant difference at P < 0.01  
NR = No response  
NA = Not applicable  
P/cmm = Parasites per cubic millimeter in blood
TABLE 8

II LEUKOCYTIC CHANGES

Comparative hematological average values, average temperatures and parasitemia in splenectomised and non-splenectomised dogs during *Trypanosoma evansi* (1 x 10⁹) trial dose.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (5-21 days)</th>
<th>Early and acute phase (22-28 days)</th>
<th>Late phase (90-1065 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>SP. dogs (n-30)</td>
</tr>
<tr>
<td>Leukocytes (10⁹/μl)</td>
<td>3.50</td>
<td>6.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Myelocytes (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Metamyelocytes (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Band Neutrophils (%)</td>
<td>2.10</td>
<td>2.00</td>
<td>2.10</td>
</tr>
<tr>
<td>Segmented Neutrophils (%)</td>
<td>19.00</td>
<td>40.00</td>
<td>18.00</td>
</tr>
</tbody>
</table>

Contd........P/2
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (5-21 days)</th>
<th>Early and acute phase (22-28 days)</th>
<th>Late phase (90-1065 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>60.00</td>
<td>50.00</td>
<td>8.36 S</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>4.89</td>
<td>3.00</td>
<td>4.87 S</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>13.00</td>
<td>4.00</td>
<td>16.03 S</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>1.10</td>
<td>1.00</td>
<td>0.89 NS</td>
</tr>
<tr>
<td>Reduction of Leukocytes (%)</td>
<td>0.52</td>
<td>0.50</td>
<td>0.40 NS</td>
</tr>
<tr>
<td>Average Temperature (°C)</td>
<td>39.00</td>
<td>38.99</td>
<td>0.65 NS</td>
</tr>
<tr>
<td>Average Parasitemia (%)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
</tr>
</tbody>
</table>

NSP. dogs = Non-splenectomised dogs  
SP. dogs = Splenectomised dogs  
NS = Non-significant difference at P < 0.05  
S = Significant difference at P < 0.01  
NR = No response  
NA = Not applicable
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (7-25 days)</th>
<th>Early and acute phase (26-30 days)</th>
<th>Late phase (100-1200 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>SP. dogs (n-30)</td>
</tr>
<tr>
<td>Erythrocytes millions /μl</td>
<td>6.80</td>
<td>6.82</td>
<td>4.20</td>
</tr>
<tr>
<td>Size of Erythrocytes μm</td>
<td>5.80</td>
<td>5.81</td>
<td>6.30</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>8.00</td>
<td>8.10</td>
<td>19.00</td>
</tr>
<tr>
<td>Reduction of Erythrocytes (%)</td>
<td>0.52</td>
<td>0.50</td>
<td>15.00</td>
</tr>
<tr>
<td>Hemoglobin Concentration g/dl</td>
<td>9.00</td>
<td>9.10</td>
<td>6.00</td>
</tr>
</tbody>
</table>

Contd.........P/2
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (7-25 days)</th>
<th>Early and acute phase (26-30 days)</th>
<th>Late phase (100-1200 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>SP. dogs (n-30)</td>
</tr>
<tr>
<td></td>
<td>t-values</td>
<td>t-values</td>
<td>t-values</td>
</tr>
<tr>
<td>Color Index</td>
<td>0.92</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>0.90</td>
<td>0.92</td>
<td>0.92 NS</td>
</tr>
<tr>
<td>Hematocrit (vol %)</td>
<td>27.00</td>
<td>27.12</td>
<td>17.00</td>
</tr>
<tr>
<td></td>
<td>0.43 NS</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Mean Corpuscular Volume (fl)</td>
<td>40.10</td>
<td>40.00</td>
<td>40.00</td>
</tr>
<tr>
<td></td>
<td>0.61 NS</td>
<td>40.11</td>
<td>0.62 NS</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin (pg/cell)</td>
<td>13.00</td>
<td>13.14</td>
<td>14.00</td>
</tr>
<tr>
<td></td>
<td>0.78 NS</td>
<td>14.13</td>
<td>0.21 NS</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin Concentration (g/dl RBCs)</td>
<td>33.14</td>
<td>33.00</td>
<td>35.00</td>
</tr>
<tr>
<td></td>
<td>0.71 NS</td>
<td>35.10</td>
<td>0.51 NS</td>
</tr>
<tr>
<td>Thrombocytes (per µl)</td>
<td>161,000</td>
<td>160,000</td>
<td>80,000</td>
</tr>
<tr>
<td></td>
<td>0.35 NS</td>
<td>100,000</td>
<td>5.50 S</td>
</tr>
<tr>
<td>Osmotic Fragility (% NaCl)</td>
<td>0.39</td>
<td>0.38</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>0.45 NS</td>
<td>0.62</td>
<td>0.92 NS</td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>0.58</td>
<td>0.96 NS</td>
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</table>

Contd.......P/3
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<th>Early and acute phase (26-30 days)</th>
<th></th>
<th>Late phase (100-1200 days)</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
</tr>
<tr>
<td>Blood pH</td>
<td>7.30</td>
<td>7.32</td>
<td>0.84 NS</td>
<td>7.40</td>
<td>7.44</td>
<td>0.93 NS</td>
</tr>
<tr>
<td>Bleeding Time (min)</td>
<td>5.00</td>
<td>4.00</td>
<td>0.70 NS</td>
<td>9.00</td>
<td>8.00</td>
<td>0.61 NS</td>
</tr>
<tr>
<td>Coagulation Time (min)</td>
<td>7.00</td>
<td>6.00</td>
<td>0.62 NS</td>
<td>8.00</td>
<td>7.00</td>
<td>0.63 NS</td>
</tr>
<tr>
<td>Prothrombin Time (Sec)</td>
<td>14.00</td>
<td>13.00</td>
<td>0.69 NS</td>
<td>15.00</td>
<td>14.00</td>
<td>0.22 NS</td>
</tr>
<tr>
<td>Average Temperature (°C)</td>
<td>39.00</td>
<td>38.99</td>
<td>0.45 NS</td>
<td>39.90</td>
<td>39.60</td>
<td>0.89 NS</td>
</tr>
<tr>
<td>Average Parasitemia (%)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>0.55</td>
<td>0.50</td>
<td>0.45 NS</td>
</tr>
<tr>
<td>P/cmm (blood)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>3.20</td>
<td>3.00</td>
<td>0.60 NS</td>
</tr>
</tbody>
</table>

NSP. dogs = Non-splenectomised dogs  
SP. dogs = Splenectomised dogs  
NS = Non-significant difference at P < 0.05  
S = Significant difference at P < 0.01
TABLE 10

II LEUKOCYTIC CHANGES

Comparative hematological average values, average temperatures and parasitemia in splenectomised and non-splenectomised dogs during *Trypanosoma brucei* (1 x 10⁶) trial dose.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (7-25 days)</th>
<th>Early and acute phase (26-30 days)</th>
<th>Late phase (100-1200 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>SP. dogs (n-30)</td>
</tr>
<tr>
<td>Leukocytes (10⁹/µl)</td>
<td>5.50</td>
<td>6.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Myelocytes (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>NA</td>
</tr>
<tr>
<td>Metamyelocytes (%)</td>
<td>1.00</td>
<td>1.10</td>
<td>0.30 NS</td>
</tr>
<tr>
<td>Band Neutrophils (%)</td>
<td>4.00</td>
<td>3.00</td>
<td>3.74 S</td>
</tr>
<tr>
<td>Segmented Neutrophils (%)</td>
<td>30.00</td>
<td>35.00</td>
<td>4.36 S</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>47.00</td>
<td>49.00</td>
<td>3.15 S</td>
</tr>
</tbody>
</table>

Contd.......P/2
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period</th>
<th>Early and acute phase</th>
<th>Late phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(7-25 days)</td>
<td>(26-30 days)</td>
<td>(100-1200 days)</td>
</tr>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>SP. dogs (n-30)</td>
</tr>
<tr>
<td></td>
<td>t-values</td>
<td>t-values</td>
<td>t-values</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2.00</td>
<td>3.26 S</td>
<td>1.00</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>15.00</td>
<td>21.00 S</td>
<td>10.00</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>1.00</td>
<td>0.89 NS</td>
<td>1.00</td>
</tr>
<tr>
<td>Reduction of Leukocytes (%)</td>
<td>0.51</td>
<td>0.40 NS</td>
<td>11.00</td>
</tr>
<tr>
<td>Average Temperature (°C)</td>
<td>39.00</td>
<td>0.45 NS</td>
<td>39.90</td>
</tr>
<tr>
<td>Average Parasitemia (%)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
</tr>
</tbody>
</table>

NSP. dogs = Non-splenectomised dogs
SP. dogs = Splenectomised dogs
NS = Non-significant difference at P < 0.05
S = Significant difference at P < 0.01
NR = No response
NA = Not applicable
### Table 11

**I. Erythrocytic Changes**

Comparative hematological average values, average temperatures, parasitemia and PPMC in splenectomised and non-splenectomised dogs during *Leishmania donovani* (1 x 10⁵) trial dose.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (100-120 days)</th>
<th>Early and acute phase (121-200 days)</th>
<th>Late phase (365-725 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>SP. dogs (n-30)</td>
</tr>
<tr>
<td>Erythrocytes (millions/µl)</td>
<td>6.80</td>
<td>6.81</td>
<td>2.00</td>
</tr>
<tr>
<td>Size of Erythrocytes (µm)</td>
<td>5.60</td>
<td>5.50</td>
<td>6.50</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>6.00</td>
<td>6.10</td>
<td>23.00</td>
</tr>
<tr>
<td>Reduction of Erythrocytes (%)</td>
<td>0.51</td>
<td>0.50</td>
<td>55.00</td>
</tr>
<tr>
<td>Hemoglobin Concentration (g/dl)</td>
<td>9.10</td>
<td>9.12</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Contd.......P/2
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (100-120 days)</th>
<th>Early and acute phase (121-200 days)</th>
<th>Late phase (365-725 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n=30)</td>
<td>NSP. dogs (n=30)</td>
<td>t-values</td>
</tr>
<tr>
<td>Color Index</td>
<td>0.90</td>
<td>0.93</td>
<td>0.99 NS</td>
</tr>
<tr>
<td>Hematocrit (vol. %)</td>
<td>27.10</td>
<td>27.13</td>
<td>0.42 NS</td>
</tr>
<tr>
<td>Mean Corpuscular Volume (fl)</td>
<td>40.11</td>
<td>40.14</td>
<td>0.82 NS</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin (pg/cell)</td>
<td>13.10</td>
<td>13.00</td>
<td>0.77 NS</td>
</tr>
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<td>Mean Corpuscular Hemoglobin Concentration (g/dl RBCs)</td>
<td>33.12</td>
<td>33.10</td>
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<td>Thrombocytes (per μl)</td>
<td>160,000</td>
<td>161,000</td>
<td>0.35 NS</td>
</tr>
<tr>
<td>Osmotic Fragility (% NaCl)</td>
<td>0.39</td>
<td>0.38</td>
<td>0.45 NS</td>
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</table>

Contd.......
p/3
<table>
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<tr>
<th>Parameters</th>
<th>Prepatent period (100-120 days)</th>
<th>Early and acute phase (121-200 days)</th>
<th>Late phase (365-725 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
</tr>
<tr>
<td>Blood pH</td>
<td>7.30</td>
<td>7.20</td>
<td>0.83 NS</td>
</tr>
<tr>
<td>Bleeding Time (min)</td>
<td>5.00</td>
<td>4.00</td>
<td>0.68 NS</td>
</tr>
<tr>
<td>Coagulation Time (min)</td>
<td>7.00</td>
<td>6.00</td>
<td>0.65 NS</td>
</tr>
<tr>
<td>Prothrombin Time (sec)</td>
<td>14.00</td>
<td>13.00</td>
<td>0.77 NS</td>
</tr>
<tr>
<td>Average Temperature (°C)</td>
<td>39.00</td>
<td>38.99</td>
<td>0.45 NS</td>
</tr>
<tr>
<td>Average Parasitemia (%)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
</tr>
<tr>
<td>PPMC</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
</tr>
</tbody>
</table>

NSP. dogs = Non-splenectomised dogs  
SP. dogs = Splenectomised dogs  
NS = Non-significant difference at P < 0.05  
S = Significant difference at P < 0.01  
NR = No response  
NA = Not applicable  
PPMC = Percent parasitized macrophage cells
### TABLE 12

**II LEUKOCYTIC CHANGES**

Comparative hematological average values, average temperatures, parasitemia and PPMC in splenectomised and non-splenectomised dogs during *Leishmania donovani* (1x10⁴) trial dose.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (100-120 days)</th>
<th>Early and acute phase (121-200 days)</th>
<th>Late phase (365-725 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n-30)</td>
<td>NSP. dogs (n-30)</td>
<td>t-values</td>
</tr>
<tr>
<td>Leukocytes (10⁶/µl)</td>
<td>3.90</td>
<td>6.00</td>
<td>5.16 S</td>
</tr>
<tr>
<td>Myelocytes (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>NA</td>
</tr>
<tr>
<td>Metamyelocytes (%)</td>
<td>1.00</td>
<td>0.99</td>
<td>0.89 NS</td>
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<tr>
<td>Band Neutrophils (%)</td>
<td>3.00</td>
<td>3.10</td>
<td>0.32 NS</td>
</tr>
<tr>
<td>Segmented Neutrophils (%)</td>
<td>20.00</td>
<td>38.00</td>
<td>24.44 S</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>60.00</td>
<td>50.00</td>
<td>8.37 S</td>
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Contd........P/2
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prepatent period (100-120 days)</th>
<th>Early and acute phase (121-200 days)</th>
<th>Late phase (365-725 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP. dogs (n=30)</td>
<td>NSP. dogs (n=30)</td>
<td>t-values</td>
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<tr>
<td>Eosinophils (%)</td>
<td>4.10</td>
<td>4.20</td>
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<td>Monocytes (%)</td>
<td>11.00</td>
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<td>11.28 S</td>
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<td>Basophils (%)</td>
<td>0.99</td>
<td>0.98</td>
<td>0.87 NS</td>
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<tr>
<td>Reduction of Leukocytes (%)</td>
<td>0.51</td>
<td>0.50</td>
<td>0.40 NS</td>
</tr>
<tr>
<td>Average Temperature (°C)</td>
<td>39.00</td>
<td>38.99</td>
<td>0.45 NS</td>
</tr>
<tr>
<td>Average Parasitemia (%)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
</tr>
<tr>
<td>PPMC</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
</tr>
</tbody>
</table>

NSP. dogs = Non-splenectomised dogs  
SP. dogs = Splenectomised dogs  
NS = Non-significant difference at P < 0.05  
S = Significant difference at P < 0.01  
NR = No response  
NA = Not applicable  
PPMC = Percent parasitized macrophage cells
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hemoglobin concentration (g/dl)</th>
<th>Mean corpuscular volume (fl)</th>
<th>Morphology of red blood cells</th>
<th>Multisegmented Neutrophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral Blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSP dogs (n=30)</td>
<td>14</td>
<td>69</td>
<td>NN</td>
<td>60</td>
</tr>
<tr>
<td>SP dogs (n=30)</td>
<td>15</td>
<td>70</td>
<td>A</td>
<td>55</td>
</tr>
<tr>
<td>Test dogs (n=30)</td>
<td></td>
<td></td>
<td>A</td>
<td>40</td>
</tr>
<tr>
<td><strong>Normal dogs</strong></td>
<td>13</td>
<td>72</td>
<td>A</td>
<td>35</td>
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effect of phenytoin sodium (0.05 S.Kg-1 IV) on hematology (therapy for convulsions during trypanosomiasis) of test dogs and comparison with normal dogs.
<table>
<thead>
<tr>
<th>Component</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>SD</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytes</td>
<td>100,000</td>
<td>120,000</td>
<td>7.35S</td>
<td>50,000</td>
<td>70,000</td>
<td>2.55S</td>
</tr>
<tr>
<td>(per µl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone Marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells per cmm x 1000</td>
<td>140,000</td>
<td>160,000</td>
<td>19.62S</td>
<td>145,000</td>
<td>166,000</td>
<td>2400</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Megaloblasts (%)</td>
<td>1.00</td>
<td>0.99</td>
<td>0.55NS</td>
<td>6.70</td>
<td>5.90</td>
<td>2400</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Legend:**
- SP.dogs = Splenectomised dogs
- NSP.dogs = Non-splenectomised dogs
- NN = Normocytic normochromic
- A = Anisocytosis
- P = Poikilocytosis
- IV = Intervenous

**Abbreviations:**
- NA = Not Applicable
- S = Significant difference at P<0.01
- NS = Non-Significant difference at P<0.05
- * = drug associated with megaloblastic anemia
- ** = Normal dogs (untreated, uninfected control dogs)
TABLE 14

Effect of *Mepacrine hydrochloride (0.040gkg^-1 IM) on hematology (therapy for babesiosis) of test dogs and comparison with normal dogs.

<table>
<thead>
<tr>
<th>Parameters</th>
<th><strong>Normal dogs</strong></th>
<th>( t )-value</th>
<th>Test dogs</th>
<th>Hours after drug inoculation</th>
<th>( t )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSP.dogs (n-30)</td>
<td></td>
<td>SP.dogs (n-30)</td>
<td></td>
<td>NSP.dogs (n-30)</td>
</tr>
<tr>
<td>Thrombocytes (per μl)</td>
<td>100,000</td>
<td></td>
<td>120,000</td>
<td></td>
<td>50,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.35S</td>
<td></td>
<td></td>
<td>40,000</td>
</tr>
<tr>
<td></td>
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<td>28,000</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5,000</td>
</tr>
</tbody>
</table>

SP.dogs = Splenectomised dogs
NSP.dogs = Non-splenectomised dogs
IM = Intramuscular

\( S \) = Significant difference at \( P<0.01 \)
\( NS \) = Non-significant difference at \( P<0.05 \)
\( \ast \) = Drug associated with thrombocytopenia
\( \ast\ast \) = Normal dogs (untreated, uninfected control dogs)
TABLE 15  
Effect of *Diminazene (3mg Kg⁻¹ IM) on hematology (therapy for babesiosis) of test dogs and comparison with normal dogs.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>** Normal dogs</th>
<th></th>
<th></th>
<th>** Test dogs</th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSP.dogs (n-30)</td>
<td>SP.dogs (n-30)</td>
<td></td>
<td>NSP.dogs (n-30)</td>
<td>SP.dogs (n-30)</td>
<td>Hours after drug inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes (10³/µl)</td>
<td>10</td>
<td>12</td>
<td>2.35S</td>
<td>6</td>
<td>9</td>
<td>24</td>
<td>3.10S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>50</td>
<td>2.75S</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>100</td>
<td>2.90S</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>200</td>
<td>0.88NS</td>
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</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>400</td>
<td>0.56NS</td>
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</table>

SP.dogs = Splenectomised dogs  
NSP.dogs = Non-splenectomised dogs  
IM = Intramuscular  
S = Significant difference at P<0.01  
NS = Non-significant difference at P<0.05  
* = Drug associated with leukocytopenia  
** = Normal dogs (untreated, uninfected control dogs)
<table>
<thead>
<tr>
<th>Parameters</th>
<th><strong>NSP.dogs (n-30)</strong></th>
<th><strong>SP.dogs (n-30)</strong></th>
<th><strong>f-value</strong></th>
<th><strong>NSP.dogs (n-30)</strong></th>
<th><strong>SP.dogs (n-30)</strong></th>
<th>Hours after drug inoculation</th>
<th>f-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral Blood</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Reduction of erythrocytes (%)</td>
<td>NIL</td>
<td>NIL</td>
<td>NA</td>
<td>16</td>
<td>10</td>
<td>24</td>
<td>4.44S</td>
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<td>50</td>
<td>5.20S</td>
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<tr>
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<td>24</td>
<td>20</td>
<td>100</td>
<td>24</td>
<td>20</td>
<td>100</td>
<td>5.52S</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>28</td>
<td>200</td>
<td>24</td>
<td>28</td>
<td>200</td>
<td>6.60S</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>43</td>
<td>400</td>
<td>24</td>
<td>43</td>
<td>400</td>
<td>4.28S</td>
</tr>
<tr>
<td>Reduction of leukocytes (%)</td>
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<td>NIL</td>
<td>NA</td>
<td>12</td>
<td>8</td>
<td>24</td>
<td>5.55S</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>10</td>
<td>50</td>
<td>24</td>
<td>10</td>
<td>50</td>
<td>9.27S</td>
</tr>
<tr>
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<td>100</td>
<td>24</td>
<td>15</td>
<td>100</td>
<td>8.42S</td>
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<td>200</td>
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<td>38</td>
<td>200</td>
<td>7.15S</td>
</tr>
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<td>40</td>
<td>400</td>
<td>24</td>
<td>40</td>
<td>400</td>
<td>6.22S</td>
</tr>
<tr>
<td>Hemoglobin concentration (g/dl)</td>
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<td>15</td>
<td>0.40NS</td>
<td>10</td>
<td>11</td>
<td>24</td>
<td>0.49NS</td>
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<td>4</td>
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<td>50</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>0.92NS</td>
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<td>4</td>
<td>100</td>
<td>10</td>
<td>4</td>
<td>100</td>
<td>0.51NS</td>
</tr>
<tr>
<td></td>
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<td>3.00</td>
<td>200</td>
<td>10</td>
<td>3.00</td>
<td>200</td>
<td>0.60NS</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
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<td>400</td>
<td>10</td>
<td>2.00</td>
<td>400</td>
<td>0.55NS</td>
</tr>
</tbody>
</table>

Contd. P...2
| Meancorpuscular volume | 69   | 70   | 0.51NS | 70   | 72   | 24   | 2.35S |
|                        | 73   | 73   | 50     | 79   | 80   | 100  | 0.20NS |
|                        | 85   | 89   | 200    | 90   | 95   | 400  | 5.30S |
|                        |      |      |        |      |      |      | 4.44S |
| Reticulocytes          | 4    | 5    | 0.22NS | 5.50 | 6.10 | 24   | 0.50NS |
|                        | 8.90 | 11.12| 50     | 15.10| 15.70| 100  | 2.12S |
|                        | 20.51| 22.90| 200    | 25.10| 30.00| 400  | 0.55NS |
|                        |      |      |        |      |      |      | 21.40S |
| Morphology of red blood cells | NN | NN | NA | Sc  | Sc  | 24 | NA |
|                        | Sc  | Sc  | 50   | Sc  | Sc  | 100  | NA |
|                        | Sc  | Sc  | 200  | Sc  | Sc  | 400  | NA |
| Osmotic fragility      | 74.10 ± 1.50 | 75.20 ± 0.98 | 0.28NS | 74.90 ± 1.63 | 75.90 ± 1.55 | 400 | 0.22NS |
| C50% (mmol/NaCl)       |      |      |       |      |      |      |      |
| Bone Marrow            |      |      |       |      |      |      |      |
| Myeloid Erythroid (%)  | 5    | 6    | 0.99NS | 8    | 9    | 24   | 0.31NS |
|                        | 16   | 18   | 50    | 20   | 24   | 100  | 2.12S |
|                        | 28   | 30   | 200   | 35   | 39   | 400  | 3.40S |
|                        |      |      |       |      |      |      | 5.55S |
|                        |      |      |       |      |      |      | 6.70S |

**SP.dogs** = Splenectomised dogs  
**NSP.dogs** = Non-splenectomised dogs  
**IV** = Intravenous  
**NA** = Not Applicable  
**NN** = Normocytic normochromic  
**S** = Significant difference at P < 0.01  
**NS** = Non-significant difference at P<0.05  
**Sc** = Spherocyte  
**** = Normal dogs (untreated, unneced control dogs)  
**C50%** = Concentration of NaCl in which 50% of hemolysis was observed.
<table>
<thead>
<tr>
<th>Parameters</th>
<th><strong>Normal dogs</strong></th>
<th>f-value</th>
<th><strong>Test dogs</strong></th>
<th>Hours after drug inoculation</th>
<th>f-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP.dogs (n-30)</td>
<td></td>
<td>NSP.dogs (n-30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>4</td>
<td>5</td>
<td>0.22NS</td>
<td>2</td>
<td>3</td>
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<tr>
<td></td>
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<td>0.25NS</td>
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<td>1</td>
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<td>NIL</td>
<td>200</td>
<td>NA</td>
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<td></td>
<td>NIL</td>
<td>NIL</td>
<td>400</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Thrombocytes (per μl)</td>
<td>100,000</td>
<td>120,000</td>
<td>7.35S</td>
<td>50,000</td>
<td>90,000</td>
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<tr>
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<td>25,000</td>
<td>50,000</td>
<td>50</td>
<td>22.11S</td>
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</tr>
<tr>
<td></td>
<td>15,000</td>
<td>28,000</td>
<td>100</td>
<td>18.10S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10,000</td>
<td>18,000</td>
<td>200</td>
<td>9.12S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>8,000</td>
<td>400</td>
<td>6.88S</td>
<td></td>
</tr>
<tr>
<td>Morphology of red blood cells</td>
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<td>NN</td>
<td>NA</td>
<td>NH</td>
<td>NH</td>
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</tr>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>200</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Monocytes (%)</td>
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<td>10</td>
<td>5.55S</td>
<td>3</td>
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**SP.dogs** = Splenectomised dogs
**NSP.dogs** = Non-splenectomised dogs
**S** = Significant difference at P<0.01
**NS** = Non-significant difference at P<0.05
**IV** = Intravenous
**NN** = Normocytic normochromic
**NH** = Normocytic hypochromic
**M** = Macrocytic
* = Drug associated with aplastic anemia
** = Normal dogs (untreated, uninfected control dogs)
SUMMARY
SUMMARY

Arthropod-transmit hemoparasitic diseases of dogs, caused by *Trypanosoma, Babesia, Leishmania*, occur throughout the world, their occurrence is greater in Pakistan, since conditions are favorable to the maintenance of vector populations. Practically nothing is known about the hemoparasites and hematology of dog in Pakistan, except Haider and his school published some information about this subject with reference to Pakistan (See review of literature). Adverse hematologic reactions to drugs with reference to Pakistan is also unknown. Therefore the present investigation were undertaken. Total of 2000 group of dogs, ranging 1-3 head of dogs (naturally and experimentally infected splenectomised and non-splenectomised dogs), were evaluated for the natural hemoparasitic infection, hematology of hemoparasitized dogs and adverse hematologic reactions to drugs were also evaluated in experimental dogs. These dogs were examined during the years 1990-1996 from Pakistan. A record of collection was kept regularly to see what hemoparasites and what hematological values of dogs are common in a certain hemoparasitic infections, and what blood dyscrasias produced by adverse reactions to drugs are diverse group. Likewise a record of collections was maintained and specific numbers are assigned to each of them for ready reference.

A total of three genera of hemoparasites including *Babesia, Trypanosoma* and *Leishmania* (*B. canis, B. gibsoni, T. evansi*, and *L. donovani* were found naturally in dogs) were studied. The hemoparasites, hematological values and adverse activity of phenytoin sodium, mepacrine
hydrochloride, diminazene, suramin and acriflavine hydrochloride are emphasized in the result section. The detailed reports have been shown in 1-17 tables.
CONCLUSION

Studies on naturally hemoparasitic infected dogs in Pakistan showed that *Babesia canis* was most prevalent hemoparasites in host, occurring 44% of dogs. Other hemoparasites were as under:

*Babesia gibsoni* (28%), *Trypanosoma evansi* (20%) and *Leishmania donovani* (8%).

There were no significant differences in the mean number of hemoparasitic species per dog on the basis of sex difference, but it was related with aging. Although there was no significant difference in the mean intensity between sexes, a significant difference was found between ages, with young dogs exhibiting higher mean intensities of babesiosis that in adult dogs with regard to ages, there was a significant higher prevalence of babesiosis in younger dogs when it was examined on overall basis, but no significant difference was found in case of other blood protozoans.

Clinical signs were found common in hemoparasitic infections, such as dehydration, anemia, anorexia, anemic anoxia, shock, icterus, febrile responses and toxic reactions.

Hematological studies showed various changes in different phases of infections, for instance no erythroid were found in splenectomised and non-splenectomised prepatent period, but these changes were hi
acute phases, during late phases these values tended to normal values.

Leukocytic changes were seen in splenectomised dogs during prepatent period, but no changes were recorded in non splenectomised dogs. During early and acute phases, both types of dogs showed leukocytic changes. During late phase these values tended to normal values. The effect on the blood cell might result from concentrations of the drug greater than those ordinarily obtained through therapeutic use. A cell deficient in a mechanism capable of protecting it against therapeutic concentrations of the drug would be especially susceptible. An example of this is the heritable deficiency of erythrocyte glucose-6-phosphate dehydrogenase, which renders the cell sensitive to certain oxidant drugs. Even normal cells might be destroyed by the same drug if an excessively high blood level were produced by overdosage, increased absorption, poor excretion or failure of normal detoxification by some other tissues, such as the liver.

The susceptible animals might have a metabolic abnormality such that a toxic product is formed from the drug to a much greater extent than in most animals.

The drug might antagonize a metabolic reaction that is not vital in most animals, but many become vital in the susceptible person because an alternative pathway fails due to enzyme deficiency.
The drug might act as a haptene which stimulates the production of an antibody capable of attacking the cell in the presence of the drug. Such a mechanism has been demonstrated in some cases of agranulocytosis and of hemolytic anemia and appears to be a frequent mode of production of drug thrombocytopenia.

Adverse hematologic reactions to drugs were recorded as follows:
1- Phenytoin Sodium associated with megaloblastic anemia.
2- Mepacrine hydrochloride associated with thrombocytopenia.
3- Diminazene associated with leukocytopenia.
4- Suramin associated with hemolytic anemia.
5. Acriflavine hydrochloride associated with aplastic anemia.
SUMMARY IN URDU

خلاصہ

کہ الہم/کلب الہم میں پاکستان کے سلسلے میں خوشی تھی، جسیں شکر خواہ، آتشینانہ کے چھپا دیکھنے کی خوشی نہیں، اوہ غول مراغہ اختیار نہیں کیا تھا۔ یہ نہیں، لوگوں کا ہاتھ ہی ہاتھ بیان کر رہے، کہ ایک ہی معاونہ کوہ پاکستان کے سلسلے میں خوشی کا اہم کردار ہے۔

عالمی سیاسی اور انسانی حقوق کا اہم کردار ہے۔ پاکستان کے سلسلے میں، خوشی کا اہم کردار ہے۔

معلومات پاکستان کے حوالے سے، اس مضمون پر کھیجیں ہوئے جو ایک کپتان تک پہنچ جاتے ہیں۔ (مالک، پریمیوم ایکٹور)

موجودہ تقریب میں کلب الہم میں تقریب میں پاکستان کا اہم کردار ہے۔ پاکستان کے سلسلے میں خوشی کا اہم کردار ہے۔

لامپوش آرڈر، مقبوضہ پاکستان کے تقریب میں بھی کلب الہم کا اہم کردار ہے۔

مزید بیکر، خوشی کا اہم کردار ہے۔

اما پاکستان کے سلسلے میں خوشی کا اہم کردار ہے۔

کلب الہم کی واقعات کا دوسرے کے واقعات کا اہم کردار ہے۔

لاہور میں واقعات کے دوسرے کے واقعات کا اہم کردار ہے۔

پاکستان کی واقعات کے دوسرے کے واقعات کا اہم کردار ہے۔

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ACKNOWLEDGMENTS

I am grateful to Prof. Dr. Imtiaz Ahmed Chairman, Department of Zoology, University of Karachi, for allowing me to undertake this project for a Ph. D. degree.

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I am greatly indebted to Dr. E. L. Pilchard, Editor Foreign animals disease reports, United States, Department of Agriculture, Col. J. Holloway, U. S. Army Medical Research Unit, Malaysia, for supplying the helpful literature. Many veterinarians and farm managers have helped me to carry out this work, and to all of them I extend my sincere thanks.

Last but not least I wish to thanks to my parents Mr. Abdul Salam and Mrs. Khair-un-Nisa for their blessings and good wishes.
PLATES
Hematological parameters of splenectomised and non-splenectomised dogs (i.e. SPD and NSPD) during prepatent periods, early and acute and late phases of different induced hemoparasitic infections, (i.e. Babesia canis; B. gibsoni; Trypanosoma evansi; T. brucei and Leishmania donovani) are presented in figure 1 to 31.
PLATE 1-A

FIGURE 1-A Erythrocytes (million/μl) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative erythrocytic values of SPD (Fig. 1-A) during prepatent periods, early and acute and late phases of five different hemoparasitic infections. Data points are given in tables 3, 5, 7, 9 & 11. For details see results.
Splenectomised Dogs

Figure 1-A

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi
PLATE 1-B

FIGURE 1-B  Erythrocytes (million/μl) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative erythrocytic values of NSPD (Fig.1-B) during prepatent periods, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 3, 5, 7, 9 & 11. For details see results.
NON-SPLENETOMISED DOGS

FIGURE 1-B

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi
PLATE 2-A

FIGURE 2-A Size of erythrocyte (μm) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative size of erythrocytes of SPD (Fig. 2-A) during prepatent period early and acute and late phases of five different hemoparasitic infections, data points are given in table 3, 5, 7, 9 & 11. For more details see results.
SPLENECTOMISED DOGS

Size of Erythrocytes (μm)

DAYS

5 7 8 9 14 15 20 21 22 25 26 30 45 90 100 120 121 200 365 725 1065 1200

Babesia canis
Babesia gibsoni
Trypanosoma brucei
Leishmania donovani
Trypanosoma evansi

FIGURE 2-A
PLATE 2-B

FIGURE 2-B  Size of erythrocyte (μm) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative size of erythrocytes of NSPD (Fig. 2-B) during prepatent period early and acute and late phases of five different hemoparasitic infections. Data points are given in table-3, 5, 7, 9 & 11. For more details see results.
NON-SPLENECTOMISED DOGS

FIGURE 2-B
PLATE 3-A

FIGURE 3-A Reticulocytes (%) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative reticulocytic values of SPD (Fig. 3-A) during prepatent period, early and acute, and late phases of five different hemoparasitic infections. Data are given in table 3, 5, 7, 9 & 11. For detail see results.
PLATE 3-B

FIGURE 3-B Reticulocytes (%) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative reticulocytic values of NSPD (Fig. 3-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections, data are given in table 3, 5, 7, 9 & 11. For detail see results.
NON-SPLENECTOMISED DOGS

![Graph showing reticulocyte levels over days for different species of parasites: Babesia canis, Babesia gibsoni, Trypanosoma brucei, Leishmania donovani, and Trypanosoma evansi.](image)

**FIGURE 3-B**
Plate 4-A

Figure 4-A Reduction of erythrocytes (%) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative percentage of erythrocytic reduction of SPD (Fig. 4-A) during prepatent period, early and acute and late phases of five different hemoparasitic infections. Data points are given in table 3, 5, 7, 9 & 11. For more details see results.
PLATE 4-B

FIGURE 4-B Reduction of erythrocytes (%) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative percentage of erythrocytic reduction of NSPD (Fig. 4-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections. Data points are given in table 3, 5, 7, 9 & 11. For more details see results.
NON-SPLENECTOMISED DOGS

Reduction of Erythrocytes (%)

50  40  30  20  10  0

DAYS

5  7  8  9  14  15  20  22  25  28  30  45  90  100  120  121  200  365  1065  1200

Babesia canis
Babesia gibsoni
Trypanosoma brucei
Leishmania donovani
Trypanosoma evansi

FIGURE 4-B
PLATE 5-A

FIGURE 5-A Hemoglobin concentration (g/dl) of SPD during different induced hemoparasitic infection.

This illustration shows the comparative values of hemoglobin concentration of SPD (Fig. 5-A) during prepatent periods, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 3, 5, 7, 9 & 11. For details see results.
SPLENECTOMISED DOGS

Hemoglobin Concentration (g/dl)

5  7  8  9  14  15  20  21  22  25  26  28  30  45  90  100  120  121  200  365  725  1165  1200

DAYS

*Babesia canis*  *Babesia gibsoni*  *Trypanosoma brucei*

*Leishmania donovani*  *Trypanosoma evansi*

**FIGURE 5-A**
PLATE 5-B

FIGURE 5-B  Hemoglobin concentration (g/dl) of NSPD during different induced hemoparasitic infection.

This illustration shows the comparative values of hemoglobin concentration of NSPD (Fig. 5-B) during prepatent periods, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 3, 5, 7, 9 & 11. For details see results.
NON-SPLENECTOMISED DOGS

DAYS

Babesia canis  Babesia gibsoni  Trypanosoma brucei
Leishmania donovani  Trypanosoma evansi

FIGURE 5-B
PLATE 6-A

FIGURE 6-A Color index of SPD during different induced hemoparasitic infection.

This illustration shows the comparative color index size of SPD (Fig. 6-A) during prepatent period early and acute and late phases of five different hemoparasitic infections, data points are given in table 3, 5, 7, 9 & 11. For more details see results.
Splenectomised Dogs

Figure 6-A

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi

Days
PLATE 6-B

FIGURE 6-B  Color index of NSPD during different induced hemoparasitic infection.

This illustration shows the comparative color index size of NSPD (Fig. 6-B) during prepatent period early and acute and late phases of five different hemoparasitic infections. Data points are given in table 3, 5, 7, 9 & 11. For more details see results.
NON-SPLENECTOMISED DOGS

![Graph showing the number of days for different species of protozoa: Babesia canis, Babesia gibsoni, Trypanosoma brucei, Leishmania donovani, and Trypanosoma evansi. The x-axis represents days, and the y-axis represents the color index.]
PLATE 7-A

FIGURE 7-A Hematocrit (vol%) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative values of hematocrit of SPD (Fig. 7-A) during prepatent period, early and acute and late phases of five different hemoparasitic infections. Data are given in table 3, 5, 7, 9 & 11. For detail see results.
Splenectomised dogs

Hematocrit (vol %)

Days

Babesia canis
Babesia gibsoni
Trypanosoma brucei
Leishmania donovani
Trypanosoma evansi

Figure 7-A
PLATE 7-B

FIGURE 7-B Hematocrit (vol%) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative values of hematocrit of NSPD (Fig. 7-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections. data are given in table 3, 5, 7, 9 & 11. For detail see results.
NON-SPLENECTOMISE DOGS

Hematocrit (vol %)

DAYS

5 7 8 9 14 15 20 21 22 25 26 28 30 45 90 100 120 121 200 365 725 1065 1200

--- Babesia canis --- Babesia gibsoni --- Trypanosoma brucei

--- Leishmania donovani --- Trypanosoma evansi

FIGURE 7-B
PLATE 8-A

FIGURE 8-A Mean corpuscular volume (fl) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative of mean corpuscular volume of SPD (Fig. 8-A) during prepatent period, early and acute and late phases of five different hemoparasitic infections. Data points are given in Table 3, 5, 7, 9 & 11. For more details see results.
SPLENECTOMISED DOGS

Mean Corpuscular Volume (fL)

DAYS

Babesia canis  Babesia gibsoni  Trypanosoma brucei
Leishmania donovani  Trypanosoma evansi

FIGURE 8-A
PLATE 8-B

FIGURE 8-B Mean corpuscular volume (fL) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative of mean corpuscular volume of NSPD (Fig. 8-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections, data points are given in table 3, 5, 7, 9 & 11. For more details see results.
PLATE 9-A

FIGURE 9-A Mean corpuscular hemoglobin (g/cell) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative values of mean corpuscular hemoglobin of SPD (Fig. 9-A) during prepatent periods, early and acute and late phases of five different hemoparasitic infections. Data points are given in tables 3, 5, 7, 9 & 11. For more details see result.
Splenectomised Dogs

DAYS

Mean Carpuscular Hemoglobin (Pg/cell)

0  5  10  15  20

5  10  15

Babesia canis  Babesia gibsoni  Trypanosoma brucei

Leishmania donovani  Trypanosoma evansi

Figure 9-A
PLATE 9-B

FIGURE 9-B Mean corpuscular hemoglobin (g/cell) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative values of mean corpuscular hemoglobin of NSPD (Fig. 9-B) during prepatent periods, early and acute and late phases of five different hemoparasitic infections. Data points are given in Tables 3, 5, 7, 9 & 11. For more details see result.
NON-SPLENECTOMISED DOGS

Mean Carpuscular Hemoglobin (Pg/cell)

DAYS

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi

FIGURE 9-B
PLATE 10-A

FIGURE 10-A Mean corpuscular hemoglobin concentration (g/dl RBCs) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative value of mean corpuscular hemoglobin concentration of SPD (Fig. 10-A) during prepatent periods, early and acute and late phases of five different hemoparasitic infections. Data points are given in tables 3, 5, 7, 9 & 11. For details see results.
FIGURE 10-B Mean corpuscular hemoglobin concentration (g/dl RBCs) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative value of mean corpuscular hemoglobin concentration of NSPD (Fig. 10-B) during prepatent periods, early and acute and late phases of five different hemoparasitic infections. Data points are given in tables 3, 5, 7, 9 & 11. For details see results.
NON-SPLENECTOMISED DOGS

DAYS

- *Babesia canis*
- *Babesia gibsoni*
- *Trypanosoma brucei*
- *Leishmania donovani*
- *Trypanosoma evansi*

**FIGURE 10-B**
PLATE 11-A

FIGURE 11-A Thrombocytes (Per µl) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative values of thrombocytes of SPD (Fig. 11-A) during prepatent period early and acute and late phases of five different hemoparasitic infections. data points are given in table 3, 5, 7, 9 & 11. For more details see results.
Splenectomised Dogs

Thousands

Thrombocytes (per μl)

DAYS

5 7 8 9 14 15 20 21 25 26 28 30 45 90 100 120 121 200 365 725 1065 1200

Babesia canis  Babesia gibsoni  Trypanosoma brucei
Leishmania donovani  Trypanosoma evansi

FIGURE 11-A
PLATE 11-B

FIGURE 11-B Thrombocytes (Per µl) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative values of thrombocytes of NSPD (Fig. 11-B) during prepatent period early and acute and late phases of five different hemoparasitic infections, data points are given in table 3, 5, 7, 9 & 11. For more details see results.
NON-SPLENECTOMISED DOGS

![Graph showing thrombocyte counts over days for different parasites.]

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi

FIGURE 11-B
PLATE 12-A

FIGURE 12-A Osmotic fragility (% NaCl) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative values of Osmotic fragility of SPD (Fig. 12-A) during prepatent period, early and acute and late phases of five different hemoparasitic infections, data are given in table 3, 5, 7, 9 & 11. For detail see results.
Splenectomised Dogs

Osmotic fragility (% NaCl)

DAYS

Babesia canis  Babesia gibsoni  Trypanosoma brucei
Leishmania donovani  Trypanosoma evansi

Figure 12-A
PLATE 12-B

FIGURE 12-B Osmotic fragility (% NaCl) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative values of Osmotic fragility of NSPD (Fig. 12-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections. Data are given in table 3, 5, 7, 9 & 11. For detail see results.
NON-SPLENECTOMISED DOGS

Osmotic fragility (% NaCl)

DAYS

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei

- Leishmania donovani
- Trypanosoma evansi

FIGURE 12-B
PLATE 13-A

FIGURE 13-A Blood pH of SPD during different induced hemoparasitic infections.

This illustration shows the comparative values of blood pH of SPD (Fig. 13-A) during prepatent period, early and acute and late phases of five different hemoparasitic infections. Data points are given in Table 3, 5, 7, 9 & 11. For more details see results.
Splenectomised Dogs

Blood pH

DAYS

- Babesia canis  - Babesia gibsoni  - Trypanosoma brucei
- Leishmania donovani  - Trypanosoma evansi

FIGURE 13-A
PLATE 13-B

FIGURE 13-B Blood pH of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative values of blood pH of NSPD (Fig. 13-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections, data points are given in table 3, 5, 7, 9 & 11. For more details see results.
NON-SPLENECTOMISED DOGS

FIGURE 13-B
PLATE 14-A

FIGURE 14-A Bleeding time (min.) of SPD during different induced hemoparasitic infection.

This illustration shows the comparative bleeding times of SPD (Fig. 14-A) during prepatent periods, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 3, 5, 7, 9 & 11. For details see results.
Splenectomised Dogs

Bleeding Time (min)

DAYS

5 7 8 9 14 20 21 22 25 26 28 30 45 90 100 120 200 365 725 1065 1200

Babesia canis  Babesia gibsoni  Trypanosoma brucei
Leishmania donovani  Trypanosoma evansi

Figure 14-A
PLATE 14-B

FIGURE 14-B Bleeding time (min.) of NSPD during different induced hemoparasitic infection.

This illustration shows the comparative bleeding times of NSPD (Fig. 14-B) during prepatent periods, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 3, 5, 7, 9 & 11. For details see results.
NON-SPLENECTOMISED DOGS

Bleeding Time (min)

DAYS

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi

FIGURE 14-B
PLATE 15-A

FIGURE 15-A Coagulation time (min.) of SPD during different induced homoparasitic infection.

This illustration shows the comparative coagulation times of SPD (Fig. 15-A) during prepatent period early and acute and late phases of five different homoparasitic infections. Data points are given in table 3, 5, 7, 9 & 11. For more details see results.
FIGURE 15-A

Splenectomised Dogs

DAYS

Coagulation Time (min)

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi
PLATE 15-B

FIGURE 15-B Coagulation time (min.) of NSPD during different induced hemoparasitic infection.

This illustration shows the comparative coagulation times of NSPD (Fig. 15-B) during prepatent period early and acute and late phases of five different hemoparasitic infections, data points are given in table 3, 5, 7, 9 & 11. For more details see results.
NON-SPLENECTOMISED DOGS

coagulation Time (min)

DAYS

Babesia canis
Babesia gibsoni
Trypanosoma brucei
Leishmania donovani
Trypanosoma evansi

FIGURE 15-B
PLATE 16-A

FIGURE 16-A Prothrombin time (Sec.) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative prothrombin times values of SPD (Fig. 16-A) during prepatent period, early and acute and late phases of five different hemoparasitic infections, data are given in table 3, 5, 7, 9 & 11. For detail see results.
Splenectomised Dogs

Prothrombin Time (sec)

DAYS

Babesia canis  Babesia gibsoni  Trypanosoma brucei
Leishmania donovani  Trypanosoma evansi

FIGURE 16-A
PLATE 16-B

FIGURE 16-B Prothrombin time (Sec.) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative prothrombin times values of NSPD (Fig. 16-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections. Data are given in table 3, 5, 7, 9 & 11. For detail see results.
NON-SPLENECTOMISED DOGS

Prothrombin Time (sec)

0 5 10 15 20

5 7 8 9 10 11 12 13 14 15 16 17 18 19 20

DAYS

Babesia canis  Babesia gibsoni  Trypanosoma brucei
Leishmania donovani  Trypanosoma evansi

FIGURE 16-B
FIGURE 17-A  Leukocytes (10^3/µl) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative leukocytic values of SPD (Fig. 17-A) during prepatent period, early and acute and late phases of five different hemoparasitic infections. Data points are given in tables 4, 6, 8, 10 & 12. For more details see result.
FIGURE 17-A
PLATE 17-B

FIGURE 17-B Leukocytes (10^3/μl) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative leukocytic values of NSPD (Fig. 17-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 4, 6, 8, 10 & 12. For more details see result.
NON-SPLENECTOMISED DOGS

Leukocytes (10³/µL)

DAYS

Babesia canis  Babesia gibsoni  Trypanosoma brucei
Leishmania donovani  Trypanosoma evansi

FIGURE 17-B
PLATE 18-A

FIGURE 18-A Myelocytes (%) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative myelocytic percentage of SPD (Fig. 18-A) during prepatent periods, early and acute and late phases of five different hemoparasitic infections. Data points are given in tables 4, 6, 8, 10 & 12. For more details see result.
Splenectomised Dogs

Myelocytes (%)

DAYS

$- Babesia canis  \quad - Babesia gibsoni  \quad \square \ Trypanosoma brucei$

$- Leishmania donovani  \quad - Trypanosoma evansi$

FIGURE 18-A
PLATE 18-B

FIGURE 18-B Myelocytes (%) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative myelocytic percentage of NSPD (Fig. 18-B) during prepatent periods, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 4, 6, 8, 10 & 12. For more details see result.
NON-SPLENECTOMISED DOGS

FIGURE 18-B
FIGURE 19-A Metamyelocytes (%) erythrocytes of SPD during different induced hemoparasitic infections.

This illustration shows the comparative metamyelocytic percentage of SPD (Fig. 19-A) during prepatent periods, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 4, 6, 8, 10 & 12. For details see results.
SPLENECTOMISED DOGS

\[ \text{Metamicrocytes (\%)} \]

\[ \begin{array}{cccccccccccccc}
\end{array} \]

\( \text{DAYS} \)

\[ \begin{array}{ccc}
\rightarrow \text{Babesia canis} & \rightarrow \text{Babesia gibsoni} & \square \text{Trypanosoma brucei} \\
\rightarrow \text{Leishmania donovani} & \rightarrow \text{Trypanosoma evansi} \\
\end{array} \]

FIGURE 19-A
PLATE 19-B

FIGURE 19-B Metamyelocytes (%) erythrocytes of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative metamyelocytic percentage of NSPD (Fig. 19-B) during prepatent periods, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 4, 6, 8, 10 & 12. For details see results.
PLATE 20-A

FIGURE 20-A Band neutrophils (%) SPD during different induced hemoparasitic infections.

This illustration shows the comparative band neutrophilic percentage of SPD (Fig. 20-A) during prepatent period early and acute and late phases of five different hemoparasitic infections. Data points are given in table 4, 6, 8, 10 & 12. For more details see results.
SPLENECTOMISED DOGS

![Graph showing the band neutrophil count over days for different parasites.]

- **Babesia canis**
- **Babesia gibsoni**
- **Trypanosoma brucei**
- **Leishmania donovani**
- **Trypanosoma evansi**

**FIGURE 20-A**
PLATE 20-B

FIGURE 20-B Band neutrophils (%) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative band neutrophilic percentage of NSPD (Fig. 20-B) during prepatent period early and acute and late phases of five different hemoparasitic infections, data points are given in table 4, 6, 8, 10 & 12. For more details see results.
NON-SPLENECTOMISED DOGS

Band Neutrophils

DAYS

0 5 7 8 9 14 15 20 21 22 25 26 28 30 45 90 100 120 121 200 365 725 1065 1200

Babesia canis
Babesia gibsoni
Trypanosoma brucei
Leishmania donovani
Trypanosoma evansi

FIGURE 20-B
PLATE 21-A

FIGURE 21-A Segmented neutrophils (%) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative segmented neutrophilic percentage of SPD (Fig. 21-A) during prepatent period, early and acute and late phases of five different hemoparasitic infections, data are given in table 4, 6, 8, 10 & 12. For detail see results.
SPLENECTOMISED DOGS

![Graph showing segmented neutrophils (%) over days for different parasites: Babesia canis, Babesia gibsoni, Trypanosoma brucei, Leishmania donovani, and Trypanosoma evansi.](image)

**FIGURE 21-A**
PLATE 21-B

FIGURE 21-B Segmented neutrophils (%) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative segmented neutrophilic percentage of NSPD (Fig. 21-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections, data are given in table 4, 6, 8, 10 & 12. For detail see results.
NON-SPLENECTOMISED DOGS

Segmented Neutrophils (%)

DAYS

1 0 5 7 8 9 14 15 20 21 22 25 28 30 45 90 100 120 121 200 365 725 1065 1200

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi

FIGURE 21-B
PLATE 22-A

FIGURE 22-A  Lymphocytes (%) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative lymphocytic percentage of SPD (Fig. 22-A) during prepertent period, early and acute and late phases of five different hemoparasitic infections, data points are given in table 4, 6, 8, 10 & 12. For more details see results.
Splenectomised Dogs

Lymphocytes (%)

DAYS

- Babesia canis
- Babesia gibsoni
- Leishmania donovani
- Trypanosoma brucei
- Trypanosoma evansi

FIGURE 22-A
PLATE 22-B

FIGURE 22-B Lymphocytes (%) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative lymphocytic percentage of NSPD (Fig. 22-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections. Data points are given in table 4, 6, 8, 10 & 12. For more details see results.
NON-SPLENECTOMISED DOGS

DAYS

Lymphocytes (%)

0 10 20 30 40 50 60 70

0 5 7 8 9 14 15 20 21 22 25 26 30 35 45 90 100 120 121 200 365 725 1065 1200

--- Babesia canis  --- Babesia gibsoni  --- Trypanosoma brucei

--- Leishmania donovani  --- Trypanosoma evansi

FIGURE 22-B
PLATE 23-A

FIGURE 23-A  Eosinophils (%) of SPD during different induced hemoparasitic infection.

This illustration shows the comparative eosinophilic percentage of SPD (Fig. 23-A) during prepatent periods, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 4, 6, 8, 10 & 12. For details see results.
Splenectomised Dogs

Eosinophils (%)

DAYS

Babesia canis  Babesia gibsoni  Trypanosoma brucei
Leishmania donovani  Trypanosoma evansi

FIGURE 23-A
PLATE 23-B

FIGURE 23-B  Eosinophils (%) of NSPD during different induced hemoparasitic infection.

This illustration shows the comparative eosinophilic percentage of NSPD (Fig. 23-B) during prepatent periods, early and acute and late phases of five different hemoparasitic infections. Data points are given in tables 4, 6, 8, 10 & 12. For details see results.
NON-SPLENECTOMISED DOGS

Eosinophils (%) vs DAYS

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi

FIGURE 23-B
PLATE 24-A

FIGURE 24-A Monocytes (%) of SPD during different induced hemoparasitic infection.

This illustration shows the comparative monocytes percentage of SPD (Fig. 24-A) during pre-patent period early and acute and late phases of five different hemoparasitic infections. Data points are given in table 4, 6, 8, 10 & 12. For more details see results.
SPLENECTOMISED DOGS

Monocytes (%)

Babesia canis  Babesia gibsoni  Trypanosoma brucei
Leishmania donovani  Trypanosoma evansi

FIGURE 24-A
PLATE 24-B

FIGURE 24-B  Monocytes (%) of NSPD during different induced hemoparasitic infection.

This illustration shows the comparative monocytes percentage of NSPD (Fig. 24-B) during prepatent period early and acute and late phases of five different hemoparasitic infections, data points are given in table 4, 6, 8, 10 & 12. For more details see results.
NON-SPLENECTOMISED DOGS

![Graph showing the progression of different parasites over days]

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi

FIGURE 24-B
FIGURE 25-A Basophils (%) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative basophilic percentage of SPD (Fig. 25-A) during prepatent period, early and acute and late phases of five different hemoparasitic infections, data are given in table 4, 6, 8, 10 & 12. For detail see results.
PLATE 25-B

FIGURE 25-B Basophils (%) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative basophilic percentage of NSPD (Fig. 25-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections. Data are given in Table 4, 6, 8, 10 & 12. For detail see results.
NON-SPLENECTOMISED DOGS

Basophils (%)

DAYS

Babesia canis
Babesia gibsoni
Trypanosoma brucei
Leishmania donovani
Trypanosoma evansi

FIGURE 25-B
PLATE 26-A

FIGURE 26-A Reduction of leukocytes (%) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative reduction percentage of erythrocytes of SPD (Fig. 26A) during prepatent period, early and acute and late phases of five different hemoparasitic infections, data points are given in table 4, 6, 8, 10 & 12. For more details see results.
SPLENECTOMISED DOGS

**FIGURE 26-A**

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi
FIGURE 26-B  Reduction of leukocytes (%) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative reduction percentage of erythrocytes of NSPD (Fig. 26-B) during prepatent period, early and acute and late phases of five different hemoparasitic infections, data points are given in table 4, 6, 8, 10 & 12. For more details see results.
NON-SPLENECTOMISED DOGS

DAYS

0  10  20  30  40  50  60

5  7  8  9  14  15  20  21  22  25  26  28  30  45  90  100  120  121  200  365  725  1065  1200

--- Babesia canis
--- Babesia gibsoni
--- Trypanosoma brucei
--- Leishmania donovani
--- Trypancsoma evansi

FIGURE 26-B
PLATE 27-A

FIGURE 27-A  Average temperature (°C) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative average temperature (°C) values of SPD (Fig. 27-A) during prepatent periods, early and acute and late phases of five different hemoparasitic infections. Data points are given in tables 4, 6, 8, 10 & 12. For more details see result.
Splenectomised Dogs

Average Temperature (°C)

DAYS

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanosoma evansi

FIGURE 27-A
PLATE 27-B

FIGURE 27-B Average temperature (°C) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative average temperature (°C) values of NSPD (Fig. 27-B) during prepatent periods, early and acute and late phases of five different hemoparasitic infections. Data points are given in tables 4, 6, 8, 10 & 12. For more details see result.
NON-SPLENECTOMISED DOGS

Average Temperature (°C)

DAYS

- Babesia canis
- Babesia gibsoni
- Trypanosoma brucei
- Leishmania donovani
- Trypanocoma evansi

FIGURE 27-B
PLATE 28-A

FIGURE 28-A Average parasitemia (%) of SPD during different induced hemoparasitic infections.

This illustration shows the comparative average parasitic percentage of SPD (Fig. 28-A) during prepatent periods, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 4, 6, 8, 10 & 12. For details see results.
Splenectomised Dogs

Average Parasitemia (%)

DAYS

5 7 8 9 14 15 20 21 22 25 26 30 45 90 100 120 121 200 365 725 1065 1200

Babesia canis  Babesia gibsoni  Trypanosoma brucei
Leishmania donovani  Trypanosoma evansi

Figure 28-A
PLATE 28-B

FIGURE 28-B Average parasitemia (%) of NSPD during different induced hemoparasitic infections.

This illustration shows the comparative average parasitic percentage of NSPD (Fig. 28-B) during prepatent periods, early and acute and late phases of five different hemoparasitic infections, data points are given in tables 4, 6, 8, 10 & 12. For details see results.
NON-SPLENECTOMISED DOGS

Average Parasitemia (%)

DAYS

Babesia canis

Babesia gibsoni

Trypanosoma brucei

Leishmania donovani

Trypanosoma evansi

FIGURE 28-B
PLATE 29-A

FIGURE 29-A Percent parasitized erythrocytes (%) of SPD during *Babesia canis* and *B. gibsoni* induced infections.

This illustration shows the comparative parasitized erythrocytic percentage of SPD (Fig. 29A) during prepatent period early and acute and late phases of *Babesia canis* and *B. gibsoni* infections, data points are given in table 3 & 5. For more details see results.
Splenectomised Dogs

FIGURE 29-A

Babesia canis

Babesia gibsoni
FIGURE 29-B Percent parasitized erythrocytes (%) of NSPD *Babesia canis* and *B. gibsoni* induced infections.

This illustration shows the comparative parasitized erythrocytic percentage of NSPD (Fig. 29-3) during prepatent period early and acute and late phases of *Babesia canis* and *B. gibsoni* infections, data points are given in table 3 & 5. For more details see results.
NON-SPLENECTOMISED DOGS

Percent Parasitised Erythrocytes

DAYS

5 7 8 9 14 15 20 21 22 25 26 28 30 45 90 100 120 200 365 725 1065 1200

Babesia canis

Babesia gibsoni

FIGURE 29-B
PLATE 30-A

FIGURE 30 (A, B) Parasites per cubic millimeter in blood of SPD during Trypanosoma evansi and T. brucei induced infections.

This illustration shows the comparative quantity of Trypanosoma evansi and T. brucei per cubic millimeter in blood of SPD (Fig. 30-A) during prepatent period, early and acute and late phases of infections. data are given in table 7 and 9. For detail see results.
FIGURE 30-A

- Trypanosoma brucei
- Trypanosoma evansi
PLATE 30-B

FIGURE 30-B Parasites per cubic millimeter in blood of NSPD during Trypanosoma evansi and T. brucei induced infections.

This illustration shows the comparative quantity of Trypanosoma evansi and T. brucei per cubic millimeter in blood of NSPD (Fig. 30-B) during prepatent period, early and acute and late phases of infections, data are given in table 7 and 9. For detail see results.
NON-SPLENECTOMISED DOGS

\[ \text{DAYS} \]

\[ 0 \quad 5 \quad 7 \quad 8 \quad 9 \quad 14 \quad 15 \quad 20 \quad 21 \quad 22 \quad 25 \quad 26 \quad 28 \quad 30 \quad 45 \quad 90 \quad 100 \quad 120 \quad 121 \quad 200 \quad 365 \quad 725 \quad 1065 \quad 1200 \]

\[ 0.5 \quad 1 \quad 1.5 \quad 2 \quad 2.5 \quad 3 \quad 3.5 \]

- Trypanosoma brucei
- Trypanosoma evansi

FIGURE 30-B
PLATE 31

FIGURE 31 Percentage macrophages cell (%) of SPD and NSPD during Leishmania donovani induced infection.

This illustration shows the comparative percentage of parasitized macrophage cells of SPD and NSPD during prepatent period, early and acute and late phases of Leishmania donovani infections. data points are given in table 11 and 12. For more details see results.
Leishmania donovani

--- Splenectomised dogs  --- Non-splenectomised dogs

FIGURE 31
Hematological parameters of splenectomised and non-splenectomised dogs (i.e. SPD and NSPD) during different time scales which are associated with different induced drugs (i.e. phenytoin sodium, mepacrine hydrochloride, diminazene, suramin and acriflavine hydrochloride) are presented in Figure 32-36.
FIGURE 32  Effect of phenytoin sodium (associated with megaloblastic anemia) on hematology of SPD and NSPD at 2400 - 4000 hours.

In Peripheral Blood

(A)  Hemoglobin concentration
(B)  Mean corpuscular volume
(C)  Morphology of red blood cells
(D)  Multi segmented neutrophils

Data point are given in Table 13. For details see results.
PLATE 32-II

FIGURE 32  Effect of phenytoin sodium (associated with megaloblastic anemia) on hematology of SPD and NSPD at 2400 - 4000 hours.

In Peripheral Blood

(E)  Thrombocytes

In Bone Marrow

(F)  Cells per cmm x 1000 and

(G)  Megaloblasts.

Data point are given in Table 13. For details see results.
PLATE 33

FIGURE 33  Effect of mepacrine hydrochloride (associated) with thrombocytopenia) on hematology of SPD and NSPD at 24 - 400 hours.

This illustration shows the scarceness of thrombocytes in SPD and NSPD. Data points are given in table 14. For details see results.
**Figure 33**

Graph showing the decline in thrombocytes over time for splenectomised dogs compared to non-splenectomised dogs.
PLATE 34

FIGURE 34 Effect of diminazine (associated with leukocytopenia) on hematology of SPD and NSPD at 24 - 400 hours.

This illustration shows the scariness of leukocytes in SPD and NSPD. Data points are given in table 15. For details see results.
FIGURE 34

Leukocytes (10^6/μL)

HOURS

Splenectomised dogs  Non-splenectomised dogs
PLATE 35-I

FIGURE 35  Effect of suramin (associated with hemolytic anemia) on hematology of SPD and NSPD at 24 - 400 hours.

<table>
<thead>
<tr>
<th>In Peripheral Blood</th>
</tr>
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<tr>
<td>(A) reduction of erythrocytes</td>
</tr>
<tr>
<td>(B) reduction of leukocytes</td>
</tr>
<tr>
<td>(C) hemoglobin concentration</td>
</tr>
<tr>
<td>(D) mean corpuscular volume</td>
</tr>
</tbody>
</table>

Data points are given in Table 16. For details see result.
PLATE 35-II

FIGURE 35  Effect of suramin (associated with hemolytic anemia) on hematology of SPD and NSPD at 24 - 400 hours.

In Peripheral Blood

(E) reticulocytes
(F) morphology of red blood cells.

In Bone Marrow

(G) myeloid erythroid.

Data points are given in Table 16. For details see result.
PLATE 36-I

FIGURE 36  Effect of Acriflavine hydrochloride (associated with aplastic anemia) on hematology of SPD and NSPD at 24 - 400 hours.

In peripheral blood

(A) reticulocytes
(B) thrombocytes
(C) morphology of red blood cells
(D) monocytes

Data points are given in Table 17. For details see result.
PLATE 36-II

FIGURE 36  Effect of Acriflavine hydrochloride (associated with aplastic anemia) on hematology of SPD and NSPD at 24 - 400 hours.

In Peripheral Blood

(E) eosinophils
(F) lymphocytes.

In Bone Marrow

(G) basophilic myelocytic
(H) basophilic metamyelocytic

Data points are given in Table 17. For details see result.
PLATE 36-III

FIGURE 36  Effect of Acriflavine hydrochloride (associated with aplastic anemia) on hematology of SPD and NSPD at 24 - 400 hours.

In Bone Marrow

(I) neutrophilic myelocytic
(J) neutrophilic metamyelocytic
(K) eosinophilic myelocytic
(L) eosinophilic metamyelocytic.

Data points are given in Table 17. For details see result.
FIGURE 37 *Babesia canis* in blood of dog.
Note the varied forms of the parasites and the characteristic pair of in this species, the pyriform cells are approximately four-fifths the diameter of the erythrocytes. Giemsa stain x 1200.

FIGURE 38 *Babesia gibsoni* in blood of dog.
Note the small size and varied shape of the parasites; annular forms are common and there are often two to four parasites in each red cell. Paired pyriform cells are infrequent. Giemsa stain x 1200.

FIGURE 39 *Trypanosoma evansi* in blood of dog.
The cells are long and narrow, tapered at the posterior end; the undulating membrane is prominent; the free flagellum is long; the kinetoplast is small and sub-terminal. Dividing cells can be seen. Giemsa stain x 1200.

FIGURE 40 *Trypanosoma brucei* in blood of dog.
The trypanosomes are broad and the undulating membrane is prominent. Note the long free flagellum, the relatively large kinetoplast which, in most of the specimens, is at the very end of the cell. Giemsa stain x 1200.

FIGURE 41 *Leishmania donovani* in smear of dog bone marrow.
Three intact macrophage cells are shown, each with the cytoplasm packed with *L. donovani* bodies. The leishmanias are recognized by the small pink colored nuclei and associated kinetoplasts. Giemsa stain x 1200.

FIGURE 42 *Leishmania donovani* in smear of dog bone marrow.
The cytoplasm of the monocytes contains numerous *L. donovani*. Giemsa stain x 1200.

FIGURE 43 *Leishmania donovani* in spleen impression smears from infected dog.
In making preparations the infected host cells often breakup scattering the parasites. In B.

FIGURE 44 *Leishmania donovani* in spleen impression smear from infected dog.
An intact cell is shown with several *L. donovani* bodies in the cytoplasm. Giemsa stain x 1200.
PLATE 38

FIGURE 45 *Leishmania donovani* from culture in NNN (Nicole, Novy and Mac Neal) medium.
This illustrates the promastigote forms with a dark staining trophonucleus and single free flagellum arising from the anterior end of the kinetoplast. May - Grunwald - stain x 1200.

FIGURE 46 Blood film Monocyte during *Babesia canis*.
This illustration shows monocytes. Hemacolor stain x 1200.

FIGURE 47 Blood film Monocyte during *Babesia gibsoni*.
This illustration shows monocytes. Hemacolor stain x 1200

FIGURE 48 Marrow film Monocytes during *Trypanosoma evansi*.
This illustration shows monocytes. Hemacolor x 1200

FIGURE 49 Marrow film Monocytes during *Leishmania donovani*.
A large promonocyte which is coarsely granular and appears to be necrobiotic. The other cells present are monocytes of normal size. Inset shows a monocyte in prophase stage of mitosis, the cytoplasm of which is markedly granular. The size of the cells in this illustration can be compared with the normal large lymphocytes. Leishman stain x 1200.

FIGURE 50 Blood film Eosinophilia during late phases of *Leishmania donovani*.
These illustrations show numerous segmented eosinophil leukocytes, several of which contain two pouch-shaped nuclear lobes. May-Grunwald-Giemsa stain x 1200.

FIGURE 51 Marrow film eosinophilia during late phases of *Leishmania donovani*.
This illustration shows all stages of maturation in the eosinophil series are present.

FIGURE 52 Blood film Thrombocytopenia (Mepacrine hydrochloride associated with thrombocytopenia).
This illustrates bizzare lymphoid cells of varying maturity (centre and upper left field), normoblastosis and thrombocytopenia. The stressed neonate frequently exhibits normoblastosis and thrombocytopenia, not indicating marrow pathology. May-Grunwald-Giemsa stain x 1200.
PLATE 39

FIGURE 53 Blood film platelet aggregates Mepacrine hydrochloride associated with thrombocytopenia.
Two areas of platelet aggregates are evident. Note the variation in size of the platelets. Platelets clump readily and may be seen in blood films as aggregates. May-Grünwald-Giemsa stain x1200.

FIGURE 54 Marrow film thrombocytopenia (Mepacrine hydrochloride associated with thrombocytopenia).
In this condition there is a marked increase in the number of megakaryocytes which are non-platelet producing. Many young forms of these cells are usually present. This illustration is a typical example of the low-power microscopic appearance in this condition. Leishman stain x 120.

FIGURE 55 Bone biopsy Megakaryocytic hyperplasia (Mepacrine hydrochloride associated with thrombocytopenia).
At low magnification, it is evident there is a well marked increase in the number of megakaryocytes. Haemalum and Eosin stain x 100.

FIGURE 56 Bone biopsy Megakaryocytic hyperplasia (phenytoin sodium associated with megaloblastic anemias).
High magnification confirms the megakaryocytic hyperplasia. Haemalum and Eosin stains x 350.

FIGURE 57 Spleen thrombocytopenia (Mepacrine hydrochloride associated with thrombocytopenia).
This field shows the characteristic development of germinal centres in lymphoid follicles which, although of normal size, are increased in number. Haemalum and Eosin stains x 40.

FIGURE 58 Thrombocytopenia (Mepacrine hydrochloride associated with thrombocytopenia).
This is a high-power view showing increased number of neutrophil and eosinophil leukocytes in the splenic pulp. A megakaryocyte can be seen in a sinusoid upper left. Haemalum and Eosin stains x 450.

FIGURE 59-60 Marrow film Megakaryocytes (Mepacrine hydrochloride associated with thrombocytopenia)
Both of the cells shown are much smaller than normal granular megakaryocytes. This is often the case in conditions where there is a marked increase in the number of cells of this type. Leishman stain x 1
FIGURE 61 Marrow film A typical Acriflavine Megakaryocytes (hydrochloride associated with aplastic anemias).
Promegakaryocyte (basophilic megakaryocyte). Note the numerous tiny vacuoles in the cytoplasm of this cell. Leishman stain x 1200.

FIGURE 62 Marrow film A typical Megakaryocytes (Acriflavine hydrochloride associated with a plastic anemia).
Promegakaryocyte. This cell is developing towards the granular megakaryocyte but the cytoplasm has not completely lost its basophilic properties. The cytoplasm contains rosette shaped vacuoles. The changes present in these two illustrations are usually seen in cases of aplastic anemia. Leishman stain x 1200.

FIGURE 63 Marrow film Trephine needle biopsy specimen Aplastic anemia (Acriflavine hydrochloride associated with aplastic anemia).
This shows a marked increase in the proportion of fat cells along with a corresponding marked decrease in the proportion of hematopoietic cells; only a very occasional blood cell is present between the fat spaces. Haemalum and Eosin stains x 95.

FIGURE 64 Marrow film Megaloblastic anemia (Phenytoin hydrochloride associated with megaloblastic anemias).
This field shows numerous megaloblasts at various stages of development, form the promegaloblast to the late megaloblast. Note the more primitive cells, to the top and left of the field, shadows of nucleoli are still present. May-Grunwald stains x 1200.

FIGURE 65 Marrow film Megaloblastic anemia. Marrow aspirate section. (Phenytoin hydrochloride associated with aplastic anemia).
This section of marrow is from the same aspirated specimen as field A and shows the marked shrinkage and distortion which takes place during fixation and processing of the tissue. While it would still be possible to give an opinion on the type of cells present it could not be stated definitely that the large cells are megaloblasts. Haemalum and Eosin stains x 800.

FIGURE 66 Marrow film Megaloblastic anemia. Marrow trephine needle biopsy. (Phenytoin hydrochloride associated with aplastic anemia).
This is a section from a trephined specimen from the same case of megaloblastic anemia. The gross distortion and disruption of the cells, due to fixation and decalcification, make a definitive diagnosis impossible. The large pleomorphic cells with large nuclei mimic the appearance of malignant epithelial cells. This pseudo-carcinomatous appearance is a well known artifact in histological rather than cytological specimen in megaloblastic erythropoiesis. For this reason the diagnosis of megaloblastic erythropoiesis should be based on cytological rather than histological preparations. Haemalum and Eosin stains x 800.

FIGURE 67 Marrow film Megaloblastic anemia (Phenytoin hydrochloride associated with aplastic anemias).
This illustration shows several early megaloblasts, which vary in size, and present along with a typical promyelocyte. May-Grunwald-Giemsa stain x 1200.

FIGURE 68 Marrow film Megaloblastic anemia (phenytoin hydrochloride associated with aplastic anemia).
This illustration shows a large early megaloblast in which shadow nucleoli can be resolved, also present are two late megaloblasts. Note also the variation in size of the red blood corpuscles. May-Grunwald-Giemsa stain x 1200.
PLATE 41

FIGURE 69-70 Blood film hemolytic anemia (suramin induced hemolysis of glucose-6-phosphate dehydrogenase deficient red cells).

In this condition, contracted and distorted cells, some of which are irregular and angular in shape are illustrated. These are known as triangular or helmet cells. Red cell fragments, some with spinous processes and polychromatic cells are also present. Wright stain x 1200.

FIGURE 71 Blood film hemolytic uraemic syndrome (suramin induced hemolysis of glucose-6-phosphate dehydrogenase deficient red cells).

In this illustration there are many polychromatic cells and prominent burr cells. Hemacolor stain x 1200.

FIGURE 72-73 Liver hemolytic anemia (suramin induced hemolysis of glucose-6-phosphate dehydrogenase deficient red cells).

In this illustration shows Kupffer cell contain large quantities of stainable iron where as hepatocytes do not. Prussian Blue x 500.

FIGURE 74 Spleen hemolytic anemia (suramin induced hemolysis of glucose-6-phosphate dehydrogenase deficient red cells).

This illustrate the lymphoid follicles have prominent germinal centres. The red pulp is engorged with blood. Haemalum and Eosin stains x 60.

FIGURE 75-76 Spleen hemolytic anemia (suramin induced hemolysis of glucose-6-phosphate dehydrogenase deficient red cells).

This composite illustration is of high power field from the dog's spleen as Table 16, showing the congested sinusoids and the presence of hemosiderin pigments in histiocytes. Fig. 39: Haemalum and Eosin stains x 450. F
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rence of glutathione instability in red blood corpuscles of the


ABBREVIATIONS
LIST OF ABBREVIATIONS

A  Anisocytosis
AMA American medical association
AP Average parasitemia
AS Absorbar,ce of the sample
AT Average temperature
ATP Adinocine tri phosphate
BC Babesia canis
BG Babesia gibsoni
BN Band neutrophil
BP Basophils
BpH Blood pH
BT Bleeding time
CDAMA Council of drug American medical association
Cl Color index
CT Coagulation Time
DF\textsuperscript{32}P Diso propylphosphofluoridate
DLC Differential leukocyte count
DNA Deoxyribose nuclic acid
DZM No. Department of zoology museum number
EC Erythrocytes
EDTA Ethylene diamine tetra acetic acid
EP Eosinophils
ERY Erythrocytes
CF  Complement fixation
Fig  Figure
Figs  Figures
G-6-PD  Glucose-6-Phosphate
Hb  Hemoglobin
HBC  Hemoglobin concentration
Hct  Hematocrit
Hg  Mecury
i.e.  that is
IFA  Indirect fluorescent antibody
IHPI  Induced hemoparasite infections
IM  Intramuscular
IV  Intravenous
LC  Leukocytes
LD  Leishmania donovani
LYC  Lymphocytes
M  Macrocyes
MC  Myelocyte
MCH  Mean corpuscular hemoglobin
MCHC  Mean corpuscular hemoglobin concentration
MCV  Mean corpuscular volume
MMC  Meta myelocytes
MOC  Monocytes
n  Number of animals
NA  Not applicable
NADAH  Nicotinamide-adenine-dinucleotide phosphate
<table>
<thead>
<tr>
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<tr>
<td>NaCl</td>
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</tr>
<tr>
<td>NBT</td>
<td>Nitroblue tetrazolium</td>
</tr>
<tr>
<td>NH</td>
<td>Normocytic hypochromic</td>
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<td>Percent parasitized macrophage cell</td>
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<td>Response</td>
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<tr>
<td>SEC</td>
<td>Size of erythrocytes</td>
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<tr>
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<tr>
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<tr>
<td>TRCV</td>
<td>Total circulating red cell volume</td>
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<td>WBC</td>
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