HEINZ BODY ANEMIA IN THE GOAT

by

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To my Parents

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

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INTRODUCTION

This thesis contains three sections. The first is a literature review of Heinz body hemolytic anemia in man and animals. The second section contains two papers for publication. The first paper describes the consequences of Heinz body formation in the red cell of the goat. The second paper compares the various hematologic alterations obtained in intact and splenectomized goats following Heinz body formation. The third section is an appendix which contains the data generated by this study, including tables of erythrocytic indices. This section also contains a description of the statistical tests used in Papers I and II of Section II.

SECTION I

LITERATURE REVIEW

History

With the development of the chemical industry in Germany in the past century, a prevalent, often severe form of toxicity appeared among workers exposed to certain coal tar derivatives.[1] This consisted of an acute hemolytic process having two characteristic features: a brown-to-green discoloration of the blood, creating a form of cyanosis in the patient, and the presence of inclusion bodies in the red cells that were evident on supravital staining.[2] The abnormal structures were described as round, oval, or serrated highly refractile granules commonly located near the margin of the cell or protruding from the cell.[3] Most of such reactions were provoked by aromatic compounds possessing amino, nitro, or hydroxy groups, of which the most notorious were aniline, nitrobenzene, phenylhydrazine and various quinones which are compounds of special importance in the industrial preparation of synthetic dyes, photographic reagents and drugs, particularly antipyretics and analgesics.[4,5] A new wave of "cyanotic" and "hemolytic" toxic syndromes occurred during World War I in workers exposed to various nitro-derivatives.[1] It was later learned that severe toxic reactions were often forestalled by routinely examining the blood of chemical workers for the appearance of small numbers of inclusion bodies as an early indication of chronic intoxication.[6,7] With the knowledge that such hemolytic processes were self-limiting, Eppinger in 1918 introduced the use of phenylhydrazine for the therapy of polycythemia vera, a practice only recently abandoned.[8]

These inclusion bodies are now referred to as Heinz bodies, after Heinz, who first described them in man, dogs, rabbits and frogs in 1890.[2,9]

They have also been named Schmauch bodies after the German pathologist who first described these inclusions in normal cats in 1899.[10,11] More recently, these bodies in cats have been referred to as erythrocyte refractile bodies or ER bodies.[12]

There has been extensive debate in the literature concerning the nature of Heinz bodies.[13,14] The most prevalent of many views has been that Heinz bodies consist, at least in part, of particles of denatured protein and are presumed to include denatured hemoglobin.[4,5] Indeed, denatured hemoglobin has been identified in the blood of animals with Heinz body anemia.[13,14] Also, Heinz bodies have been found to have staining properties of hemoglobin rather than of red cell stromal protein.[15] However, electron microscopic studies were interpreted by one investigator to indicate that these inclusions were derived from denatured red cell stromal protein.[16] Other workers have concluded that the particles derived from affected blood were too opaque to be denatured proteins.[17] It is now accepted that Heinz bodies are formed from aggregates of denatured hemoglobin.[18]

Another less specific and possibly less constant feature of Heinz body anemia is the appearance of spherocytes and increased osmotic fragility of the red cells.[19,20] There are conflicting reports as to the occurrence or absence of spherocytes in the blood,[21,22] but their absence may be in part attributed to mechanisms <u>in vivo</u>, such as splenic filtration, which tends to eliminate spherocytes from the circulating blood.[23] Oxidant drugs have been reported to produce spherocytes in addition to the formation of Heinz bodies due to pitting of the Heinz bodies from the red cells.[22]

Glutathione Metabolism of the Red Cell

The red cell contains a high concentration of the sulfhydryl-containing tripeptide, reduced glutathione (GSH).[24] Synthesis occurs in two steps:

Glutamate + cysteine + ATP ⟶ γ - glutamyl cysteine + ADP + Pi.

γ - Glutamyl cysteine + glycine + ATP ⟶ GSH + ADP + Pi.

Both steps have been shown to be catalyzed by red cell hemolysates.[25,26,27] One important function of GSH in the erythrocyte appears to be the detoxification of low levels of hydrogen peroxide which may form spontaneously, or as a result of drug administration[28] (Fig. 1).[29] In either event, the superoxide radical may be formed first, and a red cell protective enzyme, superoxide dismutase, catalyzes the dismutation of two superoxide radicals to hydrogen peroxide (H_2O_2) and oxygen.[30] Red cells also contain two enzymes, catalase and glutathione peroxidase which are capable of degrading H_2O_2.[31] GSH may also function in maintaining integrity of the red cell by reducing oxidized sulfhydryl groups of hemoglobin, membrane proteins, and enzymes which may become oxidized.[32] In the process of reducing peroxide or oxidized protein sulfhydryl groups, GSH is converted to oxidized glutathione (GSSG) (Fig. 1) or may form mixed disulfides.[28] Fortunately, the red cell has an efficient mechanism for the reduction of GSSG to GSH (Fig. 1).[29] Glutathione reductase catalyzes the reduction of GSSG, with either NADPH or NADH serving as a hydrogen donor.[33,34] In the intact mature red cell, only the NADPH appears to function through the pentose phosphate pathway (PPP).[31,35] Metabolism through the PPP is accelerated in erythrocytes in the presence of oxidizing agents due to the oxidation of NADPH.[36] NADPH formed in the pentose cycle is utilized

Fig. 1. Glutathione Metabolism in the RBC

G6PD = Glucose-6-phosphate dehydrogenase

NADP = nicotinamide adenine dinucleotide phosphate

NADPH = reduced nicotinamide adenine dinucleotide phosphate

GSH = reduced glutathione

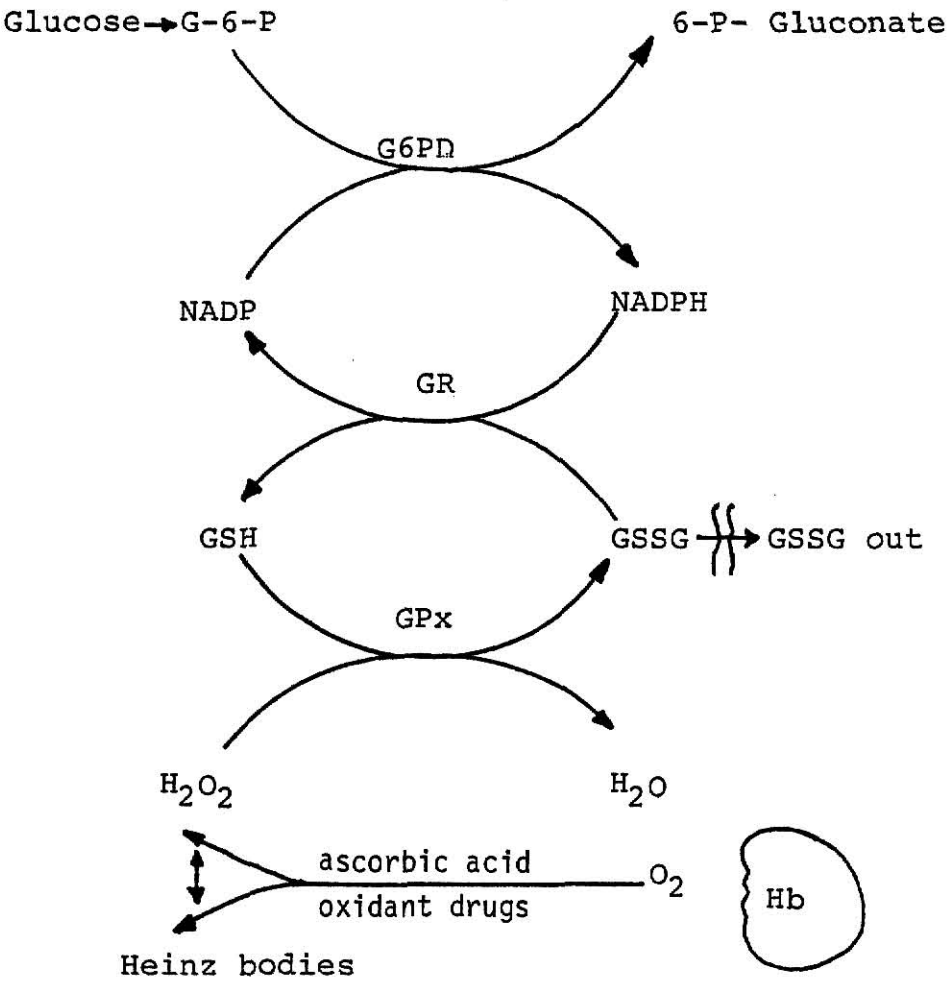
GSSH = oxidized glutathione

GR = glutathione reductase

GPx = glutathione peroxidase

H_2O_2 = hydrogen peroxide

Hb = hemoglobin



Glutathione metabolism in the RBC[29]

for the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH).[3] Reduced glutathione (GSH) in turn is required for the non-enzymatic conversion of methemoglobin to functional hemoglobin and protects erythrocytes against hemolysis by oxidant drugs through its role in the glutathione peroxidase reaction.[3] Inability of the cell to generate GSH may lead to precipitation of hemoglobin in the form of Heinz bodies by auto-oxidation or oxidation by chemical oxidants.[31]

Mechanism of Heinz Body Formation

The mechanism by which certain drugs caused Heinz body formation and resulting anemia has been resolved by the realization that such drugs catalyze a sequential denaturation of hemoglobin by oxygen.[23] The sequence is thought to occur as follows:[38]

(a) Exposure of red cells to certain drugs results in the formation of low levels of hydrogen peroxides as the drug interacts with hemoglobin.

(b) Some drugs may also form free radicals which oxidize erythrocytic glutathione (GSH) without the formation of peroxides as an intermediate. Phenylhydrazine-like drugs have also been shown to form hemochromogen directly with hemoglobin, a complex forming between the iron of ferriheme and the nitrogen bound to the benzene ring.[39]

(c) GSH may be oxidized to the disulfide form (GSSG) or the GSH may be complexed with hemoglobin to form a mixed disulfide. Such mixed disulfides are believed to form initially with the sulfhydryl group of the B93 position of hemoglobin in man.

(d) The mixed disulfide of GSH and hemoglobin is probably unstable and undergoes conformational changes exposing interior sulfhydryl groups

to oxidative and mixed disulfide formation. Once such oxidation has occurred, hemoglobin is irreversibly denatured and will precipitate as Heinz bodies. Thus, Heinz bodies are aggregates of denatured proteins, primarily hemoglobin, which form as a result of chemical insults.[18]

All of these changes occur in the red cell in a sequential manner. Thus, the formation of hydrogen peroxide and other free radicals are early transient phenomenon, whereas once Heinz bodies are formed, they persist until the affected cells are actually removed from circulation, a process continuing for days, weeks and even months.[23]

Microscopic Appearance of Heinz Bodies

Heinz bodies appear as small, rounded or angular inclusions measuring 0.3 to 2 μm in diameter by light microscopy.[18] They are easily seen using the phase contrast or the interference microscope and strongly absorb soret band (414 nm) light.[18] Vital staining with crystal violet, new methylene blue, or brilliant cresyl blue easily demonstrates these inclusions.[18] They persist after hemolysis and usually appear to be attached to the cell membrane.[18] Heinz bodies are seen in films stained with May-Grunwald-Giemsa but are poorly visible in Wright's-stained films.[3] However, if the Heinz body is sufficiently large, it appears as a white spot within the erythrocyte in the routinely stained blood film or as a projection from the surface of the cell.[3]

Using the electron microscope, the bodies appear as dense masses which begin to form in the center of the cell and then become attached to the red cell membrane.[40] Freeze-etch studies have shown Heinz bodies as dense submembrane hemoglobin aggregates affixed to the internal membrane

surface or as isolated large masses of denatured hemoglobin producing marked distortion of the overlying membrane.[41] The attachment of the Heinz body to the membrane causes a rearrangement of membrane-associated particles, with aggregation of these particles over the Heinz body regions, suggesting that denatured hemoglobin may be attached to membrane glycophorin and other proteins.[18] These Heinz body containing cells are removed by the spleen because of their rigidity.[32]

The Effect of Heinz Bodies on the Red Cell

The proper flow of an erythrocyte through the microcirculation is dependent upon the ability of an individual red cell to deform.[42] The ability of human erythrocytes to deform has been defined as "those geometric and physical characteristics that permit a cell whose greater diameter normally exceeds 8 μm to pass through 14 μm or longer segments of normal capillaries which range from 3-12 μm in diameter."[42] Species differences in the deformability of erythrocytes have been noted with the red cells of the goat being the most rigid.[43] A decrease in deformability is associated with an increase in red cell rigidity, impeded flow through the microcirculation and red cell fragmentation.[43]

The deformability of normal erythrocytes appears to depend on several factors:[43] (i) maintenance of the normal ratio of cell surface area to volume, (ii) normal internal fluidity of the cell, and (iii) intrinsic membrane deformability. The normal fluidity of the red cell depends primarily on the properties of normal hemoglobin.[42] Abnormal hemoglobins that have a predisposition to undergo intracellular crystallization cause increased rigidity of the red cell and predispose the cell to intravascular

fragmentation or removal.[42] Similarly, Heinz body formation leads to membrane depletion by fragmentation with spherocyte formation.[22] Affected red cells become less deformable and spherocytic due to the loss of cell membrane accompanying removal of the Heinz bodies and may undergo premature destruction in the microcirculation of the spleen.[44]

The splenic sinuses are lined by long, rod shaped endothelial cells arranged parallel to one another.[45] These cells are smooth surfaced and exhibit an area of nuclear swelling and tapered ends.[45,46] The endothelial cells are connected to each other by side processes.[45] Oval or spindle-shaped perforations occur between the endothelial cells and are called interendothelial slits.[46] Red cells carried across the splenic cords return to the venous circulation by squeezing through the slits in the sinus wall.[47] Deformable cytoplasm does this easily, but the rigid Heinz body is held up and ultimately detached from the red cell along with a ring of membrane and a film of hemoglobin.[47] This process is called pitting. The pitted red cells become less deformable due to loss of membrane and may undergo premature destruction.[44]

The role of the spleen in removal of Heinz bodies in the cat was studied by Jain.[48] He presented evidence that the spleen plays a minor role in the removal of Heinz bodies in the cat because splenectomy in a cat having a high, but declining Heinz body count did not prevent further reduction in numbers of Heinz bodies.[48] Also, splenectomy in the cat was unassociated with a significant elevation in Heinz body count over a period of several weeks.[48] It thus appears that the spleen may vary from species to species in its ability to remove red cells carrying inclusion bodies such as Heinz bodies.

The resistance of red cells to osmotic lysis (osmotic fragility) may be increased or decreased in disease conditions; reticulocytes and leptocytes have increased resistance while spherocytes have decreased resistance.[3] With Heinz body formation, spherocytes are formed due to membrane depletion accompanying the pitting of Heinz bodies.[44] Compounds which may form oxidants in oxidation-reduction systems namely, hydroquinones, p-aminophenol, hydroxylamine, phenylhydroxylane and phenylhydrazine have been shown to cause in vitro formation of methemoglobin and increased osmotic fragility of red cells with eventual hemolysis.[19] Each of the above compounds when injected into cats in doses of 6-50 mg daily, produced changes in the peripheral blood which included formation of methemoglobin, rapid development of anemia with hemoglobinemia, and so great an increase in osmotic fragility that erythrocytes were hemolyzed in salt solution approaching isotonicity.[19]

The hemolytic process encountered with significant Heinz body formation may be due to altered cation permeability, ATP depletion,[49] membrane damage and loss with fragmentation or spherocyte formation,[22] decreased deformability,[44] and increased osmotic fragility.[44]

Heinz Body Anemia in Domestic Animals

Heinz body hemolytic anemias due to a variety of agents have been encountered clinically or produced experimentally in various domestic animals. Heinz body formation associated with hemolytic anemia has been observed in horses receiving phenothiazine.[50,51,52] Onions, wild and domestic, contain n-propyl disulfide, a compound which produced Heinz bodies in horses, dogs, cats and cattle if ingested in high concentrations.[53,54]

Heinz body formation accompanied by hemolytic anemia and hemoglobinuria has also been observed in cows[55] and sheep[56] fed kale. Heinz body related post-parturient hemoglobinemia has also been reported in cattle.[57] Heinz bodies have also been observed in sheep following phenothiazine treatment and in association with idiopathic hemolytic anemia.[3]

A severe hemolytic anemia has been observed in cats receiving orally administered urinary antiseptics containing methylene blue.[58] Acetaminophen and phenozopyridine administered to cats have also been reported to result in Heinz body anemias.[59,60] Experimental Heinz body production in dogs has been induced by the administration of phenylhydrazine hydrochloride.[52]

From the above review, it can be recognized that several reports of Heinz body anemias in domestic animals may be found upon perusal of the literature. However, relatively little information seems to exist concerning Heinz bodies or Heinz body anemias in goats. Greenhalgh, et al. found that goats experienced a Heinz body hemolytic anemia when fed kale, but eventually returned to normal even though kale feeding was continued.[56] It thus appears that Heinz body formation may occur in goats under certain conditions and may result in anemia.

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SECTION II

PAPERS FOR PUBLICATION

PAPER I

HEINZ BODY ANEMIA IN GOATS

SUMMARY

Heinz bodies were produced in the circulating red cells of goats following the administration of phenylhydrazine hydrochloride. Heinz bodies developed rapidly with approximately 90% of the circulating red cells exhibiting one or more bodies 24 hours after administration of phenylhydrazine. The formation of Heinz bodies was followed by the development of a regenerative anemia as the Heinz body containing red cells were removed from circulation and the bone marrow responded to the rapid drop in packed cell volume and hemoglobin concentration.

Spindled red cells also appeared in the blood subsequent to phenylhydrazine treatment.

Cell deformability was found to be decreased in red cells containing Heinz bodies. Red cell deformability decreased even further with the emergence of reticulocytes into the circulation, and remained reduced throughout the study period.

Red cell osmotic fragility increased after phenylhydrazine administration and the appearance of Heinz bodies.

INTRODUCTION

Several reports of Heinz body anemias in domestic animals may be found upon perusal of the literature.[1,2,3,4,5] However, relatively little information exists concerning Heinz body anemia in goats. Greenhalgh et al. found that goats experienced a hemolytic anemia when fed kale, but returned to normal even though kale feeding was continued.[6] It thus appears that Heinz body formation may occur in goats under certain conditions and may result in anemia.

The mechanism by which certain drugs or chemical agents produce Heinz body anemia has been resolved by the realization that such drugs or chemical agents catalyze a sequential denaturation of hemoglobin by oxygen.[7] The sequence of denaturation has been described by Beutler.[8]

Several studies of Heinz body formation and the resulting anemia have been carried out using phenylhydrazine as the Heinz body inducing agent.[7,9] Once formed, Heinz bodies persist until the affected red cells are removed from circulation, a process continuing for days, weeks and even months.[10]

The objectives of the present study were (i) to determine alterations in the hemogram following Heinz body formation, (ii) to determine if red cell osmotic fragility is altered from normal following Heinz body formation and (iii) to determine if red cell deformability is affected by Heinz body formation.

MATERIALS AND METHODS

Animals -- Eight female goats of approximately 1.5 to 4 years of age were used in this study. The goats were of the Angora and Nubian breeds. The goats were judged to be clinically normal and healthy. Hematologic and blood chemistry values were normal. The goats were housed at the Animal Resource Facility on the campus of Kansas State University. Each goat was vaccinated against Clostridium perfringens types B and C and treated with an anthelmintic.[a] The animals were allowed a 5 day period to adjust to their new environment prior to beginning the study. The goats were maintained on alfalfa hay and a concentrate ration throughout the experimental period. The animals were allowed free access to water at all times.

Experimental design -- Following the 5 day adjustment period, the goats were randomly divided into two groups: Group A (n = 4) and Group B (n = 4). Blood samples for analyses were collected via jugular venipuncture using appropriate collection tubes. Sample analyses carried out on each sampling day included red blood cell count (RBC); packed cell volume (PCV); hemoglobin concentration (Hb); percentage of red cells containing Heinz bodies (% H.B.); reticulocyte count (% retic); percentage of spindled red cells (% spindle cells); red cell osmotic fragility, and red cell resistance to filtration (deformability). The erythrocytic indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were also determined.

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[a]Omnizole six. Merck Animal Health Div. Merck and Co., Rahway, N. J.

Throughout the sampling period of the study, the goats were sampled on alternate days. A total of 4 goats were sampled each day. Thus, at day 1, Group A was sampled, followed by Group B on day 2, then returning to Group A on day 3 . . . . . etc.

Animals of both groups A and B were sampled three times prior to phenylhydrazine treatment. Pretreatment sampling was carried out beginning on day 1 of the study with Group A being sampled on days 1, 3, and 5 and Group B being sampled on days 2, 4, and 6. Pretreatment samples were used to establish baseline values for each analysis performed. In the analysis and presentation of the data, the values obtained from all 8 goats during each two day sampling sequence (i.e., days 1 and 2; 3 and 4, etc.) were averaged and presented as the mean data derived for day 1, day 3, ... etc.

Following the establishment of baseline values, phenylhydrazine hydrochloride (2% sterile solution in physiologic saline) was administered subcutaneously (13 mg/kg) to groups A and B on days 8 and 9, respectively. A second dose of phenylhydrazine (6.5 mg/kg) was administered on days 12 (Group A) and 13 (Group B). Post treatment blood sampling was initiated on days 9 (Group A) and 10 (Group B) and continued on an alternating basis through day 41 of the study. Final samples were collected on days 55 and 56.

Values obtained during the post treatment period for the eight goats were compared to the corresponding mean baselines for significant changes ($p < 0.05$).

Hematologic techniques -- The following tests were performed on each set of blood samples: blood packed cell volume using a microhematocrit centrifuge;[b] percent blood reticulocyte count;[11] percent Heinz body count using new methylene blue stain on air dried blood smears;[12] and red cell osmotic fragility.[11] The percentage of spindled red cells present were determined by counting 500 red cells on an air dried smear, stained with new methylene blue and then calculating the percentage of cells exhibiting spindling. Blood hemoglobin concentration determinations were performed using the cyanmethemoglobin method set forth by Hycel, Inc.,[c] with Heinz bodies being removed by centrifugation (1785 G for 30 minutes) before absorbance readings were taken. Red blood cell counts were performed using an electron particle counter.[d] Red cell indices were calculated using the standard formulae.[11]

Red cell deformability test -- Sample preparation -- Red cell deformability was performed using a modification of Leblong and Coulombe's method.[13] Whole blood was collected into heparizined evacuated tubes[e] and stored immediately at 4C. Within 1 hour of sample collection, the blood was centrifuged (1785 G for 20 min.) and the buffy coat and plasma were carefully removed by aspiration. The remaining red cells were washed 3 times using tris-saline buffer (tris, 0.01 M; NaCl; 0.145M; pH 7.40, 300 mOsM/kg), with each washing being followed by removal of

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[b]Micro-hematocrit, Damon/IEC, div. Needham Hts., Mass.

[c]Hycel, Inc., Houston, Texas.

[d]Model ZBI, Coulter Electronics, Inc., Hialeah, Florida.

[e]Venoject, (siliconized vacutainers), Terumo Medical Corporation, Elkton, MD.

any buffy coat which formed. Following the final wash, the red cells were resuspended in buffer and the red count was adjusted to a final standard cell concentration of 10,000/µl. Red cell counts on the red cell-buffer mixture were performed using the Unopette system.[f] The total volume of red cell suspension prepared was 120 ml. The final adjusted red cell suspension (10,000 cell/µl) was tested in the filtration apparatus used to measure red cell deformability within 5 minutes of preparation.

Red cell deformability test -- filtration apparatus -- A schematic diagram of the red cell filtration-deformability apparatus used in this study may be seen in figure 1. The cell suspension to be filtered was placed in two 60 ml disposable plastic syringes (A) mounted on a syringe pump (B).[g] With the use of disposable silicone rubber tubing (C)[h] and appropriate plastic connectors, the syringes were linked to the inlet port of the filter housing (D)[i] and then to a 120 cm, 3 mm inner diameter glass tube (E) mounted onto a meter rule (F). The filter housing contained a 25 mm diameter polycarbonate membrane[j] with an average pore diameter of 3 µm and a nominal pore density of 2×10^6. All polycarbonate filters used were from the same manufacturer's lot. The filtrate was collected in a glass conical flask (G).

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[f] Unopettes, Becton, Dickinson and Co., Rutherford, N. J.

[g] Harvard Compact Infusion Pump. Harvard Apparatus. Millis, Mass.

[h] Silicone Rubber Tubing, Kraft Apparatus, Inc., Mineola, N. Y.

[i] Millipore, No. XX-3002514, Millipore Corp., Bedford, Mass.

[j] Nucleopore Corp., Pleasanton, California.

Fig. 1. Graphic Representation of the Red Cell Filtration-Deformability Apparatus

(A) disposable plastic syringes

(B) syringe pump

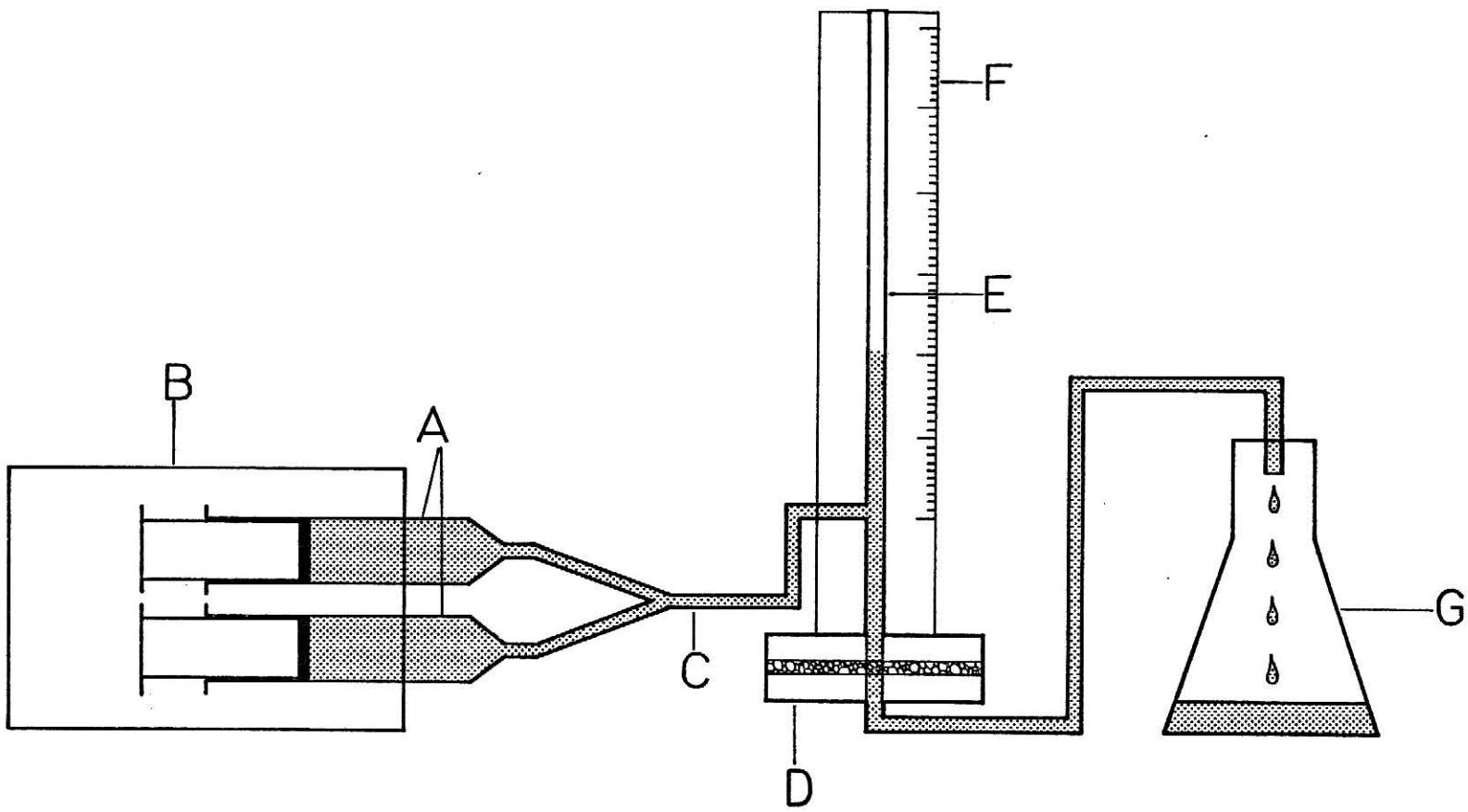
(C) silicone rubber tubing

(D) filter housing

(E) 3 mm inner diameter glass tube

(F) meter rule

(G) glass conical flask


F
E
B
A
C
D
G

Red Cell deformability test -- Filtration procedure -- The syringes and tubing were thoroughly rinsed with freshly prepared tris-saline buffer before each test. After the polycarbonate membrane was inserted into the housing and the housing closed, the fluid circuit was filled completely with buffer, with great care being taken to avoid the introduction of air bubbles especially inside the filter housing. Once filled, the system was carefully checked for any leaks and 60 ml of buffer solution was then filtered at a constant flow rate of 3 ml/min. During the filtration of the buffer solution the height attained by the fluid in the glass tube was recorded after the fluid level attained a steady state for 20 minutes. The glass tube thus served as a simple manometer with the height of the fluid column in the tube providing a relative measure of pressure within the system. The pressure produced was presumably dependent on the resistance provided by the polycarbonate membrane filter, since the infusion pump rate was held constant. The pressure attained with the buffer solution served to establish the flow characteristics of the membrane and also to provide a baseline reference value for filtration of the buffer red cell suspension.

Following the buffer filtration period, the tubing was clamped near the syringes, the syringes were removed, carefully emptied of any remaining buffer and immediately filled with the freshly prepared red cell suspension. The syringes were then connected to the system, the clamps removed and the infusion pump and a stop watch were started simultaneously. The red cell suspension was pumped through the system for a period of 30 minutes. Since the hematocrit of the cell suspension

was extremely low, ($\sim$ 0.1%) no special effort was necessary to counteract the possibility of red cell sedimentation occurring in the syringes during the 30 minute filtration period. The height (cm) of the fluid in the glass tube manometer was recorded at the end of the 30 minute filtration period. The difference between the reading attained with buffer alone and the reading attained after 30 minutes of red cell filtration was taken as a measure of the resistance to filtration by the red cells. This resistance was reported in cm of H_2O. Increasing resistance was taken as an indicator of decreased deformability.

Statistical methods -- Data derived from this study were analyzed using the repeated measure analysis which is similar to the randomized complete block method using the animal as the block and the day being the treatment. Duncan's multiple range test was used to compare animal response across days.

For statistical analysis, the alternate days for each sampling period were considered as the same day.

RESULTS

Following administration of phenylhydrazine, Heinz body formation occurred in almost 90 percent of the circulating red cells within 24 hours (Fig. 2). The percentage of Heinz body affected cells remained between 90 and 100 percent for several days and then began to fall quite rapidly on day 19 of the study. The rapid rate of fall continued through day 26 after which the number of Heinz body containing red cells disappeared at a slower rate. Heinz body containing cells never reattained the low levels found before phenylhydrazine treatment with some 21 percent of red cells exhibiting Heinz bodies on day 55. The percentage of red cells exhibiting Heinz body formation was derived by counting all red cells which contained one or more bodies. Initially the majority of red cells exhibited several (2-6) (Day 12, Fig. 3) small Heinz bodies. Over the next several sampling periods (Day 23, Fig. 4) the Heinz bodies grew larger with fewer bodies being observed per cell. By day 31, the majority of the affected cells contained small, singular Heinz bodies.

The rapid development of Heinz bodies resulted in an immediate, rapid fall in blood packed cell volume (PCV) (Fig. 5). The PCV reached a nadir on day 17, nine days after phenylhydrazine administration. A mean PCV of 13.0 was reached at that time. Similarly, rapid decreases in the RBC count (Fig. 6) and hemoglobin concentration (Fig. 7) were also observed with a low point being reached on day 17. The decreases in PCV, RBC count, and hemoglobin concentration were noted to begin concommitantly with the appearance of large numbers of Heinz body containing cells. Thus, significant reductions from baseline values for PCV, RBC count, and hemoglobin concentration were attained with values falling approximately 60 percent or more from pretreatment values.

Red cells which appeared spindle-shaped made their appearance in the circulation within 24 hours of phenylhydrazine administration (Fig. 8). No spindled red cells were observed in the pretreatment samples. The number of spindled cells reached their maximum value (7.1%) on day 13 and then rapidly disappeared with no spindled cells being observed on day 19 or thereafter (Fig. 9).

A slight rise in reticulocytes was noted on day 11 (3 days following phenylhydrazine treatment) (Fig. 10). A significant rise was noted by day 13 with a reticulocyte count of 3.4 percent being found. The mean reticulocyte count reached a maximum of 11.85 percent on day 19 (Fig. 10). Reticulocytes remained significantly elevated through day 26 after which their number returned to baseline levels.

The appearance of reticulocytes in the circulation in significant numbers followed the rapid development of anemia as indicated by the rapid decrease in PCV, RBC count and hemoglobin concentration (Fig. 5, 6, 7). Reticulocytes made a significant appearance 3 to 4 days following the development of anemia.

Following their nadir on day 17, PCV (Fig. 5) and Hb (Fig. 7) increased rapidly through day 38. The red cell count (Fig. 6) also increased during this period, but the degree of recovery of red cells was not as great as found for PCV and hemoglobin. The increases found for PCV, hemoglobin concentration, and red cell counts corresponded with the significant increase in reticulocytes (Fig. 10) and the decrease in Heinz bodies (Fig. 1).

Red cell mean corpuscular volume (MCV) was found to average 16.2 fl during the pretreatment period. As anemia developed and reticulocytes

appeared, the MCV rose rapidly with a significant rise to 25.3 fl being noted on day 15 and a peak MCV of 32.9 fl being attained by day 19. The maximum value for MCV was attained when the reticulocyte count also attained a peak (day 19). With upward correction of the PCV, the MCV began to fall slowly (days 22 through 40). The MCV for the eight goats was found to be 23.2 fl on day 55 of the study, the MCV therefore never reattained the pretreatment level of 16.2 fl.

Red cell mean corpuscular hemoglobin (MCH) also rose rapidly with the development of anemia and the appearance of reticulocytes, a significant rise to 9.6 pg. being found by day 15. The MCH reached its zenith on days 19 and 24 of the study with a level of 10.8 pg being observed. As the anemia subsided, the MCH steadily fell in a manner similar to the MCV reaching a value of 8.2 pg on day 55. The MCH at the final day of the study still remained at a level significantly greater than the pretreatment values.

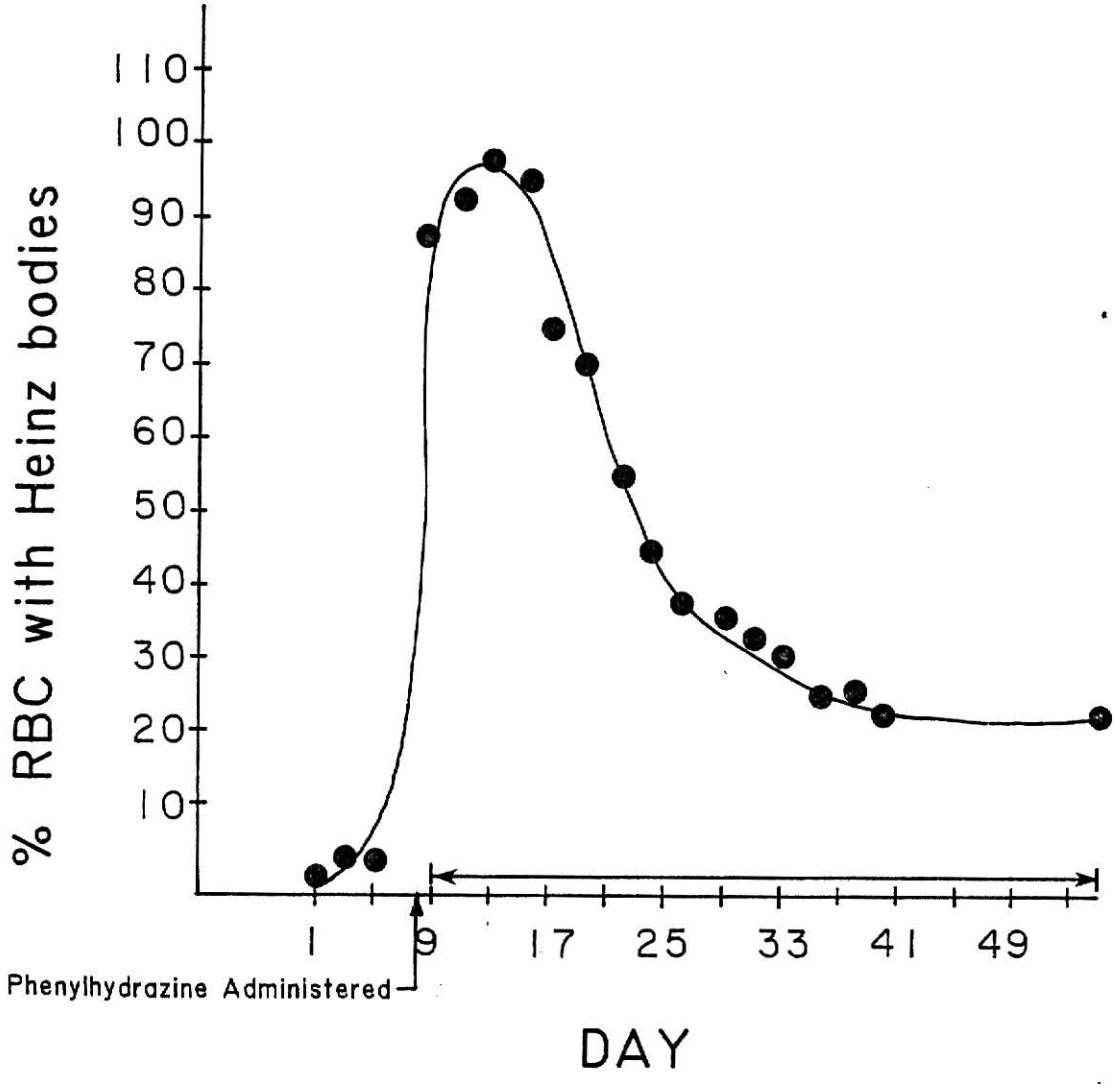
Mean corpuscular hemoglobin concentration (MCHC) during the pretreatment period averaged 36.4 percent. An initial drop in MCHC to 35.3 percent was observed on day 13, with a significant nadir of 32.9 percent being reached on day 17. The MCHC then steadily rose and attained pretreatment levels by day 33.

Figure 11 illustrates the changes found for red cell resistance to filtration. The resistance to filtration was taken as a measure of the ability of the red cell to deform. Therefore, increasing resistance meant decreased deformability. A significant increase in resistance to filtration (decreased deformability) occurred by days 9 and 11. This change occurred prior to the appearance of significant numbers of

reticulocytes in the circulation (Fig. 10), and occurred concommitantly with the appearance of large numbers of Heinz body containing cells (Fig. 2). Spindled red cells also appeared at this time (Fig. 9). Red cell resistance to filtration continued to rise dramatically, reaching a peak of 49 cm H_2O on day 19. By day 19, reticulocytes had also reached their peak (Fig. 10) and Heinz bodies although decreasing (Fig. 2) were still significantly elevated (70% of cells affected). Beginning on day 24, red cell resistance to filtration began to decrease reaching a value of 25.2 cm H_2O on day 55. Resistance to filtration did not return to the pretreatment level of 3.93 cm H_2O, remaining significantly elevated from day 9 through day 55 of the study. By day 55, reticulocytes had returned to pretreatment levels (Fig. 10) while some 21.32 percent of the red cells still contained small Heinz bodies (Fig. 2).

Red cell osmotic fragility curves are shown for days 1, 11, 19, and 38 (Figs. 12, 13, 14, 15). A significant increase in fragility was noted on days 11, 19, and 38 relative to the baseline values of day 1. Increased hemolysis was noted at saline concentrations of 0.60 to 0.85 percent. In addition, the osmotic fragility curve on day 38 (Fig. 15) indicated that a portion of the red cell population was more resistant to osmotic lysis with a significant decrease in lysis being observed in the 0.20 to 0.55 percent saline concentration range.

Fig. 2. Percentage of Erythrocytes Exhibiting Heinz Bodies in Goats Before and After Phenylhydrazine Administration



⟷ Value differs significantly ($P < 0.05$) from the mean pre-treatment value.

Fig. 3. Photograph of Small Multiple Heinz Bodies Within Erythrocytes (X500)

mH = multiple Heinz bodies

Fig. 4. Photograph of Large Heinz Bodies Within Erythrocytes (X500)

sH = single Heinz bodies

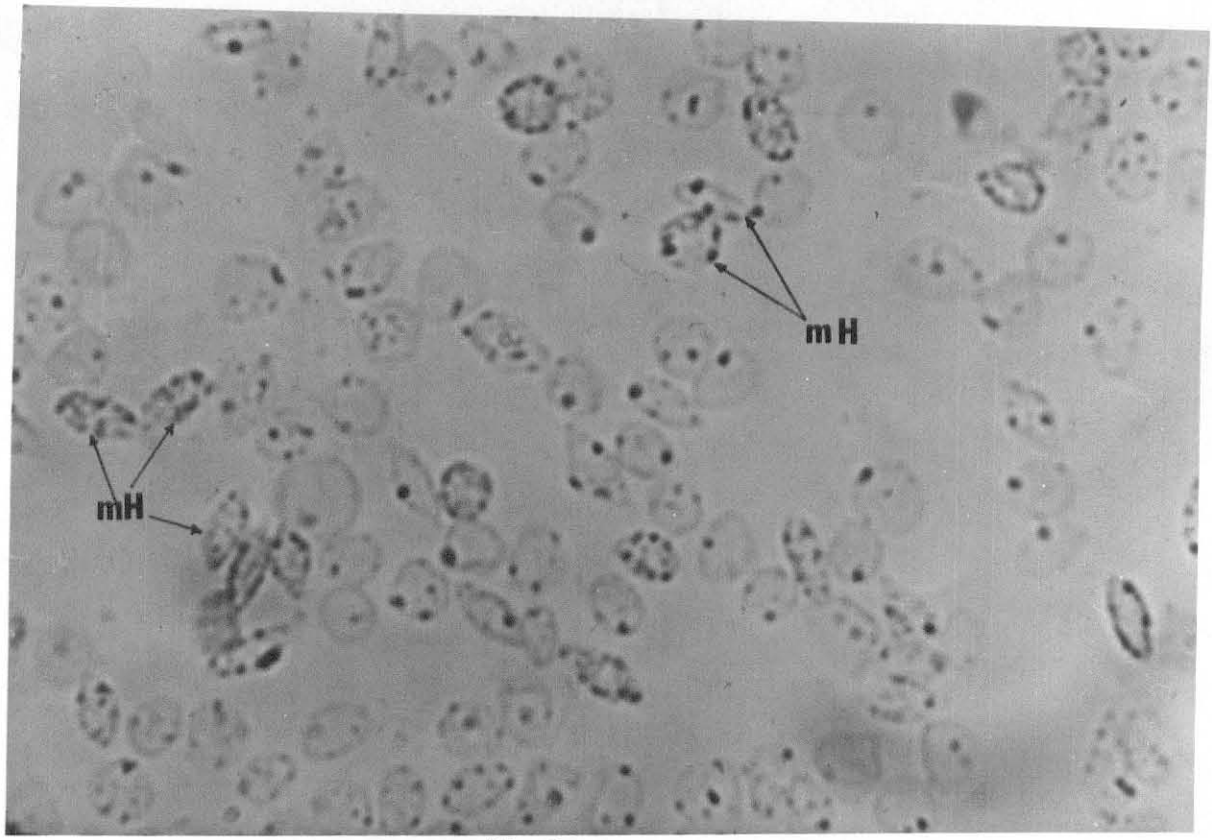


Fig 3

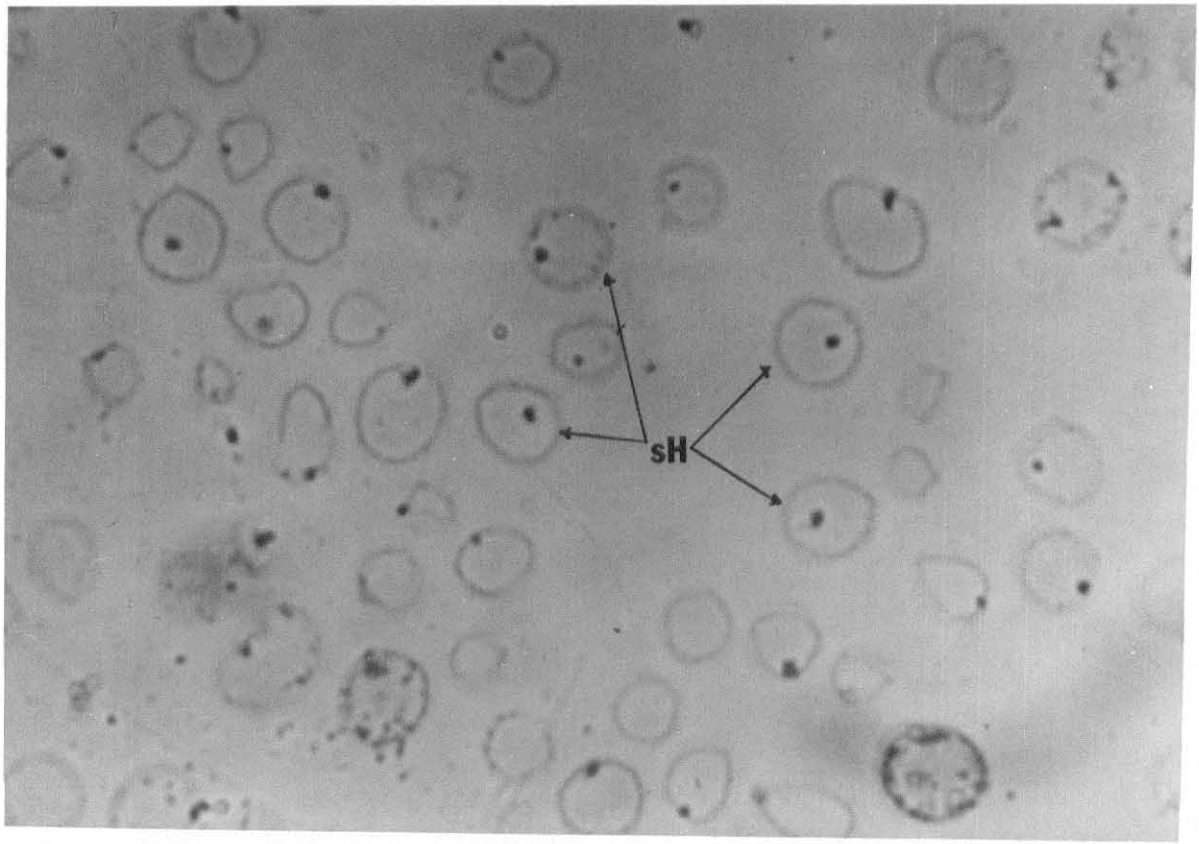
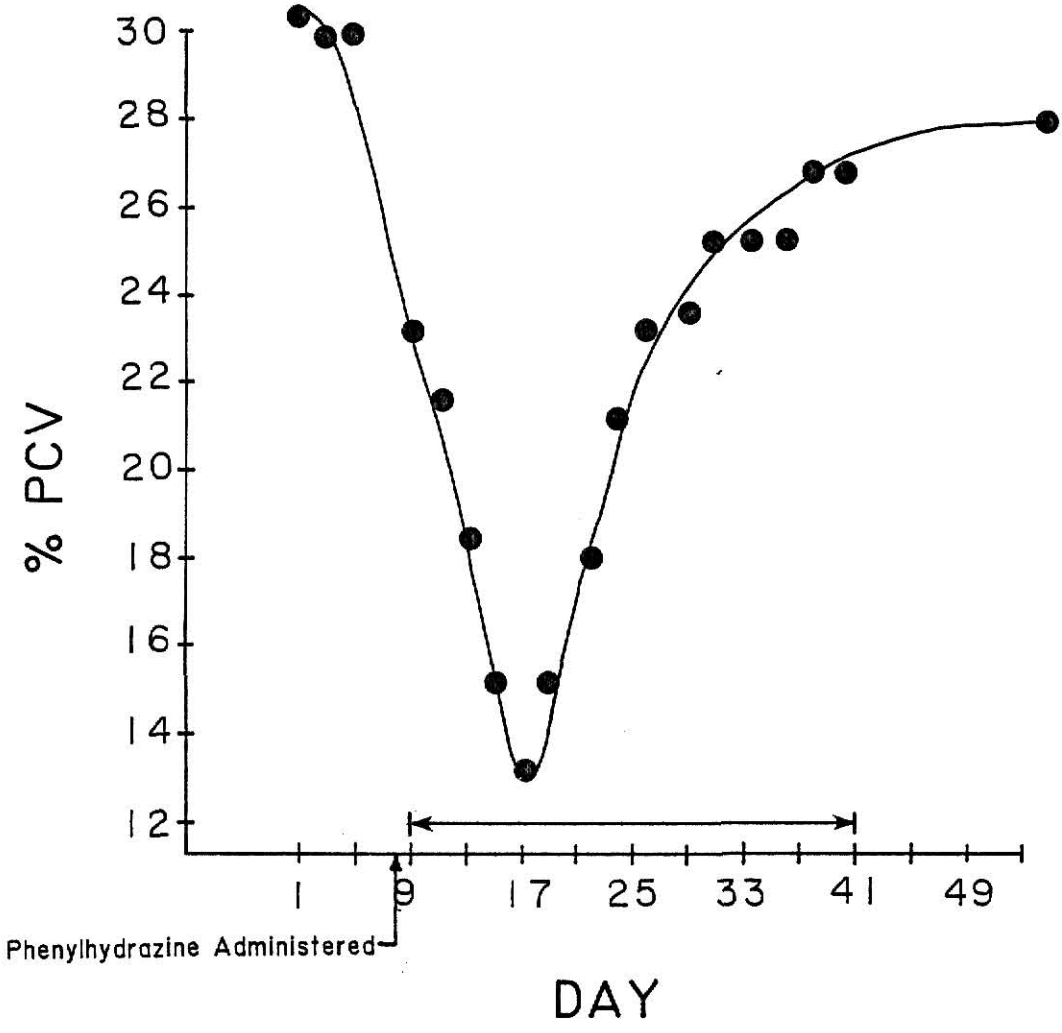


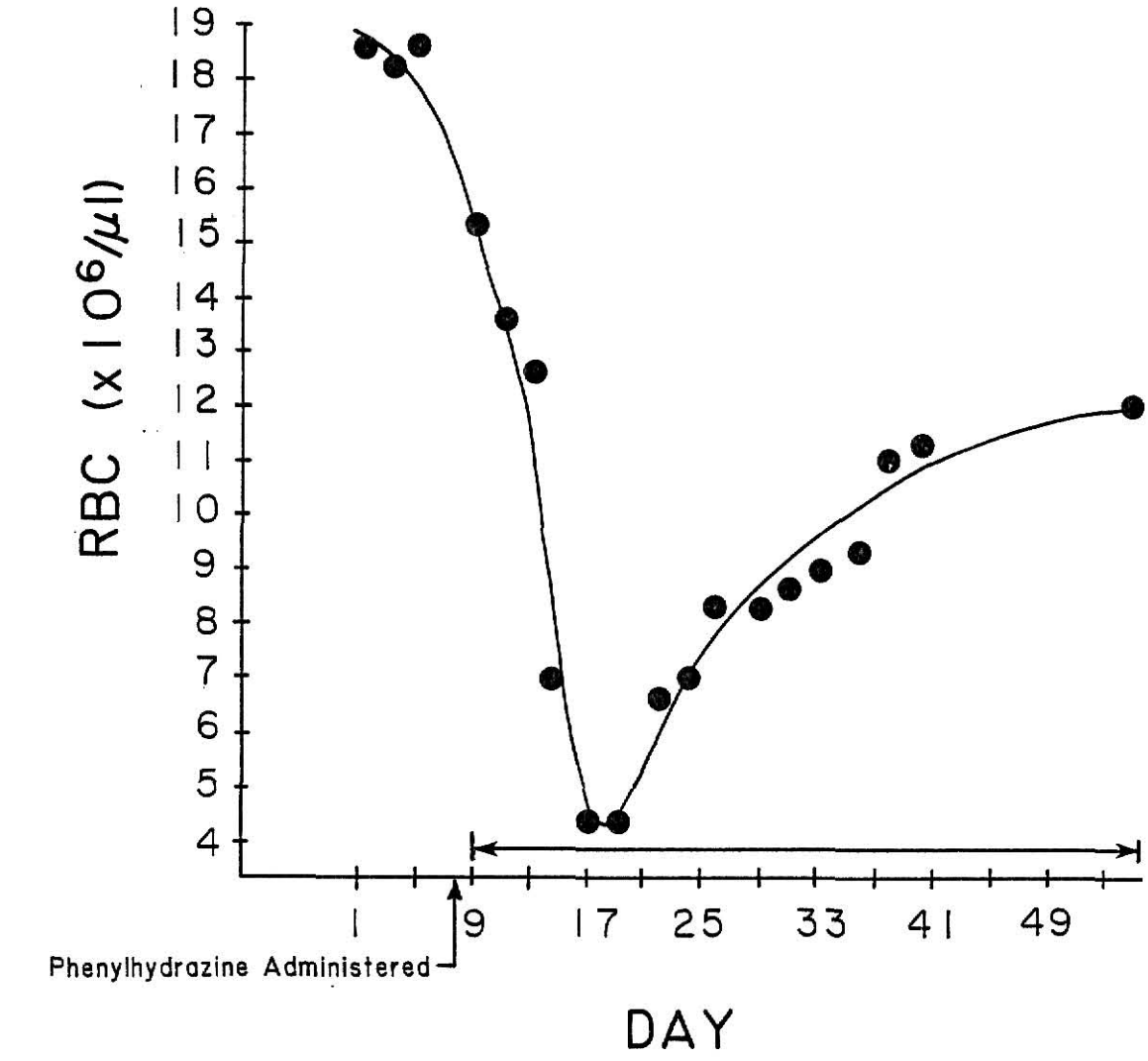
Fig 4

Fig. 5. Mean Packed Cell Volumes in Goats Before and After Phenylhydrazine Administration



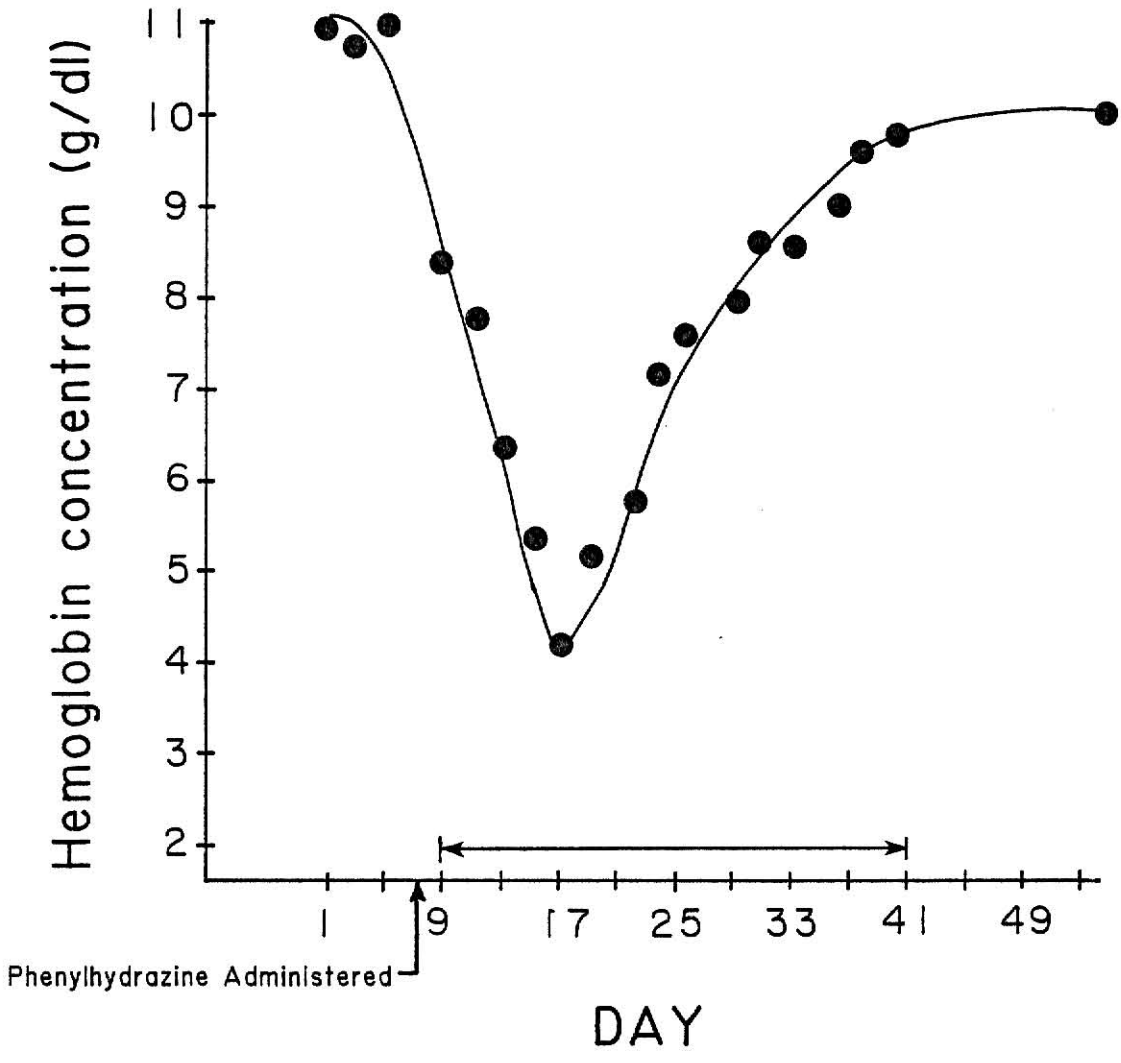
⟷ Value differs significantly ($P < 0.05$) from the mean pre-treatment value.

Fig. 6. Red Cell Counts in Goats Before and After Phenylhydrazine Administration



⟷ Value differs significantly ($P < 0.05$) from the mean pre-treatment value.

Fig. 7. Hemoglobin Concentration in Goats Before and After Phenylhydrazine Administration



⟷ Value differs significantly ($P < 0.05$) from the mean pre-treatment value.

Fig. 8. Photographs of Spindled Red Blood Cells (X500)

s = spindled red cells

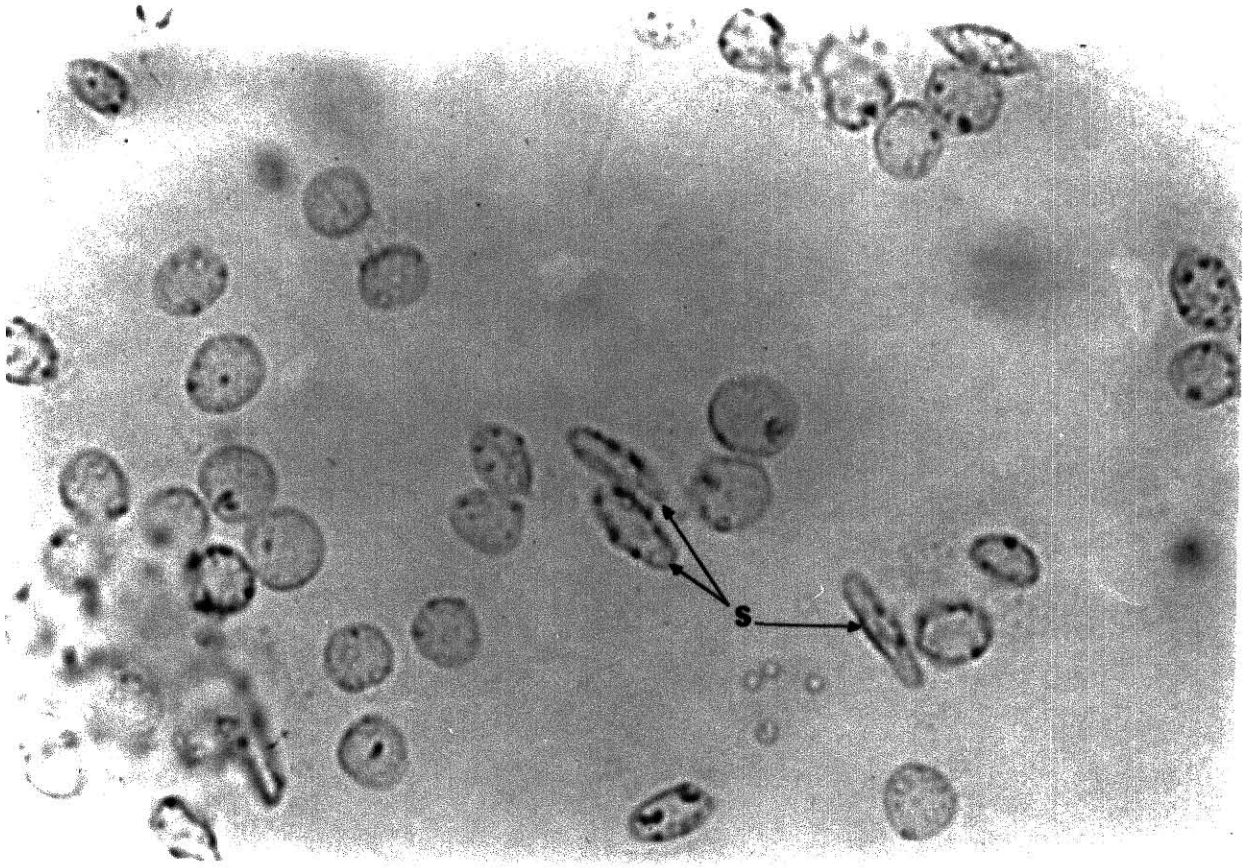
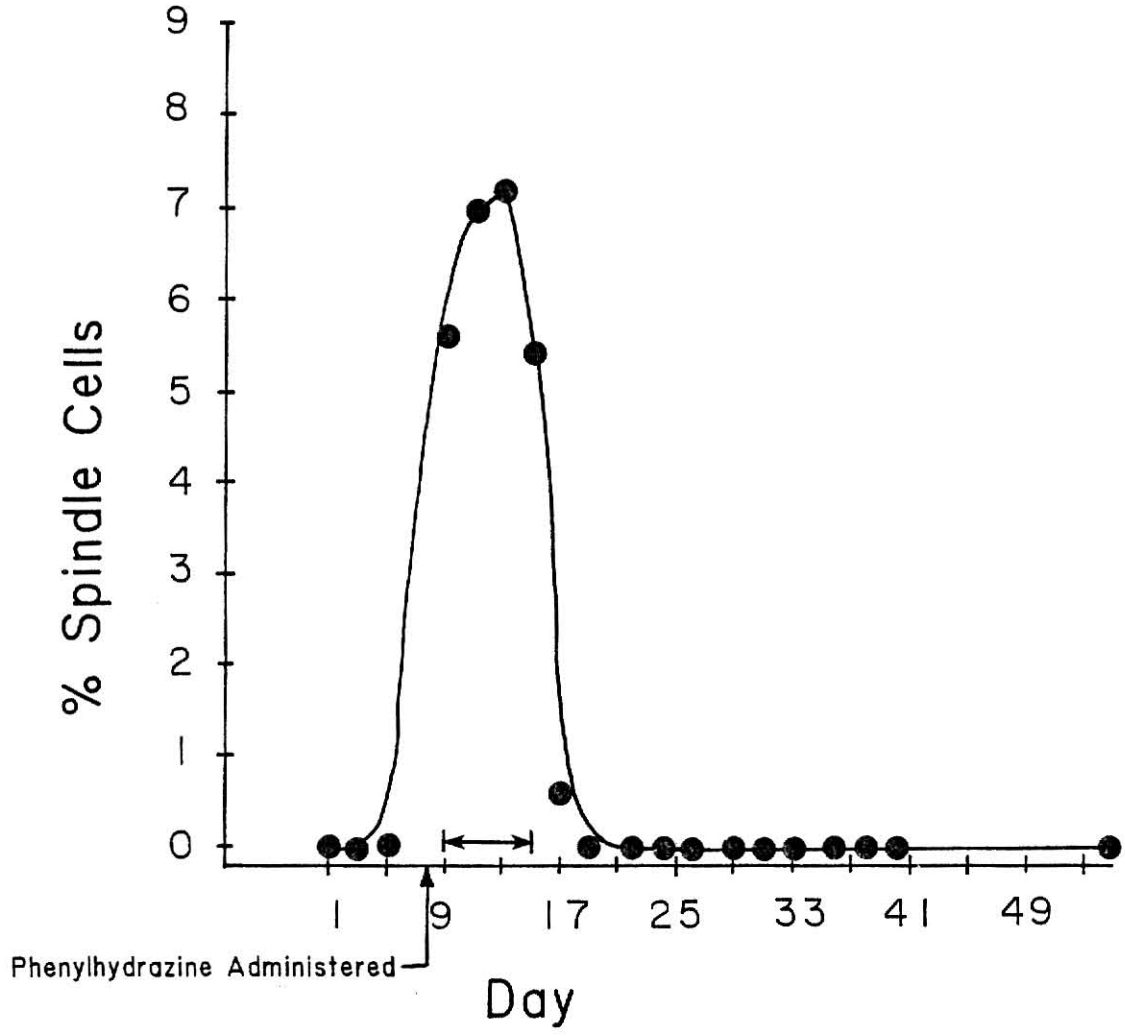
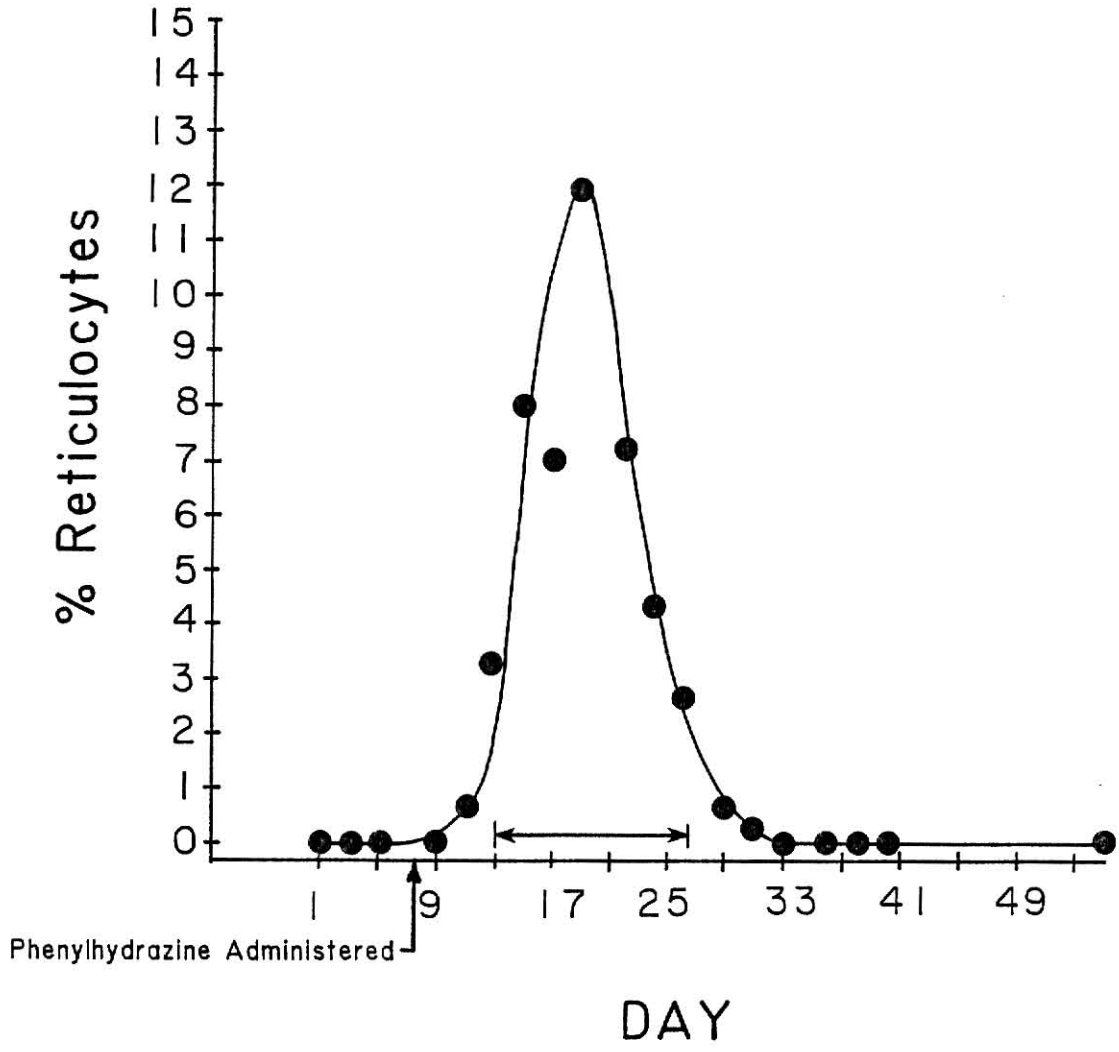

S

Fig. 9. Percentage of Erythrocytes Exhibiting Hemoglobin Crystal Formation and Spindling in Goats Before and After Phenylhydrazine Administration



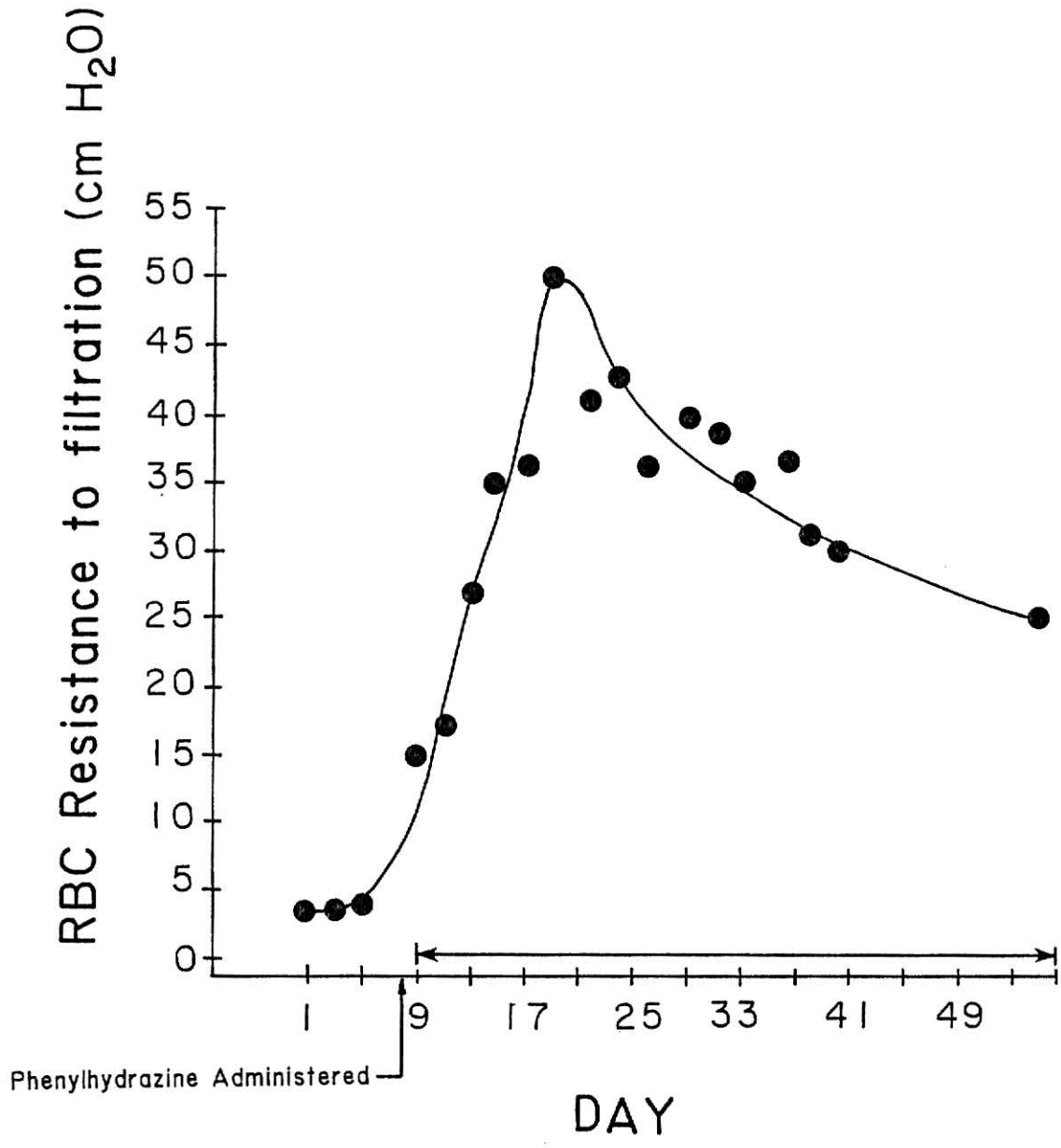
⟷ Value differs significantly ($P < 0.05$) from the mean pre-treatment value.

Fig. 10. Percentage of Reticulocytes in Goats Before and After Phenylhydrazine Administration



⟷ Value differs significantly ($P < 0.05$) from the mean pre-treatment value.

Fig. 11. Erythrocytic Filtration Resistance in Goats Before and After Phenylhydrazine Administration



⟷ Value differs significantly ($P < 0.05$) from the mean pre-treatment value.

Fig. 12. Erythrocytic Osmotic Fragility in Goats Before Phenylhydrazine Administration

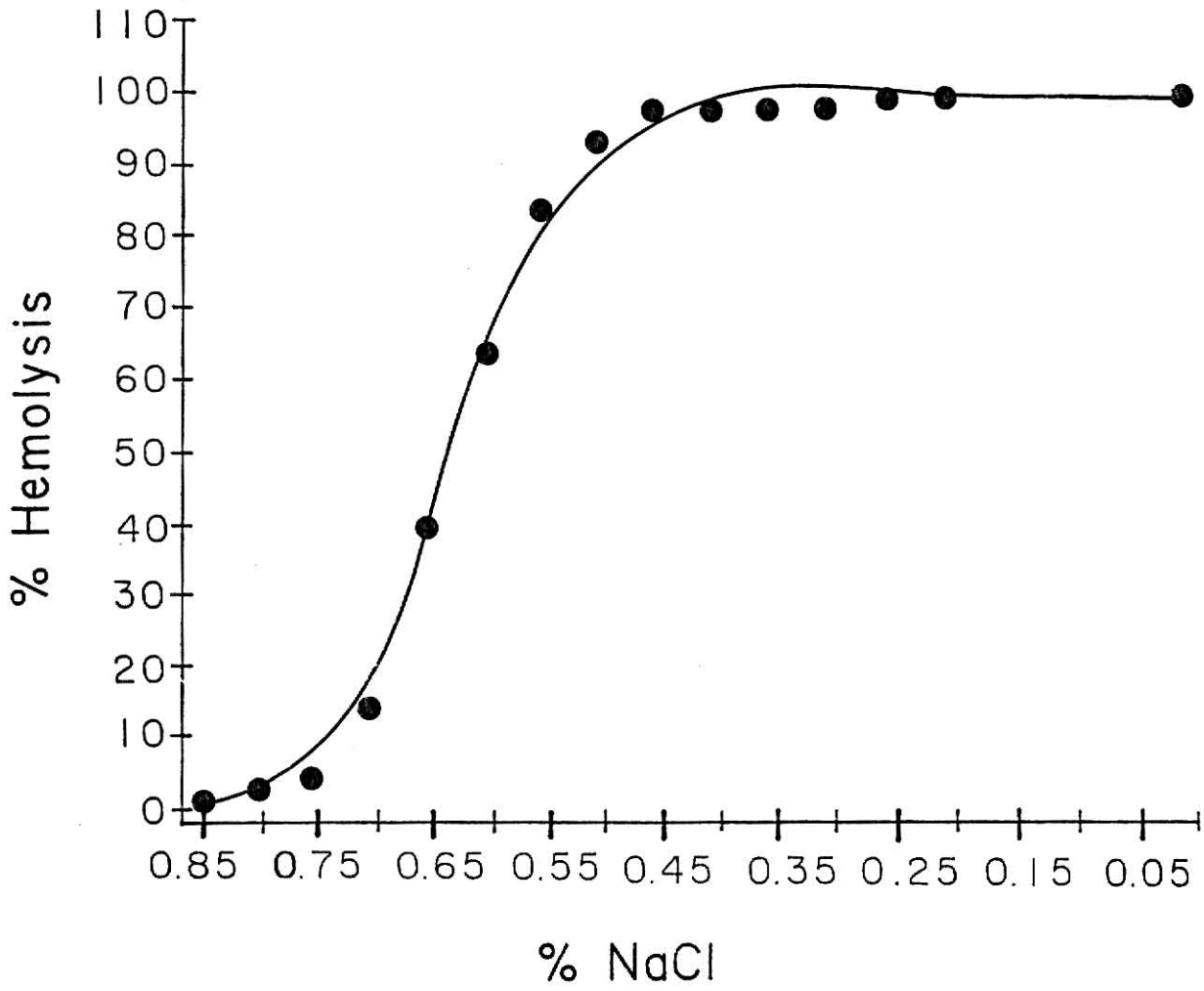
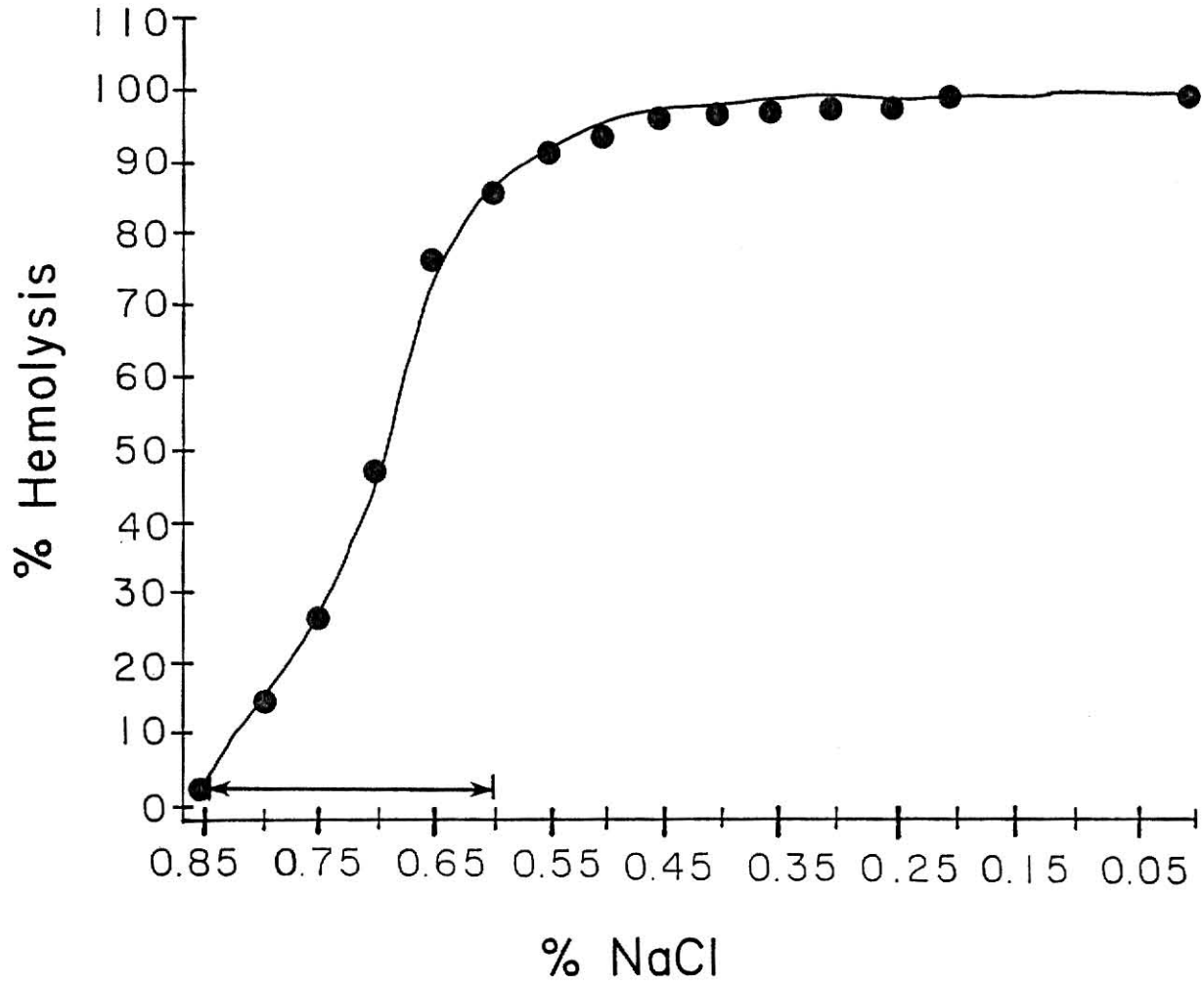
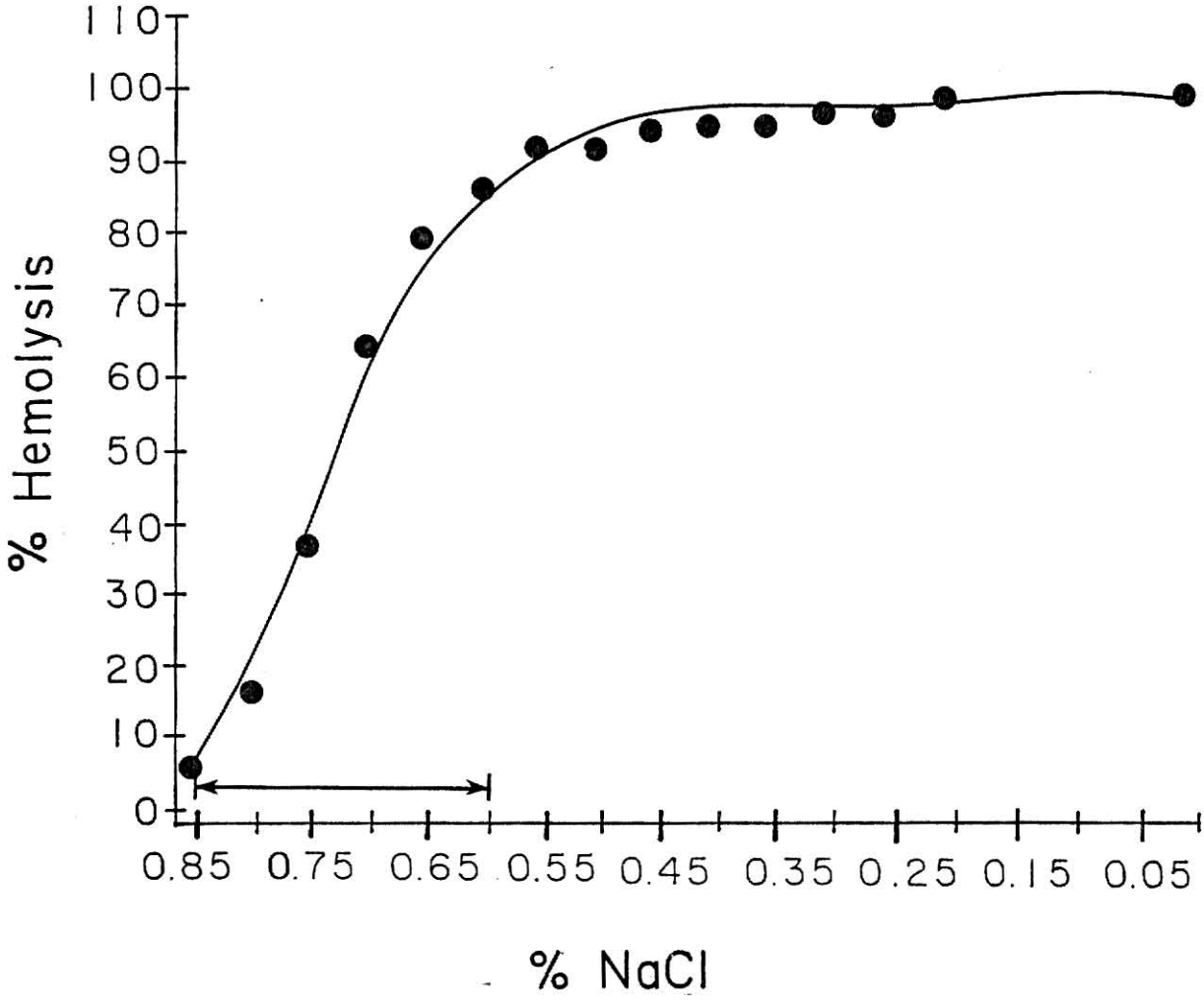

110
100
90
80
70
60
50
40
30
20
10
0
% Hemolysis
0.85 0.75 0.65 0.55 0.45 0.35 0.25 0.15 0.05
% NaCl

Fig. 13. Erythrocytic Osmotic Fragility in Goats Following Phenylhydrazine Administration (Day 11)



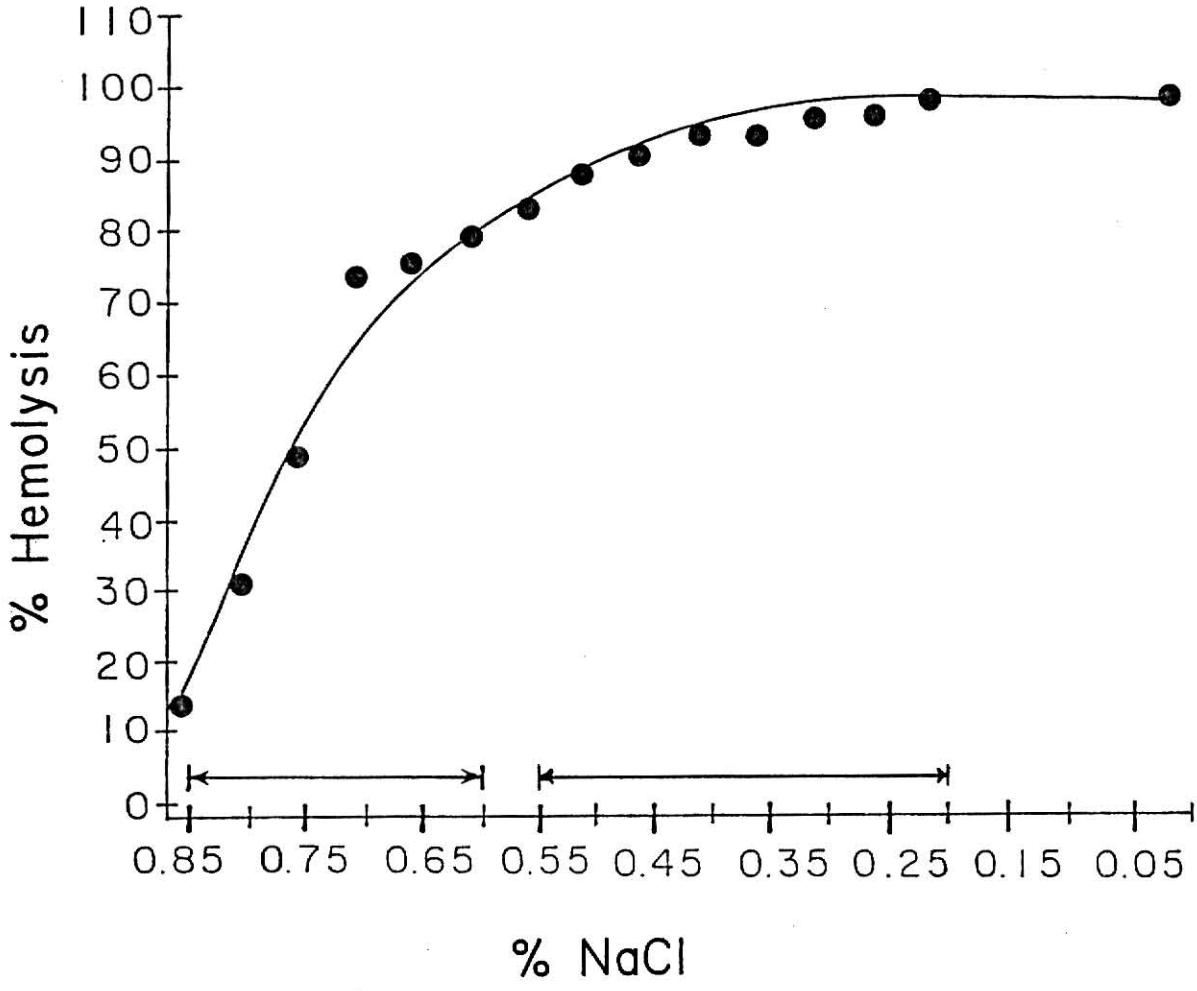
⟷ Value differs significantly ($P < 0.05$) from the mean pre-treatment value.

Fig. 14. Erythrocytic Osmotic Fragility in Goats Following Phenylhydrazine Administration (Day 29)



⟷ Value differs significantly ($P < 0.05$) from the mean pre-treatment value.

Fig. 15. Erythrocytic Osmotic Fragility in Goats Following Phenylhydrazine Administration (Day 38)



⟷ Value differs significantly ($P < 0.05$) from the mean pre-treatment value.

DISCUSSION

Numerous Heinz bodies rapidly developed in the vast majority of circulating red cells of the goats following phenylhydrazine administration. Phenylhydrazine therefore appeared to rapidly denature a portion of the hemoglobin in almost all of the circulating red cells. Jandl, working with human hemoglobin also found that over 80 percent of a hemoglobin preparation was denatured and precipitated within 12 hours of adding phenylhydrazine.[7] Clinical cases of Heinz body anemia are usually acute in nature with anemia and hemoglobinuria occurring rapidly following ingestion of the oxidant agent.[3,5,6,14]

Initially, several small Heinz bodies were observed per red cell. By day 15, the majority of Heinz body containing red cells exhibited a single large body some of which were approximately 1 μm. The small Heinz bodies initially formed thus appeared to coalesce and ultimately form a singular large body. Simpson reported similar observations while studying Heinz body formation in horse, dog, and turkey red cells.[1] It is generally considered that Heinz bodies are formed principally in mature red cells by the aggregation of fine granules of precipitated denatured hemoglobin, and ultimately appear as a dense granular structure.[15]

A perusal of the literature revealed no previous reports of spindle cell formation following phenylhydrazine treatment or accompanying Heinz body formation and anemia in man or domestic animals. However, the presence of spindled red cells in blood collected from clinically normal Angora goats has been reported by Jain.[16] Jain found that the formation of spindled red cells <u>in vitro</u> was influenced by low temperature, pH and deoxygenation. He furthermore indicated that polymerization of hemoglobin in the form of longitudinal tubular fibers was responsible

for conferring the fusiform and spindle shape to the red cell.[16] The cause of spindle cell formation following phenylhydrazine administration was not clear. The formation of the fusiform spindled cells was seemingly related to a direct effect of phenylhydrazine since the cells appeared shortly after treatment with the oxidizing agent.

The rapid falls in PCV, hemoglobin concentration, and red cell counts experienced by the goats following Heinz body formation were consistent with the findings of Schalm who noted similar hemogram results in cattle with Heinz body anemia.[11] Other investigators have also reported the acute development of anemia accompanying Heinz body formation.[3,6,14] The rapidity and the degree of development of anemia seemingly correlated with the number and size of Heinz bodies formed. Support for this comes from the observation that clinically normal cats may exhibit small Heinz bodies in up to 10 percent of circulating red cells without the development of anemia.[17] However, cats exposed to oxidant agents develop numerous large Heinz bodies and concommitantly develop severe anemia.[18] The presence of large Heinz bodies is thought to possibly reduce the deformability of the affected red cells and thus impede their passage through the microcirculation.[11] Red cells containing large Heinz bodies are sequestered with the spleen and undergo erythrophagocytosis or hemolysis.[19,20]

The MCV and MCH remained significantly elevated above baseline values at the termination of the experiment (day 55). The elevation in these indices probably reflected the relatively young mean age of the red cell population present since a majority of the circulating cells were removed following Heinz body formation and replaced with newly

formed red cells. The initial increases noted for the MCV and MCH correlated with the appearance of reticulocytes. This change would be expected since reticulocytes are decidedly larger than mature red cells.[21] Although these reticulocytes decrease in volume (MCV) while maturing in the circulation, the emergence of large numbers of young cells during and following an anemic event might be expected to influence the MCV for many days.[22,23]

The initial increase in red cell filtration resistance occurred simultaneously with the appearance of large numbers of Heinz bodies in the circulating red cells, but before the emergence of reticulocytes. This indicated that red cell deformability was decreased foblowing Heinz body formation. As reticulocytes appeared, rel cell filtration resistance increased even further, thus indicating a further decrease in deformability. This would be expected since reticulocytes have been shown to have reduced deformability compared to mature red cells.[13]

The presence of Heinz bodies within red cells would be expected to cause decreased deformability. The deformability of normal erythrocytes appears to depend on several factors:[24] (i) maintenance of the normal ratio of cell surface area to volume, (ii) normal internal fluidity of the cell, and (iii) intrinsic membrane deformability. The normal fluidity of the red cell depends primarily on the properties of normal hemoglobin.[24] Abnormal hemoglobin with a predisposition to undergo intracellular crystallization causes increased rigidity of the red cell and predisposes the cell to intravascular fragmentation or removal.[24] Similarly, Heinz body formation leads to membrane depletion by fragmentation with spherocyte formation due to the pitting of Heinz bodies from the

red cells.[25] Spherocytes are less deformable and may undergo premature destruction in the microcirculation of the spleen.[15]

The rigid nature of the Heinz body creates a hazard for the physiologic survival of the red cell.[15] Normal red cells are deformable, hence they can repeatedly pass undamaged through the microcirculation to complete their normal life span.[15] Red cells carried across the splenic cords, return to the venous circulation by squeezing through the interendothelial slits in the sinus wall.[26] The deformable cytoplasm does this easily, but the rigid Heinz body is held up and ultimately detached from the red cell along with a rim of membrane and a film of hemoglobin.[26] This process is called pitting. Observations have shown that the pitted red cell may be returned to the general circulation.[27,28] The pitted red cells are less deformable due to loss of membrane and may undergo premature destruction.[15] Alternatively, the entire red cell and its Heinz body may be phagocytized.[19,29,30] These events occur primarily in the spleen, but also in the liver and bone marrow.[29,30,31,32] Thus, the occurrence of Heinz bodies is known to promote accelerated erythrocyte destruction leading to anemia.[19,28,21]

Normal goat red cells are small (16-20 fl) and have been reported to have a low deformability relative to other species.[33] The formation of a rigid body within the red cell would further affect its ability to deform. The polycarbonate filters used in this study possessed pores varying from 2.4 to 3 micrometers in diameter and approximately 10 micrometer in length. The normal diameter of the goat red cell is 3.3 μm and the maximum length it can attain during deformation is 5.5 μm.[33] Therefore, during its passage through the polycarbonate filter, the entire

red cell would be contained within the 10 μm length of the pore. The presence of Heinz bodies apparently inhibited the ability of the goat red cells to traverse or enter the pores, thus causing increased resistance to filtration.

As reticulocytes appeared in the circulation of the goats, red cell filtration resistance increased dramatically. Due to their relatively poor ability to deform,[13] the reticulocytes apparently encountered difficulty in entering and traversing the membrane pores. Leblong utilized a 3 μm polycarbonate membrane in studying the deformability of human red cells (average diameter 8 μm).[13] An average of 99.79 percent of normal human red cells passed through the filter membrane. However, with samples containing significant numbers of reticulocytes, the average percent of red cells passing through the filter was greatly reduced.[13]

The increased red cell osmotic fragility observed in the present study seem to be related to the formation of Heinz bodies since this change initially occurred shortly after phenylhydrazine treatment and Heinz body formation (day 11). The susceptibility of a red cell to osmotic lysis is related in part to red cell size, since increasing fragility correlates with decreasing red cell volume.[11] Decreasing size limits the capacity of the red cell to expand without lysis occurring. As previously discussed, Heinz body affected red cells which have been pitted become spherocytic due to the loss of membrane accompanying the removed Heinz body.[25] Spherocytes have increased osmotic fragility.[11] The increased fragility noted for a portion of the red cell population following Heinz body formation may have been due to red cell fragmentation and spherocyte formation. Membrane alterations induced by the presence of the Heinz body may also play a

role in the increased fragility noted. In addition, oxidant compounds such as phenylhydrazine have been reported to directly increase the osmotic fragility of red cells.[34,35] The increased osmotic fragility found in this study may thus reflect a direct effect of phenylhydrazine treatment.

The increased resistance to osmotic lysis found for a portion of the circulating red cells on day 38 was assumed to be due to the presence of a young, volumetrically larger red cell population. Red cells with a larger MCV would be expected to be more resistant to osmotic lysis. The elevated MCV of 24 fl observed on day 38 would support this assumption.

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PAPER II

HEINZ BODY ANEMIA IN INTACT AND SPLENECTOMIZED GOATS

SUMMARY

Heinz bodies were produced in the circulating red cells of intact and splenectomized goats following the administration of phenylhydrazine hydrochloride. Heinz bodies developed rapidly in both groups with approximately 70 percent of the circulating red cells exhibiting one or more bodies 24 hours after phenylhydrazine administration. The formation of Heinz bodies was followed by a regenerative anemia in both groups as the Heinz body affected cells were removed from circulation.

The rate of disappearance of Heinz bodies was virtually the same in the intact and asplenic goats indicating that the spleen played a minimal role in removal of Heinz bodies either by pitting or by removal of the affected red cells.

The interendothelial slits of the goat splenic sinus were examined using scanning electron microscopy and were found to appear relatively large compared to the red cells lying within the sinus. Observation of this anatomical characteristic offered a possible explanation as to why splenectomy had little apparent effect on the rate of removal of circulating Heinz bodies.

Red cell deformability as measured by red cell resistance to filtration was found to decrease in both the intact and asplenic goats as Heinz body formation occurred. Deformability decreased even further upon the appearance of reticulocytes. As reticulocytes and Heinz body carrying cells disappeared from the circulation, red cell deformability increased in both groups during the final 18 days of the study period, with the asplenic goats exhibiting a significantly greater gain in deformability than the intact animals.

Spindled red cells also appeared in the blood of the intact and splenectomized goats subsequent to phenylhydrazine administration.

INTRODUCTION

The spleen, and only the spleen, can perform the functions of pitting and manicuring red blood cells because of a unique feature of its microanatomy.[1] The red pulp consists almost entirely of thin-walled splenic sinuses and the splenic cords which lie between them.[2] The sinuses are vascular channels lined with elongated, tapered endothelial cells arranged with their long axes parallel to that of the vessel.[2] Small gaps called interendothelial slits exist between the endothelial cells. Red blood cells traverse the sinus wall by passing through these slits.[2] Red cells carried across the splenic cords return to the venous circulation by squeezing through the slits in the sinus wall.[3] With remarkable deformability, normal erythrocytes squeeze through the narrow slits, but slightly or moderately damaged erythrocytes fail to pass through and are phagocytized by macrophages.[4]

The presence of Heinz bodies within the erythrocyte reduces deformability of the cell.[5] The deformable cytoplasm passes through the slit, but the rigid Heinz body is held up and ultimately detached from the red cell along with a rim of membrane and a film of hemoglobin.[3] The rejected inclusion is ingested by the perisinusoidal phagocytes.[3] This process is referred to as pitting. In pitting, the normal spleen removes particles from the red cell without destroying the cell.[2] Alternatively, the entire erythrocyte and its Heinz body may be phagocytized.[6,7,8]

Hematologic changes which occur in normal persons following splenectomy provide information about the normal functions of the spleen.[9] Red cells become thinner, probably because the surface area remains

somewhat greater than normal.[10] This results in the appearance of target cells in the blood smear.[11] After splenectomy, pocked red cells also appear with the pocked surface appearance actually representing vacuoles retained within the cell.[12,13] The life span of the normal red cell is neither extended nor shortened by splenectomy.[3]

Nucleated red cells and cells containing Howell-Jolly bodies may also appear in the circulation following splenectomy.[2] The percentage of reticulocytes may or may not be increased and diffuse basophilia may also be found.[2,4] The persistent presence of Heinz bodies in splenectomized individuals or persons with splenic agenesis reflects the role of the spleen in removing these intraerythrocytic inclusions, either through the pitting process or by removal of the cell itself.[2] The removal of Heinz body containing cells in great numbers may result in anemia.

The role of the spleen in removal of Heinz bodies in the cat was studied by Jain.[15] He presented evidence to indicate that the spleen plays a minor role in removal of Heinz bodies in the cat. Heinz body anemia has been reported in goats.[16] However, little if any information is currently available concerning the role of the spleen in the removal of Heinz bodies in this animal species.

The objectives of the present study were (i) to compare alterations in the hemogram of intact and splenectomized goats following Heinz body formation, (ii) to compare red cell deformability in intact and splenectomized goats following Heinz body formation, and (iii) to determine the role of the goat spleen in removing Heinz body containing red cells.

MATERIALS AND METHODS

Animals -- Eight female goats approximately 1.5 to 4 years old were used in this study. The goats were of the Angora and Nubian breeds. The goats utilized as experimental subjects were the same animals used in the first phase of a study of Heinz body anemia.[5] A rest period of 14 days was allowed between termination of the first study phase and the initiation of the present study (second phase). The animals were maintained as previously described.[5]

Experimental design -- Following the 14 day rest period, the goats were divided into two groups, Group A (n = 4) and Group B (n = 4) with 2 goats being splenectomized in each group with the remaining animals left intact. The spleens were collected and prepared for scanning electron microscopy at this time.

Following splenectomy, an additional recovery period of 14 days was allowed. At the end of this recovery period, the goats appeared clinically normal except for the presence of a surgical incision scar in the flanks of the four splenectomized goats. Hematologic values of the goats at this time were within normal limits excepting that the percentage Heinz body count and the red cell filtration resistance were still somewhat elevated above previously determined baseline values.[5] Blood samples for analyses were collected via jugular veinpuncture using appropriate tubes. Sample analyses carried out on each sampling day included red blood cell count (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), percentage of red cells containing Heinz bodies (% H.B.), reticulocyte count (% retic), percentage of spindled red cells (% spindle cells), and red cell resistance to filtration (deformability). The erythrocytic indices: mean corpuscular volume

(MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were also determined.

Throughout the sampling period of the study, the goats were sampled on alternate days. A total of four goats were sampled each day. Thus, on day 1, Group A was sampled, followed by Group B on day two, then returning to Group A on day 3, ... etc. Animals of both groups A and B were sampled three times prior to phenylhydrazine treatment. Pretreatment sampling was carried out beginning on day 1 of the study with Group A being sampled on days 1, 3 and 5 and Group B being sampled on days 2, 4 and 6. Pretreatment samples were used to establish baseline values for each analysis performed.

In analysis and presentation of the data, the values obtained from the 4 splenectomized goats and the 4 intact goats during each two day sampling sequence (i.e., days 1 and 2; days 3 and 4 . . . . etc.) were averaged and presented as the mean data derived for day 1, day 3, day 5 . . . . . etc., respectively.

Following the establishment of baseline values, phenylhydrazine hydrochloride was administered as previously described.[5] Groups A and B receiving the chemical on days 8 and 9, respectively. A second dose of phenylhydrazine was administered on days 12 (Group A) and 13 (Group B). Post treatment blood sampling was initiated on days 9 (Group A) and 10 (Group B) and continued on an alternating basis through day 41 of the study.

The values obtained during the post-treatment period for the splenectomized and the intact goats were compared against their corresponding mean baseline values and against one another for significant changes ($p < 0.05$).

Hematologic techniques -- Their analyses were performed as previously described.[5]

Splenectomy -- The goats were fasted for 24 hours before surgery. After routine surgical preparation of the left flank, each goat was sedated with acetylpromazine. Local anesthesia was induced by paravertebral blocking of T_{13}, L_1 and L_2 spinal nerves and by infiltrating the surgical site with carbicaine.[a] Splenectomy was performed as described for sheep.[17]

Preparation of the spleens for scanning electron microscopy -- Following surgical removal, the spleens were perfused with physiologic saline followed by fixation in a solution containing 2% para-formaldehyde and 3% glutaraldehyde in a 0.1 M sodium cacodylate buffer of pH 7.4.

Small tissue blocks were cut (1 cm^3) and freeze cracked.[18] The tissues were critical point dried with liquid CO_2 and mounted on stubs They were then sputter coated with gold[b] and examined using a scanning electron microscope[c] at an accelerating voltage of 20 KV. Photographs were taken using a self developing film.[d]

Statistical methods -- The methods used to analyze the data were as previously described.[5]

---

[a]Carbicaine, Winthrop Lab. Div. of Sterling Drug Inc., New York, N. Y.

[b]Model 5150 Sputter coater, Edwards High Vacuum, Grand Island, N. Y.

[c]Nissei Sangyo America, Hitachi, H-300 with H-3010 scanning electron microscope, Hitachi Scientific Instruments, Mountain View, California.

[d]Polaroid type 55 film, Polaroid Co., Cambridge, Mass.

RESULTS

Following administration of phenylhydrazine, Heinz body formation occurred in almost 70 percent of the circulating red cells of both the intact and splenectomized goats within 24 hours (Fig. 1). The percentage of Heinz body affected cells rose to between 90 and 100 percent (intact and splenectomized) and remained within this range for the next several days, before declining on day 19 of the study. The rapid rate of fall continued through day 29 after which the number of Heinz body containing red cells disappeared at a slower rate in both the intact and splenectomized goats. The number of Heinz body containing cells never reached the low levels found before phenylhydrazine treatment with approximately 20 percent of the red cells exhibiting Heinz bodies on day 40 in both groups (Fig. 1). An important feature exhibited in Figure 1 is the almost identical patterns of appearance and disappearance of Heinz body affected red cells in the intact and asplenic goats. Heinz body containing cells disappeared from the circulation at approximately the same rate in the presence or absence of the spleen.

The rapid development of Heinz bodies resulted in a rapid fall in blood packed cell volume (Fig. 2). The PCV reached a low point of 18 percent in both the intact and asplenic animals on days 17 and 19, respectively. In a similar manner, rapid decreases in the RBC count (Fig. 3) and hemoglobin concentration (Fig. 4) were observed for both groups of goats. Percentage wise, the splenectomized animals experienced a somewhat greater fall in PCV and hemoglobin concentration than did the intact goats (Figs. 2 and 4) with respective drops of approximately 40 and 30 percent occurring. The decreases in PCV, RBC counts and hemoglobin concentrations occurred beginning with the appearance of large numbers of

Heinz bodies. Thus, significant reductions from baseline values for PCV, RBC counts, and hemoglobin concentrations occurred in both the intact and splenectomized goats following Heinz body formation.

Spindled red cells appeared in the circulation within 24 hours following phenylhydrazine treatment (Fig. 5). The percentage of spindled or fusiform cells reached their maximum values (2.55 for intact goats; 1.45 for splenectomized goats) on day 11 and then eventually disappeared with no spindled cells being observed after day 19.

Significant increases in reticulocytes were observed by day 13 for both groups of goats, with reticulocytes appearing some four days following the initial falls in PCV, hemoglobin concentration and RBC counts (Fig. 2, 3, 4). Maximum mean reticulocyte counts of 1.9 percent on day 13 and 4.25 percent on day 17 were attained by the asplenic and intact goats, respectively. Reticulocytes remained elevated for several days, eventually returning to baseline levels after day 29.

Changes in the erythrocytic indices occurred in both groups of goats following the development of regenerative anemia. During the pretreatment period, the mean corpuscular volumes (MCV) were found to average 19.0 fl (intact goats) and 19.9 fl (asplenic goats). With the appearance of reticulocytes, the MCV rose steadily to the maximums of 24.6 fl (intact goats) and 25.5 fl (asplenic goats) by days 22 and 24, respectively. With remission of the anemia, the average MCV's for both groups fell back towards pretreatment levels. In a similar manner, the average mean corpuscular hemoglobin (MCH) rose in both groups following the appearance of reticulocytes. Respective peak values of 9 pg (intact goats) and 10.5 pg (asplenic goats) were attained on days 24 and 22 followed by a

steady fall toward the pretreatment range of 7.0-7.5 pg. Mean corpuscular hemoglobin concentration followed no consistent pattern in either group of goats throughout the post treatment period.

Red cell resistance to filtration for both the intact and asplenic animals is shown in Figure 7. Resistance to filtration by the red cells was taken as a measure of red cell deformability, with increasing filtration resistance meaning decreasing deformability. Pretreatment filtration resistance values were similar in both groups of goats. A significant rise in filtration resistance (decreased deformability) occurred by day 9 in the intact animals. Filtration resistance in this group reached its peak on day 19 and remained significantly elevated through day 29. Filtration resistance increased significantly in the asplenic groups by day 9 and remained significantly elevated through day 22, with a peak of 27.5 cm H_2O being reached by day 13. The initial significant rise in resistance to filtration for both groups occurred concommitantly with the appearance of Heinz bodies (Fig. 1) and before the appearance of reticulocytes on day 13 (Fig. 6). Following the emergence of reticulocytes, red cell filtration resistance increased to an even greater degree in both groups.

As can be seen in Figure 7, filtration resistance in the splenectomized animals returned to the level of pretreatment values by day 24, while values for the intact goats remained significantly elevated through day 29. From day 22 through the final day of the study (day 40), filtration resistance in the asplenic animals was significantly lower than that found for the intact group. The intact group returned at a slow rate toward pretreatment levels, while the asplenic goats exhibited a

more rapid rate of fall reaching a value of 3.7 cm H_2O on day 40. From days 31 through 40, filtration resistance for the asplenic goats was significantly lower than their pretreatment values. Thus, in the latter half of the study, the intact and splenectomized groups diverged with a fairly rapid and substantial fall in filtration resistance (increased deformability) being found for the splenectomized group. Red cells from the asplenic animals were most deformable on the final day of the study having dropped well below the pretreatment filtration resistance levels.

Using scanning electron microscopy, (Fig. 8), the endothelial cells (a) lining the splenic sinuses were found to appear as elongated, stave-like cells with their long axes parallel to that of the sinus. Between the endothelial cells, interendothelial slits (b) could be observed. The slits appeared to be relatively large compared to the red cells (c) present, suggesting that the red cells would have little trouble traversing the slits.

Fig. 1. Percentage of Erythrocytes Exhibiting Heinz Bodies in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration

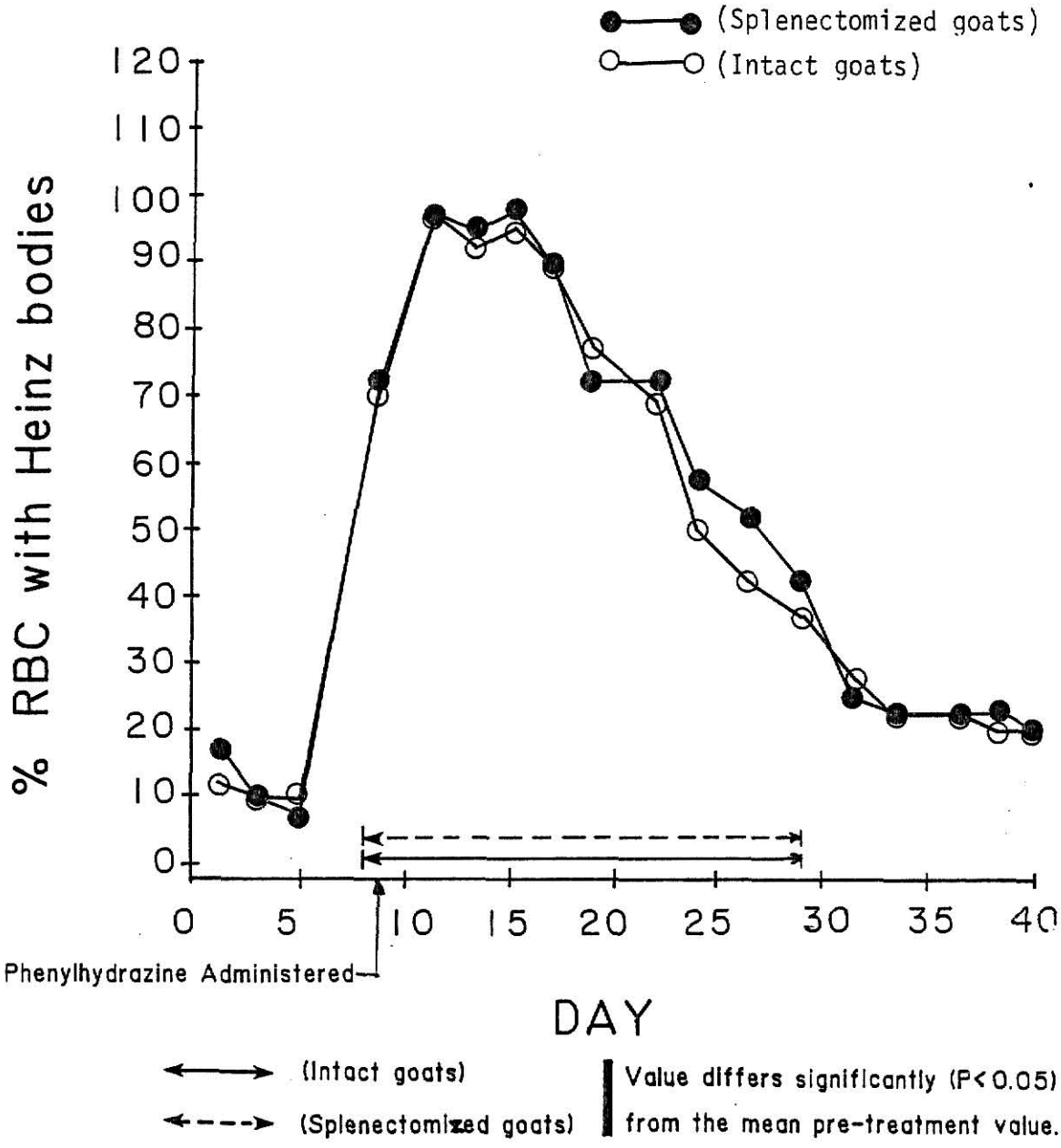

(Splenectomized goats)
(Intact goats)
% RBC with Heinz bodies
120
110
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
Phenylhydrazine Administered
DAY
(Intact goats)
(Splenectomized goats)
Value differs significantly (P<0.05)
from the mean pre-treatment value.

Fig. 2. Mean Packed Cell Volumes in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration

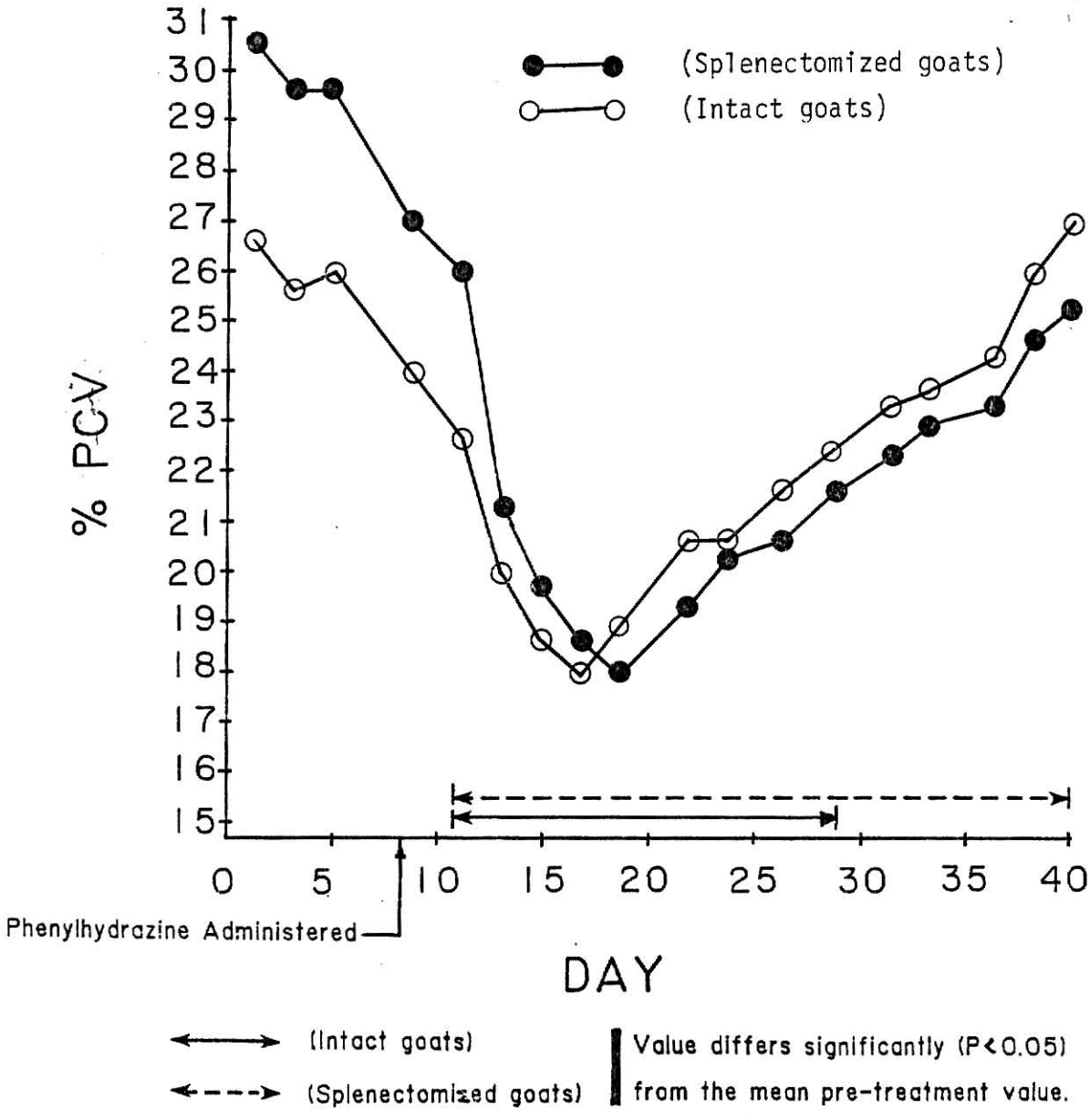

(Splenectomized goats)
(Intact goats)
% PCV
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
0
5
10
15
20
25
30
35
40
Phenylhydrazine Administered
DAY
(Intact goats)
(Splenectomized goats)
Value differs significantly (P<0.05) from the mean pre-treatment value.

Fig. 3. Red Cell Counts in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration

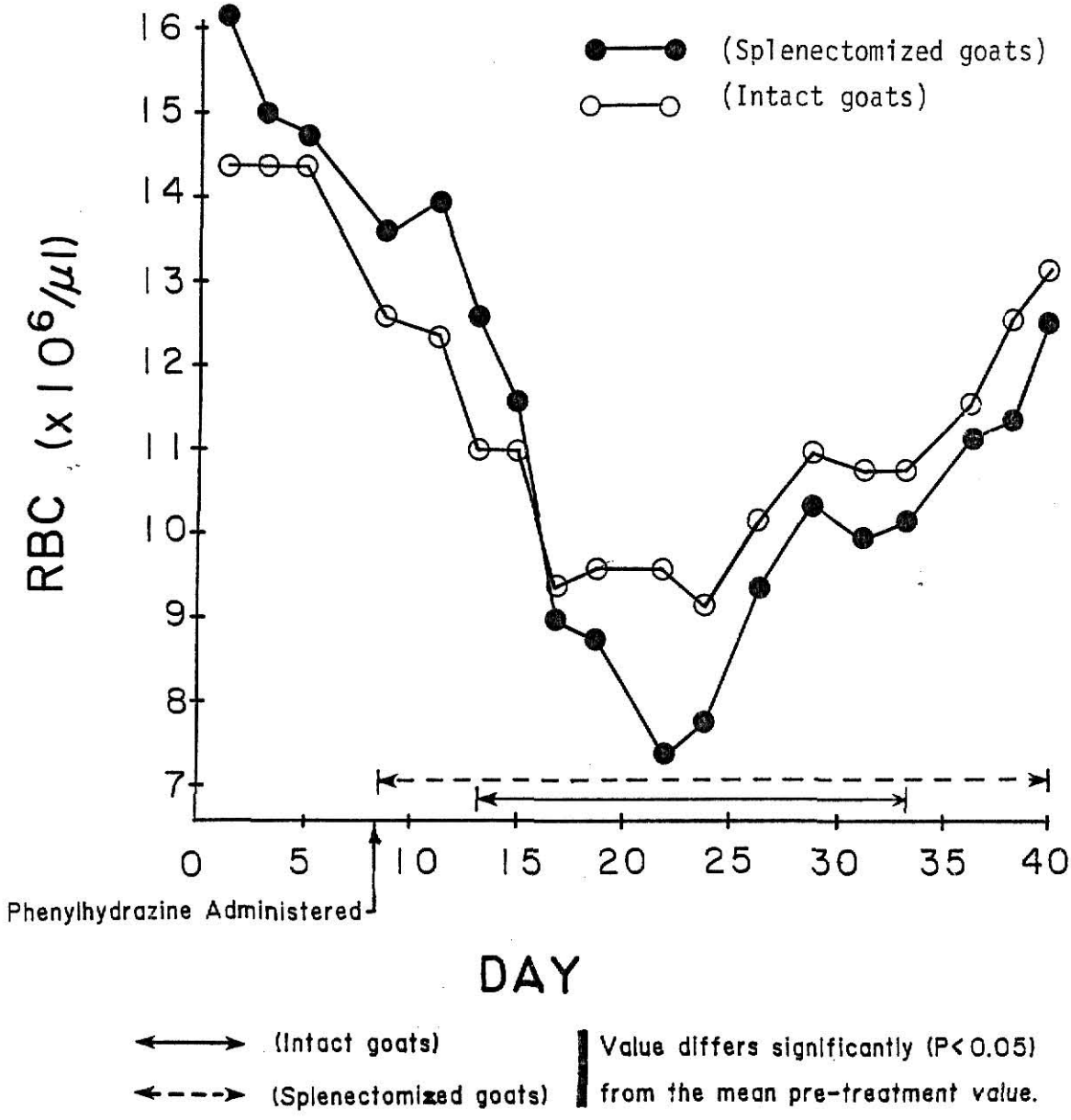

(Splenectomized goats)
(Intact goats)
RBC (x 10⁶/μl)
16
15
14
13
12
11
10
9
8
7
0
5
10
15
20
25
30
35
40
Phenylhydrazine Administered
DAY
(Intact goats)
(Splenectomized goats)
Value differs significantly (P< 0.05) from the mean pre-treatment value.

Fig. 4. Hemoglobin Concentration in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration

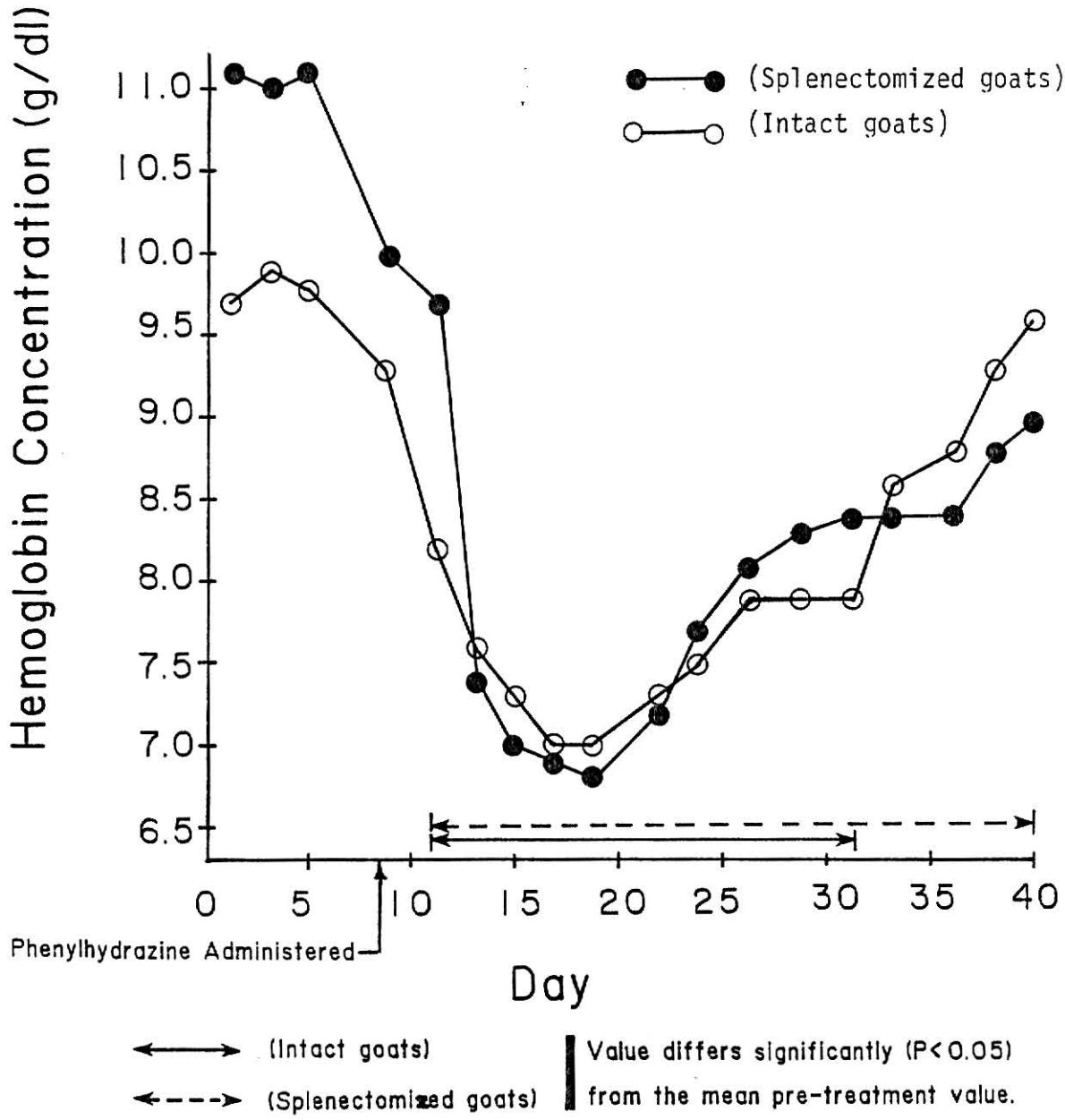

Hemoglobin Concentration (g/dl)
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
(Splenectomized goats)
(Intact goats)
0
5
10
15
20
25
30
35
40
Phenylhydrazine Administered
Day
(Intact goats)
(Splenectomized goats)
Value differs significantly (P< 0.05) from the mean pre-treatment value.

Fig. 5. Percentage of Erythrocytes Exhibiting Hemoglobin Crystal Formation and Splinding in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration

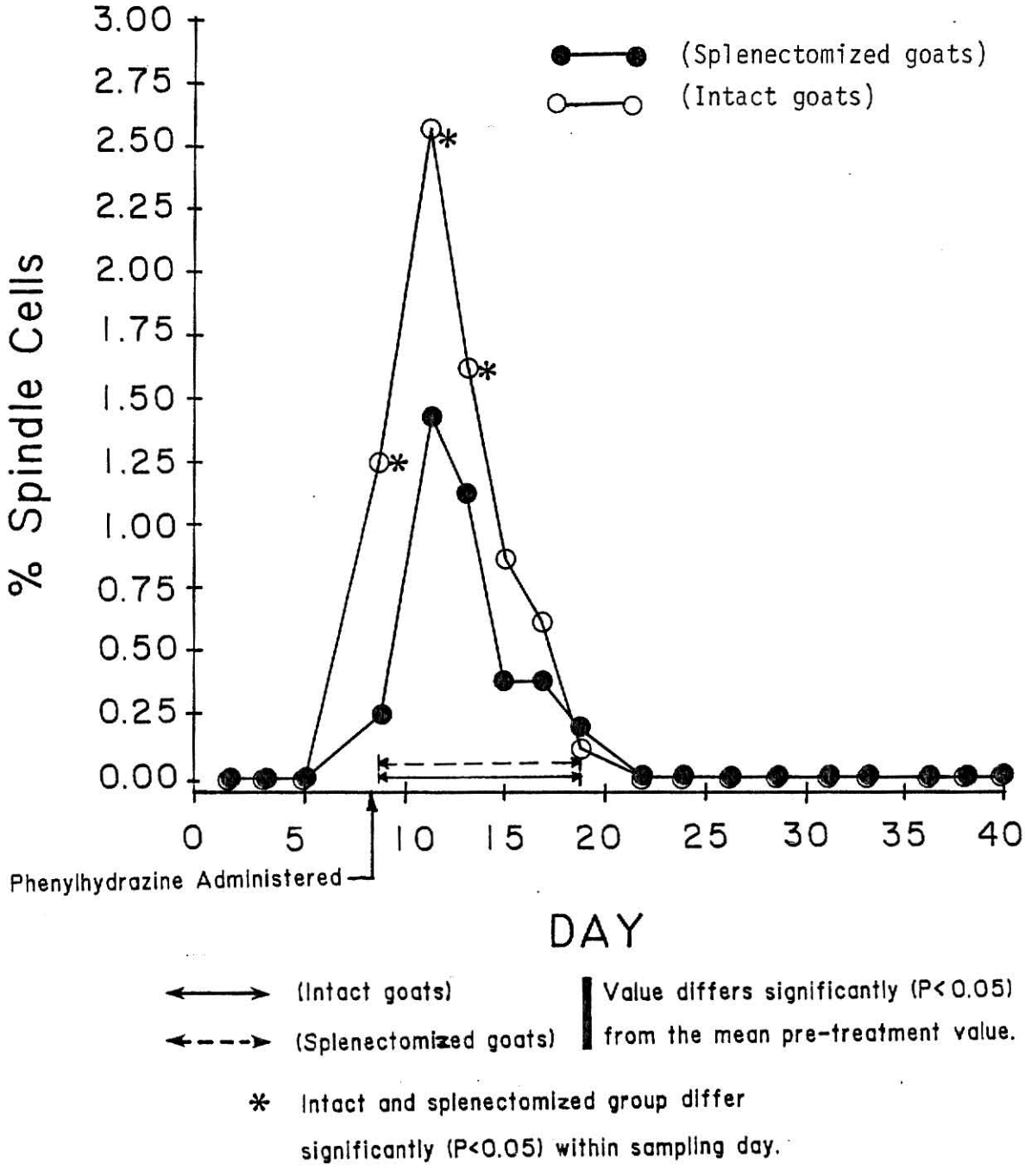

3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
% Spindle Cells
(Splenectomized goats)
(Intact goats)
0
5
10
15
20
25
30
35
40
Phenylhydrazine Administered
DAY
(Intact goats)
(Splenectomized goats)
Value differs significantly (P< 0.05) from the mean pre-treatment value.
* Intact and splenectomized group differ significantly (P<0.05) within sampling day.

Fig. 6. Percentage of Reticulocytes in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration

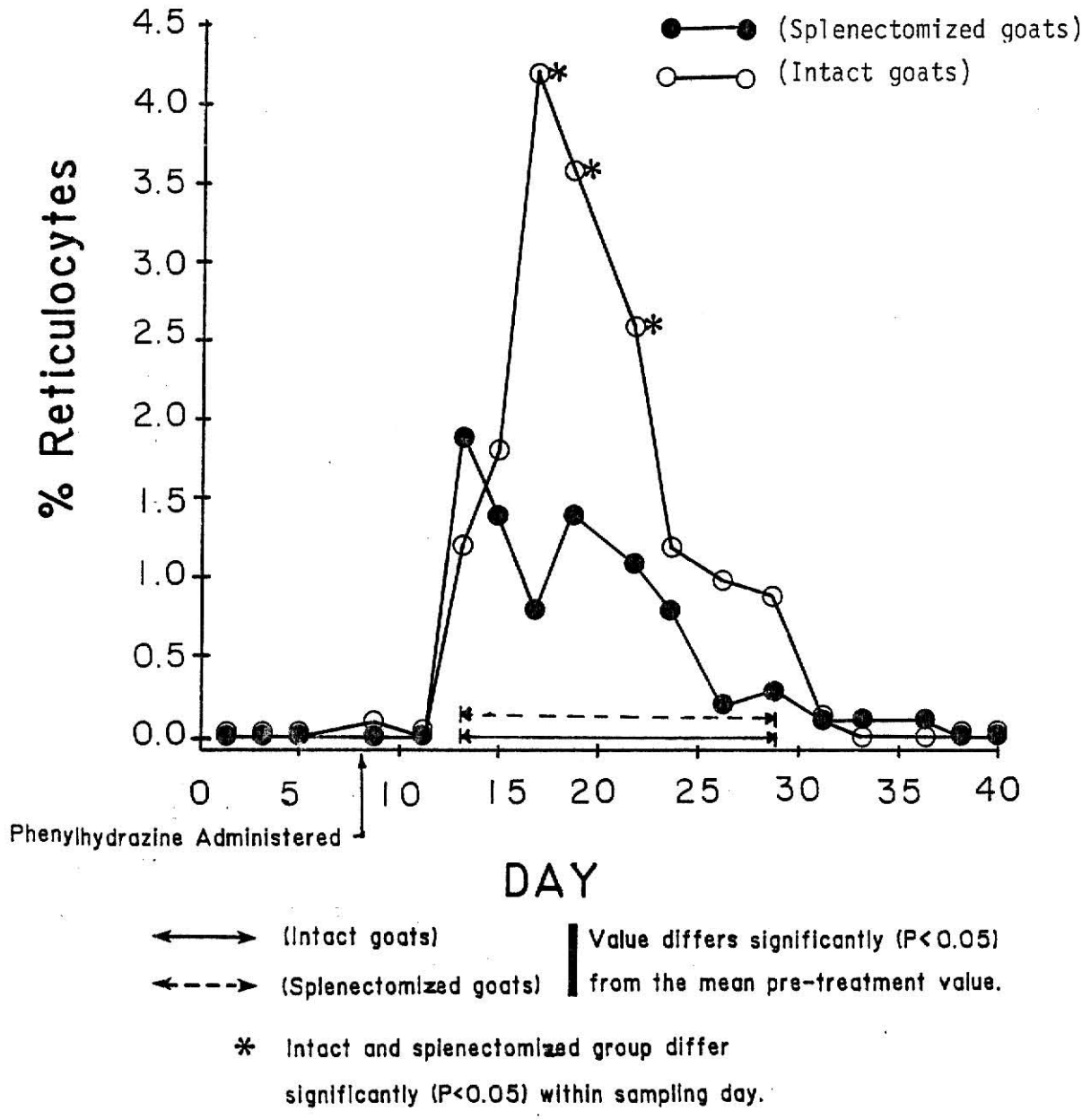

(Splenectomized goats)
(Intact goats)
% Reticulocytes
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
40
Phenylhydrazine Administered
DAY
(Intact goats)
(Splenectomized goats)
Value differs significantly (P<0.05) from the mean pre-treatment value.
* Intact and splenectomized group differ significantly (P<0.05) within sampling day.

Fig. 7. Erythrocytic Filtration Resistance in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration

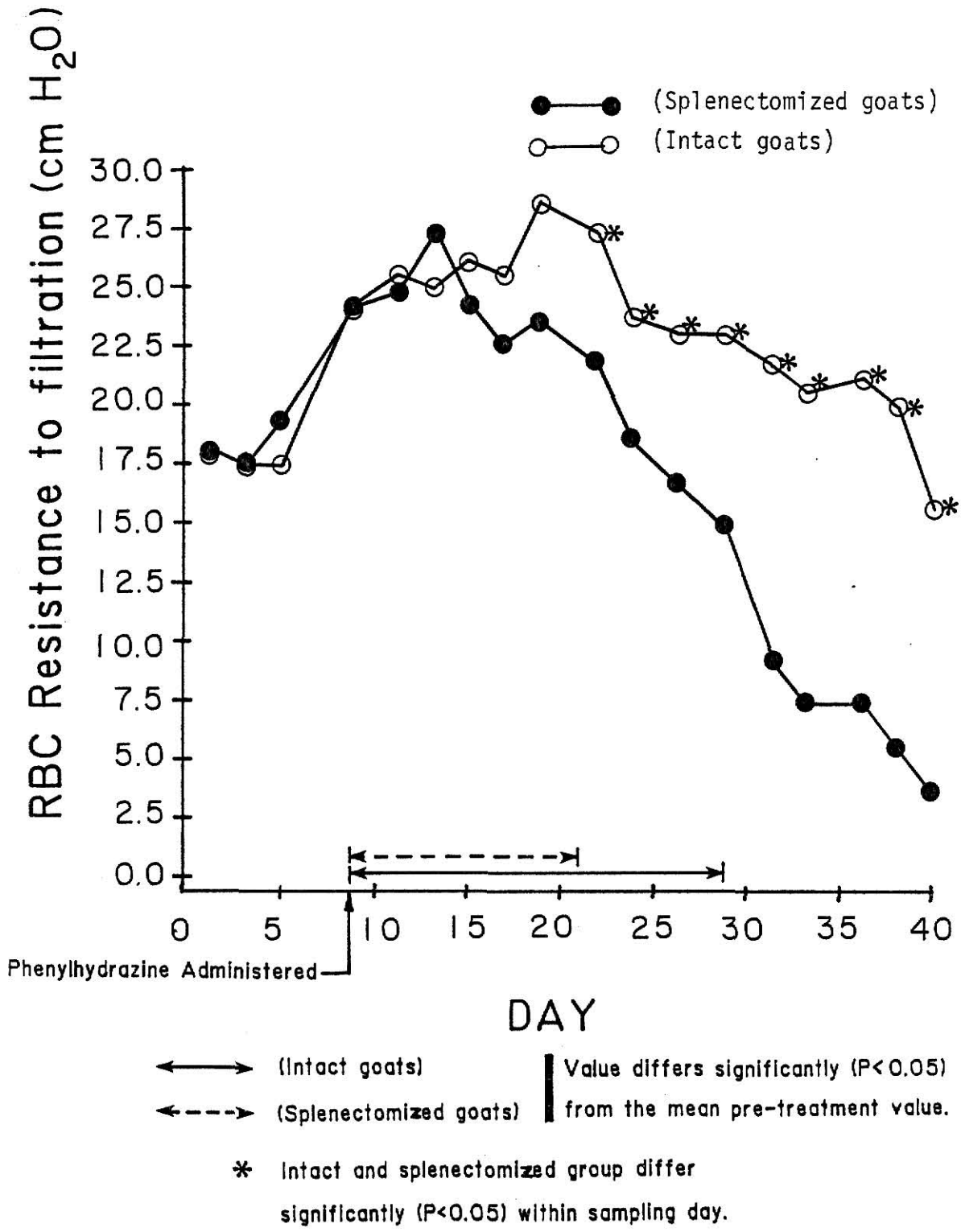
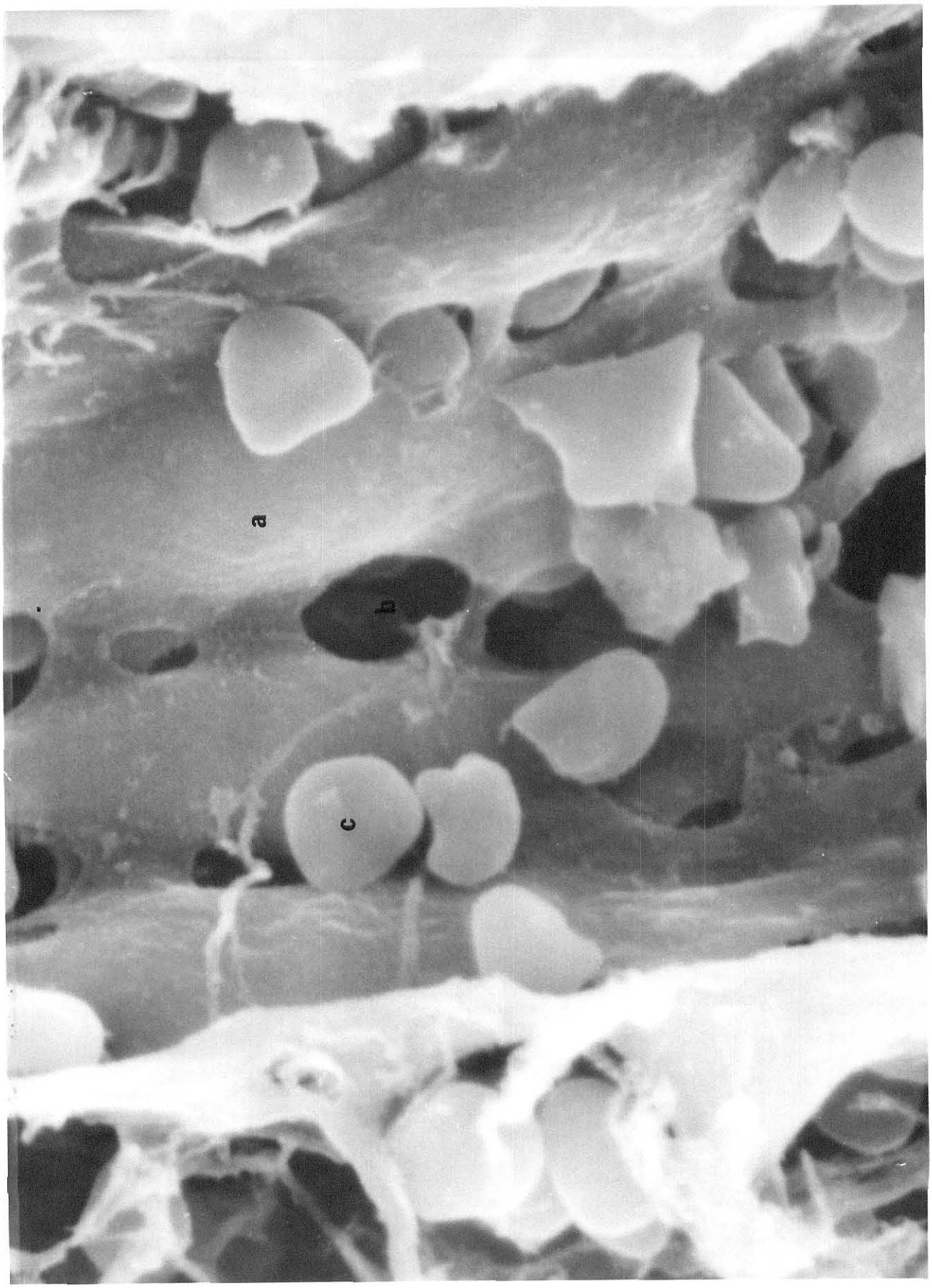

RBC Resistance to filtration (cm H_2O)
(Splenectomized goats)
(Intact goats)
30.0
27.5
25.0
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0
5
10
15
20
25
30
35
40
Phenylhydrazine Administered
DAY
(Intact goats)
(Splenectomized goats)
Value differs significantly (P< 0.05) from the mean pre-treatment value.
* Intact and splenectomized group differ significantly (P<0.05) within sampling day.

Fig. 8. Scanning Electron Photograph of a Splenic Sinus of the Goat (X5000)

(a) endothelial cells lining the splenic sinus

(b) interendothelial slits

(c) red blood cells


a
b
c

DISCUSSION

Heinz body formation and the disappearance rate of Heinz body containing red cells were strikingly similar in the intact and splenectomized goats. This finding strongly suggested that the presence of the spleen had apparently minimal effect on the rate of removal of Heinz body containing cells or the Heinz bodies themselves. The disappearance phase of the Heinz body curves obtained for both groups of goats was most likely due to the removal of affected cells and/or the pitting of Heinz bodies from the circulating cells. The rapid decreases in PCV and red cell numbers observed following Heinz body formation indicated that red cells were indeed being removed from the circulation. As reticulocytosis developed and non-Heinz body containing cells entered the circulation, the percentage of Heinz body containing cells began to fall due to the dilutional effect of the non-affected cells. During the disappearance phase, red cell removal probably continued at a significant but more subdued rate, since blood PCV, hemoglobin concentration and RBC counts rose towards pretreatment levels. This indicated that red cell production was exceeding the rate of cell removal. Since the spleen and only the spleen can perform the function of red cell pitting,[1] pitting would seemingly not account for the disappearance of Heinz bodies in the asplenic goats. If splenic pitting accounted in part for the disappearance of Heinz bodies in the intact goats, it appears to have been minimal since the disappearance rates for both groups of goats were virtually the same. The disappearance of Heinz bodies from the circulation thus appeared to be primarily due to the extraction of affected red cells with the spleen playing a minimal role in red cell and Heinz body removal.

Since the spleen seemingly had little effect on the disappearance of Heinz bodies, it might be assumed that Heinz body removal in the goat is dependent on other organs. Indeed, Crosby found that the normal hemolytic functions of the spleen were assumed by other body organs in splenectomized humans.[10] Studies have demonstrated that the entire red cell and its Heinz body may also be removed by the liver and bone marrow.[7,8,19,20] The removal of Heinz bodies in goats may therefore be primarily dependent on these organs and not the spleen.

The findings in the present study were similar to those reported for the cat by Jain.[15] Jain found that transfused Heinz body containing red cells were removed from the circulation of intact and splenectomized cats at similar rates. Furthermore, splenectomy performed on a cat with a high, but declining Heinz body count did not prevent further reduction in Heinz body numbers. Jain concluded that the spleen played a minor role in the removal of Heinz bodies in the cat.

The interendothelial slits found in the wall of the splenic sinuses play an important role in the culling of slightly or moderately damaged erythrocytes,[4] red cell containing large Heinz bodies,[6,7,8] and erythrocytes at the end of their life span,[4] because such cells are trapped within the narrow splenic slits and removed by macrophages.[4] Utilizing scanning electron microscopy, the interendothelial slits of the goat splenic sinus were found to appear large relative to red cells (Fig. 8). In the human spleen, these slits are about 1 to 2 μm in diameter.[1] Using the goat red cell as a rough micrometer and assuming the red cells viewed in the splenic sinus were approximately normal in diameter (3.3 μm as reported by Smith et al.[21]), the splenic slits appeared to be larger

than 1 to 2 μm. It thus appears that the interendothelial slits of the goat spleen may be relatively large, a finding which might explain the minor role which the goat spleen seems to play in the removal of Heinz body containing red cells or Heinz body pitting. On the other hand, the large slits may also be due to fixation or preparation artiface and a true representation of the slits may not have been attained.

The rapid falls in blood packed cell volume, hemoglobin concentration, and red cell counts for both the intact and splenectomized goats following phenylhydrazine administration were qualitatively similar to previous findings.[5] The degree of anemia produced in the goats by phenylhydrazine administration was not as marked as produced previously using the same animals.[5] Thus, it appears that phenylhydrazine when used in a repeated manner to produce separate episodes of anemia becomes attenuated in its effect.

The formation of spindled cells following phenylhydrazine treatment was similar to that found previously,[5] with both groups of goats exhibiting spindle cell formation.

Both groups of goats exhibited a mild to moderate reticulocytosis as a response to the anemia with the intact goats exhibiting a significantly greater response than the splenectomized goats. Since splenectomy does not affect reticulocyte numbers,[2] the variation between the groups may be due to extramedullary hemopoiesis by the spleen.

The changes in red cell indices for both groups of animals were basically similar, with rises in the MCV and MCH being found for both groups. Increases in MCV and MCH are expected in regenerative anemias with reticulocytosis.[22]

The increased red cell filtration resistance (decreased deformability) found in both the intact and asplenic goats following the appearance of Heinz bodies and reticulocytes was similar to that found previously.[5] Again, it appeared that the presence of Heinz bodies resulted in decreased deformability and that deformability decreased even further in degree when reticulocytes appeared. Of interest was the rapid fall in filtration resistance found for the splenectomized goats relative to the intact goats in the latter half of the study. From study day 22 through the final day of the study, the asplenic goats were significantly lower in filtration resistance than the intact group indicating that they had a red cell population of much greater deformability. Filtration resistance in the asplenic group even fell far below the pretreatment values found for the group (Fig. 7).

The absence of the spleen seemingly affected the deformability of the red cell population present. Following splenectomy, red cell volume has been found to remain normal while red cell surface area becomes larger with the cells becoming thinner.[11,23] Splenic absence in goats may also result in the formation of a red cell population consisting of cells with increased surface area relative to volume (thinner cells). These thinner cells might be expected to be more deformable. Deformability of the red cell is dependent on the maintenance of the normal ratio of cell surface area to volume.[24] Spherocytes which possess decreased surface area relative to cell volume exhibit decreased deformability.[25,26] It would seem reasonable that thinner cells might exhibit increased deformability and decreased resistance to filtration. The increased deformability found for the asplenic goats in the latter part of the study period may reflect the absence of splenic modeling of the red cells.

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SECTION III

APPENDIX

Table 1. Mean Blood Packed Cell Volumes in Goats Before and After Phenylhydrazine Administration.*

Day	Mean Packed Cell Volume (%) ± 1 Standard Deviation
1	30.37 ± 5.73
3	30.00 ± 5.63
5	30.12 ± 5.33
9	23.00 ± 4.66
11	21.62 ± 3.58
13	18.25 ± 2.25
15	15.12 ± 2.75
17	13.00 ± 2.27
19	15.00 ± 1.85
22	17.87 ± 2.35
24	21.37 ± 2.44
26	23.12 ± 4.39
29	23.62 ± 4.84
31	25.25 ± 4.92
33	25.37 ± 5.53
36	25.12 ± 5.14
38	26.87 ± 4.88
40	26.87 ± 5.69
55	27.87 ± 4.32

*Phenylhydrazine treatment commenced on day 8.

Table 2. Percentage of Erythrocytes Exhibiting Heinz Bodies in Goats Before and After Phenylhydrazine Administration.*

Day	Mean (%) Erythrocytes with Heinz bodies ± 1 Standard Deviation
1	0.90 ± 0.71
3	1.45 ± 0.99
5	1.30 ± 0.59
9	87.22 ± 4.11
11	92.37 ± 5.63
13	98.45 ± 1.24
15	95.60 ± 2.43
17	73.77 ± 12.07
19	70.22 ± 14.57
22	55.42 ± 11.99
24	44.47 ± 6.92
26	37.77 ± 3.67
29	34.82 ± 5.75
31	32.70 ± 7.94
33	30.37 ± 7.39
36	25.60 ± 3.61
38	24.37 ± 3.00
40	23.00 ± 2.81
55	21.32 ± 3.94

*Phenylhydrazine treatment commenced on day 8

Table 3. Percentage of Reticulocytes in Goats Before and After Phenylhydrazine Administration.*

Day	% Reticulocytes ± 1 Standard deviation
1	0.05 ± 0.09
3	0.05 ± 0.14
5	0.07 ± 0.15
9	0.12 ± 0.15
11	0.55 ± 0.53
13	3.42 ± 2.11
15	8.05 ± 2.07
17	7.00 ± 2.89
19	11.85 ± 2.75
22	7.27 ± 2.01
24	4.37 ± 2.03
26	2.65 ± 1.76
29	0.80 ± 0.81
31	0.37 ± 0.98
33	0.12 ± 0.15
36	0.05 ± 0.09
38	0.00 ± 0.00
40	0.00 ± 0.00
55	0.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 4. Percentage of Erythrocytes Exhibiting Hemoglobin Crystal Formation and Spindling in Goats Before and After Phenylhydrazine Administration.*

Day	% Spindled Red Cells ± 1 Standard Deviation
1	0.00 ± 0.00
3	0.00 ± 0.00
5	0.00 ± 0.00
9	5.65 ± 2.32
11	7.00 ± 4.08
13	7.12 ± 3.63
15	5.40 ± 2.99
17	0.67 ± 0.81
19	0.00 ± 0.00
22	0.00 ± 0.00
24	0.00 ± 0.00
26	0.00 ± 0.00
29	0.00 ± 0.00
31	0.00 ± 0.00
33	0.00 ± 0.00
36	0.00 ± 0.00
38	0.00 ± 0.00
40	0.00 ± 0.00
55	0.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 5. Red Cell Counts in Goats Before and After Phenylhydrazine Administration.*

Day	Mean Red Cell Count (x $10^6/\mu l$) ± 1 Standard Deviation
1	18.57 ± 2.67
3	18.42 ± 2.72
5	18.66 ± 2.13
9	15.30 ± 3.21
11	13.76 ± 2.32
13	12.51 ± 1.95
15	6.98 ± 3.59
17	4.30 ± 1.61
19	4.23 ± 1.40
22	6.53 ± 1.18
24	7.15 ± 1.68
26	8.38 ± 1.68
29	8.23 ± 1.85
31	8.60 ± 1.99
33	9.10 ± 2.27
36	9.32 ± 2.49
38	10.84 ± 1.84
40	11.22 ± 1.69
55	12.06 ± 1.72

*Phenylhydrazine treatment commenced on day 8

Table 6. Hemoglobin Concentration in Goats Before and After Phenylhydrazine Administration.*

Day	Mean Hemoglobin Conc. (g/dl) ± 1 Standard Deviation
1	10.96 ± 2.47
3	10.87 ± 1.97
5	10.99 ± 1.91
9	8.45 ± 1.43
11	7.79 ± 0.99
13	6.62 ± 0.74
15	5.34 ± 0.97
17	4.29 ± 0.87
19	5.22 ± 0.67
22	5.86 ± 0.61
24	7.16 ± 0.59
26	7.63 ± 1.34
29	7.91 ± 1.67
31	8.65 ± 2.16
33	8.57 ± 2.11
36	9.01 ± 1.83
38	9.65 ± 2.23
40	9.73 ± 2.25
55	9.92 ± 2.19

*Phenylhydrazine treatment commenced on day 8

Table 7. Erythrocytic Filtration Resistance in Goats Before and After Phenylhydrazine Administration.*

Day	Resistance to Filtration (cm H_2O) ± 1 Standard Deviation
1	4.15 ± 1.09
3	3.80 ± 1.05
5	3.84 ± 1.09
9	15.01 ± 2.46
11	17.60 ± 3.70
13	27.44 ± 2.43
15	35.59 ± 5.54
17	36.65 ± 10.85
19	49.72 ± 6.65
22	41.32 ± 8.89
24	42.59 ± 4.78
26	36.01 ± 3.56
29	39.66 ± 6.18
31	38.87 ± 4.66
33	35.42 ± 4.60
36	36.06 ± 6.66
38	31.11 ± 4.11
40	30.16 ± 3.35
55	25.20 ± 3.17

*Phenylhydrazine treatment commenced on day 8

Table 8. Erythrocytic Osmotic Fragility in Goats Before Phenylhydrazine Administration.

% Salt Concentration	% RBC Lysis in Hypotonic Saline Solution ± 1 Standard Deviation
0.85	0.90 ± 0.40
0.80	1.53 ± 1.03
0.75	4.62 ± 2.78
0.70	13.85 ± 7.06
0.65	41.22 ± 6.86
0.60	63.95 ± 10.92
0.55	85.95 ± 10.92
0.50	94.54 ± 3.17
0.45	96.31 ± 2.69
0.40	96.59 ± 2.51
0.35	97.67 ± 2.40
0.30	98.72 ± 0.58
0.25	99.08 ± 0.46
0.20	99.46 ± 0.56
0.00	100.00 ± 0.00

Table 9. Erythrocytic Osmotic Fragility in Goats Following Phenylhydrazine Administration (Day 11).*

% Salt Concentration	% RBC Lysis in Hypotonic Saline Solution ± 1 Standard Deviation
0.85	3.10 ± 1.81
0.80	14.31 ± 12.31
0.75	26.30 ± 12.70
0.70	46.47 ± 16.55
0.65	77.42 ± 18.38
0.60	86.82 ± 9.70
0.55	92.71 ± 3.79
0.50	94.49 ± 3.95
0.45	97.29 ± 2.39
0.40	97.57 ± 2.21
0.35	97.75 ± 1.99
0.30	97.93 ± 1.94
0.25	98.45 ± 1.60
0.20	99.08 ± 0.63
0.00	100.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 10. Erythrocytic Osmotic Fragility in Goats Following Phenylhydrazine Administration (Day 19).*

% Salt Concentration	% RBC Lysis in Hypotonic Saline Solution ± 1 Standard Deviation
0.85	5.10 ± 3.02
0.80	17.24 ± 8.29
0.75	37.47 ± 8.85
0.70	64.23 ± 8.46
0.65	80.96 ± 8.95
0.60	87.92 ± 8.81
0.55	92.44 ± 4.91
0.50	93.05 ± 5.20
0.45	94.58 ± 4.05
0.40	94.89 ± 4.20
0.35	96.04 ± 3.78
0.30	97.16 ± 2.62
0.25	98.40 ± 1.06
0.20	99.23 ± 0.77
0.00	100.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 11. Erythrocytic Osmotic Fragility in Goats Following Phenylhydrazine Administration (Day 29).*

% Salt Concentration	% RBC Lysis in Hypotonic Saline Solution ± 1 Standard Deviation
0.85	3.23 ± 1.40
0.80	12.96 ± 7.51
0.75	30.50 ± 9.22
0.70	51.00 ± 10.88
0.65	72.88 ± 7.46
0.60	82.72 ± 8.12
0.55	87.85 ± 6.17
0.50	92.61 ± 3.63
0.45	95.95 ± 1.57
0.40	96.50 ± 1.35
0.35	97.31 ± 1.12
0.30	97.93 ± 1.25
0.25	98.30 ± 1.25
0.20	99.25 ± 0.37
0.00	100.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 12. Erythrocytic Osmotic Fragility in Goats Following Phenylhydrazine Administration (Day 38).*

% Salt Concentration	% RBC Lysis in Hypotonic Saline Solution ± 1 Standard Deviation
0.85	3.02 ± 2.01
0.80	16.21 ± 4.63
0.75	32.15 ± 8.65
0.70	49.39 ± 4.32
0.65	73.99 ± 5.92
0.60	78.42 ± 5.32
0.55	83.10 ± 5.95
0.50	87.00 ± 5.61
0.45	90.32 ± 3.31
0.40	92.00 ± 3.98
0.35	94.39 ± 2.92
0.30	95.89 ± 2.30
0.25	96.92 ± 1.64
0.20	98.54 ± 0.88
0.00	100.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 13. Erythrocytic Mean Corpuscular Volume Attained in Goats Before and After Phenylhydrazine Administration.*

Day	Erythrocytic Mean Corpuscular Volume (fl) ± 1 Standard Deviation
1	16.42 ± 1.31
3	16.26 ± 1.43
5	15.92 ± 1.45
9	15.17 ± 1.80
11	15.88 ± 2.49
13	14.81 ± 2.44
15	25.28 ± 10.10
17	32.61 ± 9.79
19	32.85 ± 5.35
22	27.97 ± 5.06
24	31.48 ± 8.54
26	28.19 ± 5.74
29	29.22 ± 4.95
31	29.82 ± 3.70
33	28.65 ± 6.44
36	27.64 ± 4.34
38	24.92 ± 3.20
40	23.97 ± 3.93
55	23.19 ± 2.52

*Phenylhydrazine treatment commenced on day 8

Table 14. Erythrocytic Mean Corpuscular Hemoglobin Attained in Goats Before and After Phenylhydrazine Administration.*

Day	Erythrocytic Mean Corpuscular Hemoglobin (pg) ± 1 Standard Deviation
1	5.90 ± 0.72
3	5.90 ± 0.51
5	5.87 ± 0.55
9	5.60 ± 0.70
11	5.72 ± 0.66
13	5.22 ± 0.88
15	9.60 ± 2.87
17	10.72 ± 4.55
19	10.74 ± 2.87
22	9.26 ± 2.05
24	10.81 ± 3.39
26	9.36 ± 2.18
29	9.81 ± 1.68
31	10.14 ± 1.49
33	9.58 ± 1.77
36	9.94 ± 1.71
38	8.92 ± 1.59
40	8.65 ± 1.44
55	8.21 ± 1.24

*Phenylhydrazine treatment commenced on day 8

Table 15. Erythrocytic Mean Corpuscular Hemoglobin Concentration Attained in Goats Before and After Phenylhydrazine Administration.*

Day	Erythrocytic Mean Corpuscular Hemoglobin Conc.(%) ± 1 Standard Deviation
1	35.88 ± 1.68
3	36.28 ± 0.83
5	36.90 ± 1.61
9	36.95 ± 1.42
11	36.20 ± 1.60
13	35.26 ± 2.17
15	35.33 ± 1.12
17	32.88 ± 2.25
19	34.80 ± 1.46
22	33.01 ± 3.16
24	34.25 ± 4.14
26	33.09 ± 1.51
29	33.87 ± 0.91
31	34.00 ± 2.63
33	34.10 ± 2.53
36	36.09 ± 3.80
38	35.62 ± 2.30
40	36.19 ± 2.36
55	35.35 ± 3.45

*Phenylhydrazine treatment commenced on day 8

Table 16. Mean Blood Packed Cell Volumes in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration.*

Day	Mean Blood Packed Cell Volume (%) ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
1	26.50 ± 4.65	30.50 ± 1.91
3	25.75 ± 3.30	29.75 ± 2.22
5	26.00 ± 3.74	29.50 ± 2.08
9	24.00 ± 2.94	27.00 ± 2.16
11	22.50 ± 2.52	26.00 ± 2.16
13	20.00 ± 3.16	21.25 ± 3.59
15	18.75 ± 3.30	19.75 ± 2.87
17	18.00 ± 3.74	18.75 ± 3.86
19	19.50 ± 4.20	18.00 ± 3.56
22	20.50 ± 5.80	19.25 ± 3.09
24	20.75 ± 4.79	20.25 ± 2.36
26	21.50 ± 3.70	20.75 ± 1.26
29	21.50 ± 1.29	21.75 ± 1.71
31	23.25 ± 1.89	22.25 ± 2.50
33	23.75 ± 1.71	23.00 ± 2.83
36	24.25 ± 2.22	23.25 ± 2.63
38	26.00 ± 1.63	24.75 ± 2.87
40	27.00 ± 1.41	25.25 ± 3.20

*Phenylhydrazine treatment commenced on day 8

Table 17. Percentage of Erythrocytes Exhibiting Heinz Bodies in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration.*

Day	Mean (%) Erythrocytes with Heinz Bodies ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
1	13.00 ± 3.60	17.15 ± 9.80
3	11.25 ± 2.20	10.20 ± 4.49
5	9.85 ± 2.17	8.50 ± 1.67
9	70.90 ± 12.84	71.55 ± 6.43
11	96.90 ± 1.10	97.30 ± 2.13
13	92.10 ± 6.91	95.70 ± 3.89
15	94.60 ± 4.58	97.40 ± 1.30
17	90.95 ± 8.02	90.65 ± 9.91
19	76.40 ± 7.86	73.75 ± 7.12
22	70.85 ± 14.39	73.65 ± 9.61
24	48.85 ± 6.46	58.20 ± 5.51
26	42.72 ± 10.27	51.60 ± 5.29
29	38.10 ± 15.50	41.35 ± 14.79
31	27.55 ± 9.45	25.40 ± 7.60
33	23.00 ± 8.81	22.70 ± 12.07
36	23.20 ± 5.63	22.75 ± 8.33
38	20.15 ± 5.88	21.50 ± 8.01
40	19.30 ± 7.53	20.00 ± 10.58

*Phenylhydrazine treatment commenced on day 8

Table 18. Percentage of Reticulocytes in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration.*

Day	% Reticulocytes ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
1	0.00 ± 0.00	0.00 ± 0.00
3	0.00 ± 0.00	0.00 ± 0.00
5	0.00 ± 0.00	0.00 ± 0.00
9	0.05 ± 0.10	0.00 ± 0.00
11	0.00 ± 0.00	0.00 ± 0.00
13	1.20 ± 1.04	1.90 ± 2.26
15	1.85 ± 1.01	1.40 ± 0.95
17	4.25 ± 5.11	0.85 ± 1.04
19	3.60 ± 2.42	1.45 ± 1.37
22	2.65 ± 2.14	1.15 ± 1.10
24	1.25 ± 0.93	0.80 ± 0.59
26	1.00 ± 1.04	0.15 ± 0.10
29	0.90 ± 1.15	0.30 ± 0.20
31	0.05 ± 0.10	0.05 ± 0.10
33	0.00 ± 0.00	0.05 ± 0.10
36	0.00 ± 0.00	0.05 ± 0.10
38	0.00 ± 0.00	0.00 ± 0.00
40	0.00 ± 0.00	0.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 19. Percentage of Erythrocytes Exhibiting Hemoglobin Crystal Formation and Spindling in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration.*

Day	% Spindled Red Blood Cells ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
1	0.00 ± 0.00	0.00 ± 0.00
3	0.00 ± 0.00	0.00 ± 0.00
5	0.00 ± 0.00	0.00 ± 0.00
9	1.25 ± 0.91	0.25 ± 0.50
11	2.55 ± 0.82	1.45 ± 0.34
13	1.65 ± 0.47	1.10 ± 0.99
15	0.90 ± 0.38	0.40 ± 0.56
17	0.65 ± 0.91	0.40 ± 0.46
19	0.10 ± 0.20	0.20 ± 0.40
22	0.00 ± 0.00	0.00 ± 0.00
24	0.00 ± 0.00	0.00 ± 0.00
26	0.00 ± 0.00	0.00 ± 0.00
29	0.00 ± 0.00	0.00 ± 0.00
31	0.00 ± 0.00	0.00 ± 0.00
33	0.00 ± 0.00	0.00 ± 0.00
36	0.00 ± 0.00	0.00 ± 0.00
38	0.00 ± 0.00	0.00 ± 0.00
40	0.00 ± 0.00	0.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 20. Red Cell Counts in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration.*

Day	Red Cell Counts ($\times 10^6/\mu l$) ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
1	14.45 ± 3.85	16.12 ± 3.49
3	14.48 ± 3.62	15.02 ± 2.61
5	14.38 ± 3.45	14.75 ± 3.05
9	12.68 ± 2.51	13.60 ± 1.45
11	12.36 ± 2.79	13.91 ± 1.73
13	10.92 ± 1.19	12.59 ± 1.13
15	11.02 ± 2.10	11.66 ± 0.44
17	9.38 ± 3.11	9.00 ± 1.52
19	9.56 ± 3.50	8.75 ± 1.42
22	9.56 ± 3.98	7.42 ± 1.81
24	9.11 ± 3.86	7.78 ± 1.54
26	10.13 ± 4.04	9.36 ± 1.51
29	11.01 ± 4.10	10.40 ± 2.16
31	10.75 ± 3.49	10.04 ± 2.00
33	10.84 ± 2.57	10.15 ± 1.38
36	11.51 ± 3.09	11.11 ± 1.19
38	12.57 ± 3.35	11.42 ± 1.47
40	13.12 ± 2.95	12.60 ± 1.58

*Phenylhydrazine treatment commenced on day 8

Table 21. Hemoglobin Concentration in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration.*

Day	Mean Hemoglobin Concentration (g/dl) ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
1	9.71 ± 1.71	11.14 ± 0.99
3	9.92 ± 0.83	11.04 ± 0.89
5	9.79 ± 0.97	11.05 ± 0.94
9	9.27 ± 0.82	10.02 ± 0.91
11	8.22 ± 8.76	9.66 ± 0.96
13	7.62 ± 1.17	7.39 ± 1.23
15	7.30 ± 1.14	7.04 ± 1.34
17	6.96 ± 1.11	6.86 ± 1.53
19	7.05 ± 1.25	6.79 ± 1.49
22	7.34 ± 1.75	7.22 ± 1.60
24	7.49 ± 1.32	7.67 ± 0.96
26	7.90 ± 0.77	8.06 ± 0.56
29	7.91 ± 0.54	8.32 ± 0.39
31	7.95 ± 1.04	8.41 ± 0.41
33	8.64 ± 0.74	8.39 ± 0.77
36	8.80 ± 0.92	8.39 ± 1.07
38	9.34 ± 0.26	8.80 ± 0.93
40	9.64 ± 0.34	9.01 ± 0.87

*Phenylhydrazine treatment commenced on day 8

Table 22. Erythrocytic Filtration Resistance in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration.*

Day	Erythrocytic Resistance to Filtration (cm H_2O) ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
1	18.40 ± 3.58	18.32 ± 3.31
3	17.42 ± 3.00	17.75 ± 2.84
5	17.72 ± 1.98	19.07 ± 1.22
9	24.60 ± 1.91	24.30 ± 1.37
11	25.40 ± 0.62	25.15 ± 2.95
13	25.27 ± 1.33	27.60 ± 1.27
15	26.15 ± 2.33	24.15 ± 1.10
17	25.82 ± 1.96	22.62 ± 1.90
19	28.67 ± 4.75	23.72 ± 6.00
22	27.52 ± 2.06	22.07 ± 4.22
24	23.97 ± 2.62	18.77 ± 1.51
26	23.32 ± 3.05	16.77 ± 1.27
29	23.02 ± 4.34	14.92 ± 1.62
31	21.72 ± 4.00	9.32 ± 3.07
33	20.90 ± 7.08	7.70 ± 3.04
36	20.95 ± 8.88	7.30 ± 2.96
38	20.05 ± 5.48	5.45 ± 0.83
40	15.90 ± 6.54	3.72 ± 0.94

*Phenylhydrazine treatment commenced on day 8

Table 23. Erythrocytic Osmotic Fragility in Intact and Splenectomized Goats Before Phenylhydrazine Administration.

% Salt Conc.	% RBC Lysis in Hypotonic Saline Solution ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
0.85	1.98 ± 1.70	1.49 ± 0.68
0.80	4.20 ± 1.72	3.19 ± 1.84
0.75	16.64 ± 4.57	29.76 ± 16.77
0.70	61.29 ± 18.83	51.24 ± 12.36
0.65	87.02 ± 4.61	80.98 ± 4.19
0.60	91.37 ± 3.52	90.39 ± 2.10
0.55	94.40 ± 3.26	95.80 ± 0.52
0.50	96.62 ± 1.82	96.09 ± 0.68
0.45	96.76 ± 1.58	96.57 ± 0.11
0.40	96.99 ± 1.74	96.94 ± 0.17
0.35	97.30 ± 1.43	97.71 ± 0.47
0.30	97.83 ± 1.57	99.31 ± 0.44
0.25	98.48 ± 0.98	99.54 ± 0.35
0.20	98.88 ± 0.82	99.62 ± 0.19
0.00	100.00 ± 0.00	100.00 ± 0.00

Table 24. Erythrocytic Osmotic Fragility in Intact and Splenectomized Goats Following Phenylhydrazine Administration (Day 11).*

% Salt Conc.	% RBC Lysis in Hypotonic Saline Solution ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
0.85	1.16 ± 0.64	2.21 ± 2.22
0.80	5.73 ± 4.17	9.03 ± 6.08
0.75	21.50 ± 9.62	26.95 ± 9.89
0.70	49.80 ± 9.96	47.97 ± 11.60
0.65	75.90 ± 8.35	67.73 ± 9.05
0.60	80.98 ± 6.09	75.28 ± 7.60
0.55	86.47 ± 4.72	83.89 ± 10.37
0.50	89.62 ± 5.39	87.53 ± 8.67
0.45	90.13 ± 4.84	90.51 ± 6.52
0.40	91.26 ± 4.50	93.38 ± 6.64
0.35	92.43 ± 4.91	95.36 ± 3.82
0.30	95.42 ± 4.72	97.70 ± 1.20
0.25	97.13 ± 2.47	98.13 ± 1.22
0.20	98.73 ± 0.64	98.65 ± 0.93
0.00	100.00 ± 0.00	100.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 25. Erythrocytic Osmotic Fragility in Intact and Splenectomized Goats Following Phenylhydrazine Administration (Day 19).*

% Salt Conc.	% RBC Lysis in Hypotonic Saline Solution ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
0.85	2.47 ± 0.73	1.80 ± 1.54
0.80	10.52 ± 6.19	6.26 ± 2.98
0.75	18.20 ± 13.21	22.66 ± 13.21
0.70	44.13 ± 8.37	43.53 ± 11.91
0.65	76.24 ± 8.06	83.27 ± 9.01
0.60	82.90 ± 5.80	90.04 ± 4.93
0.55	91.19 ± 6.08	91.81 ± 4.94
0.50	93.66 ± 3.78	92.49 ± 4.45
0.45	94.85 ± 2.63	93.34 ± 4.70
0.40	96.69 ± 1.83	94.36 ± 3.57
0.35	97.57 ± 1.24	95.70 ± 3.89
0.30	98.34 ± 1.36	96.99 ± 3.00
0.25	98.64 ± 1.14	98.25 ± 1.28
0.20	99.13 ± 0.48	98.78 ± 0.88
0.00	100.00 ± 0.00	100.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 26. Erythrocytic Osmotic Fragility in Intact and Splenectomized Goats Following Phenylhydrazine Administration (Day 29).*

% Salt Conc.	% RBC Lysis in Hypotonic Saline Solution ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
0.85	3.19 ± 1.04	2.51 ± 1.00
0.80	12.93 ± 5.41	14.41 ± 6.22
0.75	21.17 ± 2.52	26.25 ± 6.22
0.70	40.06 ± 6.15	49.90 ± 12.59
0.65	77.12 ± 4.53	84.76 ± 6.15
0.60	80.08 ± 4.65	88.02 ± 4.35
0.55	86.41 ± 5.62	90.98 ± 2.53
0.50	88.00 ± 4.72	92.37 ± 2.32
0.45	90.30 ± 4.38	92.68 ± 2.20
0.40	92.87 ± 2.82	94.33 ± 1.72
0.35	94.45 ± 3.05	95.78 ± 1.56
0.30	95.56 ± 3.20	96.68 ± 1.17
0.25	96.66 ± 1.72	97.30 ± 0.66
0.20	98.20 ± 1.28	98.10 ± 0.74
0.00	100.00 ± 0.00	100.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 27. Erythrocytic Osmotic Fragility in Intact and Splenectomized Goats Following Phenylhydrazine Administration (Day 38).*

% Salt Conc.	% RBC Lysis in Hypotonic Saline Solution ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
0.85	1.88 ± 0.22	0.30 ± 0.39
0.80	3.88 ± 1.80	1.98 ± 1.87
0.75	14.25 ± 7.15	9.26 ± 6.45
0.70	40.08 ± 4.19	30.28 ± 12.97
0.65	79.05 ± 6.59	58.55 ± 15.68
0.60	86.34 ± 8.84	78.29 ± 6.07
0.55	92.03 ± 5.09	88.89 ± 5.76
0.50	93.34 ± 4.86	91.61 ± 3.63
0.45	93.52 ± 4.53	91.71 ± 3.44
0.40	94.65 ± 3.51	92.72 ± 4.40
0.35	95.77 ± 2.72	95.43 ± 1.48
0.30	97.09 ± 1.78	96.04 ± 1.37
0.25	98.31 ± 0.84	97.70 ± 1.16
0.20	99.15 ± 0.56	98.61 ± 0.83
0.00	100.00 ± 0.00	100.00 ± 0.00

*Phenylhydrazine treatment commenced on day 8

Table 28. Erythrocytic Mean Corpuscular Volume Attained in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration.*

Day	Erythrocytic Mean Corpuscular Volume (fl) ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
1	18.83 ± 3.85	19.38 ± 2.86
3	19.63 ± 2.85	20.05 ± 2.03
5	18.56 ± 3.27	20.39 ± 2.78
9	19.20 ± 2.59	19.97 ± 2.21
11	18.59 ± 2.72	18.83 ± 2.14
13	18.24 ± 0.96	16.94 ± 3.14
15	17.09 ± 1.02	16.93 ± 2.29
17	20.35 ± 5.95	20.89 ± 2.88
19	21.53 ± 4.24	21.06 ± 5.11
22	22.68 ± 4.13	27.51 ± 8.43
24	24.61 ± 5.99	26.97 ± 6.80
26	23.32 ± 7.69	22.70 ± 4.66
29	22.05 ± 9.21	21.63 ± 4.91
31	23.62 ± 8.92	22.62 ± 3.38
33	22.94 ± 6.14	22.79 ± 2.28
36	22.38 ± 7.22	21.03 ± 2.49
38	21.98 ± 7.01	21.79 ± 2.18
40	21.54 ± 6.02	20.17 ± 2.60

*Phenylhydrazine treatment commenced on day 8

Table 29. Erythrocyte Mean Corpuscular Hemoglobin Attained in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration.*

Day	Erythrocytic Mean Corpuscular Hemoglobin (pg) ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
1	6.93 ± 1.60	7.06 ± 0.99
3	7.58 ± 1.05	7.46 ± 0.99
5	7.04 ± 1.40	7.64 ± 1.14
9	7.45 ± 1.07	7.42 ± 0.86
11	6.79 ± 0.98	6.96 ± 0.28
13	6.96 ± 0.65	5.92 ± 1.24
15	6.66 ± 0.65	6.03 ± 1.11
17	8.26 ± 1.13	7.67 ± 1.47
19	7.87 ± 1.83	7.92 ± 2.05
22	8.24 ± 1.83	10.28 ± 3.33
24	9.06 ± 2.66	10.14 ± 2.18
26	8.77 ± 3.37	8.82 ± 1.82
29	8.01 ± 3.05	8.26 ± 1.74
31	8.16 ± 3.60	8.64 ± 1.73
33	8.34 ± 2.29	8.32 ± 0.64
36	8.03 ± 2.14	7.57 ± 0.85
38	7.80 ± 1.97	7.77 ± 0.93
40	7.56 ± 1.59	7.20 ± 0.81

*Phenylhydrazine treatment commenced on day 8

Table 30. Erythrocytic Mean Corpuscular Hemoglobin Concentration Attained in Intact and Splenectomized Goats Before and After Phenylhydrazine Administration.*

Day	Erythrocytic Mean Corpuscular Hemoglobin Conc (%) ± 1 Standard Deviation	
	Intact Goats	Splenectomized Goats
1	36.64 ± 1.06	36.47 ± 1.08
3	38.67 ± 1.69	37.14 ± 2.39
5	37.82 ± 1.78	37.45 ± 1.97
9	38.76 ± 1.42	37.15 ± 2.38
11	36.57 ± 1.02	38.20 ± 2.28
13	38.14 ± 1.05	34.90 ± 3.07
15	39.07 ± 2.48	35.52 ± 2.97
17	39.31 ± 6.63	36.50 ± 2.32
19	36.40 ± 1.64	37.59 ± 1.28
22	36.20 ± 2.47	37.24 ± 2.60
24	36.50 ± 2.66	37.92 ± 1.98
26	37.14 ± 3.04	38.84 ± 0.81
29	36.88 ± 3.11	38.32 ± 1.37
31	34.19 ± 3.91	38.02 ± 2.58
33	36.39 ± 2.12	36.62 ± 2.35
36	36.80 ± 1.28	36.04 ± 0.87
38	36.02 ± 2.52	35.60 ± 1.53
40	35.54 ± 2.40	35.79 ± 1.87

*Phenylhydrazine treatment commenced on day 8

STATISTICAL ANALYSIS

The raw data generated from this study was analyzed using repeated measure analysis (split plot), similar to randomized complete block with animal being the block and day being the treatment. Also the Duncan's multiple range test was used to compare animal response across days while the Least Significant Difference multiple test (LSD test) was used to compare responses of intact goats to splenectomized goats.

HEINZ BODY ANEMIA IN THE GOAT

by

ADEMOLA AJAYI

D. V. M., Ahmadu Bello University, Nigeria, 1977

AN ABSTRACT OF A THESIS

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Heinz bodies were produced in the circulating red cells of eight goats using phenylhydrazine hydrochloride as the inducing agent. The formation of Heinz bodies in virtually all of the circulating red cells was followed by the development of a regenerative anemia as the Heinz body containing red cells were removed from circulation.

Spindled or fusiform red cells appeared in the blood subsequent to phenylhydrazine administration.

Red cell deformability as measured by red cell resistance to filtration through a polycarbonate membrane was found to decrease following the formation of Heinz bodies. Red cell deformability decreased to an even greater degree with the emergence of reticulocytes. As Heinz bodies and reticulocytes disappeared from the circulation, red cell deformability began to increase, but never regained the deformability found during the pretreatment phase of the study.

Red cell osmotic fragility increased following phenylhydrazine administration and the appearance of Heinz bodies indicating either a direct alteration of the red cells by the chemical agent or alterations induced as a consequence of hemoglobin denaturation.

The role which the goat spleen played in the removal of Heinz bodies from the blood was determined by inducing Heinz body formation in four intact and four splenectomized goats. Heinz bodies developed rapidly in both groups following phenylhydrazine administration. Heinz body formation was followed by the development of a regenerative anemia in both groups as the Heinz body affected cells were removed.

Heinz body disappearance rates were virtually the same in the intact and asplenic goats indicating that the spleen played a minimal

role in removal of circulating Heinz bodies. Using scanning electron microscopy, the goat spleen was found to possess relatively large inter-endothelial sinus slits, a finding which could account for its minor role in Heinz body removal.

Red cell deformability in both groups of goats decreased following the appearance of Heinz bodies and reticulocytes. As reticulocytes and Heinz bodies disappeared from the circulation, red cell deformability began to increase in both groups with the asplenic goats exhibiting a significantly greater gain in deformability than the intact animals.