THE HEMATOLOGY OF BROILERS IN THE PRESENCE OF MILD STRESS, WITH REFERENCE TO THE HEMORRHAGIC SYNDROME

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Introduction

Since 1950 an apparently new and serious disease has been encountered in poultry. This disease appeared mainly in broiler flocks, but a few reports of this disease in poults and pullets in production have been made. This disease, the etiology of which is unknown, has been called "hemorrhagic syndrome" or hemorrhagic anemia."

To understand a pathological process, a knowledge of the physiology of the species involved is essential. Unfortunately avian physiology has not been extensively studied and documented; in many cases it is assumed that the physiology of the bird is very similar or identical to the physiology of mammals. This assumption may not be justified and could lead to the improper interpretation of experimental data. Even in fields such as endocrinology, where there is considerable research activity on the bird, there are wide gaps in our knowledge. Much fundamental research remains to be done in avian physiology; this in turn should simplify the study of avian pathology, particularly those diseases of non-infectious origin.

No other domestic species have been subjected to such radical changes in breeding and production techniques as have the chicken and the turkey. Intense inbreeding and development of fast-growing early-feathering strains, coupled with highly efficient growing rations and special brooding and housing methods have shortened the usual growing period of broilers and laying stock. This accelerated growth may change the pattern of response to disease and environmental change.

This experiment was designed to study the pattern of alteration of the blood, capillaries, and related tissues in the presence of mild stress and to compare these alterations with those observed in hemorrhagic anemia, in an effort to determine the role of stressing agents in the etiology of the disease.

Review of the Literature

Early history and description of hemorrhagic anemia

The first published report on the hemorrhagic syndrome of poultry was by Baker and Jaquette (1953). Their report was made after they had consistently observed the condition in birds brought to their Delaware laboratory over a period of three years. Couch et al. (1954) observed the condition in broiler flocks involving over 700,000 birds of which 30 per cent of the birds in each flock were affected. They reported the hemorrhagic syndrome to be different from avitaminosis-K in that it occurred when the ration contained 40 lbs. of alfalfa leaf per ton of feed. They expressed the opinion that the disease was not infectious since normal birds failed to contract the disease when fed the fecal contents of sick birds, or when injected with their blood. In this report they stated that certain individual pens of broilers in extensive plant operations may contract the disease, yet other birds on the same feed and from the same hatchery sources failed to show symptoms. These observations suggested that a stress factor may be involved. Stressing factors suggested were overcrowding, chilling, lack of feeder space, poor ventilation, coccidiosis, chronic respiratory disease and drug-fortified rations.

Hare et al., early in 1954, published a report in which they stated that the disease was, at that time, a threat to the very survival of the poultry industry. They pointed out that not only mortality but also carcass damage was a great financial loss. Their report indicated that the disease was active in all of the principle broiler areas, with Delaware,

Maryland, and Virginia suffering the greatest losses. These workers stated that the disease occurred from the first week of the chick's life, and that the highest incidence had occurred in chickens five to seven weeks of age. They remarked that even though this disease was not avitaminosis-K, vitamin K in the form of menadione seemed to relieve the condition. They emphasized that modern rations were lower in alfalfa leaf and other sources of K, and that sick birds may not have a high enough feed intake to meet their requirements for this vitamin. Medication of sick birds further reduced feed intake and also reduced the synthesis of vitamin K-like substances in the intestine. Solvent-extracted soy bean oil meals used in broiler rations are so low in fat that fat soluble vitamins such as vitamin K may be poorly absorbed. The work of Griminger et al. (1953), and Anderson et al. (1954) supported these theories.

Clinical symptoms and lesions

Cartrite (1954) described the clinical symptoms of hemorrhagic disease in detail and made some reference to tissue changes observed in Texas broilers. He was of the opinion that, as is often true in many disease conditions, there is an acute, sub-acute, and chronic form of the disease as it is seen in the diagnostic laboratory. Birds having the acute form of the hemorrhagic, anemic condition are usually well fleshed, die in a sitting position, are often dead on arrival at the laboratory, and have hemorrhage of any muscle or organ. The comb, muscles, liver and blood of these birds may be quite pale, and stained blood smears show many megaloblasts. Mortality may reach a certain pace and continue for days.

The subacutely anemic bird is pale with a "flop" comb and the musculature of this bird is thin. The muscles have a slimy feeling and show mild hemorrhage as compared with the acute form. There may be an erosion of the mucous membrane of the proventriculus and the horny lining of the gizzard. The subacute hemorrhagic anemic condition results in poor feed efficiency and lower market quality, although feed consumption may be good.

In the chronic anemic condition the comb is very shriveled. The feathers are not fluffed out because the birds are not preening themselves. As in the subacute condition the droppings are wet and there is an occasional fetid diarrhea. The gizzard is often eroded and reduced to one half the normal size. The small intestine may be inflamed. The blood picture reveals a change in the shape of many of the red blood cells. The failure of hemorrhagic birds to show similar lesions on autopsy is of interest.

The most detailed report on the tissue changes associated with hemorrhagic disease was published by Gray et al. (1954) of the Massachusetts Agricultural Experiment Station. Their report considered clinical, hematological, and histopathological observations. The clinical symptoms observed were similar to those reported by Cartrite. The cases reported included several thousand chickens. The majority of birds were 6-9 week old males; one flock consisted of pullets 14 weeks old. Most of the affected flocks were being raised for the broiler market, and were maintained on efficient rations and ideal conditions to promote rapid growth.

Generally the first signs of illness noted were either an increase of mortality without symptoms, or pale combs and diarrhea. Total mortality varied from a few to 40 percent, but in some cases losses eventually subsided. Increased excitability was reported by one owner. In the early stages of the disease coccidiosis was often suspected, and these workers (Gray et al., 1954) thought it significant that all of the chicks observed in this study were treated with anticoccidial drugs. Fifty percent of the flocks had received medication within the week.

The gross and microscopic findings were hemorrhage, bone marrow alteration and necrosis of the liver and intestine. Various stages of these findings were observed in several birds. Extravasated blood was commonly seen subcutaneously, within the skeletal and cardiac musculature and beneath the intestinal mucosa and subserosal connective tissue of the viscera. Occasionally the anterior chamber of the eye was filled with blood. Blood was detected in the feces and lumen of the intestine. Large hematomas developed in the necks of birds following hormone pellet implantation. Frequently however, affected birds showed little or no gross evidence of hemorrhage.

Marked alteration of the bone marrow was a prominent finding in birds during a mortality peak. Typically, the bone marrow was pink to yellow, and less frequently gray. Microscopically, the marrow of affected birds was more or less devoid of hematopoietic elements and was replaced with fatty tissue. There was conspicuous reduction of myelocytic elements, and in extreme cases only sinusoidal endothelial cells, interstitial reticular cells, and fat cells were present. A few lymphocytic foci were frequently seen.

Microscopic to massive areas of necrosis were seen in the liver. Septic thrombi were common and masses of bacteria were visible microscopically in the necrotic areas.

The involvement of the intestines was similar to that described by Cartrite.

The gross and microscopic picture of the spleen was variable. In this organ large numbers of hemosiderin-laden macrophages and focal hyaline necrosis were common findings. Commonly the capsule was thickened and occasionally subcapsular petechia were observed.

The kidneys appeared swollen and pale but hemorrhage was less fre- in this organ. Sections showed coagulation necrosis of the tubular epithelium in some areas.

Blood studies were conducted using standard techniques. No mention was made of the method of collecting blood, but a blood coagulation time of ten minutes or longer was common as shown by the capillary tube method. Table 1, taken from the article by Gray et al. (1954), is a rather detailed description of the blood picture in representative field cases of hemorrhagic anemia. This table is also a handy reference for normal blood values. The wide range of leukocyte counts shown in this table was due largely to fluctuations in the number of heterophils.

Bacteriological findings were those characteristic of secondary infections. Isolates were usually coliform, staphylococci, streptococci, or Proteus species.

A significant feature of the hemorrhagic syndrome of chickens observed by Gray et al. (1954), was the variability of findings in different flocks and in individual birds of the same flock. No lesion or sym-

Table 1. Blood Findings from a Representative Field Case of Hemorrhagic Syndrome***

BLOOD EXAMINATIONS

7-1/2 week White Rock Cockerels 4/6/54

Bird Number	1	2	3	4	Normal**
Coagulation Time, min.	40	10	15	13	4.5**
Hemoglobin, Gm./100 ml.	5.5	9.2	9.0	7.5	9.73
Red Blood Count x 10^6/mm.3	1.4	2.2	2.7	2.4	2.94
White Blood Count x 10^3/mm.3	1.0	2.0	17.0	3.0	29.4
Differential					
Cells Counted	Only 4 white	150	100	100	
Heterophils	cells found	0	25	0	20.9
Lymphocytes	in smears.	95	65	94	66.0
Monocytes	These were	4	8	5	8.1
Basophils	lymphocytes	1	2	0	3.1
Eosinphils		-	-	1	1.9
Red Cells show:					
Anisocytosis	++	++	+	++	
Polychromatophilia	+	+	-	+	
Hypochromia	+	-	-	-	
Thrombocytes x 10^3/mm.3	7.2	6.4	25.0	9.0	32.7

*Olson, Cornell Vet., 27 (1937):235-263
**Dukes. The Physiology of Domestic Animals, 6th Ed. (1947) Comstock Publishing Co., Ithaca, N. Y.
***Taken from Gray (1954)

tom was found consistently to a marked degree. Blood cell counts fluctuated during various phases of the condition so that it was necessary to evaluate the hematological data with the pathological state of the individual bird.

The symptoms and lesions described by Cartrite et al. (1954) and by Gray et al. (1954) have been generally accepted to be of diagnostic value. The number of hemorrhagic birds observed in this laboratory has been quite limited, however the symptoms and lesions compare favorably with those discussed in the literature.

Present history and occurrence of hemorrhagic anemia

Cover et al. (1954) reported that, although the course, symptomatology, and pathology have remained the same, the disease has been diagnosed less frequently since 1953. He suggested that broiler producers are now familiar with the disease and thus there are fewer requests for diagnosis. During a Symposium on Hemorrhagic Disease (1955), the panel agreed that the disease reached its peak in the late fall of 1954 and only sporadic outbreaks have occurred since then. Outbreaks in Texas were severe in the fall of 1955 according to Cartrite, and there is an occurrence of the condition in the Southeastern States at present. Members of the Symposium on Hemorrhagic Disease in 1955 also agreed that aplasia of bone marrow was common to many diseases and is not necessarily characteristic of the disease being discussed. The primary symptoms and lesions are those of anemia.

Theories on the etiology of hemorrhagic anemia

Many reports of hemorrhagic conditions, particularly those produced experimentally, have been compared with the hemorrhagic syndrome. Some

of these reports have implied that the disease had been reproduced in the laboratory. Gray et al. (1954) and Cover (1954) stated emphatically that the disease had never been produced experimentally. Gray et al. (1954) did remark, however, that the pathological and hematological findings in their paper are similar in many respects to those reported by Pritchard et al. (1952) and Sautter et al. (1952) concerning aplastic anemia of cattle. The disease in cattle was due to trichloroethylene-extracted soybean oil meal. When the same toxic meal was fed to chickens no untoward responses were observed. Balloun and Johnson (1953) and Eveleth and Goldsby (1953) did observe death, prolonged clotting time and retarded growth in chicks following the feeding of a similar soy bean oil meal. The prolonged clotting time in the latter experiment was not corrected with vitamin K. Because the hemorrhagic syndrome has appeared in flocks not fed the toxic meal, it has been concluded by most workers that the two conditions are not associated.

The first occurrence of the hemorrhagic disease of fowl was coincident with the first extensive continuous use of antibiotics and coccidiostats in poultry rations. It was therefore thought by many that these chemical substances might be the primary cause of the condition. This observation was further supported by such reports as that by Lepine et al. (1950) which indicated that tissue cultures were depressed by levels of antibiotics such as chlortetracycline and chloramphenicol that could be attained therapeutically in vivo. Many case histories of aplastic anemia in man due to chloramphenicol treatment were available at that time. Valini et al. (1950) had reported hematopoietic changes during the administration of antibiotics in man.

Asplin and Boyland (1947) noted an effect of many sulfonamides on the clotting time of blood of chickens. Work by Sweet et al. (1954) showed that clotting time could be altered by the sulfonamindes and other drugs.

Combs et al. (1954) showed that arsonilic acid in the ration could also increase the requirements for unidentified growth factors indicating that growth stimulating substances may produce a stress condition. Slinger et al. (1953) reported that the demand for known growth factors such as niacin was reduced in the presence of antibiotic growth stimulating substances such as penicillin. It is generally considered that growth-stimulating substances such as antibiotics and arsenicals act by inhibiting or altering micro-organisms (Elam et al. 1953, and Thayer 1955). The type of antibiotic or growth-stimulating substance used, the spectrum of enteric organisms present in the individual bird, and the type of ration fed can produce a variation of growth responses and nutrient demands. Rapid growth in the presence of these additives may produce critical levels of known and unknown essential nutrients and predispose to stressing conditions, rather than spare them as is ordinarily the case (Anon. 1951; Romoser et al. 1952; March and Biely 1952, and Veltre et al. 1953.)

At the time of the highest incidence of the disease most producers were using one or several chemical additives in their broiler rations. It then seemed logical to assume that this disease was the result of modern feeding methods. Klussendorf (1954) warned against the excess or improper use of drugs in poultry or livestock rations.

Yacowitz et al. (1955) fed sulfaquinoxaline to chicks at ages from 2 to 3 weeks of age. The diets were standard starter and broiler rations,

with or without penicillin added. Chicks fed .06 to .1% sulfaquinoxaline at two weeks of age showed no change in coagulation time at seven weeks. The authors commented that chicks treated early were able to overcome the stress by the seventh week, and suggested that this might explain the variation of incidence in field cases or spontaneous recovery of sick birds. Birds treated at 3, 4, or 5 weeks had prolonged coagulation times at seven weeks and some were hemorrhagic. Adding penicillin to the diet, or iodinated casein as a stress factor, increased the incidence and the degree of hemorrhage, but the addition of a vitamin supplement offered some protection from hemorrhage. Chicks fed the broiler ration grew faster and were more susceptible to hemorrhage. There was some increase in clotting time in chicks fed 5% alfalfa and 5 mg. of menadione per pound. These workers suggested that sulfaquinoxaline interferes with a mechanism not involving prothrombin, and this suggestion is supported by Jones et al. (1949). The level of sulfaquinoxaline used in this experiment is higher than the usual therapeutic level (Delaplane, 1949).

Forgacs and Carll (1955) isolated selected strains of Aspergilli, Penicillia and Alternaria species from feed taken from areas where the hemorrhagic disease had been prevalent. Chicks fed this feed showed symptoms comparable to hemorrhagic anemia. Feed obtained from an area in which hemorrhagic disease was not prevalent was only slightly contaminated and apparently did not contain toxic fungi since chicks fed such feed remained normal. Sipple (1956), working with some of the fungal strains isolated by Forgacs, noted that these strains had the ability to mutate to and from toxic forms.

Members of The Symposium on Hemorrhagic Disease (1955) agreed that the hemorrhagic syndrome of chickens had occurred in the absence of all of the stress conditions mentioned in this review. They also agreed that hemorrhage could occur when clotting time, as shown by prothrombin time, is normal. It was emphasized that anemia is the only consistent symptom of the disease. They stated, too, that the disease had not been produced in the laboratory. Nothing definite has appeared in the literature since that time to change that statement.

Treatment

Early treatment of hemorrhagic anemia was a change of feed and environment. Frost and Spruth (1955) are of the opinion that even though hemorrhagic disease does not parallel vitamin K deficiency as produced in the laboratory, it is apparent that stressing situations greatly accentuate the need for this vitamin. They advocate the use of menadione sodium bisulfite, 90-180 mg. per ton, and report that this level can be used effectively to treat hemorrhagic disease or to reduce the incidence of this disease in stress conditions. Cartrite (1955) used a liver extract, Co-Liver(R) (Silmo Chemical Co.) for the treatment and prevention of this hemorrhagic condition. Since the actual cause of hemorrhagic anemia is not known, the treatment or prevention of this disease is syptomatic and empirical.

Capillary fragility as a factor in hemorrhagic anemia

A review of the literature indicates that stressing conditions of one form or another are usually present, that hemorrhage is petechial or ecchymotic, and that the clotting mechanism is not always involved as

evidenced by hemorrhage in the presence of a normal prothrombin time. In contrast to this finding Jubelirer and Glueck (1949) observed a lack of hemorrhagic symptoms in human patients on dicumarol with unusually prolonged clotting times. This observation was also made in our preliminary experiments with chicks and indicated to us that capillary hemorrhage may not be related to the clotting ability of blood in all cases. Our attention then became directed to the role of capillaries and small vessels and their response to stresses of various types, assuming that the release of adrenal cortical hormones during stress may affect the permeability of these organs.

Best and Taylor (1955) explain that arterioles and venules are bridged by fine mono-layered ducts composed of endothelial cells adhered together by a cement substance to form the capillaries. Formed elements of the blood and the large protein molecules do not ordinarily pass through or between the endothelial cells, but water, electrolytes, oxygen and nutrients do. These substances pass in or out, depending on the osmotic, hydrostatic, or diffusion pressure of gradients, or pass on to the lymph system to be returned to the circulation. Griffith et al. (1955) stated that less importance is given to the edothelial cells and their intercellular cement, but more importance is given to the pericapillary tissue or matrix; the so-called mesenchymal ground substance. Mesenchymal ground substance, gelatinous in life, contains collagen and is sometimes referred to as collagenous ground substance; its pathologic states are spoken of as collagen diseases. Other constituents are chondroitin sulfuric acid, perhaps a cation exchanger, and reticular cells. The fibers and fibrils of fixed tissue probably represent structures not

so well defined in life. This ground substance can bind and release water, and substances therein, depending on the needs of the tissue cells or according to a variety of stimuli not now entirely understood. This ground substance may be large in amount as in subcutaneous tissue, or small as between the glomerular capillary and the capsule of Bowman. It may readily admit fluid from the capillary, which would decrease the gradient of pressure from within the capillary to the tissue outside and hence theoretically predispose to capillary hemorrhage. Such hemorrhage might be absorbed rapidly or slowly, depending upon the speed with which fluid is released by the mesenchymal ground substance to enter the lymphatics. On the other hand if pressure in the tissue outside the capillary is relatively high as it is during edema, the capillary will be somewhat supported and the development of capillary hemorrhage will be opposed. Functionally, therefore, the intercellular substance and the mesenchymal ground substances form a single unit. Among substances now thought capable of affecting the intercellular cement substance and/or the mesenchymal ground substance are endogenous and exogenous adrenal cortical hormones, rutin and other flavonoids, X-ray, hyaluronidase, probably pituitary hormones, vitamin A, vitamin E, vitamin C, certain amino acids, and perhaps antibodies.

Abnormal ground substance may fail to bind fluid and substances therein and cause death of fixed cells. If this occurs in the wall of an artery, it may lead to rupture through that wall, or if in the retina, to death of the retinal nerve cells. The release of protein molecules into the circulation may cause the production of antibodies. The substance may become less gelatinous and more fibrinous, with an absolute

decrease in bulk and therefore decrease in function.

Various stress reactions in mammals cause adrenal hypertrophy and an outpouring of cortical hormones (Tepperman et al., 1943). Very little work of this nature has been reported for birds but Jailer and Boas (1950) indicated that a similar mechanism exists in birds.

That stress might affect the mesenchymal substance and therefore capillary fragility is indicated by the experiments of Selye (1946) with desoxycorticosterone acetate (DOCA), a synthetic counterpart of the natural salt-retaining hormones of the adrenal cortex. Selye showed that this hormone, given in large doses, produced mesenchymal proliferation along with periarteritis, nephrosclerosis, and arthritis. Seifter et al. (1949) were able to show that absorption from the synovial joint cavity of the knee was increased if DOCA was given parenterally. Absorption was decreased by administration of either whole adrenal cortical extract, cortisone, or adrenalcorticotrophic hormone (ACTH). These authors suggest that "normal permeability of the synonial membrane, in this case, is in part controlled by a balance between adrenal steroids of the DOCA type and of the cortisone type". Cope et al. (1942) reported that the protein content of the lymph of adrenalectomized animals was significantly higher than in normal animals. They credited this finding to an increased capillary permeability due to an imbalance in adrenal controls. The effect of cortical hormones on pericapillary mesenchymal substance is complicated by the action of epinephrine on the capillary wall and the role of epinephrine in the release of ACTH. Link and Nalbandov (1955) reported edema in chicks injected with DOCA.

It is difficult to correlate mild anemias without hemorrhage, with possible stress reactions. The effect of the adrenal cortical hormones or ACTH on the formed elements of blood of chickens, other than erythrocytes, is interesting when compared to changes in white cells reported in cases of hemorrhagic disease.

It is known that injection of adrenal cortical extract or ACTH in the mammal causes lymphopenia and eosinopenia. Shapiro and Schechtman (1949) found that when adrenal cortical extract was injected into adult fowls, the result was a transient lymphopenia and leukocytosis. The increase was mainly in the heterophils. Weller and Schechtman (1949) also found that when adrenal cortical extract was injected into 13-15 day chick embryos, the number of lymphocytes was not changed significantly, but there was a three-fold increase in the number of polymorphonuclear cells. There was no effect upon the red cells. Gray (1954) noted fluctuations in polymorphs during hemorrhagic anemia, particularly the heterophils. Stamler et al. (1950) injected mammalian ACTH for 5 days into chicks and reported no effect upon the number of eosinophils. They remarked that perhaps mammalian ACTH does not give a true response to birds.

From this review of the literature the following hypothesis is formulated. The hemorrhagic syndrome of chickens may be the result of various complexes of stressing conditions; that is, the hemorrhagic disease in two different flocks of broilers may not be due to the same combinations of stressing environmental factors. Stresses such as drugs, disease, or moldy feed may produce a critical period in the life of a fast growing chick, wherein exposure to an additional stress might precipitate a hemorrhagic response. Each of the stresses involved may be mild alone, but in combination may not be compensated by the adaptive mechanisms during critical periods of the fast growing broiler type chick.

Methods and Materials

This experiment was designed to study the effect of combinations of some of the conditions reportedly accompanying hemorrhagic disease, and if possible, to produce this disease in the laboratory. In most cases a mild "field type" of stress or condition was produced. The criterion of response in this experiment was any statistically significant alteration in blood values, capillary fragility, and gross or microscopic lesions of organs ordinarily involved as reported by Gray et al. (1954). Alterations of blood values, even within the normal range, were observed with interest. Adrenal studies were made, and are still underway at this time, but these data are not available for this thesis.

Experimental Chicks

The chicks used in this experiment were an Oklahoma broiler strain of New Hampshire Red cockerels, developed and hatched by the Department of Poultry Husbandry, Oklahoma A. and M. College. These chicks were sexed at one day of age, and each was wing-banded with an individually numbered tag.

Experimental design

The chicks were weighed at hatching and divided into three weight groups, five chicks from each group were assigned to each pen in an effort to produce a hemogenious population. Sixteen groups of fifteen chicks each were brooded and reared in batteries. The battery trays were 30 x 48 x 14 inches, each group remained confined to its particular tray

during the entire experiment. Trays were assigned to the treatment groups in a random fashion, and the batteries were rotated in the room at frequent intervals. The room was lighted continuously, optimum temperatures were maintained and an efficient broiler rations, Experiment Station Ration #52 (Table 2) was fed to all of the experimental chicks to promote rapid growth.

Treatments used

Treatments were the following: A, liver extract, 3% of the ration; B, moldy corn meal, 6% of the ration; C, infection with the virus of encephalomyelitis; D, sulfaquinoxaline, .0125% of the ration. These treatments were given singly and in all possible combinations (Table 3). This arrangement constituted a factorial design, lending greater sensitivity to the statistical analysis (Cochran and Cox, 1950).

Treatment A

Treatment A, liver extract, 3% of the ration, Co-liver(R)(Silmo Chemical Co.) was used in this experiment to determine if it would alleviate hemorrhagic symptoms. This extract has been used effectively for prophylaxis and treatment of hemorrhagic anemias (Cartrite, 1955).

Treatment B

This treatment consisted of corn meal sterilized by autoclaving and cultured with a species of Aspergillus, Penicillia, Alternaria, and Rhizopus. The corn meal was placed in a constant temperature incubator at 25°C after wetting, and incubated for seven days. It was hoped that toxins and a high spore content would be produced. The corn meal cultured with the various molds was mixed together later and fed as 6% of

Table 2.

BASAL RATION

Ground yellow corn	56%
Pulverized oats	5%
Alfalfa meal (17% protein)	2%
Fish meal (60% protein)	5%
Soybean meal (44% protein)	22.5%
Meat and bone scrap (50% protein)	3%
Dried brewers yeast	3%
Dried whey	2%
Dicalcium phosphate (20% phosphate)	.5%
Trace mineral mix	.05%
Salt	.5%
Vit-Mix	.5%

Table 3. Experimental Design

PEN #	A	B	C	D
15 chicks in each pen	Basal ration plus Co-liver 3%	Basal ration cultured with fungi Rhizopus sp. Aspergillus sp. Alternaria sp. Penicillia sp.	Avian encephalomyelitis virus injected intracranially at one day of age	Basal ration plus sulfaquinoxaline .0125% fed continuously in the ration
I A	X			
II Basal only				
III AB	X	X		
IV B		X		
V AC	X		X	
VI C			X	
VII AD	X			X
VIII D				X
IX ABC	X	X	X	
X BC		X	X	
XI ACD	X		X	X
XII CD			X	X
XIII ABD	X	X		X
XIV BD		X		X
XV ABCD	X	X	X	X
XVI BCD		X	X	X

the ration at one week of age. This level of moldy feed was determined on the basis that this quantity of feed could be fed un-noticed in field operations where hemorrhagic disease might occur; whereas noticeably spoiled feeds would not ordinarily be fed. The cultures used were isolated as contaminants because such common contaminants cause most feed spoilage under ordinary conditions. There was some growth of other fungi in the basal ration after addition of the cultured corn meal. The work of Forgacs et al. (1954) and Forgacs and Carll (1955) provided the basis for this part of the experiment.

Treatment C

This part of the experiment was designed to test the stress of disease as a factor in hemorrhagic anemia. Because it is impossible to test the effect of the many possible diseases and disease complexes, one disease was tested with the assumption that other diseases may affect the stress mechanism in a similar manner.

Avian encephalomyelitis or "epidemic tremor" was used as a stressing disease. This particular virus was used because it is rarely transmissable by co-habitation and therefore the problem of isolation was removed. Gross lesions are not common and thus the symptoms and lesions of hemorrhagic anemia would not be masked by the symptoms and lesions of the stressing disease. Morbidity may be high but mortality is low enough to permit the use of a limited number of experimental birds. Chicks inoculated with this virus will ordinarily show symptoms of tremors at the age when hemorrhagic disease is prevalent. The strain of virus used was derived from a field outbreak, and was tested and shown to produce 30% infection with a high incidence of leg weakness and inappetence in other

birds. A virus of this nature was ideal for the type of experiment planned. The chicks were infected by intracranial injection at one day of age. One hundredth of a cc. of a brain suspension from an infected chick was injected into the brain of each of the experimental chicks, to be subjected to this treatment, through the fontanel with a 25 gauge needle. No mortality from the injection occurred after the first day (Biester and Schwarte 1952, and Jungherr 1955).

Treatment D

Because sulfaquinoxaline is the most commonly used drug in poultry rations at continuous levels, and most often associated with anemic and hemorrhagic conditions, it was used in this experiment as representative of a drug stress. Large amounts of sulfaquinoxaline, .06 to .1 percent of a ration are known to cause anemic and hemorrhagic responses (Yacowitz et al., 1955), so the level was set at .0125 percent, the usual level of sulfaquinoxaline used for continuous feeding (Jones, 1954). This level of sulfaquinoxaline was fed to the experimental chicks during the entire experiment beginning at the first day of brooding.

Collection of Data

The chicks were observed daily for evidence of symptoms of disease, and dead birds were posted and examined closely for lesions characteristic of hemorrhagic anemia as described by Cartrite (1954) and Gray et al. (1954).

Five chicks from each pen were bled weekly beginning at 20 days of age. The chicks were bled from the heart with a three inch 23 gauge needle. During the last two weeks of the experiment the birds were bled from the wing vein using a one inch 25 gauge needle. Two cc. of blood

was taken from the same five chicks each week. The balanced oxalate anticoagulant suggested by Coffin (1953), was used to prevent coagulation prior to hemoglobin and hematocrit determinations.

From the two cubic centimeter sample of blood collected weekly, the following blood values were determined: (1) erythrocytes X $10^6/mm^3$, (2) leukocytes X $10^3/mm^3$, (3) differential leukocyte count in percent, (4) hemoglobin in grams/100 cc. of blood, (5) hematocrit in percent, and (6) whole blood coagulation time in seconds.

Beginning at the 22nd day of the experiment an estimation of capillary strength was also made by means of a mercury resistometer (Fig. 1).

Techniques for Determination of Blood and Capillary Strength Values

Indirect cell counting and staining technique

The indirect counting technique of Olson (1935), employing a phloxine dye, was used for the total leukocyte count. This diluting fluid consisted of 50 mg. of phloxine, 5 cc. of neutral formalin, and 95 cc. of freshly prepared Ringer's solution. An ordinary red blood cell diluting pipette was used, and the blood was diluted 200 times. The ends of the filled pipettes were closed by means of a rubber band to prevent loss of diluting fluid. The pipettes were then placed in the refrigerator for several hours, preferably over night, until the red cells were stained a distinct pink and the acidophils, eosinophils and neutrophils (heterophils), were stained a distinct red, as viewed in a Neubauer Bright-Line hemocytometer. The pipettes were shaken vigorously for ten minutes with a mechanical shaker before counting.

Erythrocytes

The erythrocytes were then counted in the usual manner; that is,

the erythrocytes in 80 of the smallest squares were counted and the result multiplied by 10,000 as the dilution of blood in the pipettes was 1-200. The resulting figure was the erythrocyte count x $10^6/mm^3$. Normal erythrocyte counts in millions/mm^3, range from 2.25 to 3.23 (Gardner, 1936-46).

Leukocytes

The total leukocyte count was determined by counting the number of acidophilic cells in the entire ruled area of the hemocytometer (9 mm^2). A differential count of leukocytes must then be made from a stained blood smear to provide the sum of the percentage values of eosinphils and heterophils to be used in the calculation of the total number of leukocytes according to the following formula:

$$\text{Total leukocyte count} = \frac{10}{9} \text{ (Number of acidophilic cells in the hemocytometer)} \times \text{dilution (1-200)} \times \frac{100}{\text{Percentage of eosinophils in the blood smear}}$$

Normal leukocyte counts in thousands/mm^3, range from 8,400 at one day of age to 34,000 at maturity (Gardner 1946). Olson (1937) reported counts of 40,000 or more in normal mature chickens.

Differential leukocyte count

Whole blood smears were made from fresh unoxalated blood and stained with a standard Wright's stain. The length of staining or buffer pH was determined by trial and error for each batch of stain. Wright's stain is preferred for routine differential counts (DeVilliers 1938, and Olson 1935).

Differential white cell count percentages were based on 400 cells counted in each slide. Because eosinophils and heterophils are not readily distinguishable, and because the sum of the percentages of these

two cells are essential for the calculation of total leukocytes, they were counted together as acidophilic cells.

Hemoglobin

Hemoglobin values were determined colorimetrically using the acid hematin method (Cohen and Smith, 1919). Twenty cmm. of oxalated whole blood was mixed with 5.0 ml. of 1 percent hydrochloric acid solution and allowed to stand 60 minutes. This solution was then stirred with a glass stirring rod and checked for hemoglobin content in a Bausch and Lomb "Spectronic 20" colorimeter. No correction was made for turbidity due to the nuclei of the erythrocytes. The normal range of hemoglobin values uncorrected is reported to be 9.73 to 12.8 gm/100 cc. of blood, $\pm$ 1 gm (Dukes and Schwarte 1931, and Olson 1937).

Hematocrit

Packed red cell volumes in percent were determined with Van Allen hematocrit pipettes. These pipettes were used because only a small quantity of blood is needed (Denington and Lucas, 1955). The diluting fluid was an .85% saline solution. The blood had been oxalated prior to this determination. The filled pipettes were centrifuged at 3000 r.p.m. for ten minutes to insure complete packing. Readings were made at the lower margin of the buffy coat.

Coagulation

Whole blood coagulation time was determined for each sample of blood by ejecting three drops into a 35 x 12 mm Fisher specimen vial from a clean syringe. A stop-watch was released at the instant blood was withdrawn from the bird into the syringe, and was stopped the in-

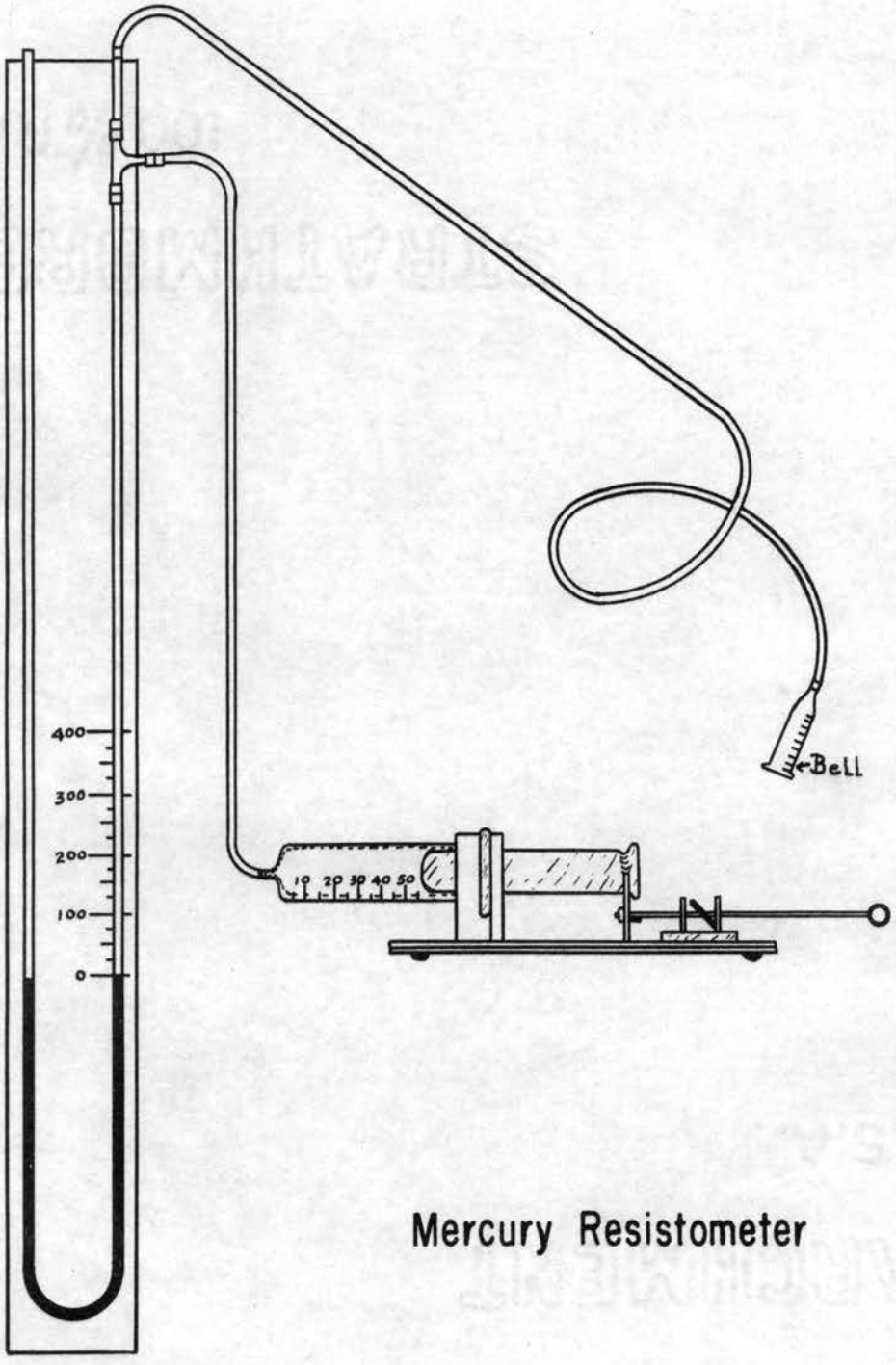
stant the blood would adhere to the side of the vial when tilted. Coagulation time was recorded in seconds.

Bainbridge and Menzies (1920) stated that, "because the blood of birds contains no true platelets it will not clot if it is drawn directly from a blood vessel without contact with the tissues". DeVilliers (1938) found also that the coagulation time of ostrich blood was prolonged and sometimes failed to clot under similar conditions. According to Dukes (1955), the average coagulation time is 4-1/2 minutes; Johnson and Conner (1933) found it to vary from 1-14 minutes with an average of six minutes. Because it is impossible to draw blood from the heart consistently free of tissue fluids, coagulation times were not determined until the last bleeding which was from the wing vein.

Capillary strength determination

An estimation of capillary strength was made by means of a mercury resistometer (Fig. 1). This is a negative pressure apparatus similar to an apparatus used on the chicken by Mushett (1955), and by Brown (1949) on the forearm of man. The bell of this instrument was applied to the breast at a point near the base of the wing, as this area is usually devoid of plummage. Mineral oil was first applied to the area, as suggested by Mushett, to make the skin more transparent. Negative pressure was then produced by withdrawing the plunger of the attached syringe until the mercury rose to the desired level. Both sides of the breast were used for these estimations and the end point recorded was the greatest negative pressure, in millimeters of mercury, at which petechiation or suffusion did not occur. It was difficult to estimate the exact degree of suffusion of blood into the aspirated area. A characteristic

Figure 1.



Mercury Resistometer

Figure 2. Breast Area of Chicken Before Applying Negative Pressure

Figure 3. Breast Area of Chicken Before Applying Negative Pressure Showing Vascular Fault

bruise appeared if vascular fault occurred (Figs. 2 and 3).

The same five birds that were bled weekly in each pen were aspirated in the manner described at 11 day intervals beginning at the 22nd day of life and ending at the 66th day of life. At 66 days, five birds in each pen, that had not been bled or aspirated at the intervals indicated, were aspirated as controls.

Tissues Collected

At the termination of the experiment liver and bone marrow sections were stained and examined for changes. Skeletal muscle sections from the site of negative pressure application were stained with special stains and examined for any histological changes. Adrenal glands were collected, weighed and sectioned. Half of the adrenals were quick-frozen and preserved for steroid assay. All birds were weighed at the end of the experiment to obtain relative adrenal weights.

Results and Discussion

Although data were collected at seven day intervals, beginning at the 20th day of the experiment, a statistical analysis was made only of data collected at 14 day intervals. This made it possible to compare blood values at the 20th, 34th, 48th, and 62nd days.

Because of the volume of data, and number of blood values studied, only those values significantly high or low, as recorded in Table 4, will be discussed; all data not mentioned should be regarded as insignificant. Furthermore the mean of a blood value for a particular pen as plotted in Figs. 4 to 11, and recorded bi-weekly in Tables 5 to 12, can not be used as a criterion of comparison of treatments. These figures are included in this paper to indicate a pattern of blood levels to be expected during the growing stage of broiler chicks, and to indicate the range of fluctuations of avian blood values encountered by us, Gray (1954) and Sturkie (1954). These fluctuations, though unexplainable at this time, are characteristic of avian blood.

The statistical analyses of a factorial design compares a particular treatment in a pen against all pens not subjected to the treatment. This type of analysis is quite sensitive but eliminates the objective comparison of mean values of each pen of treated animals (Cochran and Cox, 1950). At no time during this experiment did a group, subjected to a combination of three or more stresses simultaneously, have a significant alteration of a blood value for more than one bleeding. Because

these alterations appeared only once and did not signify a pattern of response, they were considered to be due to chance rather than interaction, in spite of the implied significance of the statistical analysis (Graybill, 1956).

Erythrocytes:

Red cell numbers were highly significantly depressed by treatment IA (basal plus liver extract) at 62 days and significantly depressed by treatment III AB (basal, mold and liver extract) at 20 days. Red cell numbers were highly significantly increased by treatment IX ABC (basal, liver extract, mold and tremors) at 48 days. There is no logical explanation for these results and they are not seriously considered as meaningful due to their sporadic occurrence and lack of reappearance in the same treatment more than once.

Erythrocytes were significantly depressed by treatment VI C (basal plus tremors) at 20 days, at 48 days, and 62 days. As this pattern of erythrocyte depression occurred consistently throughout the experiment these results are important. Tremors-infected birds were under more than a mild stress at times during the experiment and, although they ate and drank well considering their condition, they did not gain weight as rapidly as other birds. Data collected from chicks during the paralytic stages of the disease were discarded.

Treatment VIII D (basal plus sulfaquinoxaline) significantly depressed erythrocyte counts at 20 and 34 days. Cell counts returned to normal during the rest of the experiment. This result is compatible with the report of Yacowitz et al. (1955) that chicks fed sulfaquinoxaline at an early age would adapt themselves to this stress before the

seventh week of age. It is interesting to note that this low level of .0125% sulfaquinoxaline affected the hematopoietic system even at this early age.

Sulfaquinoxaline and tremors, alone or in combination, in the presence of liver extract did not depress erythrocytes; whereas sulfaquinoxaline and tremors together, as in treatments XII CD (basal, tremors and sulfaquinoxaline) and XVI BCD (basal, mold, tremors and sulfaquinoxaline) did depress the erythrocytes significantly and highly significantly at 48 days of age. No attempt is made to interpret this delay in anemic manifestation, or to interpret the mechanism of interaction between the two stresses.

Leukocytes:

In a few instances leukocytes were significantly increased by treatment V AC (basal, liver extract and tremors) at 48 days, and by treatment VII AD (basal, liver extract, and sulfaquinoxaline) at 34 days. Leukocytes were highly significantly decreased by treatment XIII ABD (basal, liver extract, mold and sulfaquinoxaline) at 34 days, and by treatment XIV BD (basal, mold, and sulfaquinoxaline) at a 3% level at 48 days. Again, these results, being sporadic, inconsistent and absent at the end of the experiment, added nothing to the over all interpretation of the final results.

Hematocrit:

Hematocrits were not significantly altered except by treatment XV ABCD (basal, liver extract, mold, tremors and sulfaquinoxaline). In this treatment packed cell columns were depressed significantly.

Hemoglobin:

The only consistent alteration of hemoglobin was by treatment VI C (basal plus tremors). Hemoglobin values were significantly increased at 34 days and highly significantly increased at 62 days of age.

Coagulation time:

Coagulation times were significantly, or highly significantly prolonged, by treatments involving epidemic tremors and epidemic tremors and sulfaquinoxaline in combination. Any treatment involving epidemic tremors or epidemic tremors and sulfaquinoxaline did not produce a prolonged coagulation time if liver extract was present.

The mechanism by which avian encephalomyelitis produced a prolonged coagulation time is not known; however the reduced feed intake of sick birds contributes to a low vitamin K intake. (Hare et al., 1954). Sulfaquinoxaline reduces the synthesis of vitamin K, and interferes with a coagulation mechanism not involving prothrombin (Yacowitz et al., 1955, and Jones et al., 1949). The mode of action of liver extract on the coagulation mechanism was not determined, but this result explains the favorable response of hemorrhagic birds to liver extract as observed by Cartrite (1955).

Coagulation time was prolonged by only one treatment not involving avian encephalomyelitis and sulfaquinoxaline. Treatment III AB (basal, liver extract, and mold) showed a significantly increased coagulation time.

This analysis shows that avian blood has a reduced ability to coagulate in the presence of tissue debris, and other predisposing conditions, after the donor has been subjected to stresses such as disease and drugs.

Table 4.

SIGNIFICANT DIFFERENCES OF BLOOD VALUES

A = basal ration plus 3% liver extract

B = basal ration cultured with fungi

C = chicks injected with virus of avian encephalomyelitis

D = basal ration plus .0125% sulfaquinoxaline

(↑) = higher or increased
(-) = lower or decreased

Pen # and Treatments	20 Days	34 Days	48 Days	62 Days
I A		Hemoglobin (-) 2.5%		Erythrocytes(-) .5%
II Basal only				
III AB	Erythrocytes (-) 2.4%			Clotting time (↑) 4.1%
IV B				
V AC			Leukocytes (↑) 2%	
VI C	Erythrocytes (-) 1.5%	Hemoglobin (↑) 3.2%	Erythrocytes (-) 5%	Erythrocytes (-) 1.5% Hemoglobin (↑) .05% Clotting time (↑) 2%
VII AD		Erythrocytes (-) 4% Leukocytes (↑) 2.5%		
VIII D	Erythrocytes (-) 2.5%	Erythrocytes (-) 2.5%		
IX ABC			Erythrocytes (↑) .08% Hemoglobin (↑) 2%	
X BC				Clotting time (↑) .7%
XI ACD				
XII CD			Erythrocytes (-) 2.5%	Clotting time (↑) .9%
XIII ABD		Leukocytes (-) 1%		
XIV BD			Leukocytes (-) 3%	
XV ABCD				Hematocrit (-) 4%
XVI BCD			Erythrocytes (-) .4%	Clotting time (↑) 4%

Figure 4. Pattern of Mean Blood Values, Treatments I A and II

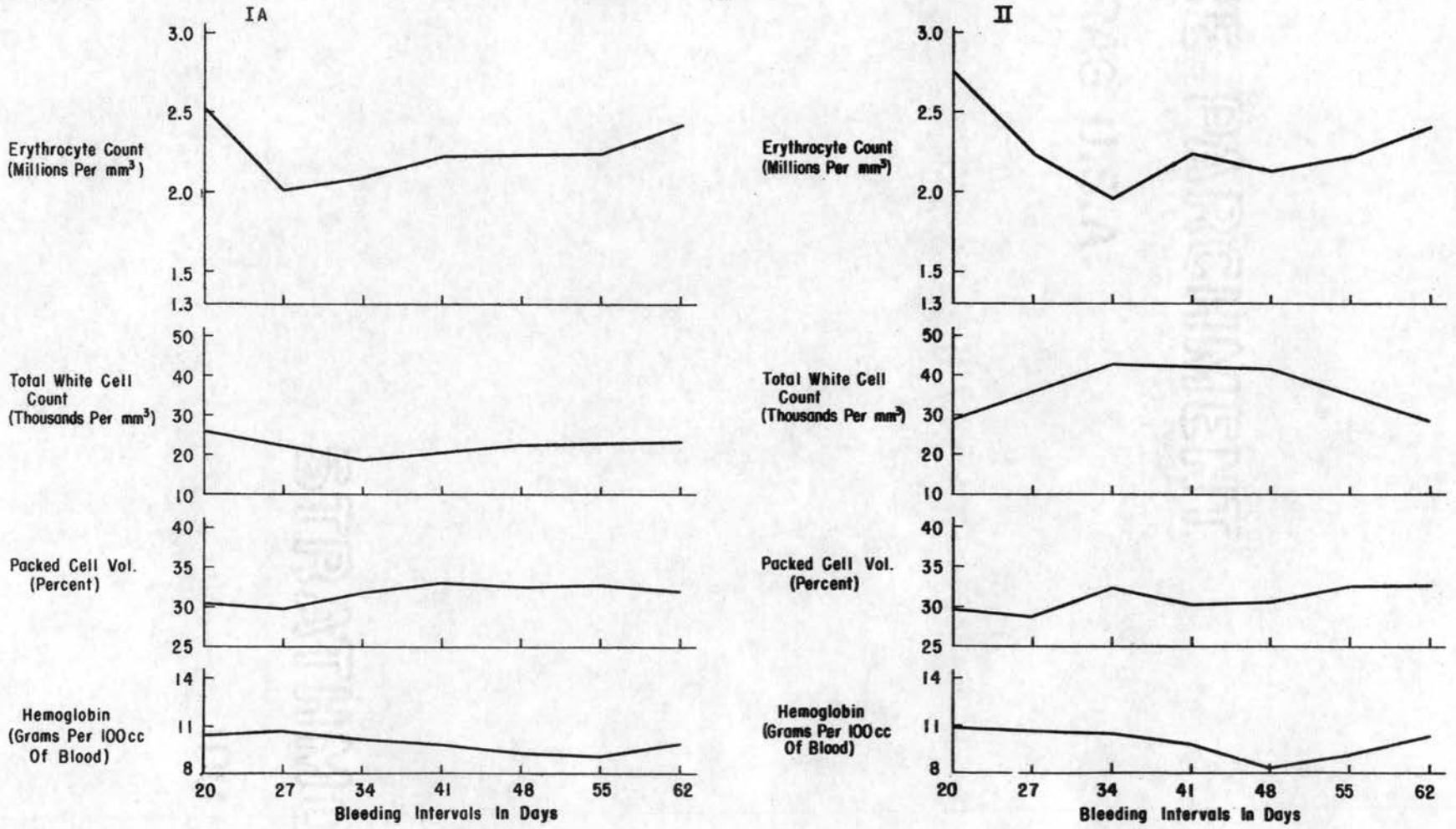


Table 5. Bi-weekly blood data, treatments IA and II.

IA						II					
Day-pen-bird	R.B.C. $X10^6/mm^3$	W.B.C. $X10^3/mm^3$	Hb gms/100cc	Hemato-crit in %	Coag. time in seconds	Day-pen-bird	R.B.C. $X10^6/mm^3$	W.B.C. $X10^3/mm^3$	Hb gms/100cc	Hemato-crit in %	Coag. time in seconds
20 - 1 - 2	2.23	39.9	10.70	31		20 - 2 - 16	—	—	—	—	
20 - 1 - 4	2.75	—	11.40	32		20 - 2 - 18	2.99	33.3	11.00	34	
20 - 1 - 8	2.01	22.2	9.90	28		20 - 2 - 22	2.61	26.6	9.40	24	
20 - 1 - 12	3.03	15.9	9.90	30		20 - 2 - 28	—	—	—	—	
20 - 1 - 15	—	—	—	—		20 - 2 - 30	2.66	25.7	12.00	31	
Mean	2.50	25.7	10.47	30.20		Mean	2.74	28.5	10.80	29.60	
34 - 1 - 2	2.70	9.3	11.40	38		34 - 2 - 16	1.67	44.4	9.40	32	
34 - 1 - 4	2.02	31.3	8.30	31		34 - 2 - 18	2.20	44.4	11.40	32	
34 - 1 - 8	1.61	26.6	8.50	25		34 - 2 - 22	—	—	—	—	
34 - 1 - 12	2.03	6.6	12.30	33		34 - 2 - 28	2.47	66.6	10.50	33	
34 - 1 - 15	—	—	—	—		34 - 2 - 30	1.48	22.2	10.70	32	
Mean	2.09	18.5	10.12	31.70		Mean	1.95	44.4	10.50	32.20	
48 - 1 - 2	2.21	26.1	11.40	32		48 - 2 - 16	1.83	31.9	8.10	31	
48 - 1 - 4	2.23	16.6	8.80	30		48 - 2 - 22	2.01	53.2	7.20	25	
48 - 1 - 8	—	—	—	—		48 - 2 - 25	2.12	71.9	8.30	34	
48 - 1 - 12	2.27	2.8	9.90	35		48 - 2 - 28	2.41	33.5	9.60	33	
48 - 1 - 15	2.26	44.4	7.20	30		48 - 2 - 30	2.27	17.7	9.00	29	
Mean	2.24	22.5	9.30	31.90		Mean	2.12	41.6	8.44	30.40	
62 - 1 - 1	2.75	18.4	11.40	37	390	62 - 2 - 16	2.37	27.3	9.90	29	600
62 - 1 - 2	1.99	66.6	9.90	34	700	62 - 2 - 19	2.49	16.6	10.20	32	330
62 - 1 - 4	2.46	18.4	8.80	30	660	62 - 2 - 22	2.18	31.5	9.00	29	1800
62 - 1 - 6	2.56	31.0	11.00	34	1800	62 - 2 - 23	2.10	17.7	10.20	31	1440
62 - 1 - 7	2.73	15.5	10.50	35	701	62 - 2 - 24	2.51	39.9	12.00	36	1140
62 - 1 - 8	2.22	22.2	8.80	29	1300	62 - 2 - 25	2.43	27.3	11.40	34	798
62 - 1 - 11	2.51	19.5	10.20	29	752	62 - 2 - 27	2.36	20.6	10.20	32	518
62 - 1 - 12	2.22	13.7	9.00	30	1320	62 - 2 - 28	2.23	25.3	9.60	31	1320
62 - 1 - 13	2.48	15.5	8.80	30	1061	62 - 2 - 29	3.13	33.3	11.00	34	370
62 - 1 - 15	2.31	18.8	9.90	32	960	62 - 2 - 30	2.32	37.7	9.90	30	1440
Mean	2.42	23.9	9.87	32.00	964	Mean	2.41	28.3	10.32	32.80	975

Figure 5. Pattern of Mean Blood Values, Treatments III AB and IV B

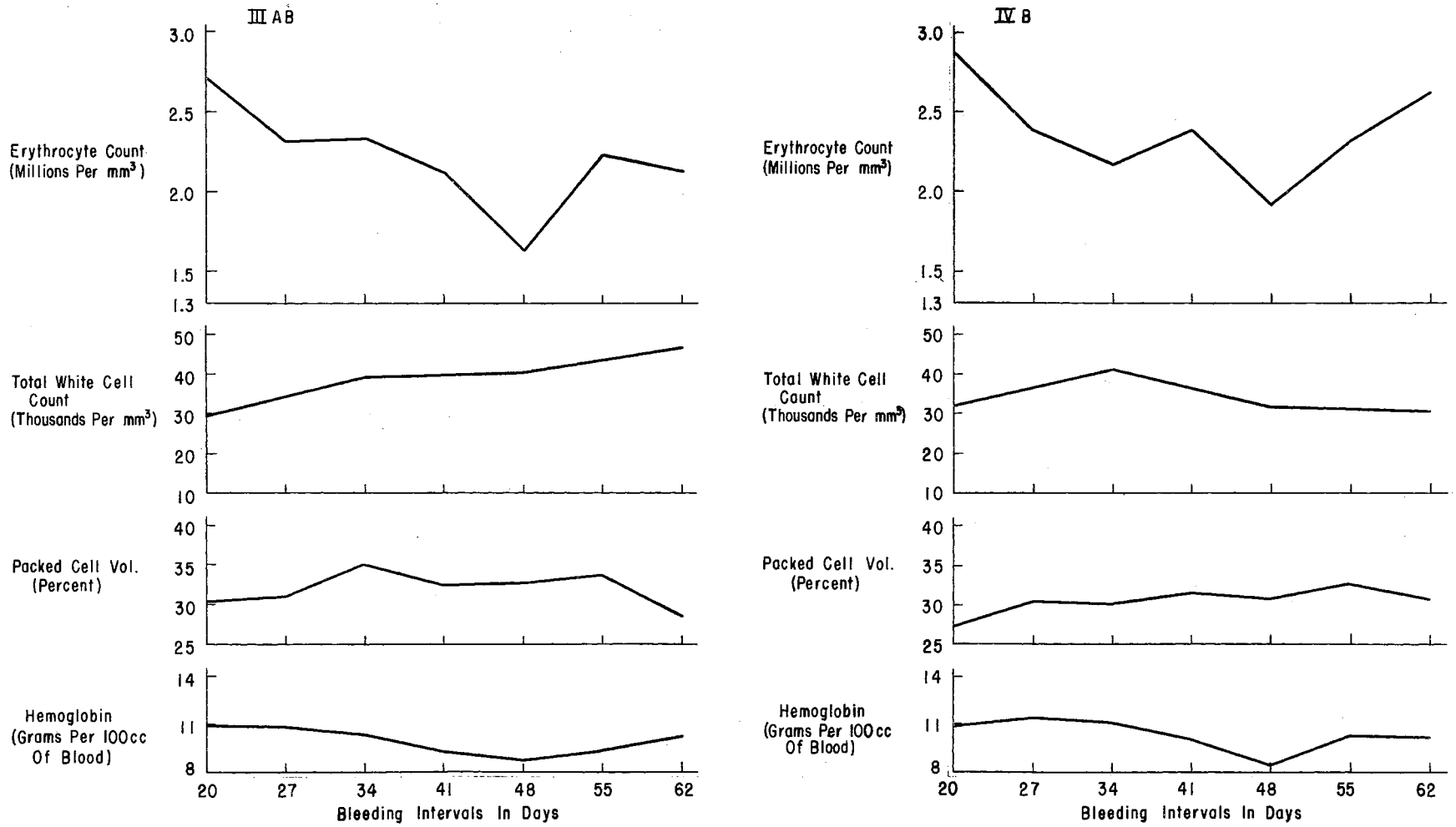


Table 6. Bi-weekly blood data, treatments III AB and IV B.

III AB						IV B					
Day-pen-bird	R.B.C. X10^6/mm^3	W.B.C. X10^3/mm^3	Hb in gm /100cc	Hemato-crit in %	Coag. time in seconds	Day-pen-bird	R.B.C. X10^6/mm^3	W.B.C. X10^3/mm^3	Hb in gm /100cc	Hemato-crit in %	Coag. time in seconds
20 - 3 - 31	2.71	21.3	10.50	30		20 - 4 - 46	2.69	41.2	10.50	22	
20 - 3 - 34	2.65	46.6	9.40	29		20 - 4 - 50	—	—	—	—	
20 - 3 - 36	2.96	17.3	10.50	30		20 - 4 - 55	2.42	44.4	11.40	30	
20 - 3 - 42	2.68	16.2	12.10	29		20 - 4 - 58	3.24	26.6	10.00	29	
20 - 3 - 43	2.55	46.6	11.60	33		20 - 4 - 60	3.09	15.7	11.40	28	
Mean	2.71	29.6	10.94	30.20		Mean	2.86	32.0	10.82	27.20	
34 - 3 - 31	—	—	—	—		34 - 4 - 46	1.86	37.7	9.00	20	
34 - 3 - 34	2.00	37.7	8.30	35		34 - 4 - 50	2.42	33.3	12.00	34	
34 - 3 - 36	2.69	26.4	8.30	32		34 - 4 - 55	2.16	48.8	10.70	33	
34 - 3 - 42	2.38	37.7	14.90	34		34 - 4 - 58	2.22	44.4	12.30	33	
34 - 3 - 43	2.26	34.1	10.20	39		34 - 4 - 60	—	—	—	—	
Mean	2.33	39.0	10.43	35		Mean	2.16	41.0	11.00	30.00	
48 - 3 - 31	1.23	32.4	7.80	33		48 - 4 - 46	1.88	20.8	6.30	28	
48 - 3 - 34	1.85	41.0	6.70	33		48 - 4 - 50	2.03	23.0	10.70	35	
48 - 3 - 36	1.45	50.0	11.40	34		48 - 4 - 55	1.82	27.7	7.80	30	
48 - 3 - 37	—	—	8.30	30		48 - 4 - 58	1.92	55.5	8.50	31	
48 - 3 - 43	2.00	36.4	9.40	34		48 - 4 - 60	1.87	32.6	8.30	29	
Mean	1.63	40.1	8.72	32.80		Mean	1.90	31.9	8.32	30.60	
62 - 3 - 31	1.99	66.6	10.20	29	585	62 - 4 - 46	1.91	35.5	8.30	24	405
62 - 3 - 32	1.66	22.2	8.80	23	660	62 - 4 - 47	2.44	23.3	10.30	29	405
62 - 3 - 36	2.27	22.2	10.20	28	1800	62 - 4 - 48	2.45	53.2	7.80	25	539
62 - 3 - 36	2.23	55.5	11.00	30	699	62 - 4 - 49	3.56	26.6	9.60	28	1800
62 - 3 - 37	1.20	82.1	10.20	25	728	62 - 4 - 50	3.19	28.8	11.60	36	190
62 - 3 - 38	1.82	17.0	8.80	33	540	62 - 4 - 53	2.83	34.6	11.60	35	510
62 - 3 - 41	2.42	57.7	12.00	31	982	62 - 4 - 55	2.94	18.7	9.90	32	1050
62 - 3 - 43	2.80	18.2	10.70	31	938	62 - 4 - 56	2.98	35.5	11.00	33	655
62 - 3 - 44	2.72	62.1	10.50	26	763	62 - 4 - 58	2.57	27.7	10.20	33	777
62 - 3 - 45	2.33	62.1	10.50	31	980	62 - 4 - 60	2.21	18.2	10.40	31	703
Mean	2.14	46.5	10.29	28.80	867	Mean	2.60	31.2	10.07	30.60	709

Figure 6. Pattern of Mean Blood Values, Treatments V AC and VI C

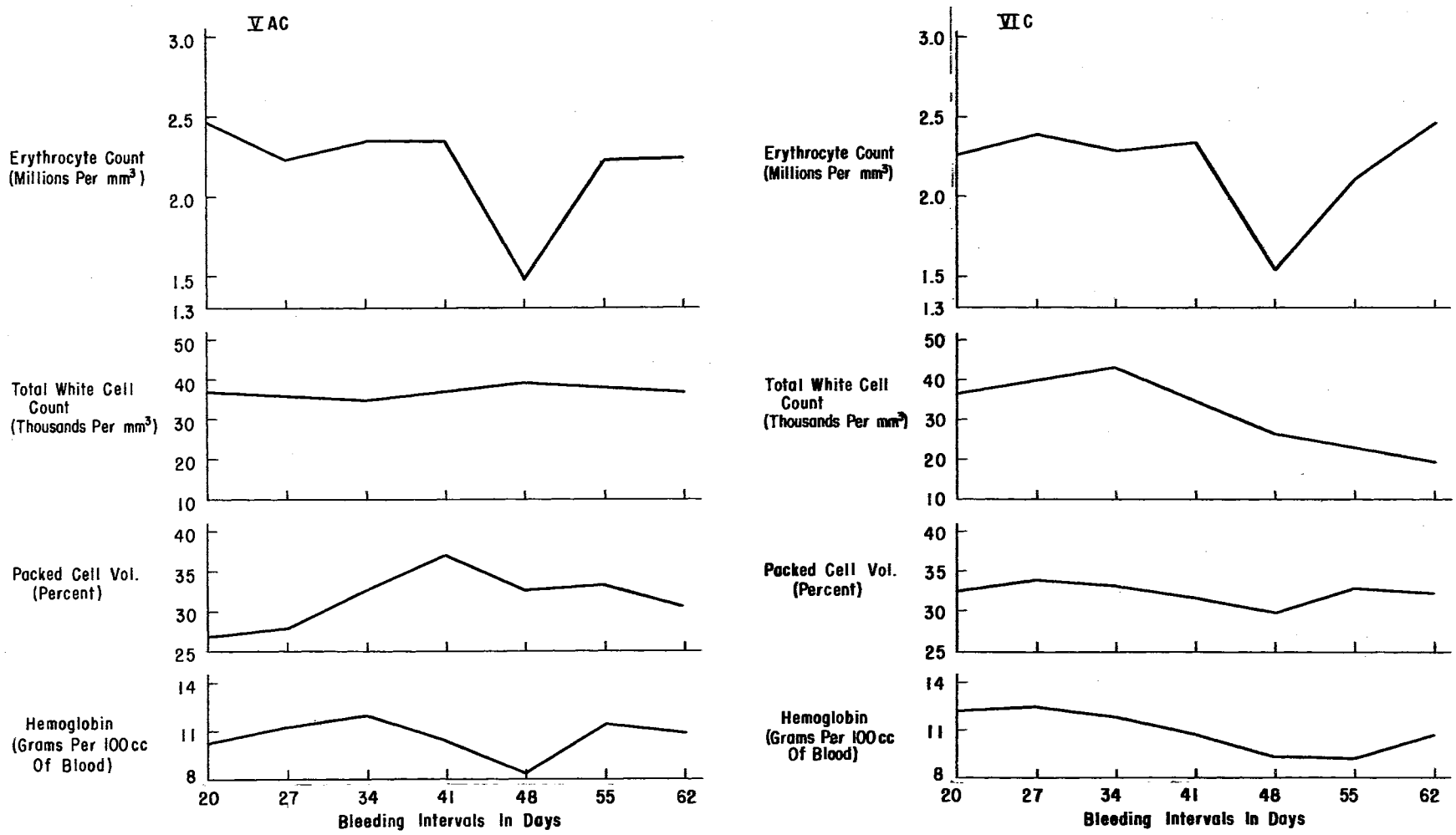


Table 7. Bi-weekly blood data, treatments V AC and VI C.

V AC						VI C					
Day-pen-bird	R.B.C. $X10^6/mm^3$	W.B.C. $X10^3/mm^3$	Hb gms/100cc	Hemato-crit in %	Coagl. time in seconds	Day-pen-bird	R.B.C. $X10^6/mm^3$	W.B.C. $X10^3/mm^3$	Hb gms/100cc	Hemato-crit in %	Coagl. time in seconds
20 - 5 - 64	2.37	32.8	7.80	22		20 - 6 - 79	2.23	71.7	12.00	30	
20 - 5 - 65	2.07	31.9	10.50	23		20 - 6 - 80	2.25	20.6	18.00	28	
20 - 5 - 69	2.60	39.2	11.60	34		20 - 6 - 85	2.29	22.4	9.60	32	
20 - 5 - 72	2.84	46.6	11.00	30		20 - 6 - 88	2.35	23.5	12.30	34	
20 - 5 - 74	2.38	31.9	10.20	25		20 - 6 - 90	2.24	43.2	9.00	28	
Mean	2.45	36.5	10.22	26.80		Mean	2.27	36.3	12.18	32.40	
34 - 5 - 64	2.15	44.4	10.20	32		34 - 6 - 79	1.96	41.9	11.60	28	
34 - 5 - 65	--	--	--	--		34 - 6 - 80	2.63	66.6	11.40	37	
34 - 5 - 69	2.38	26.8	12.00	30		34 - 6 - 85	--	--	--	--	
34 - 5 - 72	2.43	63.7	11.40	34		34 - 6 - 88	2.37	19.0	12.70	37	
34 - 5 - 74	2.08	34.1	11.60	34		34 - 6 - 90	2.16	44.4	11.60	30	
Mean	2.26	34.4	11.30	32.50		Mean	2.28	42.8	11.82	33.00	
48 - 5 - 64	1.15	13.5	8.30	34		48 - 6 - 79	1.43	41.9	9.40	28	
48 - 5 - 65	--	--	--	--		48 - 6 - 80	1.77	26.6	9.00	32	
48 - 5 - 69	1.49	62.1	10.70	43		48 - 6 - 85	1.26	6.2	8.80	32	
48 - 5 - 72	1.58	19.9	6.70	22		48 - 6 - 88	--	--	9.00	28	
48 - 5 - 74	1.72	59.9	7.20	31		48 - 6 - 90	1.72	30.4	9.40	28	
Mean	1.48	38.9	8.22	32.50		Mean	1.54	26.3	9.12	29.60	
62 - 5 - 62	2.36	41.2	10.70	32	261	62 - 6 - 78	1.76	23.9	10.20	30	1500
62 - 5 - 63	2.62	20.6	13.40	36	450	62 - 6 - 79	2.52	5.3	11.60	35	1000
62 - 5 - 64	1.90	31.3	9.90	28	600	62 - 6 - 80	2.43	11.9	10.20	31	480
62 - 5 - 65	--	--	--	--	--	62 - 6 - 83	2.83	21.3	12.70	33	1800
62 - 5 - 68	1.95	44.4	9.00	26	1170	62 - 6 - 85	2.74	12.2	10.50	35	1000
62 - 5 - 69	2.47	59.7	11.60	34	300	62 - 6 - 86	2.13	31.5	9.90	29	1440
62 - 5 - 72	1.61	19.7	10.50	27	400	62 - 6 - 87	2.78	29.7	10.70	34	1380
62 - 5 - 74	--	--	--	--	--	62 - 6 - 88	2.64	26.1	10.70	31	572
Mean	2.24	36.3	10.82	30.25	554	62 - 6 - 89	2.36	21.3	10.50	33	1290
						62 - 6 - 90	2.40	8.8	8.50	28	295
						Mean	2.45	19.2	10.55	32.00	1023

Figure 7. Pattern of Mean Blood Values, Treatments VII AD and VIII D

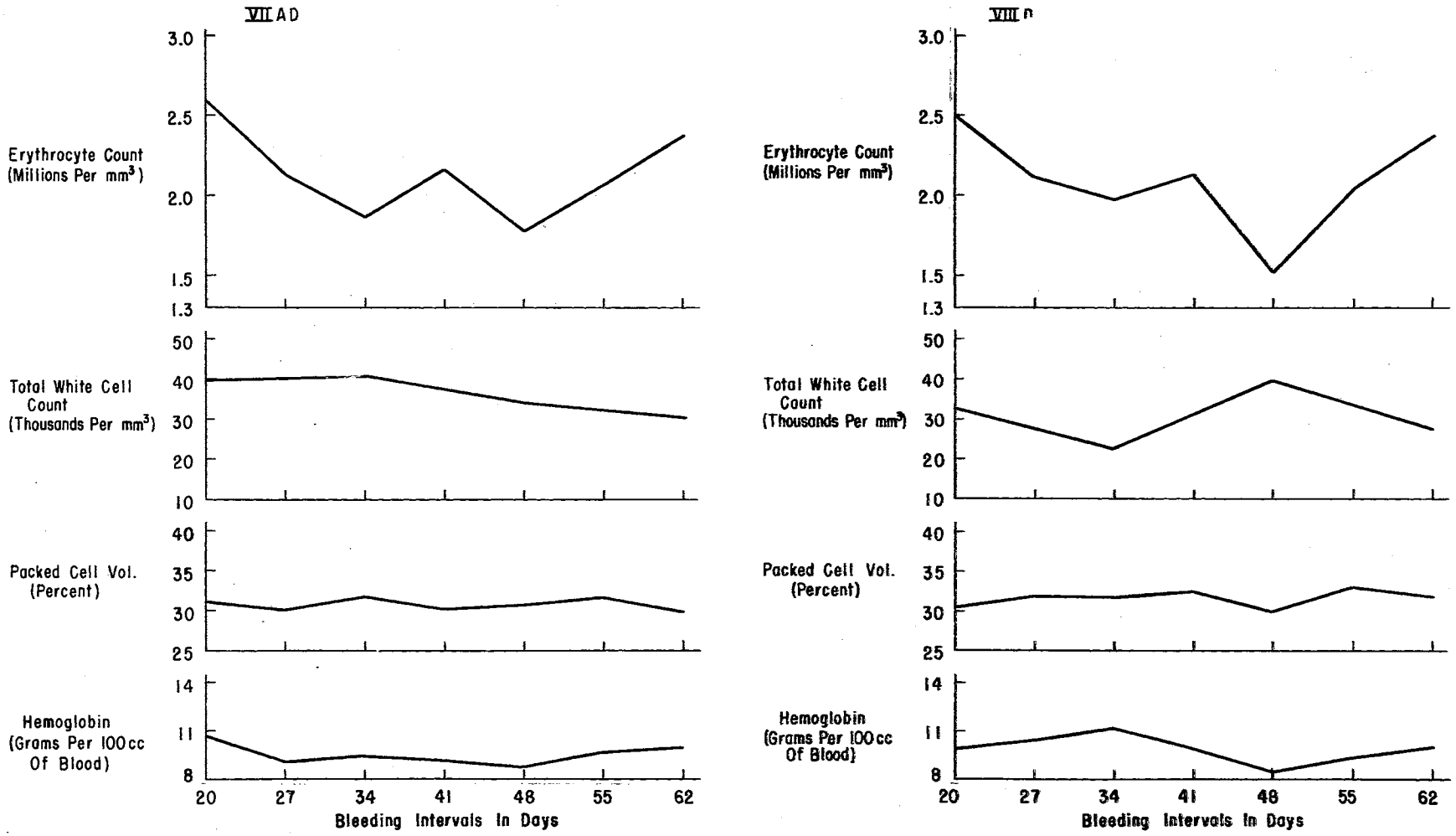


Table 8. Bi-weekly blood data, treatments VII AD and VIII D.

VII AD						VIII D					
Day-pen-bird	R.B.C. X10^6/mm^3	W.B.C. X10^3/mm^3	Hb gms/100cc	Hemato-crit in %	Coagl. time in seconds	Day-pen-bird	R.B.C. X10^6/mm^3	W.B.C. X10^3/mm^3	Hb gms/100cc	Hemato-crit in %	Coagl. time in seconds
20 - 7 - 92	2.55	51.2	9.90	29		20 - 8 - 106	2.05	33.3	10.20	31	
20 - 7 - 95	2.41	43.6	9.90	29		20 - 8 - 109	2.26	23.9	9.90	32	
20 - 7 -100	2.86	31.5	11.60	35		20 - 8 - 111	2.46	43.5	10.50	30	
20 - 7 -101	2.66	50.6	11.40	30		20 - 8 - 116	2.47	41.5	10.00	29	
20 - 7 -104	2.49	22.2	10.20	32		20 - 8 - 118	2.01	19.5	9.00	30	
Mean	2.59	39.4	10.60	31.00		Mean	2.25	32.3	9.92	30.40	
34 - 7 - 92	1.76	46.1	9.40	31		34 - 8 - 106	--	--	--	--	
34 - 7 - 95	1.94	46.1	8.10	31		34 - 8 - 109	1.88	20.8	11.40	34	
34 - 7 -100	1.75	41.7	10.70	34		34 - 8 - 111	2.05	13.9	10.50	30	
34 - 7 -101	--	--	--	--		34 - 8 - 116	1.76	16.6	11.60	29	
34 - 7 -104	1.99	25.5	11.40	30		34 - 8 - 118	2.19	38.4	10.70	34	
Mean	1.86	40.9	9.90	31.50		Mean	1.97	22.4	11.05	31.70	
48 - 7 - 92	1.34	23.9	8.50	31		48 - 8 - 106	1.32	57.0	7.80	35	
48 - 7 - 95	2.10	29.9	8.50	30		48 - 8 - 111	1.66	60.6	10.50	29	
48 - 7 -100	1.49	55.9	9.40	34		48 - 8 - 116	2.24	31.5	8.30	33	
48 - 7 -104	2.18	26.6	8.10	29		48 - 8 - 118	1.01	22.2	6.50	24	
Mean	1.77	34.1	8.62	30.50		Mean	1.55	42.8	8.27	30.25	
62 - 7 - 91	2.39	26.8	10.20	33	350	62 - 8 - 106	2.45	27.9	9.00	28	540
62 - 7 - 92	2.00	28.8	9.00	31	870	62 - 8 - 108	2.49	33.3	10.70	33	545
62 - 7 - 94	2.62	26.6	10.50	31	1260	62 - 8 - 109	2.17	29.5	9.00	30	725
62 - 7 - 95	2.80	26.8	9.90	30	180	62 - 8 - 110	2.18	11.5	9.90	28	960
62 - 7 - 96	2.09	17.9	10.70	30	1018	62 - 8 - 111	1.99	41.9	9.00	29	870
62 - 7 - 98	2.28	44.4	9.60	26	1260	62 - 8 - 113	2.44	30.6	11.00	34	510
62 - 7 -100	2.13	18.8	9.40	30	720	62 - 8 - 116	2.25	17.3	9.90	34	580
62 - 7 -102	1.99	42.1	9.40	26	750	62 - 8 - 118	2.26	34.4	9.90	33	410
62 - 7 -103	2.42	38.4	11.00	31	590	62 - 8 - 119	2.79	30.1	10.70	33	1217
62 - 7 -104	2.39	29.5	9.60	30	630	62 - 8 - 107	2.54	13.9	10.50	33	675
Mean	2.31	30.0	9.93	29.80	762	Mean	2.35	27.0	9.96	31.50	703

Figure 8. Pattern of Mean Blood Values, Treatments IX ABC and X BC

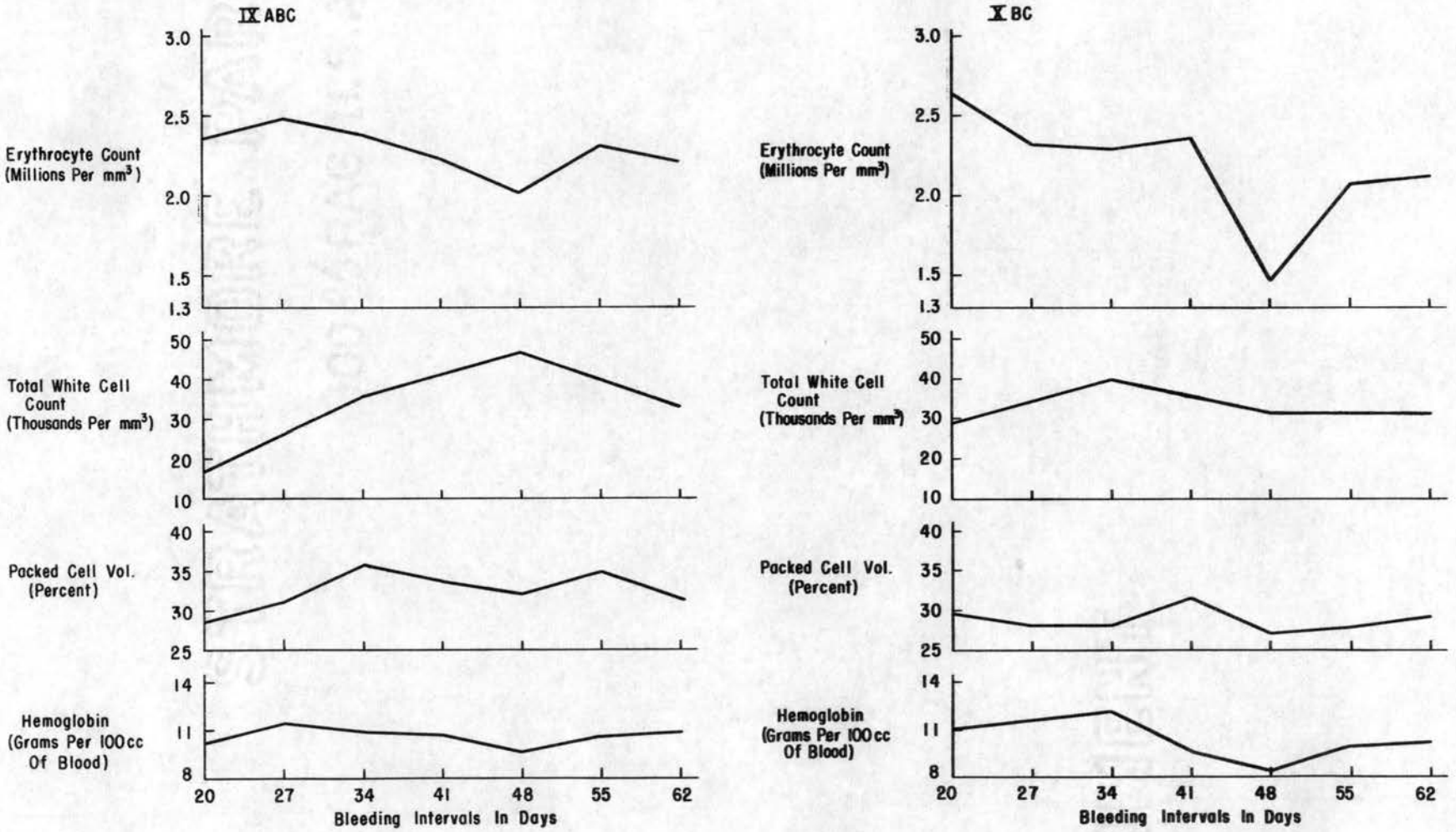


Table 9. Bi-weekly blood data, treatments IX ABC and X BC.

IX ABC						X BC					
Day-pen-bird	R.B.C. $X10^6/mm^3$	W.B.C. $X10^3/mm^3$	Hb gms/100cc	Hemato-crit in %	Coagi. time in seconds	Day-pen-bird	R.B.C. $X10^6/mm^3$	W.B.C. $X10^3/mm^3$	Hb gms/100cc	Hemato-crit in %	Coagi. time in seconds
20 - 9 - 121	2.13	15.9	11.40	30		20 - 10 -137	2.72	19.0	9.90	27	
20 - 9 - 125	--	--	--	--		20 - 10 -139	2.92	19.8	12.00	32	
20 - 9 - 126	--	--	--	--		20 - 10 -145	2.58	59.0	10.50	28	
20 - 9 - 133	2.27	11.1	8.50	26		20 - 10 -146	2.01	23.5	11.00	29	
20 - 9 - 135	2.65	23.5	10.70	30		20 - 10 -150	2.92	22.6	11.60	32	
Mean	2.35	16.8	10.20	28.60		Mean	2.64	28.8	11.00	29.60	
34 - 9 - 121	2.63	55.9	10.50	38		34 - 10 -137	1.72	26.8	9.00	27	
34 - 9 - 126	2.32	28.4	10.00	31		34 - 10 -139	2.53	39.9	13.70	35	
34 - 9 - 128	1.93	37.9	13.00	34		34 - 10 -140	2.22	29.5	11.00	28	
34 - 9 - 133	2.51	36.1	9.90	33		34 - 10 -145	2.07	21.3	15.30	31	
34 - 9 - 135	2.52	19.5	11.40	42		34 - 10 -146	2.95	32.1	11.60	26	
Mean	2.38	35.6	10.96	35.60		Mean	2.29	29.9	12.12	29.00	
48 - 9 - 121	2.00	53.2	8.80	34		48 - 10 -137	1.99	29.5	7.40	27	
48 - 9 - 126	1.71	44.4	9.40	34		48 - 10 -139	.97	34.6	8.30	30	
48 - 9 - 128	1.58	37.2	11.60	39		48 - 10 -140	--	--	--	--	
48 - 9 - 133	2.21	52.3	9.00	30		48 - 10 -145	1.63	--	9.00	27	
48 - 9 - 135	2.52	44.0	10.50	42		48 - 10 -146	1.29	32.4	9.00	23	
Mean	2.00	46.3	9.86	35.80		Mean	1.47	30.9	8.42	26.00	
62 - 9 - 121	2.51	27.3	11.40	29	1800	62 - 10 -137	1.87	20.4	9.90	27	1500
62 - 9 - 123	2.28	59.9	11.00	36	1039	62 - 10 -138	2.02	28.6	8.80	26	780
62 - 9 - 124	2.30	37.0	9.40	33	1410	62 - 10 -139	2.25	27.5	12.50	30	744
62 - 9 - 126	1.79	36.1	10.50	29	1800	62 - 10 -140	2.50	34.1	11.00	34	1000
62 - 9 - 128	2.90	59.9	11.40	30	610	62 - 10 -142	2.05	46.3	10.70	26	780
62 - 9 - 129	1.91	21.5	9.00	31	1800	62 - 10 -143	2.04	44.4	9.90	27	960
62 - 9 - 130	2.29	12.8	9.90	30	1037	62 - 10 -145	1.81	28.1	9.00	20	1440
62 - 9 - 131	2.39	35.9	11.40	32	1800	62 - 10 -146	1.87	23.9	11.00	32	960
62 - 9 - 133	1.78	19.5	11.40	30	682	62 - 10 -149	2.74	43.0	10.50	30	600
62 - 9 - 135	1.93	17.0	11.00	33	945	62 - 10 -150	2.09	15.3	8.80	28	280
Mean	2.70	32.7	10.6	31.30	1292	Mean	2.12	31.2	10.21	28.00	945

Figure 9. Pattern of Mean Blood Values, Treatments XI ACD and XII CD

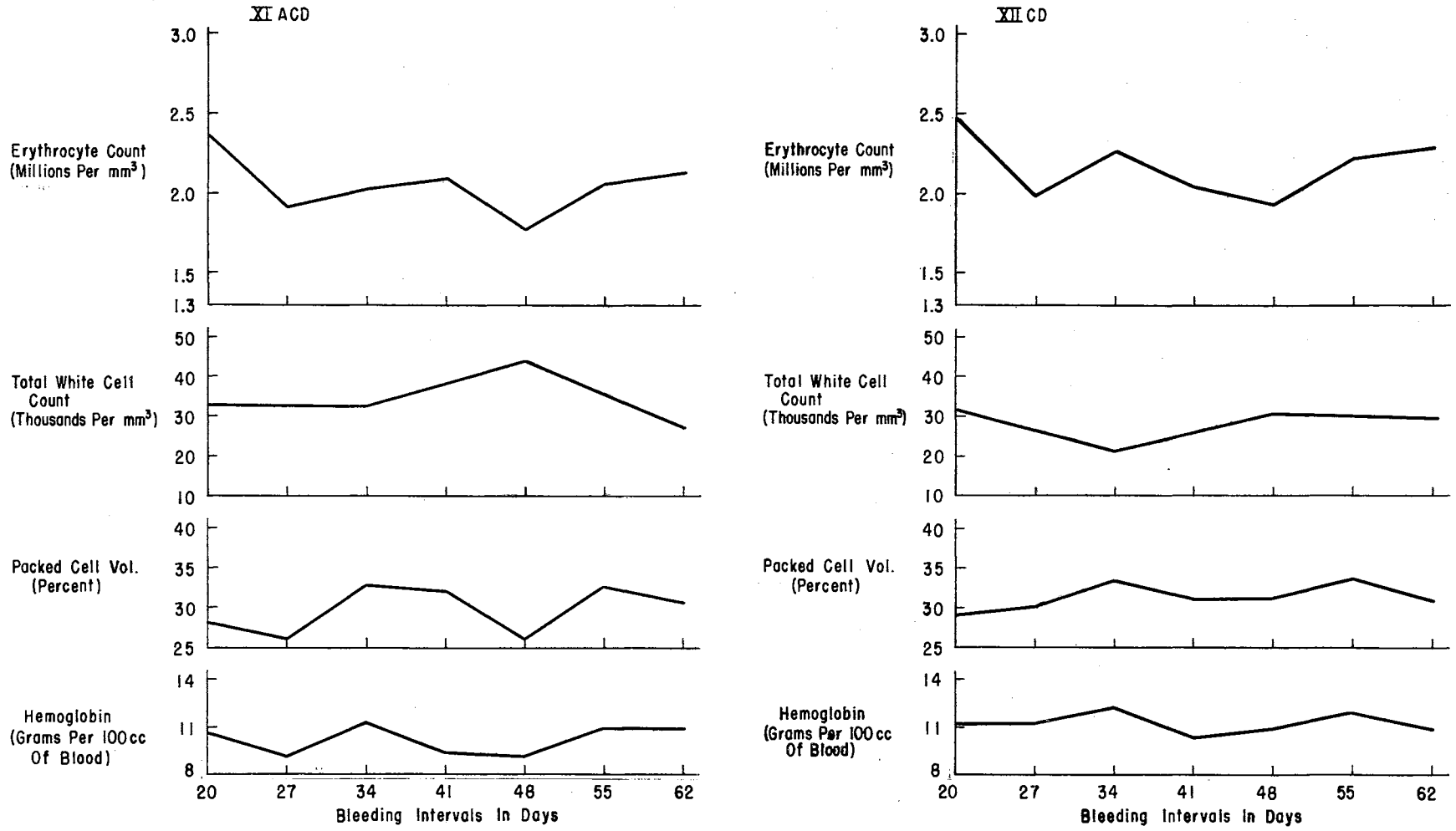


Table 10. Bi-weekly blood data, treatments XI ACD and XII CD

XI ACD						XII CD					
Day-pen-bird	R.B.C. X10^6/mm^3	W.B.C. X10^3/mm^3	Hb gms/100cc	Hemato-crit in %	Coagl. time in seconds	Day-pen-bird	R.B.C. X10^6/mm^3	W.B.C. X10^3/mm^3	Hb gms/100cc	Hemato-crit in %	Coagl. time in seconds
20 - 11 -153	—	—	—	—		20 - 12 -166	2.27	26.6	11.00	26	
20 - 11 -154	2.33	32.8	9.00	27		20 - 12 -170	2.72	51.0	11.00	33	
20 - 11 -156	2.35	32.4	11.40	29		20 - 12 -173	—	—	—	—	
20 - 11 -161	—	—	—	—		20 - 12 -178	2.73	23.7	13.40	33	
20 - 11 -165	2.38	32.2	11.40	28		20 - 12 -180	2.20	20.6	11.00	33	
Mean	2.35	32.4	10.60	28.00		Mean	2.48	30.5	11.40	29	
34 - 11 -154	1.80	31.0	9.00	32		34 - 12 -166	2.21	16.6	10.50	34.00	
34 - 11 -156	2.30	36.8	11.40	32		34 - 12 -170	—	—	—	—	
34 - 11 -161	2.38	35.5	12.30	35		35 - 12 -173	—	—	—	—	
34 - 11 -162	1.62	39.2	11.40	31		34 - 12 -178	2.39	19.5	11.40	33	
34 - 11 -165	2.01	17.6	12.00	34		34 - 12 -180	2.20	26.6	13.00	36	
Mean	2.02	32.1	11.22	32.80		Mean	2.66	20.9	12.12	33.20	
48 - 11 -154	1.60	29.5	10.20	25		48 - 12 -166	2.08	27.9	9.00	30	
48 - 11 -156	1.55	49.2	7.40	26		48 - 12 -170	2.08	21.3	11.40	31	
48 - 11 -161	1.67	44.4	9.60	28		48 - 12 -173	2.01	42.4	12.70	35	
48 - 11 -162	2.08	55.5	9.90	25		48 - 12 -178	1.66	38.8	11.00	30	
48 - 11 -165	1.63	40.8	8.30	26		48 - 12 -180	1.86	30.1	9.60	30	
Mean	1.70	43.9	9.08	26.00		Mean	1.93	30.5	10.74	31.00	
62 - 11 -153	2.61	35.7	10.70	33	1260	62 - 12 -166	2.10	41.0	11.60	25	952
62 - 11 -154	2.13	31.5	11.40	30	695	62 - 12 -167	2.07	33.3	11.40	35	1080
62 - 11 -155	2.53	22.6	10.50	32	1800	62 - 12 -168	2.16	39.9	8.80	30	1800
62 - 11 -156	1.28	30.4	9.60	29	815	62 - 12 -169	1.99	34.1	11.60	28	735
62 - 11 -157	2.38	30.1	10.20	30	1800	62 - 12 -171	2.20	31.5	9.40	30	952
62 - 11 -158	2.41	22.2	11.00	34	922	62 - 12 -172	2.32	15.3	9.90	32	738
62 - 11 -160	2.21	28.6	10.50	31	1800	62 - 12 -173	1.93	26.1	10.50	31	862
62 - 11 -161	2.20	14.6	10.70	34	1260	62 - 12 -175	2.54	22.4	10.70	32	1200
62 - 11 -162	1.31	36.6	10.20	24	1080	62 - 12 -178	2.63	28.8	14.50	34	432
62 - 11 -165	2.16	16.4	13.70	28	757	62 - 12 -180	2.89	20.4	11.40	32	480
Mean	2.42	26.6	10.85	30.50	1218	Mean	2.28	29.3	10.98	30.90	923

Figure 10. Pattern of Mean Blood Values, Treatments XIII ABD and XIV BD

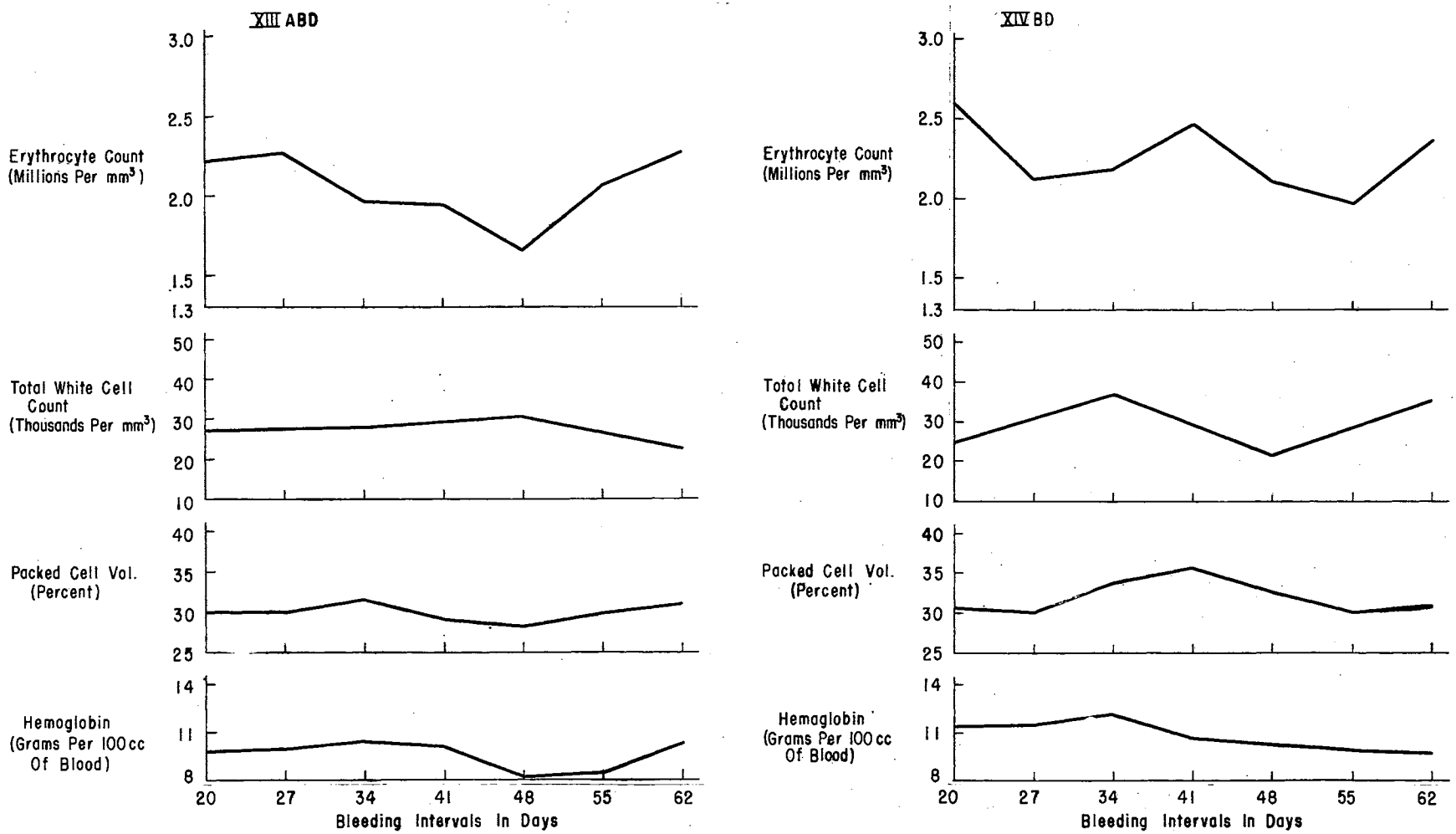


Table 11. Bi-weekly blood data, treatments XIII ABD and XIV BD

XIII ABD						XIV BD					
Day-pen-bird	R.B.C. X10^6/mm^3	W.B.C. X10^3/mm^3	Hb gms/100cc	Hemato-crit in %	Coag. time in seconds	Day-pen-bird	R.B.C. X10^6/mm^3	W.B.C. X10^3/mm^3	Hb gms/100cc	Hemato-crit in %	Coag. time in seconds
20 - 13 -182	--	--	--	--		20 - 14 -198	2.60	28.1	11.40	30	
20 - 13 -183	--	--	--	--		20 - 14 -199	2.60	21.3	12.70	30	
20 - 13 -189	2.26	42.6	9.00	30		20 - 14 -201	2.44	31.5	11.00	30	
20 - 13 -192	2.05	20.2	10.20	32		20 - 14 -209	2.60	28.1	11.40	28	
20 - 13 -195	2.31	18.4	10.00	28		20 - 14 -210	2.70	19.9	10.50	35	
Mean	2.20	27.0	9.73	30.00		Mean	2.58	24.9	11.40	30.80	
34 - 13 -182	1.85	20.2	10.50	35		34 - 14 -198	1.87	35.9	13.70	33	
34 - 13 -183	2.13	24.1	10.70	31		34 - 14 -199	2.51	51.0	12.50	36	
34 - 13 -189	--	--	--	--		34 - 14 -201	2.14	30.4	10.70	34	
34 - 13 -192	2.11	23.0	11.60	32		34 - 14 -209	--	--	13.70	35	
34 - 13 -195	1.75	44.4	8.50	28		34 - 14 -210	2.20	27.9	9.90	31	
Mean	1.90	27.9	10.32	31.40		Mean	2.18	36.7	12.10	33.80	
48 - 13 -183	1.79	23.7	9.60	28		48 - 14 -198	2.04	35.5	11.00	29	
48 - 13 -187	1.80	37.0	8.30	31		48 - 14 -199	2.05	10.8	11.40	34	
48 - 13 -189	1.20	14.2	7.80	24		48 - 14 -201	2.39	19.3	9.40	28	
48 - 13 -192	2.23	44.4	9.60	30		48 - 14 -209	1.94	35.5	11.00	29	
48 - 13 -195	1.22	30.3	6.10	27		48 - 14 -210	2.13	8.6	9.90	30	
Mean	1.65	30.1	8.28	28.00		Mean	2.11	21.9	10.26	30.00	
62 - 13 -183	2.15	25.5	8.30	33	875	62 - 14 -197	2.57	11.9	10.20	34	212
62 - 13 -184	1.95	26.6	9.90	29	1175	62 - 14 -198	2.07	55.5	9.60	29	480
62 - 13 -186	2.52	14.2	13.00	42	921	62 - 14 -199	2.18	36.8	7.40	32	870
62 - 13 -187	1.98	18.2	10.20	30	270	62 - 14 -200	2.27	64.3	9.40	28	540
62 - 13 -190	2.85	15.9	14.50	36	920	62 - 14 -201	2.30	33.7	11.00	28	780
62 - 13 -191	2.47	26.4	10.20	32	929	62 - 14 -202	2.46	21.5	9.40	30	910
62 - 13 -192	2.39	15.9	10.70	32	1000	62 - 14 -203	2.61	21.3	9.90	30	1380
62 - 13 -194	1.79	26.6	9.40	24	1800	62 - 14 -208	2.56	28.4	9.90	29	1030
62 - 13 -195	1.82	23.7	7.40	25	780	62 - 14 -209	2.49	30.4	9.40	30	1000
62 - 13 -189	1.82	28.1	7.40	25	780	62 - 14 -210	2.20	49.2	9.90	34	604
Mean	2.27	22.1	10.10	30.80	945	Mean	2.37	35.3	9.61	30.70	780

Figure 11. Pattern of Mean Blood Values, Treatments XV ABCD and XVI BCD

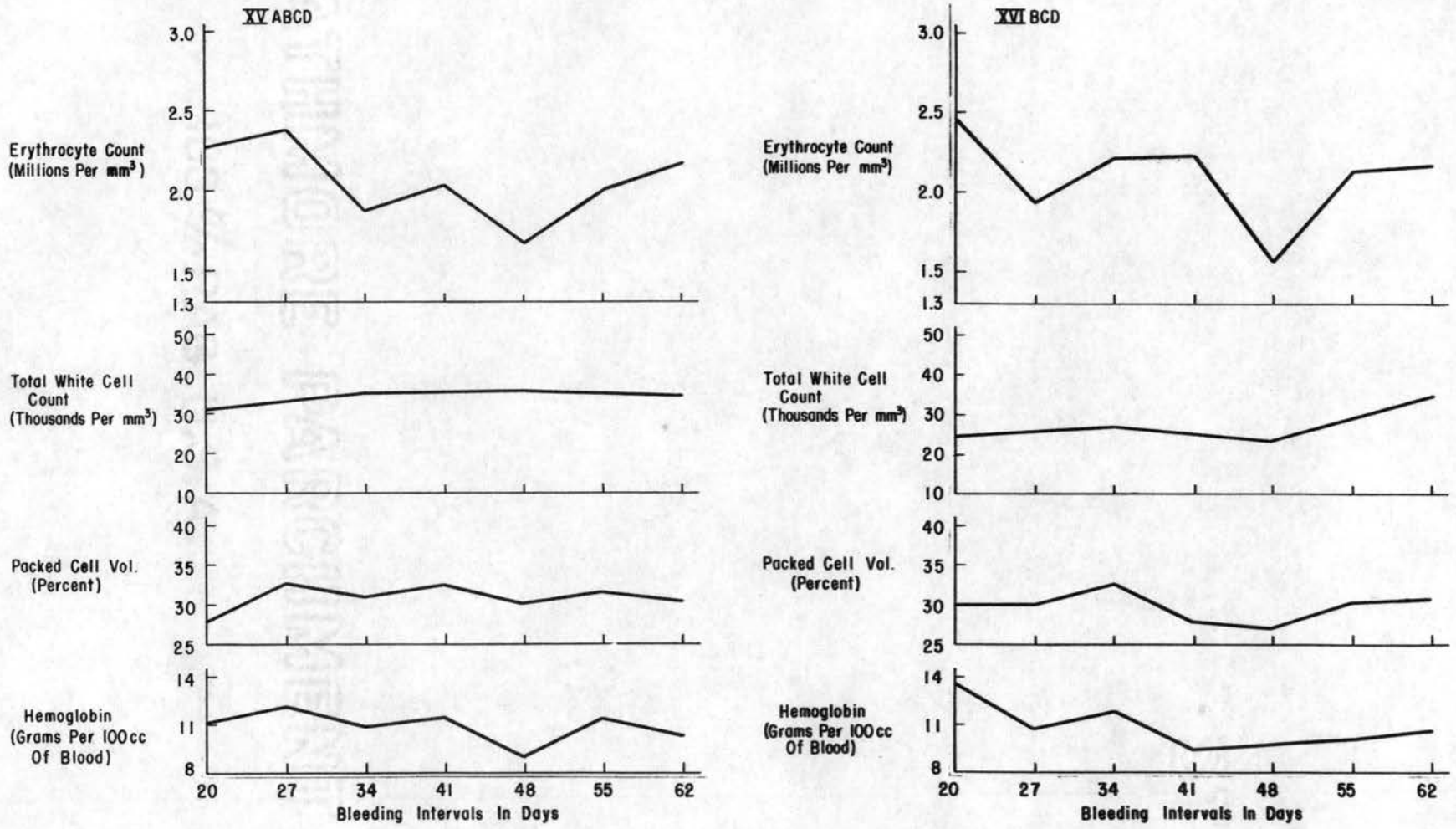


Table 12. Bi-weekly blood data, treatments XV ABCD and XVI BCD

XV ABCD						XVI BCD					
Day-pen-bird	R.B.C. X10^6/mm^3	W.B.C. X10^3/mm^3	Hb gms/100cc	Hemato-crit in %	Coag. time in seconds	Day-pen-bird	R.B.C. X10^6/mm^3	W.B.C. X10^3/mm^3	Hb gms/100cc	Hemato-crit in %	Coag. time in seconds
20 - 15 - 211	1.98	30.8	11.00	27		20 - 16 - 227	2.66	36.6	13.70	37	
20 - 15 - 212	2.35	17.3	12.70	28		20 - 16 - 229	2.61	17.0	12.30	29	
20 - 15 - 219	2.13	48.3	10.20	30		20 - 16 - 235	2.29	22.2	11.40	28	
20 - 15 - 221	2.36	41.9	11.40	28		20 - 16 - 236	2.24	22.2	16.60	26	
20 - 15 - 225	2.52	15.7	10.20	27		20 - 16 - 237	--	--	--	--	
Mean	2.26	30.8	11.10	28.00		Mean	2.45	24.5	13.50	30.00	
34 - 15 - 211	1.31	16.8	7.40	24		34 - 16 - 233	2.25	44.4	14.50	35	
34 - 15 - 212	1.48	23.5	12.00	34		34 - 16 - 229	2.55	17.7	12.00	35	
34 - 15 - 219	1.67	44.4	12.00	34		34 - 16 - 235	2.39	18.2	9.40	35	
34 - 15 - 221	2.55	42.1	11.00	31		34 - 16 - 236	1.65	27.3	11.00	25	
34 - 15 - 225	2.28	47.2	11.40	33		34 - 16 - 237	--	--	--	--	
Mean	1.85	34.8	10.76	31		Mean	2.21	26.9	11.72	32.40	
48 - 15 - 211	2.11	46.8	9.90	28		48 - 16 - 230	1.65	38.8	8.30	27	
48 - 15 - 212	1.09	27.0	9.40	32		48 - 16 - 233	1.56	32.6	9.90	21	
48 - 15 - 219	1.30	32.4	10.20	29		48 - 16 - 235	1.00	15.3	9.90	30	
48 - 15 - 221	2.14	30.4	8.50	32		48 - 16 - 236	1.52	15.5	8.30	28	
48 - 15 - 225	1.67	42.6	6.70	29		48 - 16 - 237	2.13	13.7	11.40	29	
Mean	1.66	35.8	8.94	30.00		Mean	1.57	23.2	9.56	27.00	
62 - 15 - 211	1.46	37.9	9.90	26	1200	62 - 16 - 226	2.42	31.3	12.00	35	970
62 - 15 - 212	1.99	52.1	10.00	29	840	62 - 16 - 228	2.47	46.3	10.20	30	1260
62 - 15 - 213	2.08	11.1	10.70	29	1800	62 - 16 - 230	2.11	15.5	8.30	22	600
62 - 15 - 214	2.75	30.6	9.90	33	1053	62 - 16 - 234	2.26	12.6	11.40	31	1020
62 - 15 - 216	2.11	32.4	11.40	33	600	62 - 16 - 235	1.28	32.4	10.70	32	495
62 - 15 - 217	2.57	25.5	9.60	30	1800	62 - 16 - 236	2.05	25.3	10.70	30	1200
62 - 15 - 218	2.49	16.8	9.40	32	1800	62 - 16 - 237	2.49	51.0	10.50	31	818
62 - 15 - 219	2.33	63.9	9.90	32	1500	62 - 16 - 238	2.06	44.4	11.00	31	1800
62 - 15 - 221	1.84	35.7	9.90	25	608	62 - 16 - 239	2.23	46.1	9.60	30	1520
62 - 15 - 225	2.04	37.9	10.00	32	604	62 - 16 - 240	2.50	35.5	9.60	30	1800
Mean	2.16	34.2	10.07	30.10	1180	Mean	2.18	34.0	10.40	30.20	1148

The author is in agreement with Bainbridge and Menzies (1920), that avian blood properly drawn, free of debris and tissue fluid, will not clot. In drawing blood samples for this experiment an attempt was made to produce a minimum, but uniform, amount of tissue damage in order to measure the true coagulating ability of the sample of blood. It is considered that our average coagulation time of 15 minutes and 36 seconds, from pooled data of all treatments, is not abnormal considering the techniques used to collect the blood. Blood that failed to clot was given the figure of 1800 seconds (30 minutes) as a coagulation time. This lack of clot formation occurred in apparently healthy birds and was not considered pathological. No capillary hemorrhage occurred in any bird throughout the experiment.

Capillary strength:

Estimations of capillary strength by negative pressure, were made at 11 day intervals by means of a mercury resistometer (Fig. 1). No differences in capillary strength were observed among the various treatments. The data collected from each treatment at each eleven day interval were then pooled and incorporated into a graph (Fig. 12). These pooled data were collected from a total of 80 chicks. Each bar of the graph indicates the average highest negative pressure at which the capillaries did not break down. The data from which this graph was drawn are recorded in Tables 13 to 18. The criterion of breakdown was the appearance of a bruise (Fig. 3) or suffusion of blood into the area.

From the pattern of this chart it was determined that as the chicks grew older the capillaries became more resistant to negative pressure. At 22 days an average of 217 mm of mercury was the highest negative pres-

Figure 12. Capillary Strength Measured in Millimeters of Mercury Negative Pressure for Ten Seconds

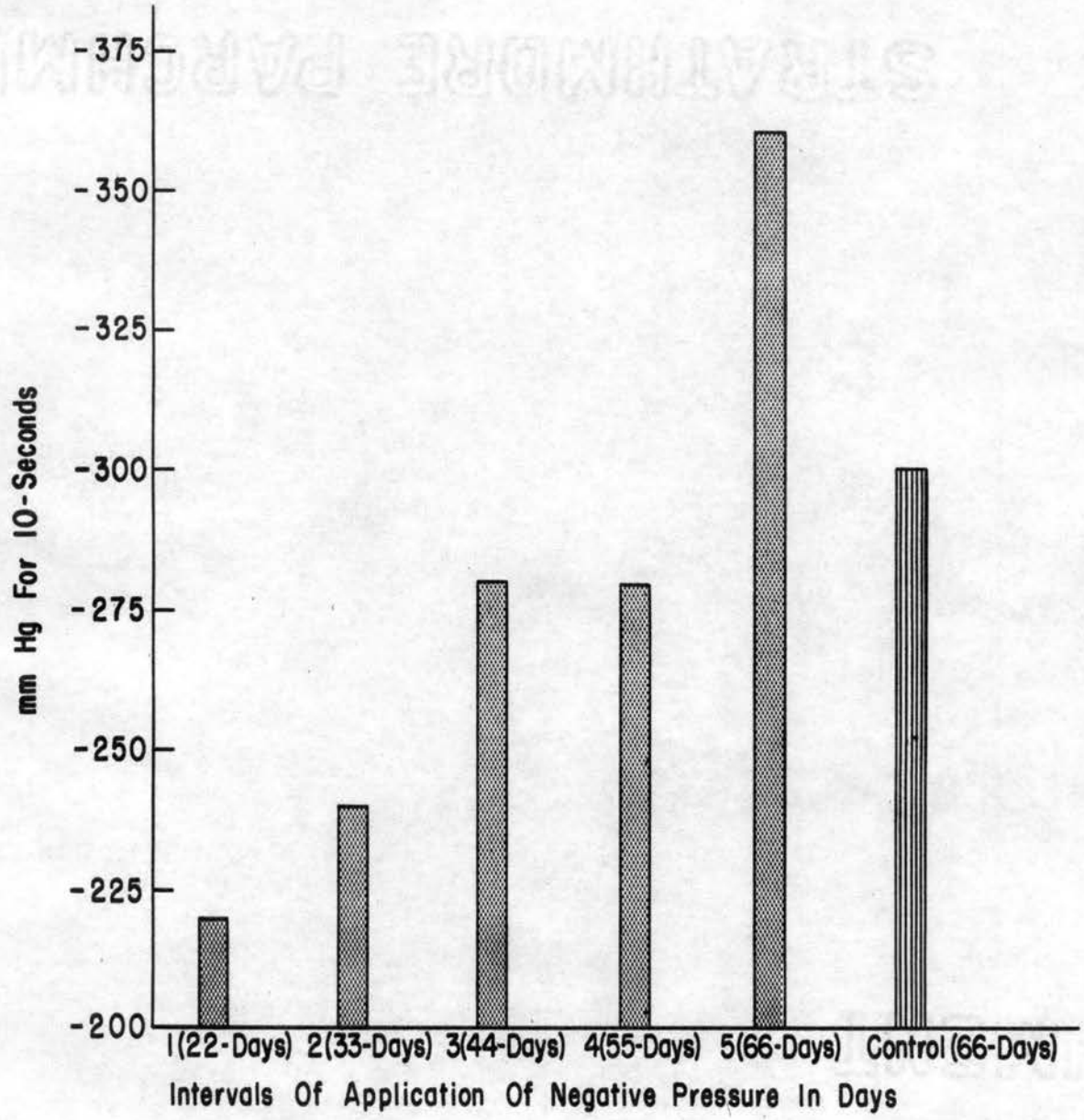


Table 13. Capillary strength at 22 days, measured in millimeters of mercury negative pressure.

Pen - bird	mm Hg negative pressure	Pen - bird	mm Hg negative pressure
1 - 2	200	9 - 121	225
1 - 4	225	9 - 126	200
1 - 8	225	9 - 133	225
1 - 12	225	9 - 135	225
1 - 15	250	10 - 137	225
2 - 16	225	10 - 139	225
2 - 18	200	10 - 145	225
2 - 22	200	10 - 146	175
2 - 28	275	10 - 150	200
2 - 30	200	11 - 153	225
3 - 31	200	11 - 154	225
3 - 34	200	11 - 156	225
3 - 36	200	11 - 161	200
3 - 42	225	11 - 165	300
3 - 43	225	12 - 166	175
4 - 46	200	12 - 170	225
4 - 50	225	12 - 173	225
4 - 55	225	12 - 178	225
4 - 58	225	12 - 180	225
4 - 60	225	13 - 182	225
5 - 64	225	13 - 183	225
5 - 65	200	13 - 189	225
5 - 69	200	13 - 192	200
5 - 72	225	13 - 195	225
5 - 74	225	14 - 198	200
6 - 70	200	14 - 199	225
6 - 80	225	14 - 201	225
6 - 85	225	14 - 209	225
6 - 88	200	14 - 210	200
6 - 90	225	15 - 211	200
7 - 92	200	15 - 212	225
7 - 95	225	15 - 219	200
7 - 100	200	15 - 221	250
7 - 101	225	15 - 225	225
7 - 104	225	16 - 227	225
8 - 106	200	16 - 229	225
8 - 109	225	16 - 235	200
8 - 111	225	16 - 236	225
8 - 116	225	16 - 237	225
8 - 118	225	Mean	217

Table 14. Capillary strength at 33 days, measured in millimeters of mercury negative pressure.

Pen - bird	mm Hg negative pressure	Pen - bird	mm Hg negative pressure
1 - 2	250	9 - 121	250
1 - 4	250	9 - 126	225
1 - 8	250	9 - 129	250
1 - 12	250	9 - 133	250
1 - 15	250	9 - 135	250
2 - 16	225	10 - 137	250
2 - 18	200	10 - 139	225
2 - 22	250	10 - 140	250
2 - 28	225	10 - 145	250
2 - 30	200	10 - 146	250
2 - 31	250	11 - 154	250
2 - 34	250	11 - 156	250
3 - 36	250	11 - 161	250
3 - 42	250	11 - 165	250
3 - 43	250	12 - 166	225
4 - 46	225	12 - 170	250
4 - 50	250	12 - 173	225
4 - 55	250	12 - 178	250
4 - 58	250	12 - 180	250
4 - 60	250	13 - 182	250
5 - 64	225	13 - 183	225
5 - 65	250	13 - 189	250
5 - 69	250	13 - 192	200
5 - 72	250	13 - 195	225
5 - 74	250	14 - 198	250
6 - 79	250	14 - 199	225
6 - 80	250	14 - 201	250
6 - 85	250	14 - 209	250
6 - 88	200	14 - 210	175
6 - 90	225	15 - 211	225
7 - 92	200	15 - 212	225
7 - 95	225	15 - 219	250
7 - 100	250	15 - 221	250
7 - 101	250	15 - 225	250
7 - 104	250	16 - 229	250
8 - 106	225	16 - 230	275
8 - 109	250	16 - 235	250
8 - 111	225	16 - 236	225
8 - 116	250	16 - 237	250
8 - 118	200	Mean	239

Table 15. Capillary strength at 44 days, measured in millimeters of mercury, negative pressure.

Pen - bird	mm Hg negative pressure	Pen - bird	mm Hg negative pressure
1 - 2	300	9 - 121	275
1 - 4	300	9 - 126	300
1 - 8	300	9 - 129	300
1 - 12	300	9 - 133	300
1 - 15	300	9 - 135	300
2 - 16	300	10 - 137	300
2 - 18	275	10 - 139	275
2 - 22	275	10 - 140	250
2 - 28	300	10 - 145	250
2 - 30	300	10 - 146	200
3 - 31	300	11 - 154	300
3 - 34	300	11 - 156	300
3 - 36	275	11 - 161	300
3 - 42	275	11 - 165	300
3 - 43	275	12 - 166	225
4 - 46	300	12 - 170	300
4 - 50	275	12 - 173	300
4 - 55	300	12 - 178	300
4 - 58	300	12 - 180	300
4 - 60	275	13 - 182	275
5 - 64	300	13 - 183	275
5 - 65	300	13 - 189	275
5 - 69	275	13 - 192	250
5 - 72	250	13 - 195	250
5 - 74	300	14 - 198	250
6 - 79	250	14 - 199	250
6 - 80	275	14 - 201	250
6 - 85	275	14 - 209	250
6 - 88	250	14 - 210	250
6 - 90	275	15 - 211	300
7 - 92	275	15 - 212	275
7 - 95	275	15 - 219	275
7 - 100	275	15 - 221	300
7 - 101	275	15 - 225	300
7 - 104	275	16 - 229	275
8 - 106	300	16 - 230	275
8 - 109	300	16 - 235	300
8 - 111	300	16 - 236	275
8 - 116	300	16 - 237	250
8 - 118	300	Mean	280

Table 16. Capillary strength at 55 days, measured in millimeters of mercury, negative pressure.

Pen - bird	mm Hg negative pressure	Pen - bird	mm Hg negative pressure
1 - 2	300	9 - 128	250
1 - 4	300	9 - 135	300
1 - 8	300	9 - 133	300
1 - 12	300	10 - 137	250
1 - 15	300	10 - 139	250
1 - 16	300	10 - 140	300
2 - 22	225	10 - 145	300
2 - 25	300	10 - 146	300
2 - 28	250	11 - 154	250
2 - 30	250	11 - 156	300
3 - 31	300	11 - 161	300
3 - 34	275	11 - 162	300
3 - 36	275	11 - 165	300
3 - 43	300	12 - 166	300
4 - 46	300	12 - 170	300
4 - 50	225	12 - 173	300
4 - 55	225	12 - 178	300
4 - 58	250	12 - 180	275
4 - 60	300	13 - 183	250
5 - 64	225	13 - 187	250
5 - 69	275	13 - 189	225
5 - 72	225	13 - 192	300
5 - 74	300	13 - 195	225
6 - 79	300	14 - 198	250
6 - 80	300	14 - 199	275
6 - 85	300	14 - 201	275
6 - 88	275	14 - 209	300
6 - 90	225	14 - 210	275
7 - 92	225	15 - 211	300
7 - 95	275	15 - 212	300
7 - 100	300	15 - 219	300
7 - 101	275	15 - 221	300
7 - 104	250	15 - 225	300
8 - 106	250	16 - 230	300
8 - 109	300	16 - 233	250
8 - 111	275	16 - 235	300
8 - 116	275	16 - 236	300
8 - 118	275	16 - 237	300
9 - 121	300		
9 - 126	300	Mean	279

Table 17. Capillary strength at 66 days, measured in millimeters of mercury, negative pressure.

Pen - bird	mm Hg negative pressure	Pen - bird	mm Hg negative pressure
1 - 2	375	9 - 126	375
1 - 4	350	9 - 128	300
1 - 8	375	9 - 133	350
1 - 12	375	9 - 135	375
1 - 15	275	10 - 137	350
2 - 16	375	10 - 139	375
2 - 22	300	10 - 140	375
2 - 25	375	10 - 145	375
2 - 28	375	10 - 146	375
2 - 30	375	11 - 154	375
3 - 31	375	11 - 156	375
3 - 35	375	11 - 161	350
3 - 36	375	11 - 162	375
3 - 39	375	11 - 165	300
3 - 43	375	12 - 166	375
4 - 46	375	12 - 169	375
4 - 50	350	12 - 173	375
4 - 55	375	12 - 178	350
4 - 58	375	12 - 180	375
4 - 60	375	13 - 183	375
5 - 64	375	13 - 187	375
5 - 69	375	13 - 189	350
5 - 72	300	13 - 192	375
5 - 74	350	13 - 195	350
5 - 77	375	14 - 198	375
6 - 79	375	14 - 199	300
6 - 85	375	14 - 201	375
6 - 88	375	14 - 209	375
6 - 90	375	14 - 210	300
7 - 92	375	15 - 211	375
7 - 95	375	15 - 212	325
7 - 100	375	15 - 220	350
7 - 101	375	15 - 221	350
7 - 104	375	15 - 225	375
8 - 106	325	16 - 230	375
8 - 109	375	16 - 234	375
8 - 111	375	16 - 235	375
8 - 116	300	16 - 236	375
8 - 118	375	16 - 237	350
9 - 121	375	Mean	361

Table 18. Capillary strength at 66 days (control), measured in millimeters of mercury negative pressure.

Pen - bird	mm Hg negative pressure	Pen - bird	mm Hg negative pressure
1 - 1	325	9 - 124	325
1 - 6	300	9 - 129	275
1 - 7	325	9 - 130	325
1 - 11	325	9 - 131	350
1 - 13	325	10 - 138	325
2 - 19	325	10 - 142	300
2 - 20	325	10 - 143	325
2 - 23	325	10 - 147	275
2 - 24	275	10 - 149	300
2 - 27	325	11 - 153	325
3 - 37	325	11 - 155	275
3 - 38	350	11 - 157	250
3 - 39	350	11 - 158	325
3 - 41	325	12 - 167	300
3 - 45	350	12 - 168	250
4 - 47	325	12 - 171	250
4 - 48	300	12 - 172	250
4 - 49	275	12 - 175	325
4 - 53	325	13 - 184	325
4 - 56	325	13 - 186	275
4 - 62	325	13 - 190	325
5 - 63	325	13 - 194	275
5 - 66	300	13 - 191	275
5 - 68	325	14 - 197	275
6 - 78	275	14 - 200	250
6 - 83	300	14 - 202	325
6 - 86	325	14 - 203	325
6 - 87	325	14 - 208	275
6 - 89	325	15 - 213	275
7 - 93	325	15 - 214	250
7 - 94	325	15 - 217	250
7 - 96	300	15 - 218	275
7 - 98	250	15 - 219	250
7 - 102	275	16 - 226	250
8 - 107	325	16 - 228	250
8 - 108	325	16 - 231	325
8 - 110	325	16 - 238	300
8 - 113	325	16 - 240	325
8 - 119	325		
9 - 123	325	Mean	303

sure tolerated by the 80 chicks; whereas at 66 days (control) an average negative pressure of 303 mm of mercury was tolerated; a difference of 86 mm of mercury.

Frequent application of negative pressure to the same area was observed to enhance capillary resistance. From the first application at 22 days to the fifth application at 66 days, a difference of 144 mm of mercury was observed. This is a difference of 58 mm of mercury as compared to a control group of the same age that had never been aspirated or bruised with the mercury resistometer. Specially stained sections of breast muscle, from controls and from frequently aspirated birds, showed no histological difference other than mild hyaline change in an occasional treated bird. The bruise, when produced, disappeared in less than a week. If any alteration of mesenchymal substance occurred it was not observed in stained sections.

The plateau of capillary strength values from 44 to 55 days as shown in Fig. 12 is coincident with the occurrence of low blood values in all treatment as shown in Figs. 4 to 11. This combined pattern of response was interpreted as indicating a critical period in the lives of these chicks; either natural or due to an unobserved environmental factor.

General observations:

Although tissues such as liver, skeletal muscle, and bone marrow were fixed and sectioned, no pathological alteration of these tissues was observed.

The mean values of blood constituents of the various treatments were within the normal range; however, an occasional mild transient anemia was observed in individual birds subjected to sulfaquinoxaline or avian

encephalomyelitis.

Birds slaughtered at the termination of the experiment (68 days) were well feathered and averaged 3.19 lbs. Uneven weight gains were observed in groups subjected to avian encephalomyelitis. The only mortality after the first few days was due to trauma of the anterior vena cava while bleeding. Two birds in pen VI C (basal and tremors) died from epidemic tremors. Five birds from the various tremors treated groups had not fully recovered from paralysis and leg weakness at the time of slaughter.

Summary and Conclusions

Since 1950 a hemorrhagic disease has occurred in young chickens. Although this condition of the blood vascular system has been described in detail by Cartrite (1954) and Gray et al. (1954), the etiology is unknown. Stresses such as chilling, overheating, disease, drugs, and moldy feed have been suggested as the cause of, or as contributing to this disease.

This experiment was designed to study the effect of mild stresses caused by low levels of mold and sulfaquinoxaline in feed, and artificial infection with avian encephalomyelitis, a low mortality disease. The ability of liver extract to alleviate the symptoms of these stresses was tested.

An estimation was made of capillary strength by the negative pressure method during the course of the experiment. Capillary strength was not altered by the various treatments, but did increase with age. Frequent application of negative pressure to the same area tended to produce a resistance to capillary breakdown. Some alteration of capillary strength with low blood values was observed at the seventh week of age in all treatments.

Birds subjected to avian encephalomyelitis showed a hyperchromic, macrocytic anemia and a reduced ability for the blood to coagulate. The latter effect was also observed in chicks subjected to tremors and sulfaquinoxaline in the absence of liver extract.

Sulfaquinoxaline fed to chicks from the first day produced an anemia through the 34th day; however they were able to adapt themselves to this stress by the 48th day.

No spontaneous hemorrhage or gross pathology was observed in any of the treated birds other than the symptoms of avian encephalomyelitis.

Tissue studies of liver, skeletal muscle, and bone marrow revealed no important histological changes.

This experiment indicated that blood values of chickens fluctuate normally within a broad range, and that disease and drugs may broaden this range of values. There is evidence that liver extract may alleviate the tendency of disease and drugs to reduce the clotting ability of avian blood.

This experiment lends support to the statement of Bainbridge and Menzies (1920) that avian blood free of tissue debris will not coagulate.

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