Sodium and potassium concentrations of erythrocytes and plasma in normal and adrenalectomized dogs

by

Larry Lowell Small

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Physiology

Iowa State University
Ames, Iowa

1971

TABLE OF CONTENTS

INTRODUCTION

In the past there have been two accepted methods used to determine the concentrations of various constituents present in the erythrocytes (RBCs) of different animal species: (a) the direct method and (b) the indirect method. The direct method involves the centrifugation of the blood sample, removal of the plasma, washing the cells in an isotonic solution, and measuring the concentrations of cellular constituents directly. The direct method, at first, appears to be an excellent approach. However, there is one major drawback to this technique. Not all RBC constituents are bound to the cell membrane; many constituents are contained within the intracellular water. When the extracellular fluids change drastically, as would occur with washing of the cells, diffusion of various RBC constituents across the RBC membrane may ensue. The indirect method, however, is not dependent upon washing of the cells. With the indirect method, the RBC constituent concentrations are calculated. The concentration of the RBC constituent in question is measured in the plasma and in a hemolyzed whole blood portion of the blood sample. The packed cell volume of the blood sample, estimated by the micro- or macro-hematocrit methods, is then used to calculate the RBC constituent concentration. The indirect method also appears to be an excellent approach, but it also has a major drawback. When estimating the packed cell volume of a blood

sample, plasma becomes trapped between the cells and causes an overestimation of the true cell volume. Consequently, when calculating the concentration of a cellular constituent, the trapped plasma error may give false results.

Many techniques have been devised to calculate the trapped plasma concentration of a blood sample. Several different radionuclides (radioisotopes) and dye dilution methods have been used to calculate the trapped plasma volume, but these methods are time consuming and the results are not always comparable among the different techniques used. Another error of the packed cell volume, not usually considered when measuring trapped plasma errors, is the effects of force and time of centrifugation on the RBCs. Because RBCs are not rigid spheres, the RBC membranes become distorted, and sometimes even rupture as the force and time of centrifugation increase. A satisfactory technique to eliminate this error has not previously been developed.

Rogoff and Stewart (35) in 1926, with an experiment concerning adrenalectomized dogs, found that the changes that occur between the plasma and cells are open to criticism because of the error associated with the methods used to determine RBC electrolyte concentrations.

This study was designed in two consecutive but interrelated parts. The first part was concerned with the evaluation of a recently developed technique (a modified indirect

method) which eliminates the major error associated with the indirect method of determining the RBC sodium and potassium concentrations. The second part dealt with the changes which occur in the plasma and RBC sodium and potassium concentrations of adrenalectomized dogs; utilizing the modified indirect method to determine the RBC sodium and potassium concentration.

LITERATURE REVIEW

The Measurement of Erythrocyte Cations

Kramer and Tisdall (28) in 1922 briefly discussed some of the first corpuscle anion and cation concentrations determined. They stated that the earliest recorded figures for the concentrations of cations and anions in human blood corpuscles and serum were those reported by Schmidt in 1850. Some years later (1876) Bunge, a pupil of Schmidt reported a number of complete analyses on the ash of blood corpuscles and serum of several animals. They also stated that Abderhalden in 1898 published the results of a similar study on many different species of domestic animals. Since the time of Schmidt, Bunge, and Abderhalden a large amount of information has been acquired concerning the errors associated with the methods used to determine RBC cation concentrations and the distribution of different cations between the plasma and RBCs of various animal species.

One of the first problems to be approached when measuring the RBC sodium and potassium concentration is deciding on the units of measurement. Coldman and Good (11) in 1967 measured RBC sodium, potassium, and glucose concentrations in eight different mammals by the indirect method. They stated that, "In the past, as a reference datum in biological work, analytical data are best expressed as weight per unit weight of

dry matter. However, it has been found with rat renal papillae that hydration and potassium depravation significantly alter the weight and density of nonaqueous material and that variation in the dry weight does not necessarily parallel concomitant changes in solute or water contents. It is, of course, usually more useful to know concentrations per unit mass of cells. This involves either a wet weight or a volume basis of measurement."

The age of the RBCs being tested should always be considered when measuring the concentrations of various RBC constituents.

Keitel et al. (27) in 1955, when measuring RBC cation and anion concentrations by direct analysis, found that different layers of centrifuged cells vary in composition. The upper cell layer is relatively rich in reticulocytes. These cells contain more water, sodium, potassium, chloride, and phosphorous than the remaining cells.

Hoffman (21) in 1958 studied certain characteristics of rabbit cells labeled with ^{59}Fe. He found that when measuring packed cell volumes, cells layer according to their physiologic age. The relative cell density increases and the osmotic resistance decreases as the cell age increases.

Valberg et al. (53) in 1965 found, with the exception of the dog, there was a negative correlation between the life span of RBCs in man, monkey, rabbit, rat, and duck and the

concentrations of total cation per µl of water and positive correlation between the RBC lifespan and the amount of zinc per µmole of hemoglobin in the cell. In 1967 Valberg et al. (54) again made an equally important study concerning various cations during the development and maturation of rabbit RBCs.

Karvonen (26) in 1958 discussed the difference in RBC electrolytes with a difference in hemoglobins and a difference in cell size.

The white blood cells (WBC) of a blood sample pose a similar problem as do the different ages of RBCs. If the WBCs have approximately the same sodium and potassium concentration as the RBCs being measured and no effort is taken to eliminate their presence when measuring RBC cation concentrations, only a small and many times negligible error will result. However, if the WBC cation concentrations are drastically different from the RBCs being measured, a significant error may result. Baron and Ahmed (2) in 1969 determined the sodium and potassium concentrations in isolated human leucocytes and found them to be high in potassium and low in sodium (the same as human RBCs). Clauvel De Mendonca et al. (10) in 1970 studied the sodium, potassium, and osmolality of human and canine RBCs. They found that canine erythrocyte and plasma fluids are more concentrated than human ones and that about 6% of the total RBC water content in both species

is bound. They also pointed out that canine leucocytes are high in potassium (like the human leucocytes).

The trapped plasma portion of a packed cell volume, which causes the overestimation of the true cell volume of a blood sample, has been calculated with many different plasma markers (element or molecule not readily permeable to the RBC membrane).

Oberst (32) in 1935 noted that packing the cells by centrifugation and directly analyzing definite volumes of cells gave more consistent results than the indirect method.

Streef (43) in 1939 measured the RBC sodium and calcium concentrations by the direct method. He stated that to account for the amount of sodium and calcium found in the cells more than 10% of the plasma would have to be trapped among the cells, which he felt was impossible.

Snyder and Katzenelbogen (39) in 1942 measured the concentrations of sodium, potassium, chloride, calcium and phosphorous in human RBCs and occasionally obtained negative results for some ions. This negativity was probably the result of their failure to correct for the trapped plasma error.

Jackson and Nutt (25) in 1951 measured the intercellular plasma present between RBCs packed under a wide range of centrifugal forces. Their data suggest that packed cell volume values decrease as the relative centrifugal force in-

creases. They felt that the use of an adequate centrifugal force insures accurate reproducible results for the Meyerstein micro-hematocrit methods.

Czaczkes et al. (13) in 1963 measured the RBC concentration of water, sodium, and potassium in normal patients and in patients with water and electrolyte disturbances. They concluded that the water and electrolyte content of RBCs, especially sodium, could be more accurately determined by measuring the amount of trapped plasma in each blood sample.

Chien et al. (8) in 1965 measured trapped plasma with radioactive ^{131}I-labeled human serum albumin (RIHSA) on elephant, man, dog, sheep, and goat RBCs after the cells had been washed in a modified Ringer's solution. They found that increasing the force of centrifugation or removal of the plasma proteins reduced the volume of fluid trapped by the packed cell volume.

Valberg et al. (52) in 1965 found that small changes in trapped plasma produced relatively large changes in the RBC calcium and sodium because of the large amounts of the respective ions present in the trapped plasma.

Beilin et al. (3) in 1966 stated that human RBC sodium concentrations could be measured accurately if: (a) trapped plasma sodium is measured with radioisotopes of sodium and a correction made for sodium entry into the cell (b) radioactive ^{14}C-sucrose is used as a standard

(c) the cells are washed with a sodium-free solution.

Trzeciack et al. (51) in 1967, with the use of radioactive ^{14}C-sucrose, found that top speed centrifugation for 10 minutes in a clinical centrifuge can leave 6-7% trapped liquid among human RBCs. He also points out that ^{14}C-sucrose does not permeate the RBC wall.

Funder and Wieth (16) in 1967 measured trapped plasma by radioactive sodium, potassium, sucrose, and albumin in packed cell volumes. It was determined that the percent trapping of ^{22}Na and ^{42}K was found to be equal to ^{14}C-sucrose, when the rate of ^{22}Na and ^{42}K uptake into the RBCs was considered. Radioactive RIHSA was found to be trapped at a significantly lower level than ^{22}Na.

Sirs (38) in 1968 measured the effects of the rate of packing, of the RBCs in the hematocrit, on the amount of trapped plasma and the amount of cell compression in a packed cell volume determination. The analysis of his data suggests that fresh blood cells are extremely flexible and can be packed very rapidly by a weak centrifugal force which causes cell membrane distortion.

Cividalli and Loker (9) in 1969 measured the trapped plasma volume of packed cell volumes using ^{59}Fe as a plasma marker. They found that the estimation of trapped plasma, with ^{59}Fe, was easier than ^{14}C-labeled compounds or RIHSA (which must be dialyzed or passed through an anion exchange

resin shortly before use).

Hunsaker (23) in 1969 used RIHSA to determine the amount of trapped plasma in the packed cell volumes of avian blood. He found that chicken RBCs trapped more plasma than geese RBCs and that chicken trapped plasma volumes were greater when compared to male turkeys but less when compared to female turkeys.

Many investigators have attempted to correct for trapped plasma volumes with the estimation of a mean trapped plasma correction factor for a species.

Hlad and Holmes (20) in 1953 also used RIHSA in normal human blood. They concluded that a constant species correction factor for trapped plasma, as applied to the packed cell volume, cannot exist.

Swan and Nelson (45) in 1968 estimated the canine trapped plasma correction factor of different microhematocrit values. They found that over a packed cell volume range of 25-55% the trapped plasma factor increased by 0.6%.

Anderson (1) 1970 found that a mean trapped plasma correction factor for swine, when using RIHSA as a plasma marker, was 0.955 ± 0.015 (S.D.).

The Movement of Cations between the Plasma and Cells

According to Tosteson (48) in 1955, the words, 'passive' and 'active' have most frequently been applied to cellular transport processes. The word passive connotating a process driven by electrochemical potential gradient of the transported molecules. The word active suggesting transport requiring free energy from reactions occurring in the cell. Transport in the direction of decreasing electrochemical potential may be termed 'passive' energetically but still involves chemical reactions with components of the cell surface and thus, must be mechanistically 'active'.

Hegnauer and Robinson (18) in 1936 measured the plasma and RBC sodium and potassium exchange which took place in adrenal insufficient cats. They found that under conditions in which the electrolyte balance of cat plasma is markedly upset, a redistribution of cations may take place across the RBC membrane. To satisfy the question as to whether the RBCs were normal and their permeabilities unchanged by the absence of cortisol, or if new cells had been formed with the new sodium and potassium content, another experiment was designed. Robinson and Hegnauer (34) in 1936 gave intraperitoneal injections of isotonic glucose and obtained a fall in sodium and chloride concentration and a general rise in potassium concentrations of both plasma and cells. This experiment led to the conclusion that when the electrolyte balance of cat

plasma is sufficiently altered, the RBC membrane may become somewhat permeable to cations.

Davson (14) in 1940 made a study concerning the permeability of cat RBCs to sodium and potassium. He demonstrated that in an isotonic solution of KCl, potassium penetrates the cell less rapidly than sodium leaves the cell. A hypotonic solution of KCl accelerates the rate of potassium penetration while inhibiting the rate of sodium loss. A hypertonic solution of KCl has the reverse effects. Davson also observed that the RBCs become less permeable to sodium with the increase in time-elapse after shedding of the blood, and may be 1/3 as permeable after standing for 2 days in an ice chest. Only slight changes in potassium permeability occur under the same conditions. Narcotics completely inhibit the escape of sodium from cat RBCs but have little effect on the potassium.

Sheppard et al. (37) in 1951 studied the sodium and potassium exchange rate in the blood of the sheep, cow, dog, and man. They concluded that the potassium exchange rate of human cells is practically unaltered by increasing the plasma potassium concentration approximately threefold. In comparing the results in the different species, the exchange rate for potassium shows a rough correlation with the intracellular amount of potassium. Canine RBC cation exchange rates were not well characterized. The presence of the rapidly

exchanging buffy coat cells (high potassium and low sodium) made characterization difficult.

In 1955 Tosteson (48) stated that the potassium and sodium transport in dog red blood cells can, in a large part, be accounted for according to the diffusion theory. Tosteson notes the possibility that a Na-K pump may not exist in canine RBCs. He also points out that one objection to this hypothesis is that, if potassium and sodium move by diffusion alone in the dog cell, colloid osmotic hemolysis of the cell would probably occur.

Harris and Prankered (17) in 1957 studied the movement of tracer sodium in human and dog RBCs. It was concluded that the permeability of the dog cell to sodium is reduced when potassium is added to the external medium (the reverse of what Davson (14) observed in the cat RBCs); which may be due to the formation of an outer K-rich region around the cell which imposes a resistance to sodium movement.

Villegas et al. (57) in 1958 used radioactive water ($THO, ^3H$) to measure water diffusion in beef and dog RBCs. He put cells into a slightly anisotonic solution and measured the rate of THO exchange. When the cells had reached equilibrium in the new environment, the rate of THO exchange was again measured. The permeability coefficients for water entrance under a pressure gradient of 1 osmol/cm^3 permitted the calculation of equivalent pore radius for RBC membrane

of 4.1Å (beef) and 7.4Å (dog).

In 1954, Bernstein (4) measured plasma and RBC (direct method) sodium, potassium and chloride content and calculated the ion ratios in man, baboon, rabbit, rat, horse, sheep, ox, cat and dog. His results indicated the presence of active cation transport in all the measured species.

Spurr and Barlow (42) in 1959 determined the RBC sodium, potassium, chloride, and water in hyperventilated dogs cooled to 25°C. for periods up to four hours, in dogs heated to either 41.5°C. or 42.5°C. for one hour, and in hyperventilated-normothermic dogs. The induced respiratory alkalemia in both normothermic and hypothermic animals appeared to result in a shift of sodium from the plasma to the RBCs.

Cell models have been theorized to explain the movement of sodium and potassium across the RBC membrane and to account for the differences between plasma and RBC sodium and potassium concentration of different animal species. One of the most accepted cell models was developed by Tosteson and Hoffman (50) in 1960 by which the cell controls its cation composition and volume by the action of a Na-K exchange pump and membrane pore leaks for both ions working in parallel. Tosteson concluded that both high potassium and low potassium sheep cells control their cation composition and volume in a manner consistent with the model-cell. Both cell types have a cation pump which exchanges one sodium ion from inside the cell

with one potassium ion from outside the cell but the pump is working 4 times faster in the high potassium cell.

Sodium fluxes were measured in swine (low sodium cells), dog (high sodium cells), and ox (intermediate sodium cells) by Sorenson et al. (40) in 1962. It was concluded that the Na-K pump is less effective in beef RBCs than in swine, and it was probable that the mechanism in dog cells is weakest of the series. Their data did not establish the existence of a pump in the dog. The pump to leak ratios were about 25 for swine cells and 3 for beef. Corresponding values for low and high sodium sheep cells are about 7 and 1 respectively. High cell sodium in both instances (ox and high sodium sheep) is due to a much less active extrusion mechanism and the rate of leak differing little from low sodium systems.

An investigation was performed by Spach and Streeten (41) in 1964 to determine the effects of aldosterone on the sodium flux of canine RBCs. They found that the rate of sodium influx into the cells of canine blood in vitro, is significantly retarded by adding aldosterone. The effect of aldosterone in retarding sodium influx increased exponentially with increasing steroid concentrations. These findings raise the interesting possibility that the action of aldosterone on the cellular sodium content might be brought about by impeding the inward movement of sodium into the cells rather

than accelerating the "Na pump" which is postulated to be actively extruding sodium from the cells.

Because of the action of aldosterone observed by Spach and Streeten (41) on the sodium influx into canine RBCs, Streeten and Moses (44) in 1968 evaluated the action of cortisone on sodium transport in canine erythrocytes. The experiment demonstrated a consistent log dose-related acceleration of sodium influx into the erythrocytes of adrenalectomized dogs. The mechanism whereby cortisol accelerates sodium influx appears to involve the enhancement of glycolysis. Thus, glucose deprivation prevented the action of cortisol on the sodium influx, whereas, the presence of adequate amounts of glucose produced parallel log dose-related increases in glucose utilization and sodium influx.

Streeten and Moses (44) summarized the effects of cortisol and aldosterone on canine RBC influx. Since aldosterone has been shown in physiological and pathological concentrations to retard sodium influx, it seems likely that the opposite actions of cortisol and aldosterone may play an important role in controlling the rate of sodium influx into canine RBCs. This possibility is supported by the findings that the mean rate of sodium influx into erythrocytes was 7.9 mmoles/liter of cells/hr in cortisol and aldosterone-deficient dogs, 22.3 mmoles/liter cells/hr in aldosterone deficient dogs, and 13.9 mmoles/liter of cells/hr in normal

dogs. Thus, the sodium influx into the RBCs of intact dogs might well reflect a balance between the opposing effects of the predominant adrenal corticoids on the energy-dependent component of sodium movement into these cells. The sodium influx of 7.9 mmoles/liter of cells/hr in the absence of cortisol appeared to result from the passive process of sodium diffusion down the slight concentration gradient from plasma into canine erythrocytes.

Duggan et al. (15) in 1965 determined plasma and RBC sodium and potassium (direct method) concentrations and RBC membrane ATPase activity in the guinea pig, horse, man, rat, rabbit, cow, cat, dog and sheep with both high and low sodium RBCs. They could not demonstrate ATPase activity in the RBCs of the horse, rabbit, or rat, all of which maintain substantial RBC-plasma gradients. Duggan stated that the data presented are not consistent with the hypothesis that sodium and potassium active ATPase is essential for active transport of cations by the RBC membrane.

In 1966, Tosteson (49) again considered the ionic composition and transport in red blood cells. He stated that the cells contain large negatively charged molecules, namely hemoglobin but also sugar phosphates and the like, contained within a membrane they cannot traverse. The cell also contains smaller ions (sodium, potassium, and chloride) as well as water, to which the cell is more or less permeable.

Rich et al. (33) in 1967 measured permeability coefficients of dog, cat, and beef RBC membranes under an osmotic pressure gradient. These data presented provide evidence supporting the existence of pores (5.9Å in radius) in canine RBC membranes.

Lubowitz and Whittam (29) made a study in 1968 which characterized the dependence of the movement of sodium and potassium in human RBCs on the external sodium with special attention given to the Na pump. They found that if the Na pump is blocked by ouabain, part of the residual ion movements can still be attributed to exchange diffusion (ouabain-insensitive ATPase). It was concluded that ethacrynic acid inhibits both the ouabain-sensitive ATPase and the ouabain-insensitive ATPase activities. Iodoacetamide decreased only the ouabain-insensitive ATPase activity.

Vieira et al. (56) in 1970, with a study concerning the diffusion of radioactive water (THO), suggested that the equivalent pore radius of the dog RBCs is some 40% greater than in man. The equivalent pore area in the human RBC is about half the amount found in the dog RBC.

Several investigations have been designed to measure the changes which occur in the RBC and plasma electrolyte concentrations during several different physiological and pathological states.

Hoffman and Jacobs (22) concluded, from an investigation

performed in 1934, that both serum and RBC potassium levels were constant in both diseased and healthy humans. A high serum potassium concentration was found, in an asthmatic patient, which was lowered by epinephrine.

Maizels (30) in 1936 stated that in microcytic anemias of man, which is the type also seen in bleeding anemia, the RBC potassium concentrations were lower than normal.

Hutt (24) in 1952 measured the RBC potassium in humans and concluded that the plasma potassium level does not necessarily reflect blood potassium concentrations. The RBC potassium seems to reflect the direction of changes in the total body stores of potassium.

McCance and Widdowson (31) in 1956 observed the following changes which occurred in the erythrocyte electrolytes of the pig and man between fetal and adult life: (a) the concentrations of water, sodium, and chloride fell in both species (b) the concentrations of nitrogen, hemoglobin, iron, and potassium rose (c) the concentration of phosphorous fell in man and rose in pig. They also stated that anemia raised the concentration of water, potassium, and probably phosphorous, lowered the concentrations of nitrogen, hemoglobin and iron, but did not alter the concentration of sodium in human RBCs. Young pigs subject to prolonged undernutrition had a lower concentration of hemoglobin, nitrogen, and iron in their cells than animals of comparable age, and a higher concentration of water and probably of potassium.

Zarkowsky et al. (59) in 1968 studied the RBCs of a child with hemolytic anemia of unknown cause and showed an intracellular sodium and potassium of 100 and 40 mEq/liter respectively. Further studies showed that the cation abnormality was due to a vast increase in permeability to monovalent cations, with a massive increase in active cation pumping.

Bugyi et al. (7), in 1969, stated that they had measured RBC sodium and potassium concentrations by both direct and the indirect methods and were able to identify, in experimental animals and humans, abnormalities in the cation concentrations in RBCs during two diseased states: hyperthyroidism and hyperaldosteronism, but not in cystic fibrosis.

Boyd (5) in 1970 measured the RBC potassium concentration in the indirect method in human RBCs. The results indicate that in malignant hypertension, the RBC potassium tends to be low.

Coulter et al. (12) in 1970 measured the RBC sodium, potassium, and chloride in fetal and maternal swine at different times during gestation. Their results indicate that fetal RBCs contain lower concentrations of sodium and potassium and a higher concentration of chloride than did those of maternal swine. The differences in the RBC sodium concentrations obtained in the experiments designed by McCance and Widdowson (31) and Coulter et al. (12) may be attributed

to the error involved in the methods used to determine RBC sodium concentration. McCance and Widdowson used the direct method while Coulter et al. used the indirect method.

Hellerstein et al. (19) in 1970 measured RBC potassium by the direct method and found there was a good correlation ($r = -0.811$) between hemoglobin and RBC potassium concentration from anemic patients. It was noted that no significant correlation was found between hemoglobin and RBC sodium or magnesium.

The Effects of Adrenalectomy in Dogs and Cats

Swingle et al. (47) in 1934 studied the plasma volume and hemoconcentration of fifteen adrenalectomized dogs by the dye dilution (congo red) method. It was concluded that the plasma volume decreased an average of 33% which paralleled an increase in blood cell concentration in the adrenalectomized dogs.

Watts et al. (58) in 1965 suggested that there is a decrease in myocardial tone and pulse with adrenal insufficiency in dogs.

Swingle and Swingle (46), in a study reported in 1967 concerning dogs which had been adrenalectomized for three years and maintained during this period on corticoids and salts, found the dog to be without accessory adrenal cortical tissue. Terminal insufficiency followed, in all dogs, within

6-15 days after steroid and salt therapy treatment was withheld. At the termination of each dog's life, it was noted the pulse became weak and fast, the plasma sodium and chloride concentrations fell, and the plasma potassium concentration increased.

Bozzini et al. (6) in 1968 studied the erythrokinetics in adrenalectomized dogs and found that there was a 40% reduction in the total circulating RBCs and a 27% reduction in the plasma volume when the dogs were maintained on DCA (desoxycorticosterone acetate). The anemia appeared to result from a diminished rate of erythropoiesis rather than an accelerated rate of RBC destruction. It was concluded that the canine adrenals may have a major erythropoietic effect.

Verrier et al. (55) in an article published in 1969 investigated the relationship between the blood volume and cardiac performance of acute and chronic adrenal insufficient cats. It was concluded that cardiac function is impaired prior to and in the absence of blood volume changes, and that this impairment is of sufficient magnitude to account for the circulatory failure observed in chronic feline adrenal insufficiency. However, it remains an enigma as to whether this impairment of the heart, following acute adrenalectomy, is a direct one or whether it is due to secondary factors such as a decreased venous return or altered sympathetic nervous activity.

Share and Travis (36) in 1970 measured the plasma vasopressin (ADH) concentration in adrenal insufficient dogs. They found that when cortisone and salt supplement were discontinued, in the adrenalectomized dogs, there was a progressive fall in body weight and plasma sodium concentrations and a progressive rise in plasma potassium and ADH concentrations.

PART I. ERYTHROCYTE Na, K, AND CELL VOLUMES CALCULATED FROM BLOOD Na AND K CONCENTRATIONS

ABSTRACT

A method was developed to measure the amount of Na and K, contributed by the plasma, in non-hemolyzed whole blood. Electrolyte concentrations were measured in canine, human, and porcine blood samples. The Na concentrations of plasma and non-hemolyzed whole blood were used to calculate the cell volume of each blood sample. Statistical analyses of the data indicate that the calculation of the cell volume, in this manner, eliminates the error associated with the packed cell volume determination. The calculated cell volume and non-hemolyzed whole blood Na and K concentrations were also employed to modify the indirect method of calculating the erythrocyte Na and K concentrations. Significant differences were found between the two indirect methods (indirect and modified indirect) used to calculate the erythrocyte Na and K concentrations of human blood samples. The modified indirect method of calculating the erythrocyte Na and K concentrations was considered to be accurate and adaptable for both clinical and research laboratories.

Key words: cell volume; packed cell volume; hematocrit; erythrocyte electrolytes; plasma sodium; plasma potassium; trapped plasma

INTRODUCTION

The indirect method of determining erythrocyte (RBC) electrolyte concentrations is not well accepted because of the error associated with the analysis (22). When calculating the RBC electrolyte concentrations by the indirect method (5, 6, 8, 9, 22) the packed cell volume (PCV) and the appropriate plasma and hemolyzed whole blood electrolyte concentrations are used to calculate the respective RBC electrolyte concentration. Beilin et al. (2) demonstrated that an error in the PCV estimation of 1%, in a human blood sample, may create a 20% error when calculating the RBC Na concentration by the indirect method.

McCance and Widdowson (15) stated that because plasma becomes trapped among the RBCs, it is impossible to separate the cells from the plasma completely by one single operation of centrifugation. The trapped plasma volume (TPV) of a blood sample, contained in a centrifuge tube, is dependent on various physical properties of the RBCs, e.g., flexibility of the RBC membranes (19) and the force and time of centrifugation (13). Therefore, a TPV correction factor would be inadequate for all animals within a species. The TPV must be determined for each sample being analyzed (10). Many different plasma markers have been used to measure TPV but discrepancies exist among the different markers being used (2, 3, 6, 7, 10).

The objective of this study was to eliminate the error associated with the PCV and thus increase the accuracy of the indirect method of calculating the RBC Na and K concentrations. Based on the assumption that the RBC Na and K ions are bound to or contained within the RBC and are not readily diffusible, non-hemolyzed whole blood (NHWB) samples diluted in an isotonic solution of LiCl, were dialyzed and the diffusible Na and K measured (that amount contributed by the plasma). The concentrations of Na in NHWB and plasma were used to calculate a cell volume unaffected by the error associated with the PCV determination.

METHODS

Venous blood samples (12 ml) were drawn from 30 mongrel dogs (4.5-18.2 kg), 32 Landrace-Yorkshire-Poland China cross-bred pigs (6.8-14.3 kg), and 21 adult Caucasian humans (46.6-98.3 kg) of both sexes. Each blood sample was placed in a test tube containing 0.1 ml of 1000 units sodium heparin/ml. The tubes were gently mixed until the whole blood analyses could be performed.

Electrolyte determinations were made in duplicate. Automated analyses[1] of Na and K concentrations were done by flame photometry. The Na and K manifold was modified to accommodate the measurement of NHWB and hemolyzed whole blood (HWB) Na and K concentrations. Two double mixing coils were added to the manifold (Fig. 1) to insure proper mixing during whole blood analyses. The blood samples were gently mixed prior to sampling.

To determine NHWB electrolyte concentrations, an isotonic solution of LiCl (6.4 g/liter) was pumped through reagent lines 2 and 4 (Fig. 1). An isotonic solution of $CaCl_2$ (11.2 g/liter) was used as the between sample wash solution to prevent hemolysis of the whole blood samples.

Canine HWB Na and K concentrations were determined

---

[1]Technicon's Auto Analyzer method file N-20b, Technicon Corp., Tarrytown, New York 10591.

on whole blood samples which were hemolyzed by pumping deionized water through reagent line 2. A hypertonic solution of $LiCl_2$ (13.2 g/liter) was pumped through reagent line number 4 (Fig. 1). Canine whole blood samples were hemolyzed in this manner to minimize the release of the high amounts of K present in canine leucocytes (1, 7). Since the RBCs of pig and man are of the high K and low Na type (opposite of dog RBCs), their HWB Na and K concentrations were measured in a protein-free filtrate to insure the release of the total RBC Na and K (3). The protein-free samples were prepared by diluting whole blood (1:10) with 5% trichloroacetic acid and the supernatant collected after centrifugation. Standards (Table 1) were prepared from NaCl and KCl solutions and treated in the same manner as the sample being analyzed.

PCVs were determined in triplicate by the microhematocrit method and the two closest values recorded. Blood filled capillary tubes were plugged with clay and centrifuged[1] at approximately 13,500-25,000 g for 6 minutes. The PCVs were measured over a fluorescent light. The packed RBC volume (PCVr) and the total PCV (PCVt, which includes the white blood cells) were calculated. The packed white cell volume (PCVw) was calculated by subtracting the PCVr from the PCVt.

---

[1]International Equipment Co., Microhematocrit centrifuge model MB, Needham Heights, Massachusetts 02192.

The following equations were used to calculate the cell volume and RBC Na and K concentrations:

$$CCV = 100 - [(NHWB\ Na/Pl Na)100]$$

$$RBCe = (HWBe - NHWBe)100/(CCV - PCVw)$$

$$RBCe' = \{HWB - [(100 - PCVt)Ple/100]\}\ 100/PCVr$$

The CCV indicates the cell volume (%) claculated from the Na concentrations of plasma and non-hemolyzed whole blood. The Ple, NHWBe, and HWBe indicate the plasma, non-hemolyzed whole blood, and hemolyzed whole blood electrolyte (Na or K) concentrations (mEq/liter) respectively. RBCe indicates the red blood cell electrolyte (Na or K) concentration (mEq/liter of RBCs) calculated by the equation from the modified indirect method. RBCe' indicates the red blood cell electrolyte (Na or K) concentration (mEq/liter of RBCs) calculated by the equation from the indirect method used by previous investigators (5, 6, 8, 9, 22).

When measuring the NHWB Na and K concentration it is readily evident that erroneous results may be obtained from the cellular efflux of Na or K. To satisfy this question as to whether RBC Na and K efflux may cause a significant error, NHWB K concentrations were measured by flame photometry and also calculated by the following equation:

$$NHWB\ K = Pl\ K(NHWB\ Na/Pl\ Na)$$

The accuracy of the calculated NHWB K concentration depends upon the accuracy of the NHWB Na and plasma Na and K concentrations.

Fig. 1. The flow diagram of the AutoAnalyzer manifold used to measure plasma, hemolyzed whole blood, and non-hemolyzed whole blood Na and K concentrations

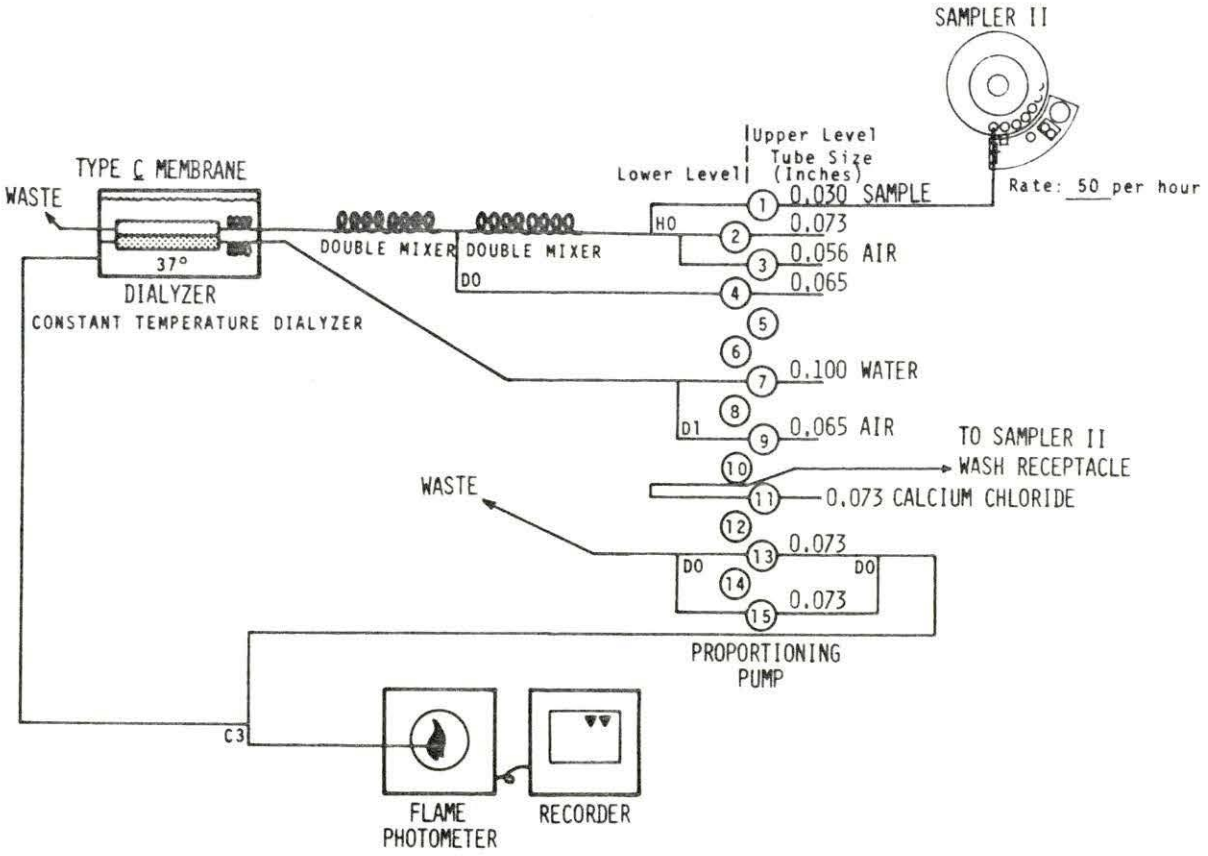

SAMPLER II
Upper Level
Tube Size
(Inches)
Lower Level
TYPE C MEMBRANE
WASTE
37°
DIALYZER
CONSTANT TEMPERATURE DIALYZER
DOUBLE MIXER
DOUBLE MIXER
Rate: 50 per hour
1 0.030 SAMPLE
HO
2 0.073
3 0.056 AIR
DO
4 0.065
5
6
7 0.100 WATER
8
D1
9 0.065 AIR
10
TO SAMPLER II
WASH RECEPTACLE
WASTE
11 0.073 CALCIUM CHLORIDE
12
13 0.073
DO
14
DO
15 0.073
PROPORTIONING
PUMP
C3
FLAME
PHOTOMETER
RECORDER

Table 1. Concentrations of standard solutions for measuring RBC Na and K concentrations

	Na	K
Pl	130-160[a]	2-8[b]
NHWB	70-100[a]	2-5[c]
HWB (Canine)	100-130[a]	2-8[b]
HWB (Human and Porcine)	70-100[a]	40-70[a]

Pl, NHWB, and HWB indicate plasma, non-hemolyzed whole blood, and hemolyzed whole blood respectively.

[a]Increments of 10 mEq/liter.

[b]Increments of 2 mEq/liter.

[c]Increments of 1 mEq/liter.

RESULTS

Means and standard deviations (means ± S.Ds.) were established for both the measured and calculated variable concentrations and statistical comparisons made (Table 2). The difference between the calculated cell volume (CCV) and the total packed cell volume (PCVt) determinations were statistically analyzed with Student's t-test (paired analysis). The CCV and PCVt determinations were significantly different in canine, human, and porcine blood samples.

The reproducibility of the two different methods used to estimate the cell volume of a blood sample (CCV and PCVt), was characterized. Means ± S.Ds. were established for the differences between duplicate PCVt (0.3 ± 0.2) and duplicate CCV (0.4 ± 0.3) determinations. No significant differences were found when comparing the CCV and PCVt reproducibilities.

When linear regressions (estimated by least squares analysis) were calculated for the CCV vs PCVt, the slopes were less than 1.000 for all species tested (Table 3) indicating that as the PCVt increases, the differences between the PCVt and the CCV determinations also increases.

The means ± S.Ds. of the electrolyte concentrations (Na and K) obtained from the modified indirect and the indirect equations (RBCe and RBCe' respectively) for the different species were evaluated with Student's t-test (Table 2).

Significant differences were found between the different equations used to calculate the RBC Na and K concentrations in human blood samples. No significant differences were found between the different equations used to calculate the RBC Na and K concentrations in canine or porcine blood samples.

The reproducibility of the modified indirect equation (RBCe), used to calculate the RBC Na and K concentrations, was statistically characterized. Means $\pm$ S.Ds. were established for the differences between duplicate RBCe determinations for dog, man, and pig (Table 4).

The NHWB K concentration was both calculated and measured by flame photometry. The means $\pm$ S.Ds. of the measured and calculated NHWB K concentration (Table 5) were compared with Student's t-test, within each species. No significant differences were found between the measured and calculated NHWB K concentrations.

Table 2. Blood variable concentrations (means ± S.Ds.) both measured and calculated

	Species		
	Dog (30)	Human (21)	Pig (32)
	mEq/liter		
Pl Na	146.4 ± 2.5	141.5 ± 1.4	142.7 ± 2.6
NHWB Na	83.9 ± 7.9	83.0 ± 5.0	93.2 ± 3.8
HWB Na	130.2 ± 3.4	85.2 ± 5.0	95.2 ± 4.2
RBC Na	110.6 ± 6.1	5.5 ± 3.0	5.9 ± 4.1
RBC Na'	112.6 ± 5.8	16.0 ± 3.6	7.6 ± 5.1
Pl K	4.1 ± 0.3	3.8 ± 0.4	5.0 ± 0.5
NHWB K	2.4 ± 0.3	2.5 ± 0.2	3.6 ± 0.5
HWB K	5.3 ± 0.4	45.0 ± 4.3	45.6 ± 2.8
RBC K	6.9 ± 1.0	104.0 ± 4.6	125.9 ± 4.1
RBC K'	6.9 ± 1.0	97.4 ± 4.2	124.2 ± 4.6
	Percent		
CCV	42.7 ± 5.7	41.3 ± 3.8	34.7 ± 2.5
PCVr	44.0 ± 5.9	44.3 ± 3.8	34.1 ± 2.6
PCVt	44.8 ± 5.9	44.8 ± 3.8	35.1 ± 2.5
PCVw	0.75 ± 0.3	0.43 ± 0.2	0.97 ± 0.3
Significant Differences (probabilities less than)			
CCV vs PCVt	0.001	0.001	0.05
RBC Na vs RBC Na'	NS	0.001	NS
RBC K vs RBC K'	NS	0.001	NS

Pl, HWB, NHWB, CCV, PCVt, PCVr and PCVw indicate plasma, hemolyzed whole blood, non-hemolyzed whole blood, calculated cell volume, total packed cell volume, packed red cell volume, and packed white cell volume respectively. The RBC Na and K concentrations were calculated by the equation from the modified indirect method. The RBC Na' and K' concentrations were calculated by the equation from the indirect method used by previous investigators. The differences between paired analysis were used to calculate Student's t-test for CCV vs PCVt. The remaining comparisons were computed on the differences between concentration means ± S.Ds. The numbers in parentheses indicate the number of animals from each species.

Table 3. Linear regression equations for the calculated cell volume versus the total packed cell volume for each species tested

Dog (30)	CCV = -0.71 + 0.969 (PCVt)
Human (21)	CCV = -2.01 + 0.968 (PCVt)
Pig (32)	CCV = 3.23 + 0.896 (PCVt)

Y = Y-intercept + slope (X). CCV indicates the calculated cell volume and PCVt indicates total packed cell volume.

Table 4. Reproducibility studies when using the modified indirect equation to calculate RBC electrolyte (RBCe, Na and K) concentrations

	mEq/liter of RBCs	
	Na	K
Dog (30)	2.0 ± 1.6	0.2 ± 0.1
Human (21)	2.0 ± 1.8	1.3 ± 1.2
Pig (32)	1.2 ± 1.1	2.1 ± 1.4

Means ± S.Ds. of the differences between duplicate electrolyte determinations.

Table 5. Measured and calculated non-hemolyzed whole blood K concentrations (means ± S.Ds.)

	Dog (30)	Man (20)	Pig (32)
NHWB K (measured)	2.4 ± 0.3	2.5 ± 0.2	3.6 ± 0.5
NHWB K (calculated)	2.4 ± 0.3	2.4 ± 0.3	3.5 ± 0.6

No significant differences were found between the measured and calculated NHWB K concentrations (Student's t-test).

DISCUSSION

The theory of calculating the cell volume of a blood sample from the Na concentrations of plasma and NHWB is based on the hypothesis that the whole blood Na is contained within the two different blood compartments (cells and plasma). The RBC Na is either bound to or contained within the cells and does not readily permeate the RBC membrane in an isotonic solution; whereas, the plasma Na is in the ionized form and is readily diffusible in an isotonic solution. Therefore, when a NHWB sample is diluted with an isotonic solution (free of Na) and the diffusible Na measured, one is actually measuring the plasma Na concentration diluted with cells.

Cation exchange studies performed on canine, human, and procine RBCs (14, 16, 17, 18, 20) support the assumption that the efflux of either Na or K through the RBC membrane (while in an isotonic solution for less than 3 minutes) would not significantly alter the accuracy of the NHWB Na and K measurements. To insure that the RBC Na or K efflux was insignificant for our purpose NHWB K concentrations were both measured and calculated by multiplying the calculated plasma volume (obtained from the Na concentrations of plasma and NHWB) times the plasma K concentration. The accuracy of the NHWB K calculation is dependent upon the accuracy of the NHWB Na and plasma Na and K measurement. When Student's t-test comparisons were made between the means $\pm$ S.Ds. of the calculated

and measured NHWB K concentrations, no significant differences were found (Table 5). These data support the hypothesis that no significant error results from the exchange of either Na or K (between cells and plasma while in an isotonic solution of LiCl) during the measurement of either NHWB Na or K concentrations of the dog, man, or pig.

Beilin et al. (2) stated that when calculating the RBC Na and K concentrations by the indirect method, a small error in the estimation of the packed cell volume may cause a large error in the calculated RBC electrolyte concentration. When determining the PCV of a blood sample, plasma becomes trapped among the cells which results in an over estimation of the actual or true cell volume. The trapped plasma volume (TPV) is largely dependent on the percentage of cells present. The greater the PCV, the greater the TPV (12, 19). Since the calculated cell volume (CCV) estimation is not affected by the TPV error, one would expect the difference between the PCVt and the CCV to increase as the PCVt increases. This expected phenomenon was demonstrated by calculating the linear regression equation (estimated by least squares analysis) for the PCVt (X-axis) vs CCV (Y-axis) for each species tested (Table 3). The estimated regression slope values were less than 1.000, in all observed species, indicating that as the PCVt increases the difference between the CCV and the PCVt also increases. Occasionally (especially when analyzing

swine blood) the CCV would be greater than the PCVt. This may be explained by previous investigations (12, 19) illustrating that fresh blood cells are extremely flexible and can be distorted by a weak centrifugal force which in turn decreases the amount of plasma trapped by the cells. To obtain a CCV greater than the PCVt, the cells must decrease in volume; consequently, the error associated with the force and time of centrifugation must be greater than the TPV error.

The CCV is an estimation of the total cell volume of a blood sample. To estimate the true red cell volume, one must correct for the white cells present. The packed white cell volume (PCVw) is estimated by subtracting the PCVr (packed red cell volume) from the PCVt (total packed cell volume). Since the PCVw is usually small (less than 1%, Table 2) the error resulting from the trapping of plasma in the buffy coat layer would be insignificant.

Beilin et al. (2) reported that with human blood samples correction for the trapped plasma volume (TPV) caused a decrease in the RBC Na concentration determined by the indirect method. Therefore, the RBC Na concentrations determined by the modified indirect method (RBC Na, Table 2), unaffected by the TPV error, were lower than those determined by the indirect method (RBC Na', Table 2). Student's t-test analyses were made between the different methods used to calculate the RBC Na and K concentrations (RBCe versus RBCe') of canine,

human, and porcine blood samples (Table 2). Significant differences were found only when calculating the RBC Na and K concentrations in the human blood samples. These findings indicated that the error associated with the indirect method of determining the RBC Na and K concentrations (RBCe', Table 2) in dog and pig causes only a small decrease in the accuracy of the results.

Although there was no statistically significant differences between the two methods used to calculate the RBC Na and K concentrations (RBCe and RBCe') in dog and pig, it would be advisable to use the modified indirect method (RBCe) for all species tested. A significant error may result from individual variations (in the rigidity of RBCs) within a species, causing erroneous RBC electrolyte results.

The RBC Na and K concentrations obtained by the modified indirect method (Table 2) agreed with those values reported by McCance and Widdowson (15), Spurr and Barlow (21), Cividalli and Loker (5), and Duggan et al. (11). The canine and porcine RBC Na and K concentrations, reported herein, do not agree with those reported by Coldman and Good (6). Coldman and Good measured the RBC Na and K concentrations by the indirect method but used the macro-hematrcrit method (Wintrobe hematocrit method) instead of the micro-hematocrit method to estimate the PCV of the blood samples. Chien et al. (4) reported that the TPV error present in the macro-

hematocrit method, was greater than the TPV error present in the micro-hematocrit method of estimating the PCV of a blood sample.

From this study it was concluded that the use of non-hemolyzed whole blood Na and K concentrations and the calculated cell volume to determine the RBC Na and K concentrations eliminates the major errors associated with the indirect method (especially in human samples). The modified indirect method of determining RBC Na and K concentrations is adaptable for most research and clinical laboratories because of the speed, accuracy, and simplicity of the method. Extraneous materials such as radionuclides and dyes are not required to eliminate the errors associated with the determination of RBC electrolyte concentrations.

REFERENCES

1. Baron, D. N. and S. A. Ahmed. Intracellular concentrations of water and the principal electrolytes determined by the analysis of isolated human leukocytes. Clin. Sci. 37: 205-219. 1969.

2. Beilin, L. J., G. J. Knight, A. D. Munro-Faure, and J. Anderson. The measurement of sodium concentration in human red blood cells. J. Gen. Physiol. 50: 61-74. 1966.

3. Bernstein, R. E. The determination of potassium and sodium in biological fluids by flame spectrophotometry. S. Afr. J. Med. Sci. 17: 101-116. 1952.

4. Chien, S., R. J. Dellenback, S. Usami, and M. I. Gregerson. Plasma trapping in hematocrit determination. Differences among animal species. Soc. Exp. Biol. and Med. Proc. 119: 1155-1158. 1965.

5. Cividalli, G. and H. Loker. Micromethod for determination of sodium and potassium in the red blood cells. J. Lab. and Clin. Med. 73: 706-710. 1969.

6. Coldman, M. F. and W. Good. The distribution of sodium, potassium, and glucose in the blood of some mammals. Comp. Biochem. Physiol. 21: 201-206. 1967.

7. Clauvel De Mondonca, M., K. Schwartz, and E. Terrier. Sodium, potassium, and osmolality of human and canine erythrocytes-interest of trapped plasma and water content for their full significance. Comp. Biochem. and Physiol. 34: 147-161. 1970.

8. Coulter, D. B., R. C. Ewan, and L. L. Small. Determination of erythrocyte Na, K, and Cl concentrations by an automated indirect method. In Advances in Automated Analysis Biomedical Profiling, Technicon International Congress 1969. Vol. III, Pp. 149-151. Mediad, Inc., White Plains, New York. 1970.

9. Coulter, D. B., R. C. Ewan, M. J. Swenson, F. X. Aherne, and D. Wyllie. Plasma and erythrocytic concentrations of electrolytes in blood of fetal and maternal swine. Am. J. Vet. Res. 31: 1179-1185. 1970.

10. Czaczkes, J. W., T. D. Ullman, L. Ullman, and Z. Bar-Kochba. Determination of the red blood cell content of

Water, sodium, and potassium in normal subjects. J. Lab. and Clin. Med. 61: 873-878. 1963.

11. Duggan, D. E., J. E. Baer, and R. M. Noll. Membrane adenosinetriphosphatase and cation composition of mammalian erythrocytes. Naturwissenschafter 52: 264. 1965.

12. Funder, J. and J. O. Wieth. Trapping of sodium, potassium, sucrose, and albumin in the packed cell column of the hematocrit. Acta Physiol. Scand. 71: 105-112. 1967.

13. Hlad, C. J., Jr. and H. H. Holmes. Factors affecting hematocrit determinations: Trapped plasma, its amount and distribution. J. Appl. Physiol. 5: 457-470. 1953.

14. Lubowitz, H. and R. Whittam. Ion movements in human red cells independent of the sodium pump. J. Physiol. 202: 111-131. 1969.

15. McCance, R. A. and E. M. Widdowson. The effect of development, anaemia and undernutrition on the composition of the erythrocytes. Clin. Sci. 15: 409-416. 1965.

16. Passow, H. Ion and water permeability of the red blood cells. In Bishop, C. and D. M. Surgenor, ed. The red blood cell. Pp. 71-145. Academic Press, New York. 1964.

17. Rich, G. T., R. I. Sha'afi, T. C. Barton, and A. K. Solomon. Permeability studies on red cell membrances of dog, cat, and beef. J. Gen. Physiol. 50: 2392-2405. 1967.

18. Sheppard, C. W., W. R. Martin, and G. Beyl. Cation exchange between cells and plasma of mammalian blood. J. Gen. Physiol. 34: 411-429. 1951.

19. Sirs, J. A. The measurement of the hematocrit and flexibility of erythrocytes with a centrifuge. Biorheology 5: 1-14. 1968.

20. Sorenson, A. L., L. B. Kirschner, and J. Barker. Sodium fluxes in the erythrocytes of swine, ox, and dog. J. Gen. Physiol. 45: 1031-1047. 1962.

21. Spurr, G. B. and G. Barlow. Plasma and erythrocyte Na, K, Cl, and water in hyperthermic and hypothermic dogs. Am. J. Physiol. 197: 648-652. 1959.

22. Streef, G. M. Sodium and calcium content of erythrocytes. J. Biol. Chem. 129: 661-672. 1939.

23. Valberg, L. S., R. T. Card, E. J. Paulson, and J. Szivek. The metal composition of erythrocytes in different species and its relationship to the life span on the cells in the circulation. Comp. Biochem. and Physiol. 15: 347-359. 1965.

PART II. PLASMA AND RBC Na AND K CONCENTRATIONS OF ADRENALECTOMIZED DOGS

ABSTRACT

The rates at which the plasma and RBC Na and K concentrations changed in adrenal insufficient (adrenalectomized) dogs were evaluated. The RBC Na and K concentrations were determined by a modified indirect method which eliminated the major errors associated with the indirect method. Five female mongrel dogs were adrenalectomized and sustained by daily injection of exogenous steroids. After the dogs had recovered from the surgical procedures, steroid therapy was discontinued. A mean of 7.2 days was required for the adrenalectomized dogs to develop severe adreanl insufficiency. The plasma and RBC Na concentrations decreased at the rate of -2.1 and -1.9 mEq/liter/day respectively, and were significantly correlated at the $P < 0.001$ level. The plasma and RBC K concentrations increased at the rate of 0.27 and 0.06 mEq/liter/day respectively, and were significantly correlated at the $P < 0.001$ level.

Key Words: adrenalectomy; adrenal insufficiency; plasma sodium; plasma potassium; erythrocyte sodium; erythrocyte potassium; Na; K

INTRODUCTION

Previous investigators have studied the changes which occur in the plasma and erythrocyte (RBC) Na and K concentrations of adrenalectomized animals (4, 6, 8, 10). Many of these experiments, which indicate the exchange or no exchange of electrolytes between plasma and cells, are open to criticism; namely, because of the error associated with the method used to determine the RBC electrolyte concentrations.

A recent technique has been developed which eliminates the major error associated with the indirect method of determining the RBC Na and K concentrations (9). The development of this technique has presented the opportunity to study more precisely the rates at which changes occur in the plasma and RBC Na and K concentrations of adrenalectomized dogs.

METHODS

Experimental Design

Five dogs were bilaterally adrenalectomized and sustained on exogenous steroid therapy. After recovery from the surgical procedures, steroid therapy was terminated and the dogs were allowed to attain the state of severe adrenal insufficiency. Blood samples were taken daily. Two normal control dogs were kept under the same environmental conditions and blood samples taken at the same time and manner as the adrenalectomized dogs. The changes which occurred in the adrenalectomized dogs were compared to the changes which occurred in the control dogs.

Experimental Protocol

Three weeks prior to the initiation of the surgical procedures, seven mature female mongrel dogs (14.8-19.1 kg) were vaccinated for distemper, given antinematodal drugs, and placed in separate cages. The dogs were fed the same diet throughout the experiment. Two of the seven dogs were randomly selected to be used as experimental controls. The remaining five dogs were bilaterally adrenalectomized (via a longitudinal abdominal incision). Following the removal of the adrenal glands, the dogs were given prednisolone sodium succinate (50 mg i.v.) to aid in the prevention of

surgical shock. The adrenalectomized dogs were subsequently sustained by daily i.m. injections of hydrocortisone acetate (1.0 mg/kg) and deoxycorticosterone (0.1 mg/kg). When the dogs had recovered from the surgical procedures, steroid therapy was terminated and each dog allowed to attain the state of severe adrenal insufficiency. Gross observation of animal appetite and general state of depression along with daily measurement of heart rate, body weight, and packed cell volume (PCV) were used to evaluate the degree of adrenal insufficiency.

Heparinized venous blood samples (12-ml) were taken daily from the jugular vein of the adrenalectomized dogs, beginning with the last day of steroid injection and continuing until each dog had attained the required pathological condition. The adrenalectomized dogs were then euthanized with an overdose of sodium pentobarbital and a necropsy done to insure the removal of all obvious adrenal tissue. The control dogs were sampled at the same time and in the same manner as the adrenalectomized dogs. Control sampling was terminated when all the adrenalectomized dogs had attained the state of severe adrenal insufficiency.

Blood Analyses

Plasma, hemolyzed whole blood, and non-hemolyzed whole blood Na and K concentrations were measured by automated flame photometry. The RBC Na and K concentrations were

calculated by a modified indirect method (9). PCVs were determined by the microhematocrit method.

Statistical Analyses

Due to the biological variation peculiar to each animal, the rate at which each adrenalectomized dog attained the state of severe adrenal insufficiency differed. To statistically analyze the measured variables, in terms of average rates of variable change, the data obtained from each dog were standardized with time. Each variable measured, on each dog tested (control and arenalectomized), was plotted on a day (abscissa)-variable (ordinate) graph (linear). When each adrenalectomized dog had attained the desired pathological condition, the abscissa of each graph was divided into nine equal time-intervals. The beginning of time-interval one coincides with the date of the last injection of steroid therapy. The length of time between the beginning of time-interval one and the end of time-interval nine was peculiar to each dog. For each adrenalectomized dog, the end of time-interval nine coincides with the day on which the desired pathological state was attained. For the control dogs, the end of time-interval nine coincides with the day at which all the adrenalectomized dogs had attained the desired pathological state. Linear regressions, correlation coefficients, and Student's t-test analyses were used to statistically characterize the results in terms of average rates of variable change per time-interval.

RESULTS

It took a range of 3 to 12 days with an average of 7.2 ± 3.7 (mean ± S.D.) days for each adrenalectomized dog, not being influenced by exogenous steroid therapy, to attain a state of severe adrenal insufficiency. As was stated previously, the results obtained daily from each variable measured on each dog was plotted on a day-variable graph. Upon the termination of the experiment, each graph was divided into nine equal time-intervals. Each time-interval was found to be equal to a mean of 0.8 days. The interpolated values for each variable measured at each time-interval for each animal tested was recorded.

Linear regression equations (estimated by least squares analysis) were calculated for each dog tested to statistically characterize the changes which occurred in the variables measured in terms of changes per time interval. The slope portion of the linear regression equation indicates the rate of variable change per time interval. The linear regression equations for each variable measured on each of the control dogs were calculated from the beginning of time-interval one through nine. The control slope values, for each of the different variables measured, were statistically grouped (means ± S.Ds., Table 1) and compared to zero with Student's t-test. None of the control dog slopes were significantly different from zero. The linear regression equations, for each variable

measured on each of the adrenalectomized dogs were calculated from time-intervals two through nine. Time-interval one includes samples taken while the dogs were being influenced by exogenous steroid therapy and presumably not susceptible to the changes which occur with adrenal insufficiency. The adrenalectomized-dog slope values, for each variable measured, were statistically grouped (means $\pm$ S.Ds.) and compared, with Student's t-test, to the respective control slope values (Table 1).

Correlation coefficients were calculated to evaluate the consistency of change per time-interval in the adrenalectomized (adrenal insufficient) dogs. The mean changes which occurred at the different time-intervals were used to calculate the correlation coefficients for each variable measured (Table 2). All correlations were significant at the 1% level indicating a relationship between the variable changes and time.

The mean variable values, at each time-interval, were also plotted to graphically demonstrate the mean variable change per time-interval (Figs. 1-2). Correlation coefficients (r), using the mean variable change at each time-interval, were also calculated to measure the relationship of the electrolyte changes which occurred between plasma and RBCs of the adrenal insufficient dogs; the plasma and RBC Na concentration changes were correlated at $r = 0.989$ and plasma and

RBC K changes were correlated at r = 0.785. Both correlations were significant at the 1% level.

Table 1. The average (means ± S.Ds.) rates of variable change per time-interval (0.8 day)

Variables	Non-adrenalectomized	Adrenalectomized
Plasma Na, mEq/liter	0.01 ± 0.40	-1.71 ± 0.9[a]
Plasma K, mEq/liter	-0.05 ± 0.03	0.22 ± 0.20
RBC Na, mEq/liter	-0.40 ± 0.50	-1.46 ± 0.47[a]
RBC K, mEq/liter	-0.11 ± 0.35	0.05 ± 0.26
PCV, %	-0.18 ± 0.19	1.91 ± 0.91[b]
Body weight, Kg	-0.02 ± 0.05	-0.19 ± 0.07[a]
Heart rate,	-0.20 ± 1.82	3.50 ± 2.94[a]

The rates of variable changes for non-adrenalectomized and adrenalectomized dogs were calculated from the beginning of time-interval one through nine and from time-interval two through nine respectively. Student's t-tests were used to compare the non-adrenalectomized and adrenalectomized groups.

[a] $P < 0.005$.

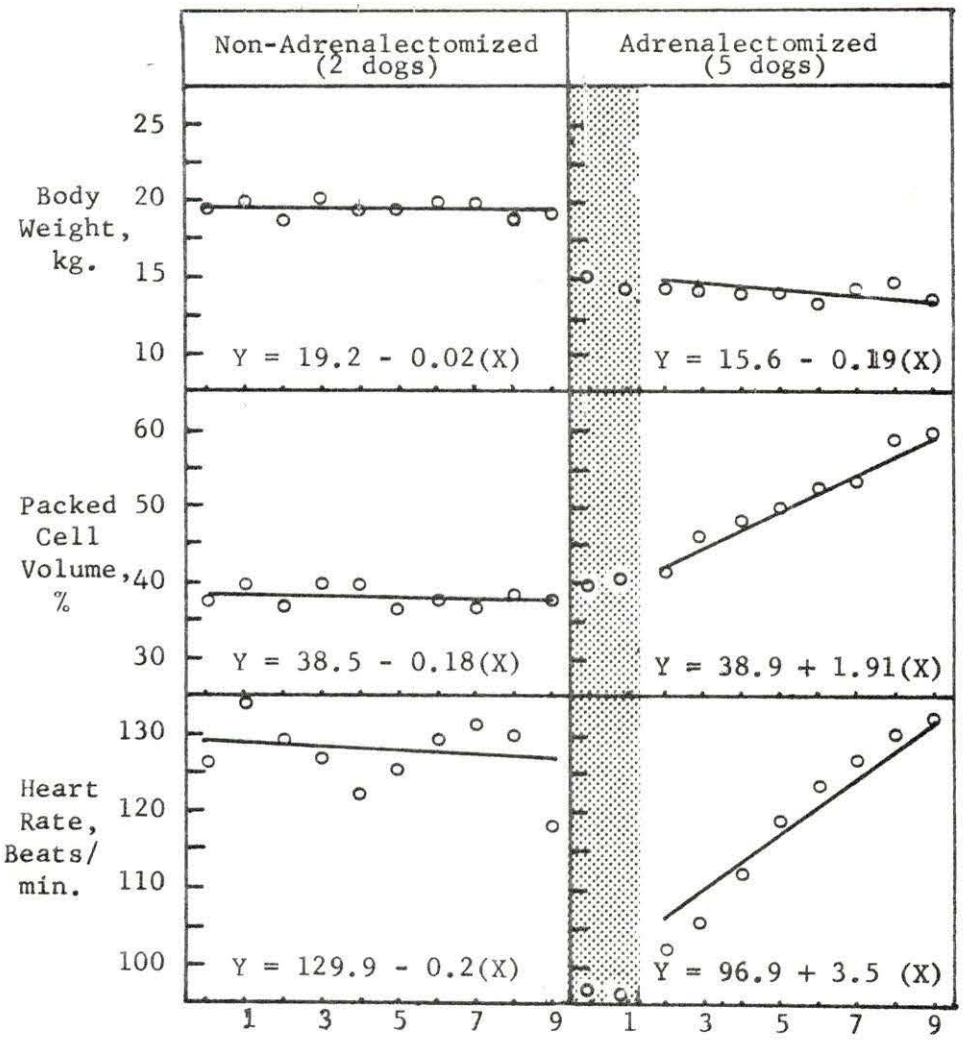
[b] $P < 0.001$.

Table 2. Correlation coefficients for the mean variable values versus the different time-intervals

Measured variable	Correlation coefficients
Plasma Na	-0.987
RBC Na	-0.989
Plasma K	0.898
RBC K	0.723
PCV	0.995
Body weight	-0.989
Heart rate	0.995

All correlations were significant at the 1% level.

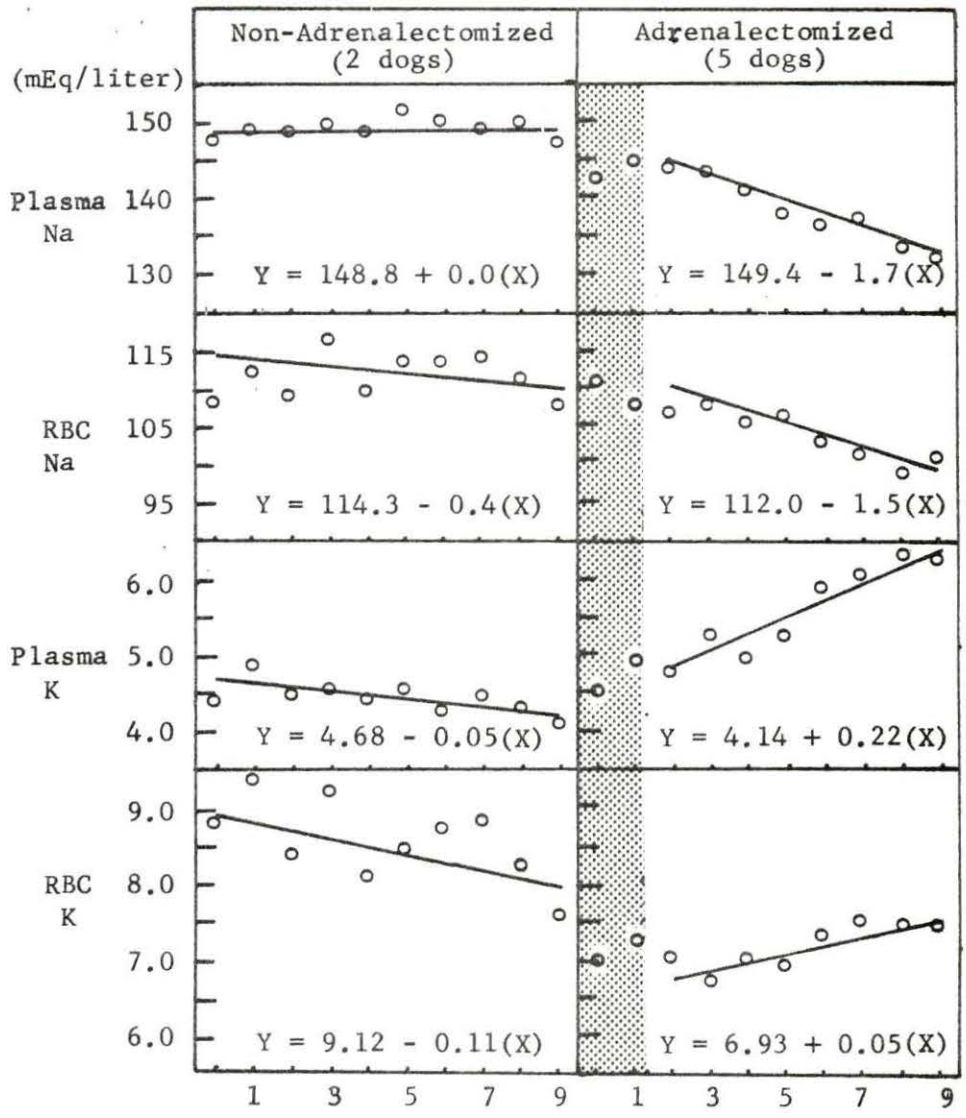
Fig. 1. A graphical illustration of the mean changes which occurred in the measured variables used to evaluate the state of adrenal insufficiency in control and adrenalectomized dogs



Adrenalectomized dogs under the influence of exogenous steroids.

Y = Y-intercept $\pm$ slope(X)

Fig. 2. A graphical illustration of the mean changes which occurred in the plasma and RBC Na and K concentrations of control and adrenalectomized dogs



Adrenalectomized dogs under the influence of exogenous steroids.

Y = Y-intercept $\pm$ slope(X)

DISCUSSION

It took 7.2 ± 3.7 (mean ± S.D.) days for the adrenalectomized dogs to attain the state of severe adrenal insufficiency. The dogs were considered to be adrenal insufficient 24 hours after the last injection of steroid therapy. Correlation coefficients (Table 2) indicate that the measured changes which occurred in the adrenal insufficient dogs were constant. The dogs lost an average of 0.19 kg/0.8 day of body weight and their packed cell volumes increased on the average of 1.91%/0.8 days (Table 1). These results coincide with data obtained by previous investigators (7, 11, 12). The heart rate of the adrenalectomized dogs began at a comparatively low rate (compared to the control dogs) and increased on the average of 3.5 beats/min/0.8 day (Fig. 1). There was no significant change in the control dogs heart rates throughout the experiment. Watts *et al.* (15) suggested that there is a decrease in myocardial tone and pulse with adrenalectomy in dogs. Verrier (14), in a study concerning adrenalectomized cats, found no increase in heart rate as the cats approached the terminal stages of adrenal insufficiency. Because heart rates were not recorded before adrenalectomy, it cannot be determined from the present study whether there was actually a decrease in heart rate with adrenalectomy or if the observed phenomenon was peculiar to these dogs.

The statistical analysis of the changes which occurred in the plasma and RBC Na concentrations of the adrenal insufficient dogs indicates that the rate of Na loss from both blood compartments (plasma and cells) was significantly increased (Table 1). Correlation coefficients, calculated to measure the relationship between the changes which occurred in plasma and RBC Na concentration of the adrenal insufficient dogs, indicate a parallel decrease. Figure 2 graphically illustrates this parallel decrease in plasma and RBC Na concentrations of the adrenal insufficient dogs.

The statistical analysis of the changes which occurred in the plasma and RBC K concentrations of the adrenal insufficient dogs indicate no significant increases in the rate of K change of either blood compartment. The inability to obtain a statistically significant increase in the rates of K change may be attributed to the small number of animals tested and the large amount of variance within the dogs sampled. The rate of plasma K change increased in four of the adrenalectomized dogs tested but was not altered in the remaining dog. However, the mean overall change in the plasma K concentrations of the adrenal insufficient dogs (1.7 mEq/liter/dog) was not as drastic as those observed by a previous investigation. Swingle and Swingle (11) in a similar experiment on ten adrenalectomized dogs observed an average increase of 3.1 mEq of K/liter/dog with terminal adrenal insufficiency.

Like the cat, the RBCs of the dog are of the high Na-low K type. Unfortunately the precise nature of Na transport is not understood (8). Tosteson (13) concluded that the Na-K transport in the dog red cells can, in a large part, be accounted for by the diffusion theory (passive transport). Hegnauer and Robinson (4) in adrenalectomized cats observed a simultaneous increase in the plasma and RBC K concentration and similar decrease in the plasma and RBC Na concentration with adrenal insufficiency. Robinson and Hegnauer (5) with a similar experiment in cats, concluded that when the electrolyte balance of plasma is sufficiently altered, the RBC membrane may become somewhat permeable to cations. Davson (2) also demonstrated the diffusion of cations from cat RBCs when placed in an isotonic solution of KCl. Harris (3) concluded that adding K to the external medium of canine RBCs caused a displacement of Na with the replacement of K.

In conclusion, there was a significant, linear, and parallel decrease in the Na concentrations of the plasma and RBCs of adrenal insufficient dogs. The changes which occurred in the K concentration of plasma and RBCs of adrenal insufficient dogs were also linear and parallel but the rates of K change were not significantly altered. The modified indirect method used to calculate the RBC Na and K concentrations proved to be very sensitive method.

REFERENCES

1. Clauvel De Mendonca, M., K. Schwartz, and E. Terrier. Sodium, potassium and osmolarity of human and canine erythrocytes interest of trapped plasma and water content for their full significance. Comp. Biochem. Physiol. 34: 146-161. 1970.

2. Davson, H. Ionic Permeability. The comparative effects of environmental changes on the permeability of the cat erythrocyte membrane to sodium and potassium. J. Cellular Comp. Physiol. 15: 317-330. 1940.

3. Harris, E. J. and T. A. J. Prankered. Diffusion and permeability of cations in human and dog erythrocytes. J. Gen. Physiol. 41: 197-218. 1957.

4. Hegnauer, A. H. and E. J. Robinson. The water and electrolyte distribution among plasma, red blood cells, and muscle after adrenalectomy. J. Biol. Chem. 116: 769-778. 1936.

5. Robinson, E. J. and A. H. Hegnauer. The water and electrolyte distribution between plasma and red blood cells following intraperitoneal injections of isotonic glucose. J. Biol. Chem. 116: 779-786. 1936.

6. Rogoff, J. M. and G. N. Stewart. Studies of adrenal insufficiency in dogs. Am. J. Physiol. 78: 683-727. 1926.

7. Share, L. and R. H. Travis. Plasma vasopresin concentration in adrenal insufficient dogs. Endocrinology 86: 196-201. 1970.

8. Spach, C. and D. H. P. Streeten. Retardation of sodium exchange in dog erythrocytes by physiological concentrations of aldosterone, in vitro. J. Clin. Invest. 43: 217-227. 1964.

9. Small, L. L. Erythrocyte Na, K, and cell volumes calculated from blood Na and K concentrations. In Sodium and potassium concentrations of erythrocytes and plasma in normal and adrenalectomized dogs. Pp. 24-47. Unpublished M.S. thesis. Library, Iowa State University, Ames, Iowa. 1971.

10. Streeten, D. H. P. and A. M. Moses. Action of cortisol on sodium transport in canine erythrocytes. J. Gen. Physiol. 52: 346-362. 1968.

11. Swingle, W. W. and A. J. Swingle. Terminal insufficiency in adrenalectomized dogs maintained for three years on substitution therapy and the apparent absence of accessory cortical tissue in this species. Endocrinology 81: 406-408. 1967.

12. Swingle, W. W., H. M. Vars, and W. M. Parkins. Study of the blood volume of adrenalectomized dogs. Am. J. Physiol. 109: 488-501. 1934.

13. Tosteson, D. C. Classification of transport processes, Theoretical section. In Shanes, A. M., ed. Electrolytes in biological systems. Pp. 124-156. Am. Physiol. Soc., Washington, D.C. 1955.

14. Verrier, R. L., M. J. Rovetto, and A. M. Lefer. Blood volume and myocardial function in adrenal insufficiency. Am. J. Physiol. 217: 1559-1564. 1969.

15. Watts, R. W., I. U. Degerli, J. D. Hardy, and M. Unal. Cardiovascular responses in adrenal insufficiency. Surgery 58: 273-282. 1965.

LITERATURE CITED

1. Anderson, D. M. The trapped plasma correction factor in the blood of pigs. J. Comp. Path. 80: 163-168. 1970.

2. Baron, D. N. and S. A. Ahmed. Intracellular concentrations of water and of the principal electrolytes determined by analysis of isolated human leukocytes. Clin. Sci. 37: 205-219. 1969.

3. Beilin, L. J., G. J. Knight, A. D. Munro-faure, and J. Anderson. The measurement of sodium concentration in human red blood cells. J. Gen Physiol. 50: 61-74. 1966.

4. Bernstein, R. E. Potassium and sodium balance in mammalian red cells. Science 120: 459-460. 1954.

5. Boyd, D. W. Red blood cell potassium and aldosteronism. Lancet 594-595. 1970.

6. Bozzini, C. E., M. E. B. Rendo, and J. A. Kofoed. Erythrokinetics in the adrenalectomized dog. Acta Physiol. Lat. Am. 18: 26-32. 1968.

7. Bugyi, H. I., E. Magnier, W. Joseph, and G. Frank. A method for measurement of sodium and potassium in erythrocytes and whole blood. Clin. Chem. 15: 712-719. 1969.

8. Chien, S., R. J. Dellenback, S. Usami, and M. I. Gregersen. Plasma trapping in hematocrit determination. Differences among animal species. Soc. Exptl. Biol. Med. Proc. 119: 1155-1158. 1965.

9. Cividalli, G. and H. Loker. Micromethod for the determination of sodium and potassium in red blood cells. J. Lab. Clin. Med. 76: 706-710. 1969.

10. Clauvel De Mendonca, M. K. Schwartz, and E. Terrier. Sodium, potassium and osmolarity of human and canine erythrocytes interest of trapped plasma and water content for their full significance. Comp. Biochem. Physiol. 34: 146-161. 1970.

11. Coldman, M. F. and W. Good. The distribution of sodium, potassium and glucose in the blood of some mammals. Comp. Biochem. Physiol. 21: 201-206. 1967.

12. Coulter, D. B., R. C. Ewan, M. J. Swenson, F. X. Aherne, and D. Wyllie. Plasma and erythrocyte concentrations of electrolytes in the blood of fetal and maternal swing. Am. J. Vet Res. 31: 1179-1185. 1970.

13. Czaczkes, J. W., T. D. Ulmann, L. Ulmann, and Z. Barkochba. Determination of the red blood cell content of water, sodium and potassium in normal subjects. J. Lab. Clin. Med. 61: 873-878. 1963.

14. Davson, H. Ionic Permeability. The comparative effects of environmental changes on the permeability of the cat erythrocyte membrane to sodium and potassium. J. Cell. Comp. Physiol. 15: 317-330. 1940.

15. Duggan, D. E., J. E. Baer, and R. M. Noll. Membrane adenosine triphosphatase and cation composition of mammalian erythrocytes. Naturwissenschaften 52: 264. 1965.

16. Funder, J. and J. O. Wieth. Trapping of sodium, potassium, sucrose albumin in the packed cell column of the hematocrit. Actas Physiol. Scand. 71: 105-112. 1967.

17. Harris, E. J. and T. A. J. Prankered. Diffusion and permeability of cations in human and dog erythrocytes. J. Gen. Physiol. 41: 197-218. 1957.

18. Hegnauer, A. H. and E. J. Robinson. The water and electrolyte distribution among plasma, red blood cells, and muscle after adrenalectomy. J. Biol. Chem. 116: 769-778. 1936.

19. Hellerstein, S., W. Spees, and L. Surapathana. Hemoglobin concentration and erythrocyte cation content. J. Lab. Clin. Med. 76: 10-24. 1970.

20. Hlad, C. J. and J. H. Holmes. Factors affecting hematocrit determinations, trapped plasma, its amount and distribution. J. Appl. Physiol. 5: 457. 1953.

21. Hoffman, J. F. On the relationship of certain erythrocyte characteristics to their physiological age. J. Cell. Comp. Physiol. 51: 415-523. 1958.

22. Hoffman, W. S. and H. R. D. Jacobs. The partition of potassium between the serum and corpuscles in health and disease. J. Lab. Clin. Med. 19: 633-644. 1934.

23. Hunsaker, W. G. Species and sex differences in the percentage of plasma trapped in packed cell volume determination on avian blood. Poultr. Sci. 48: 907-909. 1969.

24. Hutt, M. P. Effect of disease on erythrocyte and plasma potassium concentrations. Am. J. Med. Sci. 223: 176-181. 1952.

25. Jackson, M. D. and M. E. Nutt. Intercellular plasma and its effect on absolute red cell volume determination. J. Physiol. 115: 196-205. 1951.

26. Karvonen, M. J. The effect of age on the electrolytes in the red blood cells of different species. In Wolstenholme, G. E. W. and C. M. O'Connor, ed. Water and electrolyte metabolism in relation to age and sex, Ciba Foundation Colloquia on Ageing. Vol. IV. Pp. 199-208. Little, Brown, Boston, Massachusetts. 1958.

27. Keitel, H. G., H. Berman, H. Jones, and E. MacLachlan. The chemical composition of normal human red blood cells, including variability between centrifuged cells. Blood 10: 370. 1955.

28. Kramer, B. and F. Tisdall. The distribution of sodium, potassium, calcium and magnesium between corpuscles and serum of human blood. J. Biol. Chem. 53: 241-252. 1922.

29. Lubowitz, H. and R. Whittam. Ion movements in human red cells independent of the sodium pump. J. Physiol. 202: 111-131. 1969.

30. Maizels, M. The anion and cation contents of normal and anaemic bloods. Biochem. J. 30: 821-830. 1936.

31. McCance, R. A. and E. M. Widdowson. The effect of development, anaemia and undernutrition on the composition of erythrocytes. Clin. Sci. 15: 409-416. 1965.

32. Oberst, F. W. The determination of sodium in human red blood cells. J. Biol. Chem. 108: 153-160. 1935.

33. Rich, G. T., R. I. Sha'afi, T. C. Barton, and A. K. Solomon. Permeability studies on red cell membranes of dogs, cats, and beef. J. Gen. Physiol. 50: 2391-2405. 1967.

34. Robinson, E. J. and A. H. Hegnauer. The water and electrolyte distribution between plasma and red blood cells following intraperitoneal injections of isotonic glucose. J. Biol. Chem. 116: 779-786. 1936.

35. Rogoff, J. M. and G. N. Stewart. Studies of adrenal insufficiency in dogs. Am. J. Physiol. 78: 683-727. 1926.

36. Share, L. and R. H. Travis. Plasma vasopressin concentration in adrenal insufficient dogs. Endocrinology 86: 196-201. 1970.

37. Sheppard, C. W., W. R. Martin, and G. Beyl. Cation exchange between cells and plasma of mammalian blood. J. Gen. Physiol. 34: 411-429. 1951.

38. Sirs, J. A. The measurement of the hematocrit and flexibility of erythrocytes with a centrifuge. Biorheology 5: 1-14. 1968.

39. Snyder, R. and S. Katzenelbogen. The distribution of sodium, potassium, calcium, magnesium, inorganic phosphorus and chloride between the blood serum and cells of normal individuals. J. Biol. Chem. 143: 223-226. 1942.

40. Sorenson, A. L., L. B. Kirschner, and J. Barker. Sodium fluxes in the erythrocytes of swine, ox, and dog. J. Gen. Physiol. 45: 1031-1047. 1962.

41. Spach, C. and D. H. P. Streeten. Retardation of sodium exchange in dog erythrocytes by physiological concentrations of aldosterone, in vitro. J. Clin. Invest. 43: 217-227. 1964.

42. Spurr, G. B. and G. Barlow. Plasma and erythrocyte Na, K, Cl and water in hypothermic and hyperthemic dogs. Am. J. Physiol. 197: 648-652. 1959.

43. Streef, G. M. Sodium and calcium content of erythrocytes. J. Biol. Chem. 129: 661-672. 1939.

44. Streeten, D. H. P. and A. M. Moses. Action of cortisol in sodium transport in canine erythrocytes. J. Gen. Physiol. 52: 346-362. 1968.

45. Swan, H. and A. W. Nelson. Canine trapped plasma factors at different microhematocrit levels. J. Surg. Res. 80: 551-554. 1968.

46. Swingle, W. W. and A. J. Swingle. Terminal insufficiency in adrenalectomized dogs maintained for three years on substitution therapy and the apparent absence of accessory cortical tissue in this species. Endocrinology 81: 406-408. 1967.

47. Swingle, W. W., H. M. Vars, W. M. Parkins. Study of the blood volume of adrenalectomized dogs. Am. J. Physiol. 109: 488-501. 1934.

48. Tosteson, D. C. Classification of transport processes. Theoretical section. In Shanes, A. M., ed. Electrolytes in boilogical systems. Pp. 124-156. The Am. Physiol. Soc., Washington, D.C. 1955.

49. Tosteson, D. C. Some properties of the plasma membranes of high potassium and low potassium sheep red cells. N.Y. Acad. Sci. Ann. 137: 577-590. 1966.

50. Tosteson, D. C. and J. F. Hoffman. Regulation of cell volume by active cation transport in high and low potassium sheep red cells. J. Gen. Physiol. 44: 168-194. 1960.

51. Trzeciack, M. S., D. R. Krouse, and G. T. Judson. Radiotracer determination of supernatant liquid entrapped among centrifuged red blood cells. Nature 216: 510-511. 1967.

52. Valberg, L. S., J. M. Holt, E. J. Paulson, and J. Szivek. Spectrochemical analysis of sodium, potassium, calcium, magnesium, copper, and zinc in normal human erythrocytes. J. Clin. Invest. 44: 379-388. 1965.

53. Valberg, L. L., R. T. Card, E. J. Paulson, and J. Szivek. The metal composition of erythrocytes in different species and its relationship to the life span on the cells in the circulation. Comp. Biochem. Physiol. 15: 347-350. 1965.

54. Valberg, L. S., R. T. Card, E. J. Paulson, and J. Szivek. Alterations in cellular sodium, potassium, calcium, magnesium, copper, and zinc levels during the development and maturation of erythrocytes in the rabbit. Brit. J. Haem. 13: 115-125, 1967.

55. Verrier, R. L., M. J. Rovetto, and A. M. Lefer. Blood volume and myocardial function in adrenal insufficiency. Am. J. Physiol. 217: 1559-1564. 1969.

56. Vieirs, F. L., R. I. Sha'afi, and A. K. Solomon. The state of water in the human and dog red cell membrane. J. Gen. Physiol. 55: 451-467. 1970.

57. Villeges, R., T. C. Barton, and A. K. Solomon. The entrance of water into beef and dog red cells. J. Gen. Physiol. 42: 355-369. 1958.

58. Watts, R. W., I. U. Degerli, J. D. Hardy, and M. Unal. Cardiovascular responses in adrenal insufficiency. Surgery 58: 273-282. 1965.

59. Zarkowsky, H. S., F. A. Oski, R. Sha'afi, S. B. Shohet, and D. G. Wathan. Congenital hemolytic anemia with high sodium, low K red cells. I. Studies of membrane permeability. New Eng. J. Med. 278: 373-381. 1968.

ACKNOWLEDGEMENTS

I would like to thank Dr. Dwight B. Coulter who throughout the research and preparation of thesis offered his criticisms (sometimes painful), suggestions (many times needed), and appraisals (always welcome) which kept in prospective the general goals of my graduate program. I would also like to personally thank Dr. Melvin J. Swenson, Dr. Dean H. Riedesel, Miss Mary S. Arthur, Dr. Frederick B. Hembrough, Dr. Richard L. Engen, Mrs. Lois L. Rouze, Dr. Richard C. Ewan, Dr. A. Dare McGilliard, Miss Judy H. Nystrom, Mrs. Geralyn D. Sick, Mrs. Charlotte E. Peterson and Mrs. Iola M. Whitver for their cooperation and technical assistance, and a multitude of other people who offered encouragement. Financial support by Research Grant HE-05228 from the National Heart Institute of the U. S. Public Health Service is acknowledged.

I would like to dedicate this thesis to my loving wife and children who, throughout my graduate program replaced my neglect with love and understanding.

APPENDIX

Table abbreviations:

CCV	Calculated cell volume
CPV	Calculated plasma volume
CNHWB	Calculated non-hemolyzed whole blood
HWB	Hemolyzed whole blood
NHWB	Non-hemolyzed whole blood
PCVr	Packed cell volume (red)
PCVt	Packed cell volume (total)
PCVw	Packed cell volume (white)
RBCe	Red blood cell electrolyte concentration calculated by the modified indirect method
RBCe'	Red blood cell electrolyte concentration calculated by the indirect method

Table 1. Blood variables measured on 21 Caucasian humans

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
	1.	47.60	47.20	141.0	80.50	83.00	3.65	2.35	42.50
1.	2.	47.50	47.30	142.0	80.00	83.00	3.60	2.35	43.00
	X.	47.55	47.25	141.5	80.25	83.00	3.63	2.35	42.75
	1.	43.00	42.80	141.00	86.50	89.00	4.15	2.75	45.00
2.	2.	43.30	42.70	142.00	86.00	89.00	4.20	2.75	45.00
	X.	43.15	42.75	141.50	86.25	89.00	4.17	2.75	45.00
	1.	44.80	44.40	141.00	83.00	86.00	3.30	2.20	44.00
3.	2.	44.70	44.40	142.00	83.50	86.00	3.35	2.20	44.00
	X.	44.75	44.40	141.50	83.25	86.00	3.32	2.20	44.00
	1.	46.90	46.50	142.00	81.00	83.00	3.50	2.40	45.00
4.	2.	47.20	46.50	142.00	82.00	84.00	3.50	2.40	45.50
	X.	47.05	46.50	142.00	81.50	83.50	3.50	2.40	45.25
	1.	46.60	46.40	143.00	82.00	85.00	3.45	2.20	45.50
5.	2.	46.60	46.40	143.00	82.00	85.00	3.40	2.20	45.50
	X.	46.60	46.40	143.00	82.00	85.00	3.42	2.20	45.50
	1.	38.40	38.00	142.50	92.00	91.00	3.65	2.60	38.50
6.	2.	38.50	38.00	142.00	91.00	91.00	3.60	2.55	38.50
	X.	38.45	38.00	142.25	91.50	91.00	3.63	2.57	38.50
	1.	40.60	40.20	142.00	90.00	91.00	3.95	2.75	42.00
7.	2.	40.60	40.20	141.50	89.00	91.00	3.90	2.75	41.00
	X.	40.60	40.20	141.75	89.50	91.00	3.92	2.75	41.50

Table 1. (Continued)

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
	1.	47.10	46.90	140.00	80.50	82.00	3.60	2.50	47.00
8.	2.	47.10	46.90	140.00	80.50	82.00	3.65	2.50	47.00
	X.	47.10	46.90	140.00	80.50	82.00	3.63	2.50	47.00
	1.	37.80	37.20	140.00	91.50	93.00	3.20	2.30	38.50
9.	2.	37.80	37.40	139.00	91.50	93.50	3.20	2.30	38.50
	X.	37.80	37.30	139.50	91.50	93.25	3.20	2.30	38.50
	1.	34.70	34.50	137.00	95.00	97.00	3.70	2.80	34.50
10.	2.	35.00	34.60	138.50	94.00	98.00	3.70	2.80	34.50
	X.	34.85	34.55	137.75	94.50	97.50	3.70	2.80	34.50
	1.	48.90	48.70	143.00	79.50	83.00	3.30	2.20	47.00
11.	2.	49.70	49.50	143.50	79.00	82.00	3.35	2.15	46.50
	X.	49.30	49.10	143.25	79.25	82.50	3.32	2.17	46.75
	1.	44.70	44.30	142.50	84.00	86.00	3.75	2.60	43.00
12.	2.	44.70	44.00	141.50	83.50	86.50	3.70	2.55	43.00
	X.	44.70	44.15	142.00	83.75	86.25	3.72	2.57	43.00
	1.	46.60	46.40	143.00	82.00	82.50	3.35	2.40	46.00
13.	2.	46.70	46.30	143.00	81.00	83.00	3.35	2.35	46.50
	X.	46.65	46.35	143.00	81.50	82.75	3.35	2.38	46.25
	1.	47.50	47.60	142.00	81.00	84.00	4.20	2.75	47.00
14.	2.	47.20	46.80	142.00	80.50	80.40	4.25	2.70	47.00
	X.	47.35	46.90	142.00	80.75	82.20	4.22	2.72	47.00

Table 1. (Continued)

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
	1.	46.60	46.00	144.00	82.00	87.50	4.15	2.70	47.00
15.	2.	46.90	46.50	145.00	82.50	87.00	4.20	2.65	47.00
	X.	46.75	46.25	144.50	82.25	87.25	4.17	2.67	47.00
	1.	43.20	42.90	142.50	85.00	87.00	3.80	2.40	46.00
16.	2.	43.40	42.80	142.50	84.00	86.50	3.80	2.40	46.00
	X.	43.30	42.85	142.50	84.50	86.75	3.80	2.40	46.00
	1.	45.40	44.80	142.00	80.50	83.00	4.70	2.80	44.00
17.	2.	45.10	44.80	141.00	80.00	82.00	4.65	2.80	44.00
	X.	45.25	44.80	141.50	80.25	82.50	4.67	2.80	44.00
	1.	48.70	47.70	141.00	76.00	79.50	4.65	2.55	53.00
18.	2.	48.70	48.00	141.50	76.00	79.00	4.70	2.55	53.00
	X.	48.70	47.85	141.25	76.00	79.25	4.67	2.55	53.00
19.	1.	44.70	44.40	140.50	80.50	83.00	4.20	2.50	48.00
	2.	44.70	44.30	140.00	80.00	83.00	4.20	2.50	48.00
	X.	44.70	44.35	140.25	80.25	83.00	4.20	2.50	48.00
	1.	49.10	48.40	140.50	75.50	76.00	3.65	2.00	50.00
20.	2.	48.90	48.50	140.00	75.50	76.50	3.65	2.00	50.00
	X.	49.00	48.45	140.25	75.50	76.25	3.65	2.00	50.00
	1.	46.70	45.80	140.50	78.50	80.00	3.60	2.05	51.00
21.	2.	46.50	45.80	140.00	77.50	80.00	3.60	2.00	51.00
	X.	46.60	45.80	140.25	78.00	80.00	3.60	2.03	51.00

Table 2. Blood variables calculated on 21 Caucasian humans

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter RBC	Sodium, mEq/liter RBC'	Potassium, mEq/liter RBC	Potassium, mEq/liter RBC'	CNHWB	PCVw
	1.	57.09	42.91	5.88	19.31	93.53	86.91	2.08	0.40
1.	2.	56.34	43.66	6.90	17.86	93.53	86.91	2.03	0.20
	X.	56.71	43.29	6.40	18.59	93.98	86.45	2.06	0.30
	1.	61.35	38.65	6.50	20.16	109.88	99.61	2.55	0.20
2.	2.	60.56	39.44	7.72	19.87	108.79	99.81	2.54	0.60
	X.	60.95	39.05	7.12	20.02	109.33	99.71	2.55	0.40
	1.	58.87	41.13	7.36	18.40	102.61	95.00	1.94	0.40
3.	2.	58.80	41.20	6.11	16.83	102.21	94.93	1.97	0.30
	X.	58.63	41.17	6.74	17.62	102.41	94.96	1.96	0.35
	1.	57.04	42.96	4.70	16.34	100.10	92.78	2.00	0.40
4.	2.	57.75	42.25	4.81	19.41	103.72	93.88	2.02	0.70
	X.	57.39	42.61	4.76	17.87	101.89	93.33	2.01	0.55
	1.	57.34	42.66	7.07	18.62	101.98	94.09	1.98	0.20
5.	2.	57.75	42.25	7.13	19.77	102.96	94.15	1.96	0.20
	X.	57.54	42.46	7.10	19.19	102.47	94.12	1.97	0.20
	1.	64.56	35.44	-2.85	8.47	102.46	95.40	2.36	0.40
6.	2.	64.08	35.92	0.00	9.66	101.51	95.49	2.31	0.50
	X.	64.32	35.68	-1.42	9.07	101.98	95.44	2.33	0.45
	1.	63.38	36.62	2.76	16.55	108.37	98.64	2.50	0.40
7.	2.	62.90	37.10	5.45	17.29	104.22	96.23	2.45	0.40
	X.	63.14	36.86	4.11	16.92	106.28	67.43	2.48	0.40

Table 2. (Continued)

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter RBC	Sodium, mEq/liter RBC'	Potassium, mEq/liter RBC	Potassium, mEq/liter RBC'	CNHWB	PCVw
8.	1.	57.50	42.50	3.55	16.93	105.20	96.15	2.07	0.20
	2.	57.50	42.50	3.55	16.93	105.20	96.10	2.10	0.20
	X.	57.50	42.50	3.55	16.93	105.20	96.12	2.08	0.20
9.	1.	65.36	34.64	4.41	15.91	106.34	98.14	2.09	0.60
	2.	65.83	34.17	5.92	18.83	107.19	97.62	2.11	0.40
	X.	65.59	34.41	5.16	17.38	106.76	97.88	2.10	0.50
10.	1.	69.34	30.66	6.57	21.85	104.08	93.00	2.57	0.20
	2.	67.87	32.13	12.61	23.05	99.91	92.76	2.51	0.40
	X.	68.60	31.40	9.65	22.45	101.94	92.88	2.54	0.30
11.	1.	55.59	44.41	7.92	20.38	101.34	93.05	1.83	0.20
	2.	55.05	44.95	6.70	19.84	99.11	90.54	1.84	0.20
	X.	55.32	44.68	7.31	20.11	100.22	91.78	1.84	0.20
12.	1.	58.95	41.05	4.92	16.25	99.38	92.38	2.21	0.40
	2.	59.01	40.99	7.45	18.75	100.40	93.08	2.18	0.70
	X.	58.98	41.02	6.18	17.49	99.89	92.73	2.20	0.55
13.	1.	57.34	42.66	1.18	13.23	102.69	95.28	1.92	0.20
	2.	56.64	43.36	4.66	14.65	102.78	96.58	1.90	0.40
	X.	56.99	43.01	2.93	13.94	102.73	95.93	1.91	0.30
14.	1.	57.04	42.96	7.07	20.11	104.22	95.31	2.40	0.50
	2.	56.69	43.31	-0.23	11.59	103.24	95.63	2.41	0.40
	X.	56.87	43.13	3.40	15.86	103.73	95.47	2.40	0.45

Table 2. (Continued)

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter RBC	Sodium, mEq/liter RBC'	Potassium, mEq/liter RBC	Potassium, mEq/liter RBC'	CNHWB	PCVw
	1.	56.94	43.06	12.95	23.05	104.34	97.36	2.36	0.60
15.	2.	56.90	43.10	10.54	21.52	103.86	96.28	2.39	0.40
	X.	56.92	43.08	11.74	22.28	104.10	96.81	2.38	0.50
	1.	59.65	40.35	3.99	14.13	108.86	102.19	2.27	0.30
16.	2.	58.95	41.05	6.18	13.66	107.78	102.45	2.24	0.60
	X.	59.30	40.70	5.59	13.89	108.32	102.32	2.25	0.45
	1.	56.69	43.31	5.85	12.21	96.46	92.49	2.66	0.60
17.	2.	56.74	43.26	4.66	10.25	95.90	92.52	2.64	0.30
	X.	56.71	43.29	5.25	11.22	96.18	92.50	2.65	0.45
	1.	53.90	46.10	7.76	15.03	111.86	106.11	2.51	1.00
18.	2.	53.71	46.29	6.58	13.36	110.66	105.39	2.52	0.70
	X.	53.81	46.19	7.17	14.19	111.26	105.75	2.52	0.85
	1.	57.30	42.70	5.90	11.94	107.30	102.88	2.41	0.30
19.	2.	57.14	42.86	7.07	12.60	107.17	103.11	2.40	0.40
	X.	57.22	42.78	6.48	12.27	107.23	102.99	2.40	0.35
	1.	53.74	46.26	1.10	9.27	105.35	99.47	1.96	0.70
20.	2.	53.55	46.45	2.17	9.17	104.23	99.25	1.95	0.40
	X.	53.64	46.36	1.64	9.22	104.78	99.36	1.98	0.55
	1.	55.87	44.13	3.47	11.16	113.24	107.16	2.01	0.90
21.	2.	55.36	44.64	5.69	11.14	111.51	107.15	1.99	0.70
	X.	55.61	44.39	4.59	11.15	112.37	107.16	2.00	0.80

Table 3. Blood variables measured on 30 mongrel dogs

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
	1.	40.60	39.90	144.00	88.00	127.00	4.00	2.55	5.85
1.	2.	40.50	39.60	144.00	88.00	126.50	4.00	2.55	5.80
	X.	40.55	39.75	144.00	88.00	126.75	4.00	2.55	5.82
	1.	47.50	47.30	149.00	82.00	131.00	4.10	2.30	4.60
2.	2.	47.70	47.30	149.50	82.50	131.00	4.10	2.30	4.70
	X.	47.60	47.30	149.25	82.25	131.00	4.10	2.30	4.65
	1.	48.20	47.60	146.50	80.00	129.00	4.00	2.25	5.20
3.	2.	48.30	47.60	147.00	80.00	128.00	4.00	2.25	5.15
	X.	48.25	47.60	146.75	80.00	128.50	4.00	2.25	5.17
	1.	40.40	39.80	146.50	90.00	134.50	4.50	2.80	5.00
4.	2.	40.40	39.80	146.50	90.50	135.00	4.50	2.85	5.05
	X.	40.40	39.80	146.50	90.25	134.75	4.50	2.82	5.02
	1.	37.10	36.10	140.00	90.50	129.00	4.10	2.80	5.40
5.	2.	37.10	36.10	139.50	91.00	127.00	4.05	2.80	5.25
	X.	37.10	36.10	139.75	90.75	128.00	4.07	2.80	5.32
	1.	56.40	55.60	145.00	67.00	124.50	3.60	1.80	4.80
6.	2.	56.30	55.60	145.00	67.00	124.00	3.60	1.80	4.85
	X.	56.35	55.60	145.00	67.00	124.25	3.60	1.80	4.82
	1.	46.80	45.70	146.00	80.50	131.00	3.90	2.15	5.00
7.	2.	46.30	45.90	146.00	81.00	131.50	3.85	2.20	5.00
	3.	46.55	45.80	146.00	80.75	131.25	3.88	2.17	5.00

Table 3. (Continued)

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
	1.	45.30	44.40	151.50	85.50	137.00	4.05	2.35	4.90
8.	2.	45.30	44.40	152.00	85.00	136.00	4.05	2.35	4.80
	X.	45.30	44.40	151.75	85.25	136.50	4.05	2.35	4.85
	1.	50.10	49.60	149.50	76.00	129.00	3.80	2.10	6.20
9.	2.	50.40	49.60	149.00	76.50	130.00	3.80	2.10	6.25
	X.	50.25	49.60	149.25	76.25	129.50	3.80	2.10	6.22
	1.	39.80	39.20	146.50	91.50	138.50	4.35	2.85	5.40
10.	2.	40.30	39.50	146.50	91.00	137.00	4.30	2.80	5.25
	X.	40.05	39.35	146.50	91.25	137.75	4.32	2.82	5.32
	1.	41.00	40.30	145.00	88.00	129.00	3.80	2.40	4.95
11.	2.	41.30	40.20	144.00	89.00	128.00	3.85	2.40	4.90
	X.	41.15	40.25	144.50	88.50	128.50	3.82	2.40	4.92
	1.	32.50	31.90	147.50	101.00	136.00	4.40	3.20	6.00
12.	2.	32.50	31.90	147.00	101.00	136.00	4.45	3.20	6.00
	X.	32.50	31.90	147.25	101.00	136.00	4.42	3.20	6.00
	1.	47.30	46.20	148.00	82.00	129.00	3.95	2.30	5.20
13.	2.	47.40	46.20	148.00	81.50	127.00	3.90	2.30	5.05
	X.	47.35	46.20	148.00	81.75	128.00	3.92	2.30	5.13
	1.	51.10	50.50	149.00	77.00	130.00	3.90	2.05	4.70
14.	2.	51.00	50.30	149.00	76.00	131.00	3.95	2.10	4.80
	X.	51.05	50.40	149.00	76.50	130.50	3.92	2.07	4.75

Table 3. (Continued)

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
	1.	43.90	43.00	149.00	87.00	132.00	4.10	2.50	5.40
15.	2.	43.90	42.70	149.00	86.50	130.00	4.05	2.45	5.40
	X.	43.90	42.85	149.00	86.75	131.00	4.07	2.47	5.40
	1.	41.00	40.40	144.00	88.00	128.00	3.95	2.50	4.80
16.	2.	41.00	40.40	144.00	88.00	126.00	3.90	2.45	4.70
	X.	41.00	40.40	144.00	88.00	127.00	3.92	2.47	4.75
	1.	47.50	47.10	144.00	79.00	125.00	4.60	2.60	5.25
17.	2.	47.80	47.10	144.00	78.50	125.00	4.65	2.60	5.30
	X.	47.65	47.10	144.00	78.75	125.00	4.63	2.60	5.27
	1.	47.10	46.40	145.00	79.50	124.00	4.30	2.40	4.90
18.	2.	47.20	46.80	145.00	79.50	124.00	4.25	2.40	4.90
	X.	47.15	46.60	145.00	79.50	124.00	4.27	2.40	4.90
	1.	41.70	40.90	142.00	86.00	126.00	4.45	2.75	5.40
19.	2.	41.70	40.80	142.00	86.00	126.00	4.50	2.80	5.30
	X.	41.70	40.85	142.00	86.00	126.00	4.47	2.77	5.35
	1.	35.40	34.90	145.00	96.00	132.00	4.75	3.30	5.35
20.	2.	35.50	34.90	146.00	95.50	134.00	4.80	3.30	5.35
	X.	35.45	34.90	145.50	95.75	133.00	4.77	3.30	5.35
	1.	44.10	43.70	146.00	86.50	135.00	4.25	2.50	5.20
21.	2.	44.30	43.80	146.00	86.00	134.00	4.20	2.45	5.25
	X.	44.20	43.75	146.00	86.25	134.50	4.22	2.47	5.22

Table 3. (Continued)

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
	1.	38.70	38.40	145.00	92.50	134.00	3.80	2.45	5.30
22.	2.	38.70	38.10	146.00	92.50	134.00	3.80	2.45	5.25
	X.	38.70	38.25	145.50	92.50	134.00	3.80	2.45	5.27
	1.	41.10	40.10	146.00	89.00	134.00	4.05	2.50	4.90
23.	2.	41.00	39.90	147.00	89.00	134.50	4.05	2.50	4.90
	X.	41.05	40.00	146.50	89.00	134.25	4.05	2.50	4.90
	1.	42.20	40.60	149.00	89.00	136.00	3.70	2.40	5.70
24.	2.	42.20	40.60	148.50	87.50	134.00	3.75	2.35	5.65
	X.	42.20	40.60	148.75	88.25	135.00	3.72	2.38	5.65
	1.	44.80	43.50	149.00	85.50	132.00	3.90	2.30	5.40
25.	2.	44.80	44.10	150.00	84.50	132.00	3.85	2.30	5.35
	X.	44.80	43.80	149.50	85.00	132.00	3.88	2.30	5.38
	1.	42.90	42.00	147.50	87.00	131.50	4.30	2.55	5.20
26.	2.	42.90	42.20	147.00	87.00	131.50	4.30	2.60	5.10
	X.	42.90	42.10	147.25	87.00	131.50	4.30	2.57	5.15
	1.	56.20	55.40	149.00	68.50	125.50	4.25	1.90	5.80
27.	2.	56.00	55.40	148.00	68.50	126.00	4.20	1.90	5.85
	X.	56.10	55.40	148.50	68.50	125.75	4.22	1.90	5.82
	1.	48.40	47.90	144.00	79.00	128.50	4.30	2.30	5.30
28.	2.	48.20	47.90	144.00	79.00	128.00	4.30	2.30	5.40
	X.	48.30	47.90	144.00	79.00	128.25	4.30	2.30	5.35

Table 3. (Continued)

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	PLasma	NHWB	HWB
	1.	57.20	56.60	148.00	66.50	128.00	4.50	1.95	5.35
29.	2.	57.20	56.80	148.00	65.50	128.00	4.50	1.95	5.30
	X.	57.20	56.70	148.00	66.00	128.00	4.50	1.95	5.32
	1.	46.10	45.40	143.50	80.50	127.50	4.40	2.40	5.30
30.	2.	46.50	45.40	143.50	81.00	126.00	4.40	2.40	5.30
	X.	46.30	45.40	143.50	80.75	126.75	4.40	2.40	5.30

Table 4. Blood variables calculated on 30 mongrel dogs

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter RBC	Sodium, mEq/liter RBC'	Potassium, mEq/liter RBC	Potassium, mEq/liter RBC'	CNHWB	PCVw
	1.	61.11	38.89	102.12	103.92	8.64	8.27	2.44	0.70
1.	2.	61.11	38.89	101.35	103.08	8.56	8.21	2.44	0.90
	X.	61.11	38.89	101.74	103.50	8.60	8.24	2.44	0.80
	1.	55.03	44.97	109.46	111.57	5.14	4.86	2.26	0.20
2.	2.	55.18	44.82	109.19	111.65	5.40	5.07	2.26	0.40
	X.	55.11	44.89	109.33	111.61	5.27	4.97	2.26	0.30
	1.	54.61	45.39	109.39	111.58	6.59	6.20	2.18	0.60
3.	2.	54.43	45.58	106.96	109.25	6.46	6.09	2.18	0.70
	X.	54.51	45.49	108.17	110.41	6.52	6.14	2.18	0.65
	1.	61.43	38.57	117.21	118.56	5.79	5.53	2.76	0.60
4.	2.	61.77	38.23	118.27	119.81	5.85	5.53	2.78	0.60
	X.	61.60	38.40	117.74	119.19	5.82	5.53	2.77	0.60
	1.	64.64	35.36	112.06	113.41	7.57	7.20	2.65	1.00
5.	2.	65.23	34.77	106.61	108.74	7.26	6.79	2.64	1.00
	X.	64.94	35.06	109.36	111.07	7.41	6.99	2.65	1.00
	1.	46.21	53.79	108.50	110.22	5.66	5.40	1.66	0.80
6.	2.	46.21	53.79	107.36	109.06	5.74	5.49	1.66	0.70
	X.	46.21	53.79	107.93	109.64	5.70	6.44	1.66	0.75
	1.	55.14	44.86	115.39	116.69	6.51	6.24	2.15	1.10
7.	2.	55.48	44.52	114.46	115.68	6.35	6.10	2.14	0.40
	X.	55.31	44.69	114.92	116.19	6.43	6.17	2.14	0.75

Table 4. (Continued)

Animal No.	Test No.	CPV	Sodium, mEq/liter CCV	RBC	RBC'	Potassium, mEq/liter RBC	RBC'	CNHWB	PCVw
	1.	56.44	43.56	120.71	121.91	5.98	5.74	2.29	0.90
8.	2.	55.92	44.08	118.11	119.04	5.67	5.52	2.26	0.90
	X.	56.18	43.82	119.40	120.48	5.82	5.63	2.28	0.90
	1.	50.84	49.96	108.91	109.68	8.43	8.27	1.93	0.50
9.	2.	51.34	48.66	111.79	113.10	8.67	8.37	1.95	0.80
	X.	51.09	48.91	110.34	111.39	8.55	8.32	1.94	0.65
	1.	62.46	37.54	127.22	128.33	6.90	6.51	2.72	0.60
10.	2.	62.12	37.88	124.04	125.42	6.61	6.20	2.67	0.80
	X.	62.29	37.71	125.63	126.87	6.75	6.35	2.69	0.70
	1.	60.69	39.31	106.19	107.82	6.60	6.33	2.31	0.70
11.	2.	61.81	38.19	105.14	108.14	6.74	6.22	2.38	1.10
	X.	61.25	38.75	105.67	107.98	6.67	6.27	2.34	0.90
	1.	68.47	31.53	113.18	114.22	9.05	8.78	3.01	0.60
12.	2.	68.71	31.29	114.03	115.28	9.12	8.78	3.06	0.60
	X.	68.59	31.41	113.60	114.75	9.09	8.78	3.04	0.60
	1.	55.41	44.59	108.06	110.40	6.67	6.28	2.19	1.10
13.	2.	55.07	44.93	104.04	106.39	6.29	5.95	2.15	1.20
	X.	55.24	44.76	106.05	108.39	6.48	6.11	2.17	1.15
	1.	51.68	48.32	111.06	113.15	5.55	5.25	2.02	0.60
14.	2.	51.01	48.99	113.89	115.29	5.59	5.37	2.01	0.70
	X.	51.34	48.66	112.48	114.22	5.57	5.31	2.02	0.65

Table 4. (Continued)

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter RBC	Sodium, mEq/liter RBC'	Potassium, mEq/liter RBC	Potassium, mEq/liter RBC'	CNHWB	PCVw
	1.	58.39	41.61	110.54	112.58	7.12	6.74	2.39	0.90
15.	2.	58.05	41.95	106.76	108.69	7.24	6.91	2.35	1.20
	X.	58.22	41.78	108.65	110.64	7.18	6.83	2.37	1.05
	1.	61.11	38.89	104.47	106.53	6.01	5.69	2.41	0.60
16.	2.	61.11	38.89	99.25	101.58	5.88	5.57	2.38	0.60
	X.	61.11	38.89	101.86	104.06	5.94	5.63	2.40	0.60
	1.	54.86	45.14	102.82	104.88	5.92	5.63	2.52	0.40
17.	2.	54.51	45.49	103.83	105.80	6.03	5.73	2.53	0.70
	X.	54.69	45.31	103.32	105.34	5.98	5.68	2.53	0.55
	1.	54.83	45.17	100.06	101.93	5.62	5.39	2.36	0.70
18.	2.	54.83	45.17	99.39	101.37	5.58	5.34	2.33	0.40
	X.	54.83	45.17	99.73	101.65	5.60	5.36	2.34	0.55
	1.	60.56	39.44	103.53	105.66	6.86	6.48	2.70	0.80
19.	2.	60.56	39.44	103.80	105.92	6.49	6.13	2.73	0.90
	X.	60.56	39.44	103.66	105.79	6.67	6.30	2.71	0.85
	1.	66.21	33.79	108.13	109.83	6.16	5.87	3.14	0.50
20.	2.	65.41	34.59	113.27	114.13	6.03	5.87	3.14	0.60
	X.	65.81	34.19	110.72	111.98	6.09	5.87	3.14	0.55
	1.	59.25	40.75	120.19	122.16	6.69	6.18	2.52	0.40
21.	2.	58.90	41.10	118.24	120.27	6.90	6.39	2.47	0.50
	X.	59.08	40.92	119.21	121.22	6.79	6.55	2.50	0.45

Table 4. (Continued)

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter RBC	Sodium, mEq/liter RBC'	Potassium, mEq/liter RBC	Potassium, mEq/liter RBC'	CNHWB	PCVw
	1.	63.79	36.21	115.58	117.49	7.94	7.74	2.42	0.30
22.	2.	63.36	36.34	115.14	116.80	7.77	7.67	2.41	0.60
	X.	63.57	36.43	115.35	117.15	7.85	7.70	2.42	0.45
	1.	60.96	39.04	118.29	119.72	6.31	6.27	2.47	1.00
23.	2.	60.54	39.46	118.63	119.72	6.26	6.29	2.45	1.10
	X.	60.75	39.25	118.46	119.72	6.28	6.28	2.46	1.05
	1.	59.73	40.27	121.55	122.85	8.53	8.13	2.21	1.60
24.	2.	58.92	41.08	117.79	118.64	8.36	8.13	2.21	1.60
	X.	59.33	40.67	119.65	120.74	8.45	8.13	2.21	1.60
	1.	57.38	42.62	112.54	114.37	7.50	7.13	2.24	1.30
25.	2.	56.33	43.67	110.55	111.66	7.10	6.92	2.17	0.70
	X.	56.86	43.14	111.52	112.96	7.30	7.02	2.20	1.00
	1.	58.98	41.02	110.93	112.57	6.61	6.31	2.54	0.90
26.	2.	59.18	40.82	110.93	112.71	6.23	5.92	2.54	0.70
	X.	59.08	40.92	110.93	112.64	6.42	6.12	2.54	0.80
	1.	45.97	54.03	107.09	108.73	7.33	7.04	1.95	0.80
27.	2.	46.28	53.72	108.25	109.89	7.44	7.13	1.94	0.60
	X.	46.13	53.87	107.67	109.31	7.38	7.08	1.95	0.70
	1.	54.86	45.14	110.98	113.14	6.72	6.26	2.36	0.50
28.	2.	54.86	45.14	109.28	111.50	6.91	6.47	2.36	0.30
	X.	54.86	45.14	110.08	112.32	6.82	6.37	2.36	0.40

Table 4. (Continued)

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter		Potassium, mEq/liter		CNHWB	PCVw
				RBC	RBC'	RBC	RBC'		
	1.	44.93	55.07	112.91	114.23	6.24	6.01	2.02	0.60
29.	2.	44.26	55.74	112.93	113.83	6.05	5.90	1.99	0.40
	X.	44.59	55.41	112.92	114.03	6.15	5.95	2.01	0.50
	1.	56.10	43.90	108.79	110.47	6.71	6.39	2.47	0.70
30.	2.	56.45	43.55	106.00	108.43	6.83	6.39	2.48	1.10
	X.	56.27	43.73	107.41	109.45	6.77	6.39	2.48	0.90

Table 5. Blood variables measured on 32 Yorkshire-Landrace-Poland China crossbred pigs

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
	1.	36.60	36.00	142.50	93.00	94.00	4.75	3.30	46.50
1.	2.	36.50	35.60	142.50	93.50	94.00	4.70	3.30	46.50
	X.	36.55	35.80	142.50	93.25	94.00	4.72	3.30	46.50
	1.	36.20	34.90	154.00	95.50	97.00	4.60	3.35	45.00
2.	2.	36.20	34.80	145.50	95.00	97.00	4.60	3.35	45.00
	3.	36.20	34.85	145.25	95.25	97.00	4.60	3.35	45.00
	1.	31.40	30.40	141.00	98.50	99.00	4.50	3.40	41.00
3.	2.	31.40	30.40	142.00	98.00	99.00	4.60	3.40	40.50
	3.	31.40	30.40	141.50	98.25	99.00	4.55	3.40	40.75
	1.	36.10	34.70	144.50	95.50	96.50	5.60	3.95	46.00
4.	2.	35.80	34.80	144.00	95.00	96.00	5.50	3.90	46.00
	3.	35.95	34.75	144.25	95.25	96.25	5.55	3.92	46.00
	1.	33.50	32.90	135.00	88.50	88.00	4.30	3.05	44.00
5.	2.	35.70	34.80	135.00	88.50	88.00	4.30	3.00	44.00
	3.	34.60	33.85	135.50	88.50	88.00	4.30	3.02	44.00
	1.	42.70	41.50	143.00	83.00	83.00	6.00	3.90	53.00
6.	2.	42.80	41.70	143.50	83.00	83.00	6.00	3.85	52.50
	3.	42.75	41.60	143.25	83.00	83.00	6.00	3.88	52.75
	1.	34.40	33.50	142.00	92.50	94.00	4.40	3.20	45.00
7.	2.	34.40	33.50	142.00	92.50	94.00	4.40	3.20	45.00
	X.	34.40	33.50	142.00	92.50	94.00	4.40	3.20	45.00

Table 5. (Continued)

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
	1.	40.30	39.70	147.00	89.00	91.00	6.15	4.20	50.00
8.	2.	40.50	39.60	146.00	88.00	91.00	6.15	4.15	50.00
	X.	40.40	39.65	146.50	88.50	91.00	6.15	4.17	50.00
	1.	41.30	40.30	138.00	81.00	83.00	4.55	2.90	51.00
9.	2.	41.30	40.70	137.00	81.00	83.00	4.50	2.90	50.00
	X.	41.30	40.50	137.50	81.00	83.00	4.52	2.90	50.50
	1.	35.00	33.70	143.50	94.00	96.00	5.20	3.80	43.00
10.	2.	35.00	33.70	143.50	94.50	97.00	5.20	3.75	43.50
	X.	35.00	33.70	143.50	94.25	96.50	5.20	3.77	43.25
	1.	33.50	31.90	140.50	93.00	94.00	4.90	3.50	42.00
11.	2.	33.80	32.20	141.00	93.00	94.00	4.90	3.50	42.50
	3.	33.65	32.05	140.75	93.00	94.00	4.90	3.50	42.25
	1.	33.90	33.10	145.00	94.00	95.50	4.90	3.50	44.00
12.	2.	33.90	33.10	145.00	94.00	96.00	4.90	3.50	44.50
	X.	33.90	33.10	145.00	94.00	95.75	4.90	3.50	44.25
	1.	33.60	32.60	144.00	96.00	95.50	5.35	4.10	43.50
13.	2.	33.60	32.90	144.00	95.50	95.50	5.35	4.05	43.00
	X.	33.60	32.75	144.00	95.75	95.50	5.35	4.07	43.25
	1.	36.70	36.00	144.50	92.00	93.00	4.70	3.40	45.50
14.	2.	36.90	36.40	144.50	91.50	93.00	4.80	3.40	45.00
	X.	36.80	36.20	144.50	91.75	93.00	4.75	3.40	45.25

Table 5. (Continued)

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
	1.	31.60	30.60	142.00	96.00	97.00	4.90	3.55	40.00
15.	2.	31.70	30.60	143.50	96.00	96.50	4.90	3.60	39.50
	X.	31.65	30.60	142.75	96.00	96.75	4.90	3.57	39.75
	1.	34.50	33.50	145.00	95.00	96.50	5.30	3.80	45.00
16.	2.	34.60	33.50	146.00	95.00	96.50	5.30	3.80	45.00
	X.	34.55	33.50	145.50	95.00	96.50	5.30	3.80	45.00
	1.	34.70	33.80	143.00	92.50	94.50	4.70	3.35	44.50
17.	2.	35.00	34.10	142.00	93.00	95.00	4.65	3.35	44.50
	X.	34.85	33.95	142.50	92.75	94.75	4.67	3.35	44.50
	1.	36.50	35.40	142.00	90.50	92.00	4.80	3.40	49.00
18.	2.	36.50	35.70	142.00	90.00	92.50	4.80	3.40	49.00
	X.	36.50	35.55	142.00	90.25	92.25	4.80	3.40	49.00
	1.	33.30	32.00	141.00	94.00	98.50	4.40	3.30	45.50
19.	2.	33.20	32.10	142.00	95.00	98.50	4.40	3.30	45.50
	X.	33.25	32.05	141.50	94.50	98.50	4.40	3.30	45.50
	1.	37.00	35.90	143.50	91.00	95.00	5.20	3.65	50.00
20.	2.	37.00	35.80	143.50	91.00	94.50	5.20	3.60	50.00
	X.	37.00	35.85	143.50	91.00	94.75	5.20	3.63	50.00
	1.	33.60	31.80	138.00	93.00	96.00	5.10	3.65	45.50
21.	2.	33.60	31.80	139.00	92.00	96.50	5.05	3.65	45.50
	X.	33.60	31.80	138.50	92.50	96.25	5.07	3.65	45.50

Table 5. (Continued)

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
	1.	35.10	34.00	142.00	92.00	95.50	4.60	3.35	47.00
22.	2.	35.10	34.30	142.00	92.50	95.50	4.60	3.35	47.00
	X.	35.10	34.15	142.00	92.25	95.50	4.60	3.35	47.00
	1.	34.10	33.00	143.50	95.00	98.50	4.90	3.65	46.00
23.	2.	34.10	33.50	143.00	94.50	99.00	4.90	3.65	46.00
	X.	34.10	33.25	143.25	94.75	98.75	4.90	3.65	46.00
	1.	33.90	33.00	144.00	96.00	98.50	4.90	3.65	45.00
24.	2.	33.90	33.00	144.50	95.00	98.00	4.90	3.65	45.00
	X.	33.90	33.00	144.25	95.50	98.25	4.90	3.65	45.00
	1.	32.70	31.80	144.00	96.00	98.50	4.80	3.50	45.00
25.	2.	32.70	31.80	144.00	96.50	98.50	4.80	3.50	45.00
	X.	32.70	31.80	144.00	96.25	98.50	4.80	3.50	45.00
	1.	35.70	35.00	145.00	96.50	98.50	5.20	3.80	48.00
26.	2.	35.80	35.00	144.50	96.50	98.50	5.20	3.80	48.00
	X.	35.75	35.00	144.75	96.50	98.50	5.20	3.80	48.00
	1.	33.00	32.40	144.00	95.00	100.00	5.20	3.75	44.00
27.	2.	32.90	32.80	145.00	95.00	100.00	5.20	3.80	44.00
	X.	32.95	32.60	144.50	95.00	100.00	5.20	3.77	44.00
	1.	33.70	32.90	143.50	95.00	96.00	6.00	3.95	45.00
28.	2.	33.90	32.90	142.00	95.00	96.00	6.00	3.95	45.00
	X.	33.80	32.90	142.75	95.00	96.00	6.00	3.95	45.00

Table 5. (Continued)

Animal No.	Test No.	PCVt	PCVr	Sodium, mEq/liter			Potassium, mEq/liter		
				Plasma	NHWB	HWB	Plasma	NHWB	HWB
29.	1.	34.90	34.10	147.00	97.50	102.00	4.20	4.10	47.00
	2.	35.00	33.80	147.00	97.50	101.50	4.20	4.35	47.00
	X.	34.95	33.95	147.00	97.50	101.75	4.20	4.23	47.00
30.	1.	33.70	33.30	143.00	94.50	97.50	5.10	3.70	45.50
	2.	34.10	33.30	143.00	94.50	97.50	5.10	3.70	45.50
	X.	33.90	33.30	143.00	94.50	97.50	5.10	3.70	45.50
31.	1.	32.30	31.50	141.50	97.00	98.00	5.20	3.80	42.00
	2.	32.10	31.30	142.00	97.00	98.00	5.20	3.80	42.00
	X.	32.20	31.40	141.75	97.00	98.00	5.20	3.80	42.00
32	1.	34.80	33.60	137.00	90.00	93.00	5.40	3.85	46.00
	2.	34.50	33.40	137.00	90.50	93.00	5.40	3.90	46.00
	X.	34.65	33.50	137.00	90.25	93.00	5.40	3.88	46.00

Table 6. Blood variables calculated on 32 Yorkshire-Landrace-Poland China crossbred pigs

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter RBC	Sodium, mEq/liter RBC'	Potassium, mEq/liter RBC	Potassium, mEq/liter RBC'	CNHWB	PCVw
	1.	65.26	34.74	2.93	10.15	126.55	120.80	3.10	0.60
1.	2.	65.61	34.39	1.49	9.87	129.01	122.23	3.08	0.90
	X.	65.44	34.56	2.22	10.01	127.77	121.51	3.09	0.75
	1.	65.86	34.14	4.57	12.87	126.83	120.53	3.03	1.30
2.	2.	65.29	34.71	6.00	11.99	125.05	120.88	3.00	1.40
	X.	65.58	34.42	5.29	12.43	125.93	120.70	3.02	1.35
	1.	69.86	30.14	1.72	7.48	129.02	124.71	3.14	1.00
3.	2.	69.01	30.99	3.33	5.22	123.72	122.84	3.17	1.00
	X.	69.43	30.57	2.54	6.35	126.33	123.78	3.16	1.00
	1.	66.09	33.91	3.08	12.00	129.34	122.25	3.70	1.40
4.	2.	65.97	34.03	3.03	10.21	127.47	122.04	3.63	1.00
	X.	66.03	33.97	3.05	11.10	128.40	122.14	3.66	1.20
	1.	65.07	34.93	-1.46	-7.42	119.30	125.05	2.80	0.60
5.	2.	65.56	34.44	-1.49	3.43	122.23	118.49	2.82	0.90
	X.	65.31	34.69	-1.47	-1.82	120.74	121.68	2.81	0.75
	1.	58.04	41.96	0.00	2.56	120.47	119.43	3.48	1.20
6.	2.	57.84	42.16	0.00	2.20	118.48	117.67	3.47	1.10
	X.	57.94	42.06	0.00	2.38	119.47	118.55	3.48	1.15
	1.	65.14	34.86	4.42	2.53	123.09	125.71	2.87	0.90
7.	2.	65.14	34.86	4.42	2.53	123.09	125.71	2.87	0.90
	X.	65.14	34.86	4.42	2.53	123.09	125.71	2.87	0.90

Table 6. (Continued)

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter RBC	Sodium, mEq/liter RBC'	Potassium, mEq/liter RBC	Potassium, mEq/liter RBC'	CNHWB	PCVw
	1.	60.54	39.46	5.15	8.16	117.87	116.70	3.72	0.60
8.	2.	60.27	39.37	7.73	10.43	118.09	117.02	3.71	0.90
	X.	60.41	39.59	6.44	9.30	117.98	116.86	3.72	0.75
	1.	58.70	41.30	4.96	4.95	119.34	119.92	2.67	1.00
9.	2.	59.12	40.88	4.97	6.34	116.94	116.36	2.66	0.60
	X.	58.91	41.09	4.96	5.65	118.14	118.13	2.67	0.80
	1.	65.51	34.49	6.03	8.09	118.09	117.57	3.41	1.30
10.	2.	65.85	34.15	7.61	11.05	121.02	119.05	3.42	1.30
	X.	65.68	34.32	6.81	9.57	119.55	118.31	3.42	1.30
	1.	66.19	33.81	3.10	1.78	119.54	121.45	3.24	1.60
11.	2.	65.96	34.04	3.08	2.04	120.21	121.91	3.23	1.60
	X.	66.07	33.93	3.09	1.91	119.87	121.68	3.24	1.60
	1.	64.83	35.17	4.36	-1.04	117.83	123.15	3.18	0.80
12.	2.	64.83	35.17	5.82	0.47	119.28	124.66	3.18	0.80
	X.	64.83	35.17	5.09	-0.29	118.55	123.90	3.18	0.80
	1.	66.67	33.33	-1.55	-0.36	121.86	122.54	3.57	1.00
13.	2.	66.32	33.68	0.00	-0.35	118.10	119.90	3.55	0.70
	X.	66.49	33.51	-0.77	-0.35	119.96	121.21	3.56	0.85
	1.	63.67	36.33	2.81	4.25	118.15	118.12	2.99	0.70
14.	2.	63.32	36.68	4.15	5.00	114.99	115.31	3.04	0.50
	X.	63.49	36.51	3.48	4.63	116.56	116.71	3.02	0.60

Table 6. (Continued)

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter RBC	Sodium, mEq/liter RBC'	Potassium, mEq/liter RBC	Potassium, mEq/liter RBC'	CNHWB	PCVw
	1.	67.61	32.39	3.19	-0.42	116.10	119.77	3.31	1.00
15.	2.	66.90	33.10	1.56	-4.94	112.18	118.15	3.28	1.10
	X.	67.25	32.75	2.37	-2.68	114.12	118.96	3.30	1.05
	1.	65.62	34.48	4.48	4.55	123.05	123.97	3.47	1.00
16.	2.	65.07	34.93	4.43	3.03	121.78	123.98	3.45	1.10
	X.	65.29	34.71	4.46	3.79	122.41	123.97	3.46	1.05
	1.	64.69	35.31	5.81	3.32	119.57	122.58	3.04	0.90
17.	2.	65.49	34.51	5.95	7.92	122.44	121.63	3.05	0.90
	X.	65.09	34.91	5.88	5.63	120.99	122.10	3.04	0.90
	1.	63.73	36.27	4.37	5.17	129.66	129.81	3.06	1.10
18.	2.	63.38	36.62	6.98	6.53	127.30	128.72	3.04	0.80
	X.	63.56	36.44	5.63	5.85	128.47	129.26	3.05	0.95
	1.	66.67	33.33	14.05	13.92	131.74	133.02	2.93	1.30
19.	2.	66.90	33.10	10.94	11.35	131.88	132.59	2.94	1.10
	X.	66.78	33.22	12.49	12.63	131.81	132.80	2.94	1.20
	1.	63.41	36.59	11.27	12.80	130.62	130.15	3.30	1.10
20.	2.	63.41	36.59	9.89	11.44	131.13	130.51	3.30	1.20
	X.	63.41	36.59	10.58	12.12	130.87	130.33	3.30	1.15
	1.	67.39	32.61	9.74	13.74	135.84	132.43	3.44	1.80
21.	2.	66.19	33.81	14.06	13.22	130.73	132.54	3.34	1.80
	X.	66.79	33.21	11.94	13.48	133.22	132.48	3.39	1.80

Table 6. (Continued)

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter RBC	Sodium, mEq/liter RBC'	Potassium, mEq/liter RBC	Potassium, mEq/liter RBC'	CNHWB	PCVw
	1.	64.79	35.21	10.26	9.83	127.97	129.45	2.98	1.10
22.	2.	65.14	34.86	8.81	9.74	128.16	121.32	3.00	0.80
	X.	64.96	35.04	9.53	9.79	128.06	128.89	2.99	0.95
	1.	66.20	33.80	10.70	11.92	129.52	129.61	3.24	1.10
23.	2.	66.08	33.92	13.51	14.22	127.12	127.67	3.24	0.60
	X.	66.14	33.86	12.12	13.08	128.31	128.63	3.24	0.85
	1.	66.67	33.33	7.71	10.05	123.97	126.55	3.27	0.90
24.	2.	65.74	34.26	8.99	7.53	123.97	126.55	3.22	0.90
	X.	66.20	33.80	8.36	8.79	123.97	126.55	3.24	0.90
	1.	66.67	33.33	7.71	4.99	127.95	131.35	3.20	0.90
25.	2.	67.01	32.99	6.23	4.99	129.34	131.35	3.22	0.90
	X.	66.84	33.16	6.97	4.99	128.64	131.35	3.21	0.90
	1.	66.55	33.45	6.11	15.04	134.97	127.59	3.46	0.70
26.	2.	66.78	33.22	6.17	16.37	136.34	127.60	3.47	0.80
	X.	66.67	33.33	6.14	15.71	135.65	127.60	3.47	0.75
	1.	65.97	34.03	14.96	10.86	120.41	125.05	3.43	0.60
27.	2.	65.52	34.48	14.54	8.25	116.92	123.51	3.41	0.10
	X.	65.74	34.26	14.75	9.55	118.64	124.27	3.42	0.35
	1.	66.20	33.80	3.03	2.61	124.40	124.69	3.97	0.80
28.	2.	66.90	33.10	3.12	6.50	127.89	124.72	4.01	1.00
	X.	66.55	33.45	3.07	4.56	126.11	124.71	3.99	0.90

Table 6. (Continued)

Animal No.	Test No.	CPV	CCV	Sodium, mEq/liter RBC	Sodium, mEq/liter RBC'	Potassium, mEq/liter RBC	Potassium, mEq/liter RBC'	CNHWB	PCVw
	1.	66.33	33.67	13.69	18.48	130.50	129.81	2.79	0.80
29.	2.	66.33	33.67	12.32	17.60	131.34	130.98	2.79	1.20
	X.	66.33	33.67	13.01	18.05	130.92	130.39	2.79	1.00
	1.	66.08	33.92	8.95	8.08	124.72	126.48	3.37	0.40
30.	2.	66.08	33.92	9.06	9.80	126.22	126.54	3.37	0.80
	X.	66.08	33.92	9.00	8.94	125.46	126.51	3.37	0.60
	1.	68.55	31.45	3.26	7.00	124.64	122.16	3.56	0.80
31.	2.	68.31	31.69	3.24	5.05	123.66	122.90	3.55	0.80
	X.	68.43	31.57	3.25	6.03	124.15	122.53	3.56	0.80
	1.	65.69	34.31	9.06	10.94	127.32	126.43	3.55	1.20
32.	2.	66.06	33.94	7.61	9.78	128.19	127.13	3.56	1.10
	X.	65.88	34.12	8.34	10.36	127.75	126.78	3.56	1.15

Table 7. Variables measured on control dog number 1. Day number 1 coincides with the day on which the last steroid injections were given to the adrenalectomized dogs

Day	Body temp. (°C)	Body weight (kg)	Heart rate (BPM)	PCVr (%)	CCV (%)	Sodium (mEq/liter)		Potassium (mEq/liter)	
						Plasma	Cell	Plasma	Cell
1.	102.6	17.9	132	33.9	33.3	147	105	4.4	10.4
2.	103.7	18.2	160	36.6	34.6	149	107	4.4	12.5
3.	102.9	17.9	136	36.7	35.1	149	115	4.5	13.0
4.	102.9	18.5	156	35.3	33.4	146	108	4.2	9.8
5.	102.1	18.3	160	35.7	33.7	148	115	4.6	11.1
6.	102.1	18.6	160	34.4	32.7	147	103	4.1	9.4
7.	102.3	18.4	156	34.4	32.0	150	120	4.7	9.2
9.	103.0	18.6	156	36.1	34.4	150	111	4.2	9.7
11.	103.5	18.6	160	33.5	32.1	148	117	4.5	9.4
13.	102.9	16.9	116	36.0	35.3	144	105	3.7	7.5

Table 8. Variables measured on control dog number 2. Day number 1 coincides with the day on which the last steroid injections were given to the adrenalectomized dogs

Day	Body temp. (°C)	Body weight (kg)	Heart rate (BPM)	PCVr (%)	CCV (%)	Sodium (mEq/liter)		Potassium (mEq/liter)	
						Plasma	Cell	Plasma	Cell
1.	102.1	20.4	100.0	45.4	43.8	147	111	4.3	6.5
2.	103.0	20.2	136	44.3	42.5	149	110	4.3	6.5
3.	102.0	20.0	112	41.1	40.1	150	118	4.5	6.8
4.	102.3	19.2	108	49.6	46.8	145	110	4.2	6.4
5.	102.1	20.5	92	42.7	40.6	148	122	4.3	7.7
6.	102.1	19.6	88	45.1	42.2	148	107	4.1	6.8
7.	102.0	19.6	96	41.6	39.0	154	110	4.6	7.1
9.	101.7	19.9	104	40.0	38.5	152	114	4.5	7.9
11.	102.4	20.0	132	42.1	40.1	151	112	4.5	8.3
13.	102.2	20.4	116	42.9	41.6	148	108	3.9	7.0

Table 9. Blood variables measured on adrenalectomized dog number 1. Day number 1 coincides with the day on which the last steroid injection was given

Day	Body temp. (°C)	Body weight (kg)	Heart rate (BPM)	PCVr (%)	CCV (%)	Sodium (mEq/liter)		Potassium (mEq/liter)	
						Plasma	Cell	Plasma	Cell
1.	102.9	12.5	68	36.9	35.8	148	110	4.4	8.2
2.	103.0	12.4	72	36.0	35.1	149	108	4.6	8.0
3.	102.7	12.1	100	39.9	39.1	149	108	5.2	9.9
4.	102.4	11.8	112	43.3	41.7	142	108	5.3	9.7
5.	102.1	11.3	144	50.8	47.8	141	109	5.5	9.8
6.	102.1	11.3	132	50.7	47.1	134	94	4.8	8.7
7.	102.4	11.1	124	50.4	46.9	133	94	5.5	8.9
9.	100.9	10.8	136	50.7	48.9	132	93	5.7	8.1
11.	102.3	10.3	140	52.4	49.5	133	101	6.5	7.8

Table 10. Blood variables measured on adrenalectomized dog number 2. Day number 1 coincides with the day on which the last steroid injection was given

Day	Body temp. (°C)	Body weight (kg)	Heart rate (BPM)	PCVr (%)	CCV (%)	Sodium (mEq/liter)		Potassium (mEq/liter)	
						Plasma	Cell	Plasma	Cell
1.	101.7	14.5	88	43.7	41.2	150	110	4.1	6.4
2.	100.8	14.6	80	41.8	39.9	148	107	4.5	6.0
3.	101.0	14.5	64	45.0	43.5	150	109	5.1	6.5
4.	101.3	14.2	68	46.6	44.3	144	109	5.0	6.4
5.	101.1	14.2	80	51.1	48.5	145	113	5.3	7.3
6.	101.4	13.6	72	55.2	51.3	141	100	5.2	6.9
7.	101.6	13.4	100	56.3	52.9	141	98	5.6	7.3
9.	101.4	13.1	100	55.4	52.5	137	98	5.6	7.4
11.	100.7	12.9	128	55.6	54.3	133	102	5.3	7.6
13.	98.8	12.5	124	67.0	68.7	126	89	5.0	5.6

Table 11. Blood variables measured on adrenalectomized dog number 3. Day number 1 coincides with the day on which the last steroid injection was given

Day	Body temp. (°C)	Body weight (kg)	Heart rate (BPM)	PCVr (%)	CCV (%)	Sodium (mEq/liter)		Potassium (mEq/liter)	
						Plasma	Cell	Plasma	Cell
1.	101.4	18.5	76	42.5	38.9	146	114	3.9	6.3
2.	101.8	18.8	92	41.1	39.1	147	104	4.7	5.7
3.	102.3	18.1	112	48.2	45.3	148	114	5.4	5.8
4.	102.7	17.6	136	55.7	52.5	140	107	5.9	5.9
5.	101.6	17.3	128	59.7	55.7	139	103	7.5	7.2
6.	102.1	17.2	132	61.7	59.9	135	95	7.4	7.3

Table 12. Blood variables measured on adrenalectomized dog number 4. Day number 1 coincides with the day on which the last steroid injection was given

Day	Body temp. (°C)	Body weight (kg)	Heart rate (BPM)	PCVr (%)	CCV (%)	Sodium (mEq/liter)		Potassium (mEq/liter)	
						Plasma	Cell	Plasma	Cell
1.	102.3	18.0	132	47.3	45.1	137	104	4.9	8.7
2.	102.6	18.0	132	46.6	43.9	139	99	5.2	8.3
3.	102.2	17.7	100	45.3	42.7	144	106	5.1	8.3
4.	102.0	18.0	124	44.9	43.8	141	106	4.8	7.1
5.	102.0	17.6	120	48.3	46.4	141	110	6.6	8.2
6.	101.8	17.2	120	52.3	48.3	134	99	6.9	9.1
7.	102.6	17.3	128	53.5	48.9	130	95	7.5	10.7

Table 13. Blood variables measured on adrenalectomized dog number 5. Day number 1 coincides with the day on which the last steroid injection was given

Day	Body temp.	Body weight (kg)	Heart rate (BPM)	PCVr (%)	CCV (%)	Sodium (mEq/liter)		Potassium (mEq/liter)	
						Plasma	Cell	Plasma	Cell
1.	102.5	13.1	120	46.0	44.5	138	107	4.3	5.9
2.	103.2	12.5	104	44.8	43.5	318	101	4.9	5.3
3.	102.5	12.3	128	49.6	46.3	139	101	5.0	5.8
4.	102.5	12.0	132	55.1	52.3	135	99	5.1	5.8

Table 14. Body temperatures (°C) of control and adrenalectomized dogs from time-intervals 1 through 10

Time-interval	Control		Adrenalectomized				
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
1.	102.9	102.3	102.9	101.5	101.4	102.2	102.5
2.	103.3	102.5	102.9	100.9	101.5	102.4	102.5
3.	102.9	102.3	102.6	100.3	101.7	102.6	102.6
4.	102.1	102.1	102.3	101.1	102.0	102.3	102.9
5.	102.2	102.1	102.1	101.5	102.4	102.1	103.2
6.	102.5	101.9	102.3	101.5	102.6	102.0	102.9
7.	103.0	101.7	101.8	101.3	102.3	102.0	102.6
8.	103.3	102.2	101.0	100.9	101.8	101.9	102.5
9.	103.3	102.3	101.7	100.0	101.8	102.0	102.5
10.	102.9	102.2	102.3	98.8	102.1	102.6	102.5

Table 15. Body weight (kg) of control and adrenalectomized dogs from time-intervals 1 through 10

Time-interval	Control		Adrenalectomized				
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
1.	18.00	20.32	12.50	14.50	18.50	18.00	13.90
2.	18.05	20.14	12.32	14.55	18.50	17.95	13.90
3.	18.36	19.36	12.05	14.27	18.73	17.91	12.95
4.	18.32	20.36	11.64	14.18	18.50	17.77	12.73
5.	18.50	19.55	11.32	13.55	17.95	17.91	12.55
6.	18.41	19.68	11.18	13.32	17.50	17.86	12.45
7.	18.50	19.91	11.05	13.14	17.23	17.59	12.36
8.	18.50	19.95	10.86	13.03	17.32	17.36	12.27
9.	17.86	20.14	10.55	12.77	17.27	17.27	12.14
10.	16.86	20.36	10.32	12.55	17.18	17.32	12.05

Table 16. Heart rate (BPM) of control and adrenalectomized dogs from time-intervals 1 through 10

Time-interval	Control		Adrenalectomized				
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
1.	141	110	68	86	76	132	120
2.	146	122	78	72	80	132	120
3.	154	108	104	67	89	129	116
4.	160	94	124	80	101	107	110
5.	159	90	138	80	114	112	104
6.	156	96	127	100	128	123	114
7.	156	103	131	100	134	120	124
8.	159	124	135	118	129	120	129
9.	143	125	138	127	130	122	130
10.	150	116	140	124	132	128	132

Table 17. The packed cell volume (PCVr, %) of control and adrenalectomized dogs from time-intervals 1 through 10

Time-interval	Control		Adrenalectomized				
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
1.	34.9	45.0	36.8	43.1	42.5	47.3	46.0
2.	36.7	42.5	36.9	43.6	42.2	47.0	46.0
3.	35.5	46.4	41.8	46.2	41.4	46.6	45.8
4.	35.6	42.9	46.2	51.1	44.2	45.6	45.4
5.	34.4	44.0	50.7	55.8	48.7	45.1	45.0
6.	35.0	41.1	50.7	56.0	53.2	45.6	46.9
7.	36.1	40.0	50.5	55.4	56.8	48.0	48.8
8.	34.1	41.6	50.7	55.6	60.2	50.3	50.8
9.	34.5	42.4	51.5	60.6	61.6	52.9	53.0
10.	36.0	42.9	52.4	68.7	62.7	53.5	55.1

Table 18. The calculated cell volume (CCV, %) of control and adrenalectomized dogs from time-intervals 1 through 10

Time-interval	Control		Adrenalectomized				
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
1.	33.8	43.3	35.7	40.8	39.0	45.1	44.5
2.	35.4	41.1	36.2	42.0	39.0	44.7	44.5
3.	33.7	45.1	40.4	44.4	39.0	43.8	44.3
4.	33.6	40.8	44.4	48.8	41.8	43.0	43.9
5.	32.4	41.7	47.4	52.2	45.7	43.2	43.5
6.	32.8	40.2	36.9	52.7	50.0	44.4	44.9
7.	34.3	38.6	47.7	52.5	53.2	46.5	45.8
8.	32.6	39.6	48.7	52.7	55.2	47.7	47.6
9.	33.3	40.6	49.2	58.4	57.5	48.5	50.0
10.	35.1	41.6	49.5	67.0	60.9	48.9	52.3

Table 19. Plasma sodium concentration (mEq/liter) of control and adrenalectomized dogs from time-intervals 1 through 10

Time-interval	Control		Adrenalectomized				
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
1.	148	148	148	149	146	137	138
2.	149	150	149	149	146	138	138
3.	147	146	147	145	147	139	138
4.	148	148	142	144	147	143	138
5.	148	150	138	141	148	142	138
6.	150	154	134	140	143	141	138
7.	150	152	133	137	140	141	139
8.	149	151	132	134	139	136	138
9.	147	150	132	130	137	133	136
10.	144	148	133	126	135	130	135

Table 20. RBC sodium concentration (mEq/liter) of control and adrenalectomized dogs from time-intervals 1 through 10

Time-interval	Control		Adrenalectomized				
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
1.	105	110	110	109	114	104	107
2.	111	114	108	108	111	103	107
3.	108	111	108	109	106	99	107
4.	113	121	109	112	108	104	106
5.	108	107	102	102	114	106	106
6.	117	110	94	98	110	107	104
7.	112	113	94	98	106	110	102
8.	115	112	93	100	104	103	100
9.	112	110	97	96	100	98	100
10.	105	108	102	89	95	95	99

Table 21. Plasma potassium concentration (mEq/liter) of control and adrenalectomized dogs from time-intervals 1 through 10

Time-interval	Control		Adrenalectomized				
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
1.	4.4	4.3	4.4	4.2	3.9	4.9	4.3
2.	4.5	5.1	4.7	4.8	4.1	5.0	4.3
3.	4.3	4.4	5.2	5.0	4.5	5.2	4.4
4.	4.6	4.3	5.4	5.3	5.0	5.1	4.7
5.	4.3	4.3	5.2	5.3	5.4	4.9	4.9
6.	4.6	4.6	5.2	5.6	5.7	5.1	5.0
7.	4.3	4.5	5.6	5.6	6.3	6.4	5.0
8.	4.4	4.5	5.7	5.4	7.2	6.8	5.0
9.	4.2	4.3	6.1	5.2	7.4	7.1	5.1
10.	3.7	3.9	6.5	5.0	7.4	7.5	5.1

Table 22. RBC potassium concentration (mEq/liter) of control and adrenalectomized dogs from time-intervals 1 through 10

Time-interval	Control		Adrenalectomized				
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
1.	11.1	6.5	8.2	6.2	6.3	8.7	5.9
2.	12.8	6.7	8.5	6.3	6.1	8.5	5.9
3.	10.4	6.6	9.8	6.4	5.7	8.3	5.7
4.	10.8	7.1	9.7	7.3	5.7	8.3	5.5
5.	9.3	6.9	9.2	7.1	5.8	7.7	5.3
6.	9.4	7.3	8.8	7.3	5.8	7.3	5.5
7.	9.7	7.8	8.6	7.4	6.2	8.1	5.7
8.	9.5	8.2	8.2	7.5	7.0	8.8	5.8
9.	8.6	7.9	8.0	6.8	7.3	9.6	5.8
10.	7.5	7.0	7.8	5.4	7.3	10.7	5.8