CARDIOVASCULAR STUDIES IN ADRENAL INSUFFICIENT DOGS

Thesis for the Degree of Ph. D.

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Clarence F. Decker

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This is to certify that the

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Cardiovascular Studies in
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Clarence F. Decker

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Wm Dwayne Collings
Major professor

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By

Clarence F. Decker

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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An attempt was made to make certain cardiovascular measurements in a group of five trained, unnarcotized, bilaterally adrenalectomized dogs. Heart rate, blood pressure, stroke index and cardiac index were followed simultaneously in the same animal in different states of mild adrenal insufficiency. The cardiac index was determined from the contour of the pressure pulse curve according to the method of Hamilton and Remington. In this method the arterial pressure curve, produced by cardiac ejection of blood, is analyzed in terms of the volume of blood necessary to elicit the pressure fluctuation. To convert a pressure curve to blood volume requires consideration of pulse wave transmission times to various parts of the expansible arterial tree. This is justifiable on the basis that the volume of blood distending the vessel alters the rate of movement of the compression wave. The Hamilton-Remington tables for pulse wave transmission times at various pressures were used in the present work. A strain gage manometer and direct recording galvanometer were used to record the central pulse from the carotid artery.

The earliest noted circulatory alterations following bilateral adrenalectomy were a decrease in systolic and an increase in diastolic pressure with a corresponding reduction in the pulse pressure. A reduction in both stroke index and cardiac index was evident. These changes occured before marked alterations in the electrocardiogram, blood non-

protein nitrogen, plasma sodium or potassium had taken place. When adreno-cortical replacement therapy was withdrawn a progressive fall in cardiac output occured which was roughly proportional to the severity of the adrenal insufficient state as indicated by blood chemistry and hematocrit changes. Re-administration of therapy resulted in an improvement in stroke index and cardiac index. This improvement often occured even before marked improvement in blood chemistry had occured.

It was suggested that the initial increase in diastolic pressure observed could be explained on the basis of an increased peripheral resistance. An increased peripheral resistance coupled with the observed reduction in carddac output could be indicative of an inadequate blood volume, but the present data do not permit positive evaluation of this factor. The improved flow obtained when replacement therapy was again administered could be explained on the basis of a lowered peripheral resistance.

It was concluded that the present data indicate that considerable alteration in the output of the heart occurs in adrenal insufficiency before marked changes in blood chemistry and the electrocardiogram take place. It was suggested that a direct action of adrenal steroids on heart muscle might be implicated, but the possibility of their indirect effect upon the peripheral resistance is not ruled out by the data.

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INTRODUCTION

The importance of the adrenal glands in the maintenance of an adequate circulatory status has been repeatedly demonstrated. A decline in blood pressure following adrenalectomy has been reported by numerous workers. Derangement of the capillaries and arterioles has also been reported as a consequence of severe adrenal insufficiency. Terminal collapse of adrenal insufficient animals is almost always accompanied by circulatory failure, characterized by extremely low blood pressure levels, decreased heart size, and atonic and dilated capillaries. In addition, hemoconcentration, serum electrolyte disturbances and electrocardiographic abnormalities may be present.

Little work has been done to determine the effect of the circulatory disturbances in the adrenalectomized animal on the output of the heart. Harrison et al. (36) reported that the adrenals were essential for regulating the output of the heart, and that in adrenal deficiency crisis induced by acute removal of both glands, the output was severely decreased. These authors attributed the failure of the adrenalectomized dog to the depression in the output of the heart. The experiments upon which Harrison based his conclusions however were acute terminal ones. The procedure employed by this investigator was to exclude the adrenals from the circulation by placing a loose ligature about the lumbar adrenal veins. An hour or so after the operation, when the dog had "recovered" the ligatures were twisted to exclude the adrenals from

the circulation, and observations were made on the cardiac output determined by the direct Fick method. In the first place, it is highly unlikely that the dog had "recovered" from the operation in this short time interval. In addition, one might question whether such a procedure would appreciably decrease the level of circulating adrenal hormones. Furthermore, the removal of the small amount of blood necessary for determining the cardiac output by this method could in itself conceivably be sufficient to precipitate circulatory collapse in the animals.

More recently, Remington (84) has studied circulatory factors in adrenal crisis in sedated anesthetized dogs, and also reported a decrease in the output of the heart. However, all of Remington's experiments were also terminal ones. Blood pressure levels and cardiac indices of dogs still on therapy were shown to be nearly normal whereas crisis levels were almost always indicated in dogs off therapy for one to four days. Remington stated that, "Once the animal was sedated, and the surgery accomplished, it declined more or less rapidly into a fatal circulatory collapse." It is difficult to separate the effect of the adrenalectomy per se and that of experimental procedures on the circulation of adrenalectomized animals in experiments such as these two investigators have reported. Such investigations may give information concerning the effect of induced adrenal circulatory failure upon the output of the heart but can be of only questionable value with respect to obtaining quantitative data on the cardiac output of adrenal insufficient animals not in a crisis state induced by the experimental procedure.

The present work was undertaken in an attempt to quantitate certain circulatory variables such as cardiac index, heart rate, blood pressure and electrocardiograms in adrenal insufficient dogs without any recourse to general anesthesia, and with a minimum of surgery. An attempt was made to obtain several sets of data in the same animal in different states of insufficiency. The work was particularly restricted by the extreme sensitivity of the adrenalectomised animal to any kind of experimental procedure. It is realised that considerable variability exists in the state of insufficiency induced in different animals when subjected to the same treatment. Moreover, replacement therapy may vary greatly from one animal to the next. The expenditure of both time and material essential to an investigation of this type precludes the use of large numbers of animals. A total of twelve dogs was studied during the course of this work, five of these survived for completion of the experiments. Since it was impossible to treat these five dogs in exactly the same manner, no attempt has been made to group the animals for the purpose of a formal statistical analysis.

While it is readily admitted that some of the measurements may lack quantitative accuracy it is believed that the data show accurate directional trends with at least a good approximation to absolute values.

HISTORICAL REVIEW

A complete review of the literature dealing with the functions of the adrenal gland is not only impractical but almost impossible. The tremendous volume of literature relating various aspects of adrenal function to other physiological processes necessarily places a limitation on the length of this review.

Certain features of adrenal function must be arbitrarily omitted. Some of those which have been omitted or only briefly mentioned in this review are: the interrelationships between the adrenal gland and other endocrine glands; histological changes which the adrenal gland undergoes under various experimental conditions; and regulation of salt and water metabolism. The role which the adrenal cortex plays in intermediary metabolism has also been omitted.

In this review emphasis has been placed upon those functions of the adrenal gland most directly concerned with maintenance of an adequate circulatory status of the animal. To the extent that they affect this aspect of the total physiology the other functions of the gland have been considered as carefully as possible. To facilitate the review, the cardiovascular effects of bilateral adrenalectomy have been listed under the following headings: (1) blood pressure and arterioles, (2) capillaries and blood volume, and (3) potassium and electrocardiographic changes.

BLOOD PRESSURE AND ARTERIOLES

In 1855 Addison (1) described a syndrome resulting from impaired function of the suprarenal glands, one symptom of which was a lowering of the blood pressure. Harrop et al. (43) reported that systolic pressure changes did not appear in the dog until after other evidence of circulatory failure had become evident. Britton and Silvette (9) described the pressure changes as a "late" and indirect manifestation of adrenal removal. Loeb (62) however attached a greater significance to the pressure changes. According to this investigator the decrease in pressure was slow in onset and secondary to alterations in renal function and electrolyte balance.

Several hypotheses were offered to explain the blood pressure decline. Elliot (23) believed the muscles of the vessel walls lose their ability to contract, but offered no explanation for this hypothesis. Harrop et al. (40) believed the pressure fall was due to a reduction in circulating blood volume resulting from excessive fluid loss because of impaired kidney function. Loeb et al. (63) attributed the blood pressure fall to a specific ion effect upon the blood vessels following sodium depletion. Swingle et al. (114), on the other hand, showed that adrenalectomised dogs maintained on saline without hormone preparations appear normal in most respects but have lowered blood pressures. Swingle et al. (80,102,106,108,110) attributed the blood pressure decline to a reduction of circulating blood volume because of transudation of plasma fluid into the tissues.

This same group elaborated upon the concept of a critical blood pressure below which spontaneous recovery was supposed to be impossible, an idea first set forth by Porter (81). The terminal collapse of the animal was always associated with extremely low blood pressure levels. Although no simple relation between blood pressure and blood sugar reduction was found; it was noted that those procedures in the adrenalectomized dog against which desoxycorticosterone offered no protection, but adrenal cortex extract was effective, were the same procedures following which alteration in blood sugar were most marked (107). Britton (8) reported a direct relationship between glycogen depletion and blood pressure decline, and considered the pressure changes to be a latent manifestation of general carbohydrate metabolism impairment.

Thorn (119) stated that "anemia, reduced plasma volume, and alteration in peripheral vascular response" contribute to the production of hypotension in adrenal cortical insufficiency.

In a more recent study of the effect of cortisone on plasma volume and arterial pressure in adrenalectomized dogs, Swingle et al.(104) have shown that large doses of this steroid given for prolonged periods of time can elevate the arterial pressure in adrenalectomized dogs. The effect however, is only slight compared to that of whole extract or desoxycorticosterone. These findings are in agreement with clinical reports on the effect of cortisone upon arterial pressure in non Addisonian patients (100).

It has been often demonstrated that hypertension cannot be produced in the adrenalectomised animal even though salt is given (7,15, 24,31,79). The adrenalectomised dog is also insensitive to injected renin; the sensitivity to this substance can be restored, however, by either cortical extract or desoxycorticosterone (29,86,129). Collings et al. (16) observed that the concentration of hypertensinogen was decreased after adrenalectomy and Gaudino (33) suggested that the adrenals were involved in the synthesis of hypertensinogen.

Splanchnic stimulation fails to evoke a measurable rise in blood pressure in adrenal deficient dogs (23). Barium chloride and pitressin are also ineffective as pressor agents in this experimental animal (5, 23,86). Adrenalin was shown to exert a full pressor response (5,23), however, the dose of adrenalin must be large and large doses of renin show the same effect (86).

Coombs (17) and Langsdorf (59) independently presented evidence which they felt indicated a decreased sympathetic tone in adrenalectomised animals. Secker (94,95) found that the nicitating membrane of the adrenalectomised cat rapidly became unresponsive to motor stimulation, but would respond to injected epinephrine. Based on these findings he postulated a failure of sympathin formation, which would also explain the failure of pressor response to afferent sciatic stimulation. Later workers failed to confirm the Secker hypothesis (5,48,65). Despite this, Secker insisted that his findings were correct.

Fowler and Cleghorn (27) could find no failure in splanchnic constriction in animals in acute adrenal insufficiency, and concluded

that the absence of pressure rise could be attributed to a failure of the heart to respond to the increased peripheral resistance.

In a more recent approach to the problem of circulatory failure in adrenalectomised animals, Remington (85) has followed heart rate, cardiac index and peripheral resistance simultaneously in anesthetised, sedated adrenalectomised dogs. Adrenalectomised dogs of three kinds were studied by this author; (a) animals in terminal adrenal insufficiency, (b) animals in acute crisis produced by minor amounts of trauma or hemorrhage and (c) dogs in acute crisis following bilateral adrenal removal at a single operation. The results can be summarised as follows: In all cases the circulatory crisis was initiated by a sharp fall in resistance and blood pressure. In contrast to the normovolemic, intact dog an increase in cardiac index did not follow the resistance fall. The pressor response to epinephrine did not obviously diminish until shortly before death, however, the pressor response to more weakly active agents was lost in early crisis. The pressor response to afferent nerve stimulation was also lost early, although respiratory response was still present. An exaggerated, or prominent because unopposed, vagal reflex was common. The author concluded that, "The most reasonable explanation of the findings may lie in a metabolic failure of the nervous system, one characteristic of which is a loss of sympathetic activity." It is apparent that the findings of Remington are in agreement with the Secker hypothesis.

CAPILLARIES AND BLOOD VOLUME

A considerable amount of evidence indicates a faulty capillary bed may be at least partly responsible for the circulatory derangement in adrenalectomized animals. Most of the evidence seems to indicate that the capillaries of the untreated adrenalectomized animal are atonic, dilated and abnormally permeable. Such findings have been used to explain the fact that the reduction in blood volume in adrenalectomized animals is greater than one might expect if there were a free transfer of fluid from the interstitial reservoirs (83,111). Grandinescu (32) noted a steady rise in the number of red blood cells per cubic centimeter, and believed a change in permeability of the blood vessel wall resulted in escape of plasma to the tissues. Dale (21,22) thought the increase in blood concentration to be associated with an increased susceptability to histamine after adrenal ablation. Kellaway and Cowell (55) believed a cortical effect to be responsible for the increased blood concentration. These authors did not support the theory of Grandinescu that loss of plasma from the blood took place; they noted there were no collections of fluid in the serous spaces, and that the tissues always appear dryer than usual. Joelson and Shorr (53) found that the blood concentration usually but not invariably increased after adrenalectomy. It was also shown that a serum transfusion could precipitate circulatory collapse in an adrenalectomized dog, accompanied by an excess edema (107), and that this edema formation could be counteracted by cortical extract or desoxycorticosterone.

An abnormal leakage of protein or of injected dye also indicated a faulty capillary bed. Since hemoconcentration is not an invariable finding in adrenalectomy, this cannot be the sole factor.

Menkin reported that cortical extract and corticosterone were able to prevent the leakage of injected trypan blue into extravascular tissues after the injection of leucotaxin (71).

Several workers have reported conflicting results on the efficacy of desoxycorticosterone in preventing dye leakage after peptone or leucotaxin injections. Freed and Lindner (28) reported that cortical extracts and corticosterone prevented dye leakage but that desoxycorticosterone was without effect. This work, however, was criticised by Menkin (71) on the basis of the experimental technique employed. Shleser and Freed (96) reported favorable results with cortical extracts in preventing dye leakage after peptone injections, but negative results with the corticosterones.

Desoxycorticosterone was reported to act like the sex steroids in increasing capillary permeability in the uterus, but was without effect in this regard in other tissues of the body (49).

Many reports have indicated an increase in the protein content of the lymph following adrenalectomy. Cope et al. (19) stated that the protein content of the lymph was almost doubled following adrenalectomy. Cope (18) also reported an increased activity in the peripheral lymph when radioactive colloid was injected into adrenal insufficient dogs, and compared to suitable controls. Levin et al. (61) and Hartman et al.

(46) both believed the increase in plasma protein in adrenalectomized dogs was due to an increase in the globulin fraction with little change in the albumin fraction. This was attributed by Levin to a failure in albumin metabolism (60). Swingle et al. (105) believed that the circulatory failure in adrenal insufficient dogs could be attributed to collapse of the peripheral circulation. When it was shown that circulatory failure could be induced by "shocking procedures" before marked changes in blood volume or composition became evident, the suggestion was made that the adrenal cortical hormone is necessary for maintenance of capillary tone. The capillaries of dogs succumbing from circulatory collapse were described as being asthenic, atonic and abnormally dilated. It was postulated that in the absence of the hormones of the adrenal cortex a huge peripheral reservoir opens, into which blood flows and stagnates causing capillary damage and a resultant increase in capillary permeability.

More recently Overman (78) has studied the effects of cortisone and D C A[1] on radiosodium transport in normal and adrenalectomized dogs, and concluded that both these steroids effect sodium turnover dynamics, particularly across capillary membranes. The direction of the effects of D C A was shown to be opposite to that of cortisone. This author concluded that the data supported the hypothesis of antagonism between 11-desoxycorticosterone and 11-,17-oxysteroids as they affect membrane permeability.

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[1] Desoxycorticosterone acetate.

A reduction in the plasma volume of untreated adrenalectomized animals (44,55,112,117,132) and of patients with Addison's disease (64,90) has been reported often. Cortical extract was shown to restore the volume to normal, while D C A actually increased the plasma volume above normal in the Addison's disease patients (26,64,124) and in intact or adrenalectomized dogs (14,107). The increased volume is associated with a marked retention of sodium and chloride as well as water.

The effect of the cortical steroids on plasma volume is probably more specific than a simple production of positive water balance. Cortical extract was shown to prevent the reduction in plasma volume which follows prolonged etherization in the dog (70).

It is apparent that evidence for interaction of adrenal hormones in insuring homeostasis with respect to blood volume is still fragmentary and inferential.

POTASSIUM METABOLISM AND ELECTROCARDIOGRAPHIC CHANGES

A rise in serum potassium level following removal of the adrenal glands was first noted almost thirty years ago (6), and has been confirmed often. Numerous workers have also shown that the adrenalectomized animal is exceedingly sensitive to injected potassium salts (4,13,125, 131,133,136). Intact animals administered quantities of potassium salts sufficient to raise the plasma concentration to those typical of terminal insufficiency showed many of the symptoms of the adrenalectomized animals and often died (47,126,134,135).

Diets low in potassium have been shown to be of therapeutic value in the treatment of Addison's disease, or for maintenance of adrenalectomized animals (2,3,4,76,91,128). The increase in plasma potassium concentration was shown to be largely a reflection of a decreased renal capacity to excrete the ion (40). This kidney failure could be corrected by cortical extract (38,40). Harrison and Darrow (37) attributed the renal dysfunction to a disturbance in tubular function, so that potassium was not concentrated in the urine in a normal manner.

Several other groups of investigators presented data which indicated that renal failure could not account for the increase in plasma potassium. Marenzi (66) found that injected potassium was fixed by the tissue cells less readily in adrenalectomized animals. Winkler and associates (131) found that plasma potassium levels were elevated by a smaller amount of injected potassium than that required for the intact dog. It was postulated by Marenzi (66) that cortical extract was concerned in binding

potassium in the tissue cells, in regulating the potassium equilibrium between tissues and plasma and in regulating the excretion of excess plasma potassium.

The action of D C A upon potassium metabolism is even greater than that of cortical extract. The effect is probably largely upon the kidney, for this steroid will cause an increase in potassium excretion even in the intact animal (58).

On the other hand, Talbott and co-workers (118) found quite low potassium clearances in patients with Addison's disease even after long periods of D C A therapy. D C A was shown to prevent the usual increase in intracellular potassium which accompanies adrenal insufficiency, and it would actually lower the tissue potassium in intact animals (10,25, 74). The toxic effect of injected potassium seemed to be directly related to elevation of the serum concentration and only indirectly to the rise in muscle potassium (73). Ferrebee et al. (25) have suggested that the muscle weakness which follows prolonged treatment with D C A may be associated with the decreased muscle potassium, and its replacement with sodium.

It is generally agreed that the accumulation of potassium in the serum in itself cannot account for the symptoms of adrenal insufficiency (116). Keith and Binger (54) reported that induced high levels of serum potassium in normal human subjects, and equally high levels in diseased patients, do not necessarily produce toxic symptoms. Schamp (93) was unable to obtain symptoms resembling those of adrenal insufficiency in

normal dogs injected for long periods of time with potassium salts. Patients with Addison's disease may show little or no potassium retention even in crisis, and on the other hand, may show greatly elevated serum potassium levels and yet remain symptom free (62). Loeb (62) has reported cases of Addison's disease where the electrolyte pattern of the blood is virtually normal at death. "Adrenalectomized animals dying of circulatory failure after trauma show no consistent serum potassium change." (111)

"Electrolyte imbalance does not seem directly concerned in the production of circulatory collapse except as far as it throws a strain upon an asthenic peripheral circulation." (116)

Irregular heart rhythms have been shown to be more or less frequent in adrenal insufficiency. Nicholson and Soffer (75) were among the first to note such effects and believed they might be due to the rise in serum potassium concentrations. Cleghorn and his co-workers (11,12) studied adrenalectomized dogs exhibiting high serum potassium when showing symptoms of adrenal insufficiency, and concluded that at least part of the circulatory collapse could be attributed to cardiac failure. Cortical extract and D C A were both shown to be able to correct the cardiac abnormalities (12).

More recently Roberts (88) and Roberts and Pitts (89) have reported on electrocardiographic changes occurring in the adrenalectomized dog in insufficiency. The first change in the electrocardiogram (E C G) pattern noted was an increase in height and peaking of the T wave, which

was often seen in the early stages of insufficiency even before electrolyte changes were noted. With the progression of severe insufficiency and marked alteration in plasma electrolyte concentrations, there occurred widening of the T wave, bradycardia, disappearance of the P wave and various irregularities in cardiac rhythm. They concluded that the changes in E C G pattern paralleled the alteration in plasma sodium and potassium in adrenal insufficiency and could be corrected only if adequate concentrations of these ions were restored. The same workers showed that adequate doses of cortisone could prevent such changes but could not correct them once they had occurred. Sodium chloride administration either alone or with cortisone lowered the serum potassium and restored the E C G pattern to normal.

In a long term study conducted on adrenalectomized dogs maintained on cortisone at 0.93 mg per kg per day, Swingle et al. (103) showed that this dosage was adequate to maintain adrenalectomized dogs in an active and vigorous state, free from symptoms of insufficiency. This dosage of cortisone was not adequate, however, to prevent changes in the serum electrolyte pattern, the sodium level decreased and the potassium level became markedly elevated. These workers claim a positive correlation between states of hyperpotassemia in these animals and electrocardiographic changes. Massive doses of cortisone, 1.86 mg per kg per day, were reported to restore the electrolyte and E C G patterns to normal (68).

As pointed out by Swingle, the changes in E C G noted in adrenal insufficient dogs exhibiting hyperkalemia are very similar to the

changes observed by Winkler, Hoff and Smith (130) on E C G records taken during infusion of KCl in dogs.

Similar studies on Addisonian patients during crisis and recovery or while maintained on various types of therapy, i.e., salt, D C A in oil, cortical extract and implanted D C A pellets (92,98,121) confirm these findings.

Currens et al. (20) on the other hand caution against indiscriminate use of E C G changes in animals as an index to alterations in plasma potassium level, and conclude that there is probably a better correlation between intracellular potassium and E C G changes.

EXPERIMENTAL

Animals and Care

Healthy male and female mongrel dogs were used in the experimental work. The dogs had been in the laboratory for a period of time ranging from 4 to 12 months. During this period each animal was trained to lie quietly on its back with a minimum of restraint while various experimental procedures were carried out. Each animal was kept in an individual cage, with a constant supply of food and water. The diet consisted of dry Borden's (Chunx), and canned Rival dog food. Each dog was adrenalectomized in two stages. The right adrenal was removed soon after bringing the dog to the kennel. The second adrenal was removed just prior to beginning the experimental observations. The time elapsing between operations varied from 3 (one dog) to 12 months.

All operations were performed using 30 mg of sodium pentabarbital per kilogram of body weight as the anesthetic. Immediately prior to removal of the second adrenal gland each dog received 5 mg of D C A[2] and 1 cc of Lipo adrenal cortical extract[3] intramuscularly. Immediately after the operation each animal was given 5 cc of aqueous adrenal cortex extract[4] (A C E) intravenously. Replacement therapy following removal

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[2] Percorten (Ciba) courtesy of Dr. E. Oppenheimer.

[3] Lipo-Adrenal Cortex (The Upjohn Company) courtesy of Dr. William Haines.

[4] Adrenal Cortex Extract (The Upjohn Company) courtesy of Dr. William Haines.

of the second gland was gauged to suit the requirements of the particular animal. The exact dosage of extract used is listed in the detailed results for each dog. In cases of crisis additional Lipo or A C E were given.

During the period of training of each animal several blood samples were taken to determine the control levels of certain blood constituents such as the non-protein nitrogen, plasma sodium and potassium. Several control E C G and blood pressure records were taken during this period also. About two weeks prior to removal of the second gland, control values for the cardiac index, stroke index, blood pressure and heart rate were determined.

After removal of the second gland each animal was supported for at least seven days with Lipo adrenal cortex. The exact length of time necessary to return the animal to an active and apparently healthy state was somewhat variable from one animal to another. In any case no experimental work was begun until the animal was in an active post-operative condition as indicated by the physical appearance and body weight. When the dog was in an active "normal" or near "normal" state, measurement of the blood pressure, cardiac index, stroke index, heart rate and E C G were made simultaneously.

Immediately following the vascular measurements, the dog was injected intravenously with 5 cc of A C E and 1 cc of the Lipo adrenal cortex extract. This was considered necessary because of the minor surgery accompanying these measurements. At least one week on full therapy was allowed between sets of experimental determinations.

After a sufficient length of time had elapsed to insure that the animal had recovered from the previous experiment, therapy was withdrawn for variable lengths of time and another experiment performed.

Criteria for State of Adrenal Insufficiency

Five measurements were made to determine the state of adrenal insufficiency existing in any particular dog at the time experimental work was begun.

(a) Hematocrit. As an index to hemoconcentration the hematocrit was determined in duplicate. Two Wintrobe hematocrit tubes were filled and centrifuged for thirty minutes at 3000 revolutions per minute, after which the hematocrit in each tube was read and the average value recorded.

(b) Non-protein nitrogen of the blood. A protein free blood filtrate was prepared by transferring 3 cc of heparinized whole blood to a 50 cc graduated centrifuge tube using an Ostwald-Folin pipette. Twenty-one cc of distilled water were added to this by means of a burette, and the mixture allowed to stand until the blood was hemolyzed. After hemolysis was complete the protein was precipitated by adding 6 cc of 20% trichloroacetic acid, while gently shaking the centrifuge tube. The protein material was separated by centrifuging for about ten minutes, a clear supernatant remained. An aliquot of the above protein-free filtrate was used to determine the non-protein nitrogen according to the method of Koch and McMeekin (57). All samples were read in a

Coleman model 11 universal spectrophotometer at a wavelength of 480 mu. A standard curve was prepared and is given in Figure 10 of the Appendix.

(c) Plasma sodium and potassium. Plasma sodium and potassium were determined by flame photometry using a Beckman D U spectrophotometer with flame attachment model no. 9200. The method used was a modification of that described in Beckman Instrument technical bulletin DU-12-B. In order to eliminate interferences due to variable amounts of protein, the plasma proteins were precipitated. The protein precipitant contained 5% trichloroacetic acid and 10% isopyropyl alcohol. About 45 cc of the precipitant were added to a 50 cc graduated centrifuge tube from a burette, and 1 cc of heparinized plasma was carefully added. It was then brought to volume with the precipitating reagent. It was necessary to avoid violent agitation which might give rise to floating particles or particles which adhere to the walls. The mixture was allowed to stand for ten minutes, after which time it was centrifuged for ten minutes. The supernatant which was crystal clear was then analyzed for sodium and potassium. A sample of a synthetic serum[6] as a standard was prepared at the same time as the unknown.

1. Spectrophotometric procedure: using the standard oxy-hydrogen flame the percent emission (on the transmittance scale) of the sample was read in comparison to that of the synthetic serum standard prepared above. The instrument settings employed were: for sodium, wave length

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[6] Synthetic serum contains 1.683 g NaCl and 0.071 g KCl per 200 cc distilled water. This is equal to 144 meq Na/liter and 4.8 meq K/liter.

592 mu, ultraviolet sensitive photo tube with 10,000 megohm resistor, selector switch at 0.1, sensitivity at about midpoint, and slit .04 mm.

For potassium, wave length 770 mu, red sensitive phototube with 10,000 megohm resistor, selector switch at 0.1, sensitivity at 1 1/2 turns clockwise and slit 0.02 mm.

2. Gas pressure: the optimum pressure settings for analyses under these conditions for our instrument were 18 pounds of oxygen and 4 pounds of hydrogen. Calibration curves for sodium and potassium were determined and the exact instrument settings adjusted to stay on this calibration curve each time an unknown sample was read. The curves are shown in Figure 11 of the Appendix. When the instrument settings were adjusted using the synthetic standard sample, a reagent blank consisting of the precipitating reagent was read and the reading was subtracted from both the reading of the standard and of the unknowns (net luminosities).

3. Calculation of results: the response for potassium is linear in the range of samples encountered in this work, so that the concentration of the unknown was determined either from the calibration curve or calculated from net luminosities of the standard and unknown. Since the range of variation of plasma sodium is so small the sodium line was also considered to be linear and sodium concentration as the unknown determined in the same manner.

(d) The body weight of the animals to the nearest 0.2 kilogram was checked daily after removal of the second gland.

(e) Blood pressure. The blood pressure was determined by means of a strain gage manometer discussed in detail under method of measuring the cardiac index.

(f) Electrocardiograms were determined using a Sanborn Polyviso recorder, model 67-1200. The three standard limb leads were recorded simultaneously. Simultaneous recordings of the three unipolar leads AVR, AVL and AVF also were recorded.

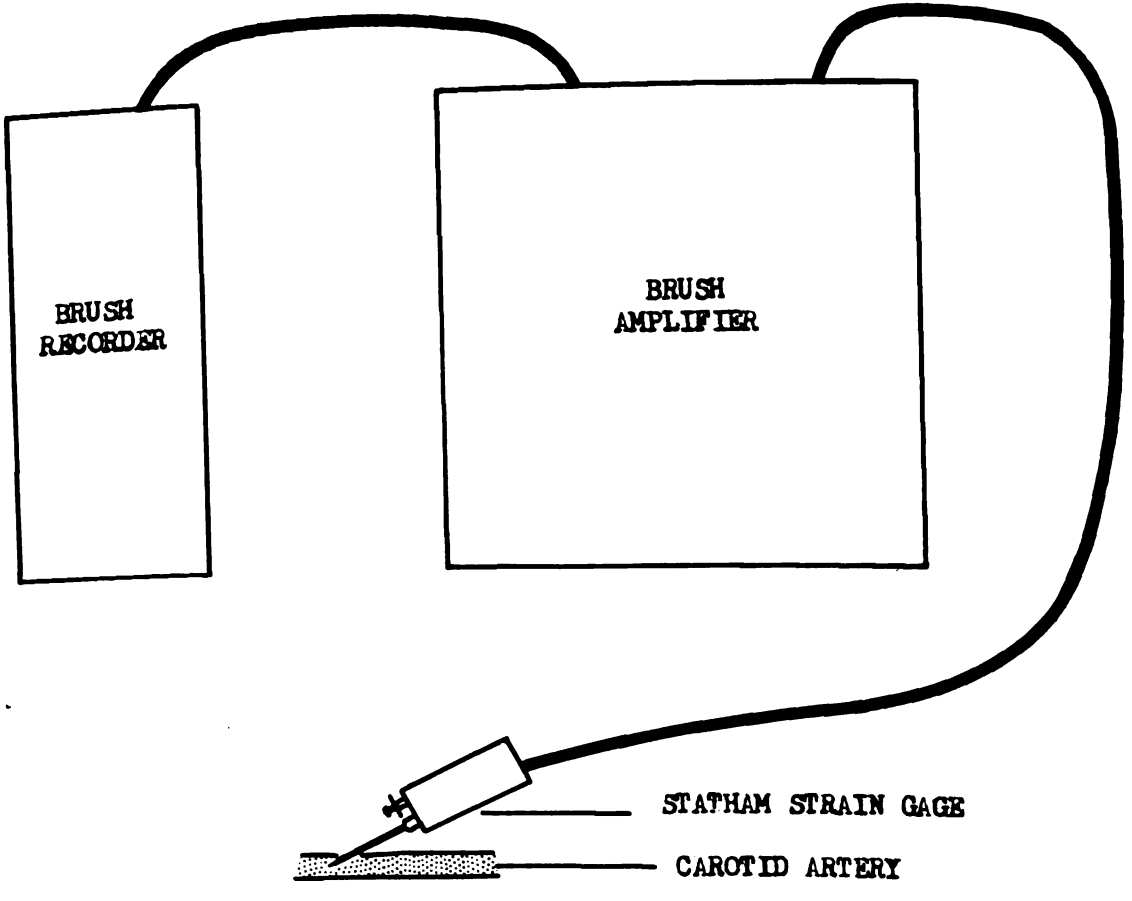
In addition, the clinical condition of the animals was noted daily when maintenance therapy was being administered, and at least twice daily when therapy was suspended. The amount of voluntary activity exhibited, and the presence of gastro-intestinal symptoms such as anorexia, diarrhea and vomiting were observed.

Cardiac Index, Stroke Index, Heart Rate and Blood Pressure

These four measurements were made simultaneously using a strain gage, amplifier system and recorder. The pickup unit of the system consisted of a twenty gauge hypodermic needle attached to a Statham strain gage, model P 23A. The strain gage was connected to a Brush universal analyser amplifier, model BL 320, which was in turn connected to a Brush model 202, direct recording galvanometer. A schematic diagram of the system is shown in Figure 1. The strain gage was filled with heparinised saline and the system calibrated against a mercury manometer. To prevent errors in pressure measurements, the system was calibrated just prior to running each record. It was possible to repeat the calibration values

FIGURE 1.

SCHEMATIC DIAGRAM OF STRAIN GAGE AMPLIFIER SYSTEM



from day to day. A calibration curve is shown in Figure 8 of the Appendix.

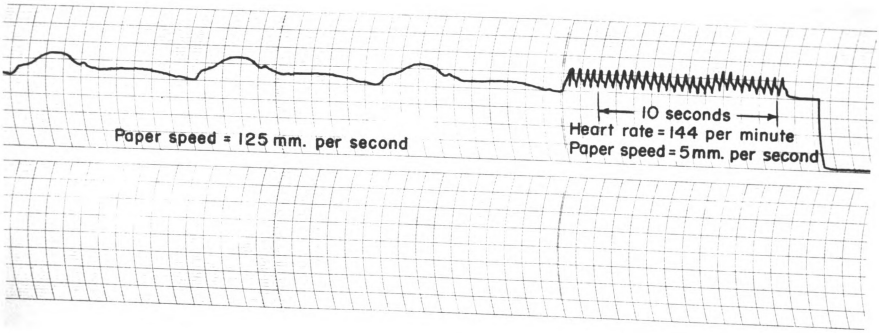
It has been established by Hamilton and Remington (35), that the central pulse, properly recorded, is the only pressure pulse adequate for calculation of the cardiac index. The same authors have also shown that the central pulse obtained from the carotid artery low in the neck is only slightly different from that obtained from the root of the aorta.

Method of Obtaining Central Pulse Record

The carotid artery of the dog was exposed through a one inch incision low in the neck under local anesthesia (procaine sulfate). A loose ligature was placed under the artery to help in locating it when about to record. When the dog was lying quietly and apparently undisturbed, the hypodermic needle (20 g.) attached directly to the strain gage, was inserted into the carotid artery. The recorder could be run at three speeds 5, 25 and 125 mm per second. To obtain a record of the central pulse adequate for computing the stroke index and cardiac index the fastest paper speed, i.e., 125 mm per second was used. To obtain a large number of individual pulses and thus assure a statistically valid measurement of cycle length, systolic, and diastolic blood pressures, paper speed of the recorder was changed during the process of recording to the slow speed, i.e., 5 mm per second Figure 2 illustrates the record obtained.

Fig. 2 - CENTRAL PULSE RECORD

Obtained by a Variable Speed Brush Recorder and Statham P 23 A Strain Gage

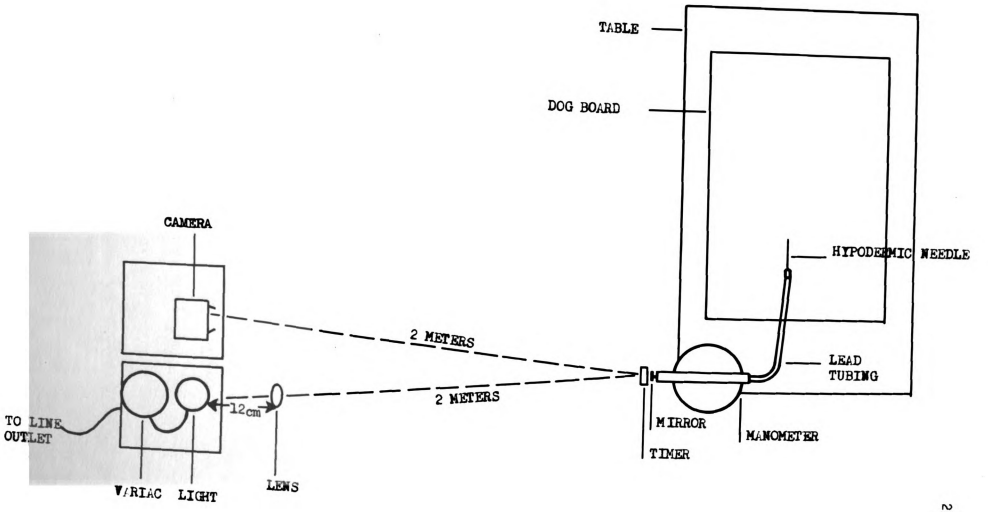


Strain gage manometer systems have been criticised as being inadequate to record faithfully the central pulse (77). Others have adapted such systems to a quantitation of stroke index in man (127). The criticism of the former is based upon the low frequency response of such systems as compared to optical manometers. While this may be a valid objection in cases where catheters are attached to the strain gage decreasing the frequency response of the system it is not believed to be valid in the system as employed in the present work. The natural frequency of the system described was 75 cycles per second.

In order to ascertain the adequacy of the strain gage system for recording the central pulse a direct comparison was made between this system and an optical one. The optical system employed was one assembled in this laboratory. The manometer was a beryllium-copper alloy membrane type described by Hamilton (34). The pickup unit consisted of a twenty gage hypodermic needle attached to a lead tube which was connected to the manometer. The light source consisted of a galvanometer lamp with a narrow slit Power for the light was furnished by a Variac which was plugged into the 120 volt laboratory outlet. The camera used for recording was one salvaged from an old Hindle electrocardiograph. The paper used was Kodak Electrocardiograph 553. Paper speed was 60 mm per second. In order to have a time record a small synchronous motor driving a five blade propellor was placed in front of the baseline mirror of the manometer to interrupt the baseline beam. Power for the motor was supplied by a 6 volt stepdown transformer connected to the 120 volt outlet in the laboratory. The system is shown schematically in Figure 3, and the calibration curve in Figure 9 of the Appendix.

FIGURE 3.

SCHEMATIC DIAGRAM OF OPTICAL SYSTEM



A group of seven anesthetized and four unanesthetized dogs were used to compare the two manometer systems. The measurements were not made simultaneously but within a few minutes of each other. When the dog was adequately prepared for recording the central pulse a record was made using one or theother system. After an adequate record had been obtained, the dog was moved into position to record with the other system. Bleeding of the carotid artery needle puncture usually stopped within a few minutes. Table I shows the results of this comparison. The data show excellent agreement between the two systems with respect to the values obtained for the cardiac index and stroke index in both the anesthetized and unanesthetized dogs. It was decided that the strain gage system was the one to use in the present work because of certain advantages such as, a variable speed recorder and ease of operation.

Figure 4 is a reproduction of the central pulse record obtained from one dog using (a) the optical manometer and (b) the strain gage manometer system described. Especially noteworthy are the similarities in the contour of the records. The apparent difference in cycle length of the individual pulses in the two records is, of course, due to the difference in paper speeds of the two recording systems.

Method of Analyzing the Central Pulse Record

The subdivision of a central pulse for computing the stroke volume is shown in Figure 5. Stroke index is a correction of the heart stroke volume output for the size of the animal and is expressed as cc per

TABLE I

COMPARISON OF A STRAIN GAGE MANOMETER AND OPTICAL MANOMETER SYSTEM

Dog	Weight Kg.	Blood Pressure		Cardiac Index		Percent Diff.	Stroke Index		Percent Diff.
		Strain Gage	Optical	Strain Gage	Optical		Strain Gage	Optical	
Anesthetized*									
Chow	11.8	148/116	156/120	2.90	3.20	10.3	15.9	17.4	9.3
Spitz	7.6	151/123	152/123	2.28	2.20	3.6	15.6	15.1	3.2
Jupiter	10.6	148/115	159/120	2.02	2.20	8.9	17.5	19.5	11.4
Exeter	13.2	158/123	164/122	2.59	2.73	5.4	18.7	19.6	4.8
Julius	14.2	150/123	152/125	2.25	2.35	4.4	13.7	13.5	1.5
Spot-2	12.0	160/123	162/126	2.84	2.81	1.1	17.7	16.6	6.6
Champ	15.5	154/135	148/127	2.76	2.62	5.3	19.3	18.6	3.8
Black & White	13.0	140/108	---	2.70	--	--	19.8	--	--
Blackie	13.5	144/118	---	2.66	--	--	13.8	--	--
Means		150/120	156/123	2.55	2.58	5.6	16.8	17.2	5.8
Unanesthetized									
Hound	12.0	148/90	159/100	4.05	4.01	1.1	36.8	34.9	5.44
Collie	14.5	140/80	145/85	4.16	3.74	11.2	41.6	40.5	2.70
Brutus	15.2	178/105	180/104	3.70	3.70	0	32.0	37.0	15.0
Pinto	16.5	151/95	165/98	4.35	4.41	1.4	34.2	39.6	15.8
Pup	10.6	156/102	158/100	3.08	3.38	9.7	33.0	35.1	6.3
Means		154/94	160/97	3.86	3.85	4.7	35.5	37.4	7.91

* 30 mg sodium pentobarbitol per kg body weight.

Fig. 4a - CENTRAL PULSE RECORD
Obtained Using an Optical Manometer System

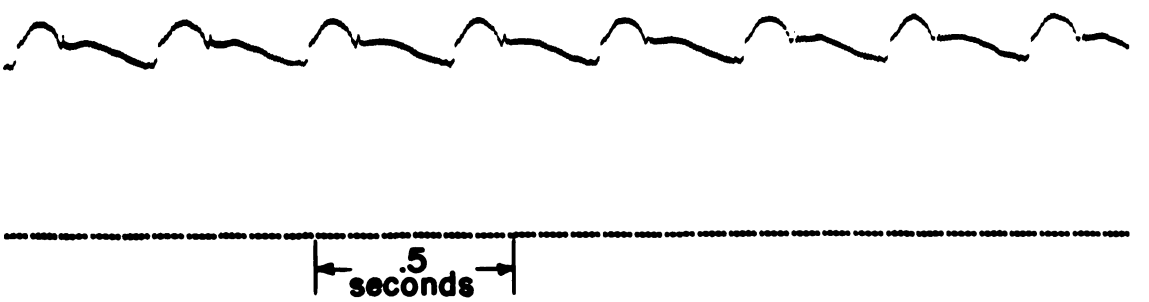


Fig. 4b - CENTRAL PULSE RECORD
Obtained Using a Strain Gage Manometer System

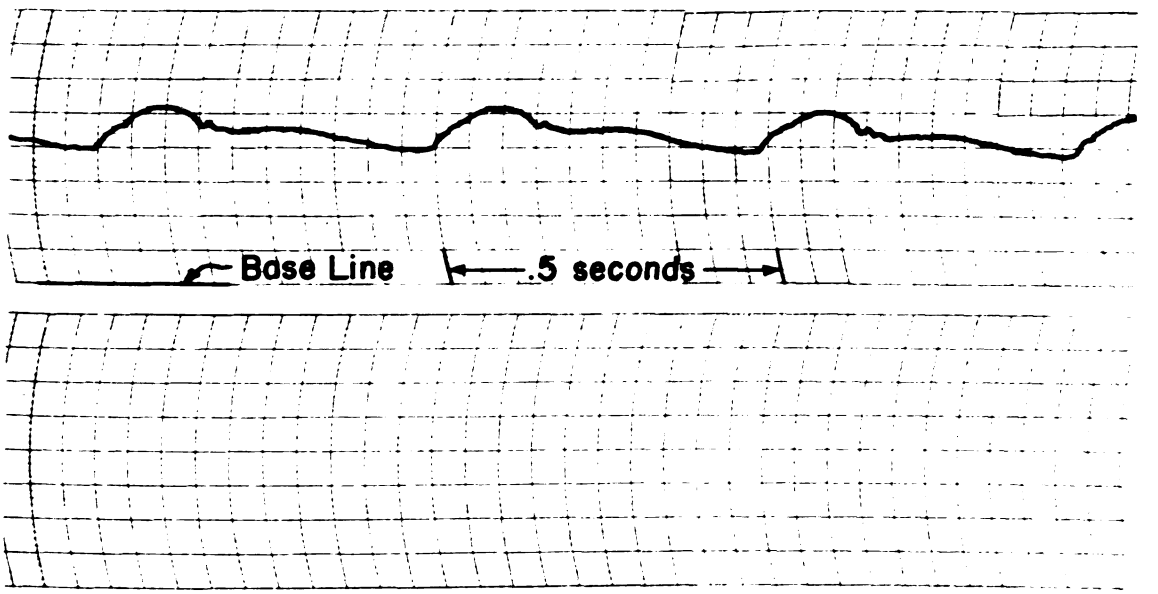
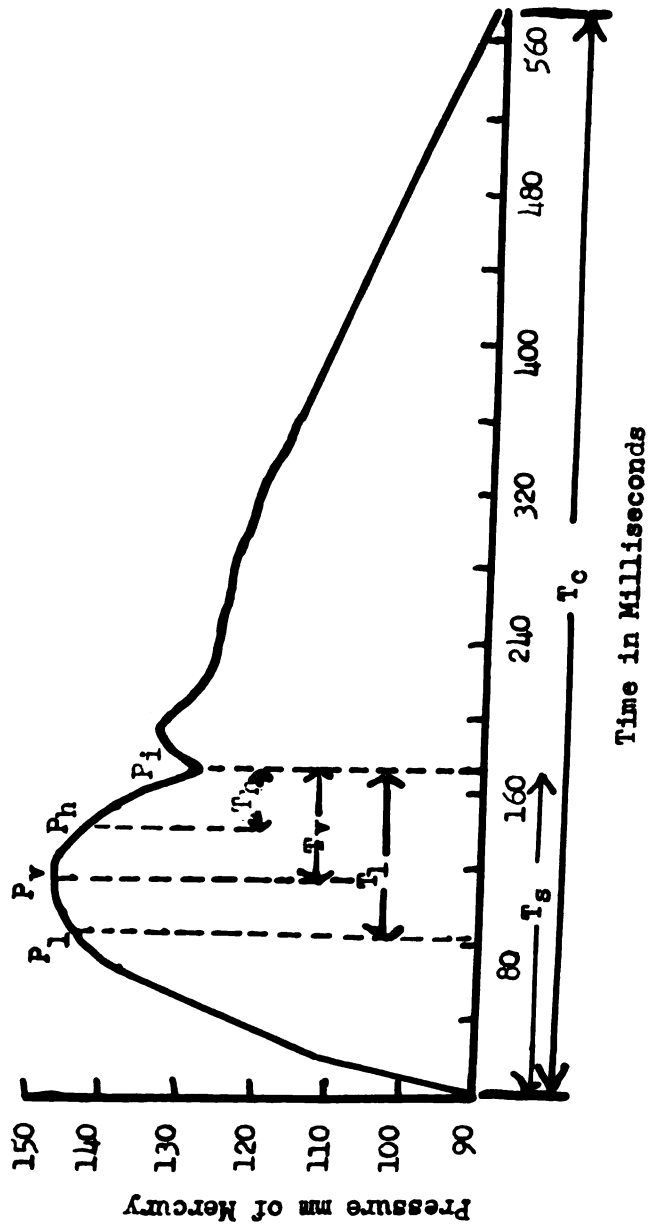


FIGURE 5.

SUBDIVISION OF A CENTRAL PULSE FOR CALCULATING STROKE VOLUME

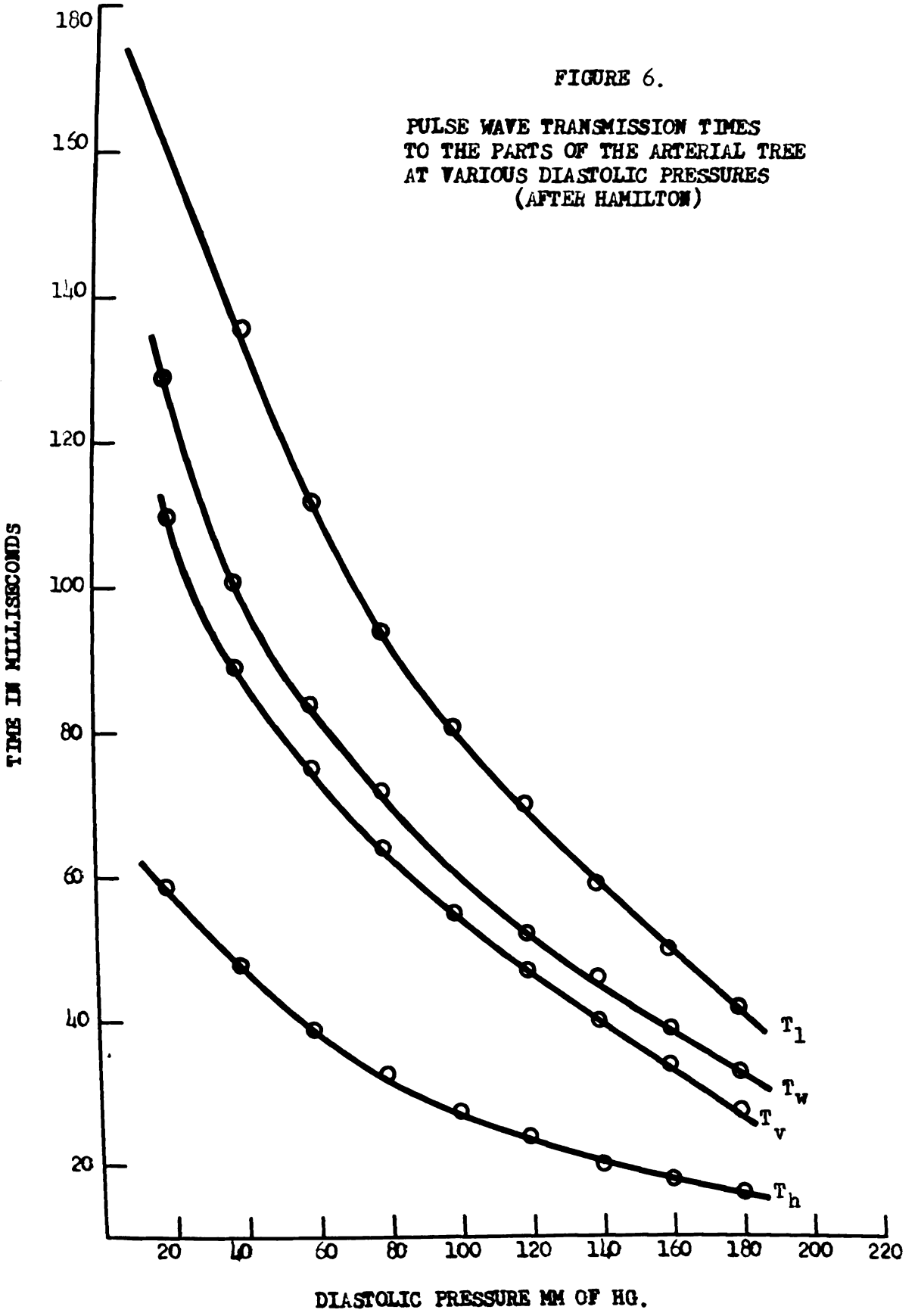


beat per square meter body surface area. Cardiac index is a correction for the minute volume output of the heart for the size of the animal and is expressed as liters per minute per square meter body surface area. The stroke index and cardiac index were determined from the central pulse record according to the method of Hamilton and Remington (35) using the revised arterial uptake tables (85).

The stroke volume is the sum of the arterial uptake, U, plus arteriolar-drainage during systole, S_d. The factor U is calculated from the contour of the central pulse as follows: the uptake of the large arteries by the end of cardiac ejection is calculated for each of the four divisions of the aorta and its major branches, the arch, the head and thorax, the viscera and abdomen, and the legs. Uptake of the arch, U_a, is calculated from the difference in the volume of the arch at incisural pressure, P_i, (pressure at time of aortic semi-lunar valve closure) and the volume at diastolic pressure, P_d. The uptake of the other three beds is calculated next. This involves laying back into systole from the incisura the pulse wave transmission times, T_h, T_v and T_l. It is necessary to lay these times back into systole in order to determine the pressures obtaining in each of the three systems at the instant of closure of the semi-lunar valves, that is, incisural time. These times are obtained by referring to the series of curves in Figure 6 relating transmission time to diastolic pressure level (as established by Hamilton and Remington, 80), using the diastolic pressure obtained from the experimental pulse record. The simultaneous pressures

FIGURE 6.

PULSE WAVE TRANSMISSION TIMES
TO THE PARTS OF THE ARTERIAL TREE
AT VARIOUS DIASTOLIC PRESSURES
(AFTER HAMILTON)



obtaining in each of the beds are then noted from the record and are recorded as P_h, P_v and P_l. The uptake of blood by each of these beds is then calculated from the difference between the volume of the particular bed at this pressure and the volume at diastolic pressure. These figures are obtained by referring to the series of curves in Figure 7 relating the capacity of the arterial tree to arterial pressure. The individual uptakes are recorded as U_h, U_v and U_l. The total arterial uptake then is the sum of these four, i.e. $U = U_a + U_h + U_v + U_l$.

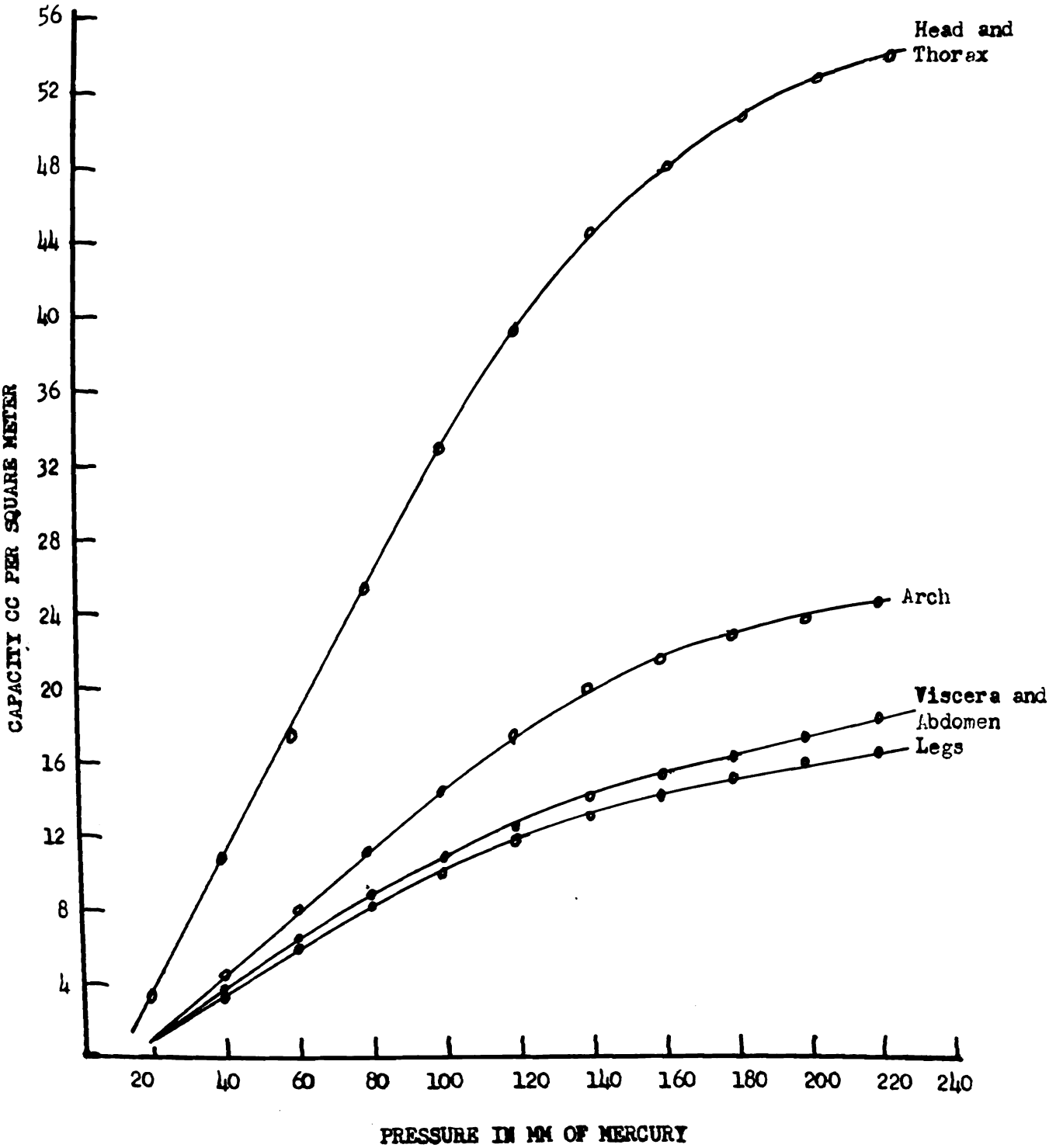
Remington (81) has also recently simplified the calculation of systolic drainage, S_d. If one assumes that the integrated mean pressures during the systolic and the diastolic portions of the pressure pulse are equal for all practical purposes, then the ratio of systolic drainage to diastolic drainage should be equal to the ratio of their respective durations, i.e.,

$$\frac{S_d}{D_d} = \frac{(T_s - T_w)}{(T_d + T_w)} \qquad (1)$$

where S_d = systolic drainage volume, D_d = diastolic drainage volume, T_s = apparent duration of systole, T_d = apparent duration of diastole, $(T_s - T_w)$ = effective duration of systole, $(T_d + T_w)$ = effective duration of diastole. The factor T_w is an average weighted transmission time and is also obtained from Figure 6. T_w is derived from average transmission times to the various arterial beds weighted according to their relative drainages. Adding T_w to diastolic time accounts for the fact that systolic drainage begins when the pulse wave reaches the periphery and ends at the time of closure of the aortic semi-lunar valves. All drainage

FIGURE 7.

CAPACITY PER SQUARE METER BODY SURFACE OF THE SEVERAL PARTS OF THE ARTERIAL TREE AT DIFFERENT PRESSURES (AFTER HAMILTON)



thereafter is at the expense of blood already accounted for. This correction is used because the actual duration of systole is less than that recorded, by an amount of time equal to the average length of time required for the pulse wave to reach the terminal arterioles of each bed. By the same reasoning adding T_w to diastolic time accounts for the diastolic drainage which occurs after ejection begins and before the pulse wave reaches the periphery.

Equation (1) can be written:

$$S_d = D_d \frac{(T_s - T_w)}{(T_d + T_w)} \qquad (2)$$

and since $D_d = U$, we have

$$S_d = U \frac{(T_s - T_w)}{(T_d + T_w)} \qquad (3)$$

or since $(T_d + T_w) = (T_c - T_s + T_w)$, then

$$S_d = U \frac{(T_s - T_w)}{(T_c - T_s + T_w)} \qquad (4)$$

where T_c = entire cycle length or $T_s + T_d$

The equation for the stroke volume can then be written: $S_v = U + S_d = U + \frac{U(T_s - T_w)}{(T_c - T_s + T_w)}$ or dividing through by the common denominator and combining terms $S_v = \frac{U\ T_c}{(T_c - T_s + T_w)}$. T_c and T_s are measured from the pulse contour as shown in Figure 5. T_w is obtained from the curve in Figure 6. If flow is to be expressed as cardiac index, hence, liters per minute per square meter body surface, then $F = \frac{60\ U}{(T_c - T_s + T_w)}$.

A sample calculation will illustrate the method. Having obtained the central pressure pulse contour shown in Figure 5, the diastolic pressure in this case, 90 mm of mercury, is referred to the series of curves in Figure 6, and the pulse wave transmission time (milliseconds) to the divisions of the aorta are noted. In this example: $T_h = 30$, $T_v = 59$, $T_l = 87$. Pressures (mm Hg) existing in these portions of the arterial tree are read from the pulse record: $P_h = 144$, $P_v = 148$, $P_l = 143$. The pressure at the incisura = 128. The uptakes are then calculated as follows:

U_a = volume at P_i - volume at P_d = 18.4 - 13.0 = 5.4 cc
U_h = volume at P_h - volume at P_d = 45.2 - 28.5 = 16.7 cc
U_v = volume at P_v - volume at P_d = 14.6 - 9.8 = 4.6 cc
U_l = volume at P_l - volume at P_d = 13.3 - 9.2 = 4.1 cc
U = total arterial uptake = 30.8 cc

The total arterial uptake during systole is then $U = 30.8$. The cycle length is measured from the initial systolic upswing of the pressure pulse curve being studied to the initial upswing of the next following pulse. In the example: $T_c = 576$, $T_s = 172$, and $T_w = 66$ milliseconds. T_w is obtained from figure 6. The stroke index is then obtained as follows:

$$S_v = \frac{U\ T_c}{(T_c - T_s + T_w)} = \frac{30.8 \times 576}{460} = 38.4 \text{ cc.}$$

And the flow or cardiac index $F = \frac{60\ U}{(T_c - T_s + T_w)} = 4.01$ liters per minute per square meter.

RESULTS

Since it was not possible to treat each of the bilaterally adrenalectomized dogs in the same manner with respect to replacement therapy, the results obtained on each dog are presented in protocol form in Tables II through VI. Exact dosages of A C E, Lipo and D C A used for each dog are indicated below.

HOUND DOG

As shown in Table II preoperative control values for blood and cardiovascular observations on this dog are within commonly reported limits for normal healthy animals (104). The first experiment (I) was conducted one week after removal of the second adrenal gland while the dog was still on therapy, 3 cc A C E per day. As indicated in Table II there was a slight decrease in systolic pressure and an increase in diastolic pressure resulting in a decreased pulse pressure. The cardiac index and stroke index were reduced along with the decreased pulse pressure. The heart rate was not a factor in the decreased cardiac index since it was slightly (but probably not significantly) greater than in the preoperative state. There was a slight decrease in R wave voltage in all leads of the E C G but no other significant alteration in the electrocardiogram.

The second experiment (II) was performed eleven days after the first; the dog received 3 cc of A C E per day for seven days and then no extract for the last four days. The dog was in a mild state of adrenal insufficiency as indicated by all data used for its estimation.

Both systolic and diastolic blood pressures had fallen to low levels, the pulse pressure being further reduced below that observed in experiment I. The heart rate did not change, but the cardiac index and stroke index both were further depressed. The E C G showed a slight further decrease in R wave voltage in all leads and a rounding of the P wave in lead I only. Following this experiment the dog was weak but still able to walk. Dramatic recovery by the following morning followed the administration of 10 cc of A C E intravenously plus 2 cc Lipo extract intramuscularly.

The third experiment (III) was performed 11 days after the second experiment while the dog was still on therapy. The dog received 3 cc of A C E per day for eight days then 1 cc Lipo per day for three days. All values indicated an improved condition except plasma potassium which was still elevated. The heart rate was slightly slower than in previous records. The systolic pressure was increased relatively more than the diastolic blood pressure, resulting in an increased pulse pressure. The cardiac index and stroke index were both increased. The E C G showed little change except a less severe rounding of the P wave in lead I.

The fourth and final experiment (IV) on this dog was performed six days after the third one and after the dog had been off therapy for two days. During this time on therapy, 1 cc of Lipo plus 3 cc A C E for one day and then 1 cc of Lipo for three days were administered and then no extract for two days. The data show a decrease in systolic blood pressure, with a decrease in pulse pressure.

Again the cardiac index and stroke index were both depressed. The E C G showed a marked rounding and widening of the P wave in several leads.

Following this experiment the dog was no longer supported but allowed to decline into a terminal adrenal insufficiency. Within six and one-half days after complete removal of therapy and four and one-half days after completion of the final experiment the animal died. During this period off replacement therapy the animal exhibited a progressive loss of appetite, decline in body weight and reduction of activity.

TABLE II

HOUND DOG

Treatment	Body Weight kg.	NPN mg%	Na meq/liter	K meq/liter	Hemato-crit	Blood Pressure mm. Hg	Heart Rate	Cardiac Index	Stroke Index	Electro-cardiogram	Remarks
Preoperative*	12.0	36	144	4.62	47	148/90 175/90**	110	4.05	36.8	Normal record	Dog was easily trained, very quiet on board.
Postoperative											
On therapy for 7 days. EXPERIMENT I	11.5	40	143	4.65	48	140/105	130	2.92	22.5	Slight depression in R wave voltage all leads	Dog was still apparently strong, able to walk around lab.
On therapy for 7 days then no therapy for 4 days. EXPERIMENT II	10.5	55	132	5.20	45	76/55	130	2.40	17.0	Slight depression R wave voltage all leads. Rounding of P wave lead I only	Dog was noticeably weak, but able to walk.
On therapy for 11 days. EXPERIMENT III	10.8	48	139	5.25	46	100/63	100	2.95	29.5	Less severe rounding of P wave lead I	Dog much improved, strong and alert.
On therapy for 4 more days then off for 2 days. EXPERIMENT IV	10.2	58	134	5.40	50	90/55	103	2.64	25.6	Marked rounding and widening of P wave in several leads.	Dog very weak, hind legs unsteady.

* Average of three individual measurements, except heart rate, cardiac index and stroke index which are single values.

** Average of five individual measurements. (Femoral artery)

SMOKEY

The first post-operative experiment (I) was performed on this dog seven days after removal of the second adrenal gland and while the dog was still on therapy consisting of 1 cc of Lipo daily for seven days. All data indicated an adrenal insufficient condition. Systolic and diastolic pressures were both decreased. The pulse pressure was low with an accompanying reduction in the cardiac index and stroke index despite an increased pulse rate. A slight reduction in the R wave voltage in all E C G leads was noted.

Experiment II was performed eight days after the first experiment and while the dog was receiving therapy of 1 cc Lipo per day for three days and then 2 cc Lipo plus 5 mg D C A for one day, then 1 cc Lipo plus 2 mg D C A per day for four days. The blood N P N was higher than in the previous experiment, with little change in plasma sodium and potassium values. The heart rate was much lower than before. Despite the fall in both systolic and diastolic pressures, the pulse pressure was increased. Also the stroke index and cardiac index were both increased. No significant change in the electrocardiogram was noted. Experiment III was performed eight days after the second. The dog had received 1 cc Lipo per day for six days and then no therapy for the last two days just prior to the experiment. All measurements indicated a severe adrenal deficient state. The hematocrit at this time was quite high, namely, 60. The pulse pressure was greatly reduced with a corresponding decrease in stroke index and cardiac index. Except for a further reduction of the R wave voltage no further change in the E C G record was noted.

Experiment IV was performed twelve days after the third experiment. During this interval the dog was given therapy consisting of 1 cc Lipo plus 5 cc A C E plus 5 mg of D C A per day for four days, then 1 cc Lipo plus 2 cc A C E and 2.5 mg of D C A per day for eight days. The data, except body weight, indicated an improved condition since the previous experiment. M P M was reduced, sodium was increased and potassium decreased. The blood pressure was improved by the greatest increment obtained in any experiment. Cardiac index was markedly increased. The hematocrit had decreased to 40; no further alteration in the E C G was noted.

After completion of the fourth experiment this dog was taken off therapy and allowed to decline. On the fourth day off therapy the final experiment (V) was performed. At this time the animal was noticeably weak and appeared to be suffering from gastrointestinal disturbances as indicated by retching. The animal was able to walk about the laboratory but would slip and appeared unsteady. As shown in Table III, the data indicate severe adrenal insufficiency except for the hematocrit. However, the hematocrit had returned from the unusually low value of the previous experiment to the control value indicating some hemoconcentration. Heart rate was unchanged; cardiac index and stroke index were reduced. The R wave voltage of the E C G had almost completely disappeared.

No further therapy was administered to this dog and the following morning the animal was found dead in the cage.

TABLE III

SMOKEY

Treatment	Body Weight kg.	NPN mg%	Na meq/liter	K meq/liter	Hemato-crit	Blood Pressure mm. Hg	Heart Rate	Cardiac Index	Stroke Index	Electro-cardiogram	Remarks
Preoperative*	10.6	38	143	4.90	48	170/110 180/113**	130	4.29	31.5	Normal record	Normal healthy dog. Not too friendly but well trained.
Postoperative											
On therapy for 7 days. EXPERIMENT I	10.5	52	140	4.85	52	150/125	156	2.06	13.0	Slight depression of R wave voltage all leads.	Dog appeared healthy and strong, eating well, not too active.
On therapy for 8 more days. EXPERIMENT II	10.6	68	139	5.02	50	114/75	102	2.61	25.6	No significant change.	Dog appeared well, active and eating reduced ration.
On therapy for 6 days then no therapy for 2 days. EXPERIMENT III	9.2	175	138	5.95	60	73/50	135	1.92	14.3	No change	Dog showed signs of weakness, reduced eating and spontaneous activity.
On therapy for 12 days. EXPERIMENT IV	9.2	85	141	4.95	40	138/105	156	3.25	20.8	No further change.	Dog in very good condition, active eating well, alert.
Off therapy for 4 days. EXPERIMENT V	8.8	190	139	6.52	48	105/80	156	2.33	14.9	Severe depression R wave voltage all leads	Very weak, showed signs of muscular weakness.

* Average of three individual measurements, except heart rate, cardiac index and stroke index which are single values.

** Average of five individual measurements. (Femoral artery)

SPOT

Except for being slightly obese this dog was very healthy and active. As can be seen in Table IV all control data indicated a normal dog.

After removal of the second adrenal gland the dog was supported with replacement therapy for ten days and then experiment I was performed. The therapy was 1 cc Lipo per day for three days then 0.5 cc Lipo per day for seven days. Of the data used to evaluate the state of adrenal deficiency, B P N and hematocrit showed the most significant changes. The systolic blood pressure was unchanged but diastolic pressure was elevated, resulting in a decreased pulse pressure. Cardiac index and stroke index were both markedly decreased although the heart rate was slightly increased.

Experiment II was performed six days after experiment I. The dog received 0.5 cc Lipo for 4 days, then no extract for two days. The data indicate the animal was in a state of adrenal insufficiency: plasma potassium was very high, plasma sodium was low, and blood B P N was more than double the control value. Both systolic and diastolic blood pressures had fallen and heart rate was decreased. The cardiac index and stroke index were depressed to about half the control values. The E C G showed a rounding and widening of the P wave in all leads.

Experiment III was performed nine days after the second one. The dog had received 1 cc Lipo plus 2 cc A C E for three days, then 1 cc Lipo for six days. The B P N was elevated still further. However, all other measurements indicated a slightly improved condition. The cardiac output was especially improved.

The final experiment (IV) was performed five days after experiment III, the dog receiving 1 cc Lipo for 3 days, then no therapy the last two days. All measurements again indicated an adrenal insufficiency. Blood pressure had fallen again; heart rate was decreased. Cardiac index and stroke index both were again decreased. A very wide P wave and severe reduction of the R wave voltage were noted in the E. C. G record.

TABLE IV

SPOT-1

Treatment	Body Weight kg.	NPN mg%	Na meq/liter	K meq/liter	Hemato-crit	Blood Pressure mm. Hg	Heart Rate	Cardiac Index	Stroke Index	Electro-cardiogram	Remarks
Preoperative*	15.6	35	143	4.85	48	150/98 175/105**	138	4.28	31.0	Normal record	Dog slightly obese
Postoperative											
On therapy for 10 days. EXPERIMENT I	15.4	45	140	4.90	58	150/115	150	2.80	18.6	Reduction of R wave voltage all leads.	Dog appeared well and active. Appetite good.
On therapy for 4 days then no therapy for 2 days. EXPERIMENT II	14.7	80	136	6.80	63	80/50	110	1.88	17.3	Rounding and widening of P wave all leads	Dog noticeably weak. Not eating well.
On therapy for 9 days EXPERIMENT III	14.0	125	139	6.00	61	105/72	125	2.78	22.2	No further significant change	Dog much stronger, appetite improved.
On therapy for 3 days then no extract for 2 days. EXPERIMENT IV	13.5	133	137	6.43	60	84/55	110	2.00	18.2	Very wide P wave lead I	Dog exhibits characteristic weakness and muscular asthenia of adrenal insufficiency.

* Average of three individual measurements, except for heart rate, cardiac index and stroke index which are single values.

** Average of five individual measurements. (Femoral artery)

BROWNIE

This was the smallest dog in the group, but was very active and well trained. The dog was maintained for eight days on 0.5 cc Lipo adrenal extract per day, following removal of the second adrenal, then suddenly developed an acute circulatory crisis. Peripheral veins were almost completely collapsed. Cerebral depression was apparent as indicated by a semi-comatose appearance of the animal. As indicated in Table V the dog was revived by the administration of a large amount of adrenal extract. Five days after this circulatory collapse and after having received 1 cc of Lipo plus 3 cc of A C E per day for two days and then 1 cc of Lipo per day for two days, the animal appeared well and strong and experiment I was performed. The body weight was 1 kg below pre-operative weight, but the blood chemistry data indicated the animal was in a near-normal condition. Systolic blood pressure was decreased about 10 mm Hg, the diastolic pressure was elevated about 15 mm Hg and the pulse pressure was reduced accordingly. Heart rate was markedly increased compared to control values. The stroke index was greatly reduced, whereas the cardiac index was near the control value.

Experiment II was conducted six days after experiment I, the dog receiving 0.5 cc Lipo per day for four days and then no extract for two days. The data indicated adrenal insufficiency. Despite the fall in blood pressure, the pulse pressure was unchanged since the previous experiment. However, due to a decreased heart rate, the cardiac index was decreased.

Experiment III was conducted seven days after experiment II during maintenance therapy (0.5 cc Lipo per day). A slight improvement in the condition of the animal was indicated by all measurements except M P M. The cardiac index and stroke index were improved.

TABLE V

BROWNIE

Treatment	Body Weight kg.	NPN mg%	Na meq/liter	K meq/liter	Hemato-crit	Blood Pressure mm. Hg	Heart Rate	Cardiac Index	Stroke Index	Electro-cardiogram	Remarks
Preoperative*	10.4	35	135	4.85	49	150/90 155/87**	100	3.58	35.8	Normal record	Smallest dog in group.
Postoperative											
On therapy for 8 days.***											
On therapy for 5 more days. EXPERIMENT I	9.4	40	140	4.70	47	140/106	168	3.44	20.4	Slight depression R wave voltage.	Dog appeared strong alert, able to run about lab.
On therapy for 4 days, then no extract for 2 days. EXPERIMENT II	9.0	78	130	5.6	54	105/72	110	2.45	20.5	No record	Not eating well. Dog noticeably weak after this experiment.
On therapy for 7 days. EXPERIMENT III	9.2	120	134	4.95	52	110/65	110	2.70	24.6	Further depression R wave voltage	Dog somewhat stronger, eating improved.

* Each value represents the average of three individual determinations, except heart rate, cardiac index and stroke index which are single values.

** Average of five individual measurements. (Femoral artery puncture.)

*** Appeared well and active, then suddenly developed acute circulatory crisis, revived by administration of 8 cc of A C E intravenously and 1 cc Lipo plus 5 mg. desoxycorticosterone intramuscularly.

CHAMP

This dog had the slowest heart rate and lowest blood pressure of any of the bilaterally adrenalectomized dogs studied. The control cardiac index and stroke index also were respectively the lowest in the group. None of the other control data indicated the animal was not a healthy active dog. As a matter of fact, this dog had the largest appetite and was one of the most active in the group.

The first post-operative experiment was performed one week after removal of the second adrenal gland. For these seven days, 1.5 cc Lipo per day were administered. The M P M and hematocrit were elevated. A slight decrease in the systolic blood pressure and an increase in the diastolic blood pressure resulted in a reduced pulse pressure. Heart rate was unchanged but the cardiac index and stroke index were decreased. There was a reduction in R wave voltage in all leads in the E C G record. Following the first post-operative experiment the dog was again placed on therapy for 6 days (1.5 cc Lipo per day), then therapy was withdrawn for 2 days and experiment II performed. All criteria used indicated an adrenal insufficient condition. Heart rate was significantly increased. Systolic and diastolic blood pressures had both decreased since the last experiment with a resulting low pulse pressure. Although the stroke index was further reduced the cardiac index was little changed since the last experiment. There was a slight further reduction in R wave voltage in all leads.

Experiment III was performed twelve days after experiment II and while the dog was still on therapy (1.5 cc Lipo per day for the twelve days).

All criteria indicated an improved condition since the previous experiment. Systolic pressure was unchanged but diastolic pressure was slightly lower, the pulse pressure being increased. Stroke index was significantly improved and cardiac index was slightly but probably not significantly greater than the control value. The E C G record showed no further change from that of experiment II.

The dog received 1.5 cc Lipo again for six days, then no extract for two days. Experiment IV was then performed. Body weight decreased one kg; M P M further elevated; and plasma sodium was unchanged, whereas plasma potassium was elevated. The hematocrit was about the same. Both systolic and diastolic blood pressures were greatly decreased. Heart rate was the highest value obtained in any of the experiments. Cardiac and stroke indices were both decreased. Observation of the E C G record showed a slight further depression of the R wave voltage in all leads. Following the fourth experiment, the dog was no longer supported with replacement therapy but allowed to decline into a terminal adrenal insufficiency.

TABLE VI

CHAMP-2

Treatment	Body Weight kg.	NPN mg%	Na meq/liter	K meq/liter	Hemato-crit	Blood Pressure mm. Hg	Heart Rate	Cardiac Index	Stroke Index	Electro-cardiogram	Remarks
Preoperative*	15.8	35	142	4.92	48	145/93 162/90**	87	2.60	30.2	Normal record	Very active dog, huge appetite. Very easily trained
Postoperative											
On therapy for 7 days. EXPERIMENT I	15.4	60	140	4.85	52	136/102	84	1.55	18.5	Reduced voltage R wave, all leads.	Dog recovered rapidly from operation, looked strong and active, eating well.
On therapy for 6 days, no extract for 2 days. EXPERIMENT II	14.5	78	137	5.28	58	111/93	145	1.70	11.7	Slight further reduction R wave voltage all leads.	Dog still looked good, eating well, somewhat thinner.
On therapy for 12 days. EXPERIMENT III	14.0	62	140	5.12	52	108/80	145	2.80	19.4	No further change	
On therapy for 6 days, then no extract for 2 days EXPERIMENT IV	13.0	90	139	6.50	53	72/55	156	1.77	11.3	Further reduction R wave, voltage all leads.	Noticeably weak, reduced appetite.

* Average of three individual measurements, except for heart rate, cardiac index and stroke index which are single values.

** Average of five individual measurements. (Femoral artery)

DISCUSSION OF RESULTS AND CONCLUSIONS

The results will be discussed in two parts. The first is concerned with observations on changes in body weight and blood chemistry, while the second part is concerned with the data pertaining to cardiovascular measurements.

BODY WEIGHT AND BLOOD CHEMISTRY

Body Weight

In the five bilaterally adrenalectomized dogs studied body weight changes paralleled the alterations in appetite and activity of the animals. In all five of the dogs a loss in weight occurred within two to three days after removal of the second adrenal gland. In each instance the dog's weight remained quite constant at the decreased level as long as replacement therapy was being administered. However, in no instance was the pre-operative weight restored during therapy. Withdrawal of replacement therapy even for as little as two days again caused a loss in body weight.

Numerous factors contribute to weight loss in adrenal insufficiency. Withdrawal of therapy often results in a rapid loss of weight due to excessive renal excretion of water and salts. In more advanced insufficiency, vomiting and diarrhea may contribute to the dehydration (52). Reduction of food and water intake and impaired intestinal absorbtion may also lead to weight loss. Faulty fat metabolism may be implicated in the body weight reduction (120). Reduced intestinal

absorption usually occurs in chronic insufficiency of long duration. Vomiting was not observed in any of the animals studied until terminal complete removal of replacement therapy, and then in only two animals. No cases of diarrhea were noted.

Blood Non-Protein Nitrogen

Pre-operative N P N values ranged from 32 to 40 milligrams percent, with a maximum range for any one dog of 5 milligrams percent. Individual values for each dog are included in Table IX of the Appendix. These values are in close agreement with accepted values for normal dogs. In the present work, the N P N tended to increase steadily after removal of the second adrenal gland. An elevation of the N P N was noted within two days after removal of the second adrenal gland despite supportive treatment. Complete withdrawal of replacement therapy caused a sharp increase in the N P N in every case. When maintenance therapy was again administered to three of the dogs the N P N decreased; Hound Dog, experiment III; Smokey, experiment IV; and Champ, experiment III. In two others the N P N rose steadily until the terminal experiment was performed, Spot and Brownie. In general, it was noted that changes in N P N tended to lag behind responses of hematocrit and blood pressure to supportive therapy.

Marshall and Davis (68) were the first to observe consistent rises in blood urea (the chief component of the excess N P N) associated with impaired kidney function and on the basis of experiments on fasting animals stated that the changes did not involve increased protein

catabolism. The findings of these authors were confirmed by Harrop et al. (39) and Stahl et al. (101). Swingle et al. (112,113,115) reported a consistent reciprocal relationship between changes in blood urea and blood pressure throughout the development and recovery from adrenal insufficiency in the dog, and attributed the urea changes as secondary to renal impairment which resulted from reduced blood pressure and blood volume. This group also stressed the importance of protein catabolism as a factor in adrenal insufficiency. Loeb (62) supported the idea of renal origin of the increased N P N resulting from reduced blood flow through the kidney, reduced filtration pressure or impaired tone of the renal efferent arterioles. Hartman (45) suggested that the rise in N P N was a result of kidney damage caused by salt depletion.

It was hypothesized by Kerple-Fronius (56) that dehydration and not electrolyte unbalance was the major factor in causing increased blood nitrogen. Harrop (39), however, found a closer relationship between blood potassium and blood urea than between either sodium or chloride and urea and stated that electrolyte changes are not the cause of the raised N P N.

Hematocrit and Plasma Sodium

Changes in water content and plasma sodium are so closely related that the two can easily be discussed together. Hematocrit and plasma sodium determinations were obtained on all dogs each time an experiment was performed.

Pre-operative hematocrit readings for the five bilaterally adrenalectomized dogs averaged 48 percent red blood cells with a group range

of 47 to 49 percent. Plasma sodium values averaged 142 milliequivalents (meq) per liter with a range of 137 to 144 meq for the group and a range of not more than ±3 meq for any one animal.

Hematocrit readings were approximately 5 percent higher than those generally reported for normal dogs. No explanation can be offered for this. However, since all hematocrits were treated in the same manner the relative changes are valid regardless of the absolute values for the control figure. The values for plasma sodium are in good agreement with those reported elsewhere for normal dogs (41).

In the five animals studied the hematocrit afforded a somewhat variable index to the condition of the animal. An early elevation was found in four of the five dogs. In four of the five dogs hematocrit readings taken when therapy was suspended were, with one exception Hound Dog, Experiment II, higher than those obtained when therapy was being administered. The hematocrit in the fifth dog (Spot) was elevated very soon after removal of the second adrenal and remained high throughout the course of the experimental work.

Salt and water depletion of the blood during the course of adrenal insufficiency has been established beyond doubt. Restoration of normal values follows the administration of adequate quantities of A C E, D C A, or other steroid preparations (30,40,51,63,97,99,110,112). Changes in the total plasma content of sodium are always demonstrable, but alterations in sodium concentration may be counteracted by simultaneous loss of fluid in excess of electrolytes (122,123).

Harrop et al. (40,44,45) and Loeb (62,63) were among the first to emphasize the importance of excessive renal excretion of water and sodium salts during the onset of adrenal deficiency in dogs. A specific regulatory action upon kidney function (41,42,45,51,107) particularly upon the tubular reabsorption of sodium has been postulated (112,118).

Swingle (110,113) has repeatedly stressed the importance of the regulation by adrenal hormone of the distribution of fluid and electrolytes between the plasma and the tissues, an action not directly related to renal function.

Harrop (40,41) believed that the regulation is brought about directly through alterations in tissue permeability. As pointed out early in the review of literature pertaining to capillaries, Swingle (43) regarded whatever capillary permeability changes which can be demonstrated as secondary to stagnation of blood and localized anoxia.

The results obtained in the present experiment with respect to hematocrit and plasma sodium are in general in good agreement with the literature reports of alterations of these two variables in adrenal insufficient animals.

Plasma Potassium

In the twelve dogs studied the group mean for the pre-operative plasma potassium values was 4.74 meq. per liter with a range of $\pm$ 0.6 meq. per liter. The range for any one animal was not more than $\pm$ 0.6 meq/liter (one animal) with an average range of $\pm$ 0.25 meq. for the group. These values are well within the normal limits for dogs (103).

A rise in plasma potassium in adrenal insufficiency has been established unequivocally in the past (4,13,125,131,133,136). Considerable difference in opinion exists concerning the time of onset and the primary cause of the elevated potassium. The significance of the excess plasma potassium with respect to its effect on the cardiovascular system in the adrenal insufficient animal has by no means been adequately established. As pointed out in the literature review, acute circulatory collapse in both laboratory animals and humans often occurs even before significant electrolyte alterations have occurred. In the present work plasma potassium values were not elevated in any of the experiments to levels as high as those usually found in more severe, prolonged adrenal insufficiency, yet circulatory disturbances were evident. In one animal an acute circulatory crisis occurred before electrolyte or other changes were apparent. It is concluded that the elevation of plasma potassium is not a necessary prerequisite for the observed circulatory abnormalities although it may aggravate them once they have occurred. As shown in Table VIII B page 69, the cardiac index was sharply reduced below controls, although individual protocols (Tables II-VI) show that all dogs had normal plasma potassium levels.

The data obtained in the present series of experiments with respect to body weight and blood chemistry changes in adrenal insufficiency add little to the information already known. These measurements were made primarily to help provide an evaluation of the state of adrenal insufficiency existing in any particular animal at the time cardiovascular measurements were made.

CARDIOVASCULAR MEASUREMENTS

Arterial Blood Pressure

The average blood pressure values obtained for a total of 40 femoral artery punctures in twelve intact unanesthetized dogs were 157, 106 and 50 mm mercury for systolic, diastolic and pulse pressure, respectively. The ranges were 130 to 180 systolic, 75 to 110 diastolic and 55 to 60 pulse pressure. Individual values are included in Table IX of the appendix. The average pre-operative femoral artery blood pressure values for the five bilaterally adrenalectomized dogs were 171 systolic, 97 diastolic and 74 pulse pressure, with ranges of 160 to 180, 90 to 113 and 68 to 85 respectively. It will be noted that systolic and pulse pressures are somewhat higher than those ordinarily obtained on dogs when less sensitive methods of measurement are used.

The blood pressures as measured at the carotid artery in nine intact unanesthetized dogs and nine intact dogs anesthetized with sodium pentabarbital are shown in Table VII. The most obvious difference in these two sets of data is the higher diastolic pressures resulting in a lower pulse pressure in the anesthetized dogs. This is at least partially due to the increased heart rate in the anesthetized dogs, that is, an average of 153 for the anesthetized dogs compared to 106 for the unanesthetized dogs.

In four of the five bilaterally adrenalectomized dogs studied the pre-operative carotid artery pressures ranged from 145 to 150 systolic, 90 to 98 diastolic and 52 to 60 pulse. In the fifth dog the pressure

TABLE VII

HEART RATE, BLOOD PRESSURE,* CARDIAC INDEX AND STROKE INDEX OF ANESTHETIZED AND UNANESTHETIZED DOGS

Dog	Weight Kg.	Heart Rate	Blood Pressure mm. Hg	Cardiac Index lit/min/ sq. met.	Stroke Index cc/beat/ sq. met.
			Anesthetized**		
Chow	11.8	182	148/116	2.90	15.9
Spitz	7.6	146	151/123	2.28	15.6
Jupiter	10.6	115	148/115	2.02	17.5
Exeter	13.2	138	158/123	2.59	18.7
Julius	14.2	164	150/123	2.25	13.7
Spot-2	12.0	160	160/123	2.84	17.7
Champ-1	15.5	143	154/135	2.76	19.3
Black & White	13.0	136	140/118	2.70	19.8
Blackie	13.5	192	144/118	2.66	13.8
Mean	12.3	153	150/120	2.55	16.8
			Trained-Unanesthetized		
Hound Dog	11.6	110	148/90	4.05	36.8
Collie	14.5	89	140/80	4.16	41.6
Brutus	15.2	95	178/105	3.70	32.0
Pinto	16.5	116	151/95	4.35	34.2
Pup	10.6	95	156/102	3.08	33.0
Smokey	10.6	130	170/110	4.29	31.5
Spot	15.6	138	150/98	4.28	31.0
Champ-2	15.5	87	145/93	2.60	30.2
Brownie	10.4	100	150/90	3.58	35.8
Mean		106	154/94	3.78	34.0

* Measured at the caretid artery.
** 30 mg. Sodium Pentobarbital per kg. body weight.

was 180/113, but this animal showed no signs of being abnormal in any other respect.

The first change noted in blood pressure recorded at the carotid was a decrease in systolic and an increase in diastolic pressure, resulting in a reduced pulse pressure. Withdrawal of therapy for two to four days in every case caused a marked decrease in both systolic and diastolic pressures, and when therapy was again administered, pressure was improved in almost every case, although in these experiments the magnitude of the increase was not great, probably due to the short time interval between periods off and on therapy. During recovery systolic pressure increases in every case preceded diastolic improvement and were of greater magnitude than diastolic pressure changes with a consequent increase in pulse pressure. In experiment IV, Smoky, a combination of D C A and Lipo extract was given for twelve days and the greatest increase in pressure was obtained. The lowered hematocrit indicated a possible overdose of D C A.

One dog, Brownie, appeared to be well on the way to recovery from the second adrenal removal then suddenly developed an acute circulatory crisis with a precipitous fall in blood pressure and was revived only by the administration of a large dose of A C E intravenously.

In general, in the present work blood pressures tended to reflect most accurately and conclusively the condition of the animal, and the adequacy or inadequacy of therapy.

As cited in the literature review, a lowering of the blood pressure has been associated with adrenal cortical insufficiency since the time

of Addison (1). Today all investigators in the field agree that the blood pressure is reduced following adrenalectomy, but there is considerable disagreement as to the importance of this finding, relative to other disorders which develop, the time of onset of the hypotension, and the nature of the underlying mechanisms.

In the present work, changes in blood pressure afforded the earliest and most sensitive indication of alterations in the physiological condition of the animal. This agrees with the findings of Swingle, et al. (102,111,112,114) who reported pressure changes in adrenalectomized dogs as early as twenty-four hours after withdrawal of A C E injections, followed by a steady decline over a period of days, terminating in circulatory collapse and death. The return of blood pressure to normal was described (109) as one of the first and most dramatic changes following the administration of A C E to animals in severe insufficiency. Harrop et al. (43,44), Britton and Silvette (9) and Loeb (62) all believed that the pressure changes did not occur until after other signs of circulatory failure had become evident. The relative insensitivity of the methods of measurement employed by these investigators may explain in part their failure to observe early changes.

In the five dogs studied in the present work the data show conclusively that alterations in systolic, diastolic and pulse pressures occur within a day or two after removal of the second adrenal and often even while the dog is still on therapy.

The several hypotheses which have been offered to explain the blood pressure decline in adrenal insufficiency were presented in detail in

the literature review. The contention of Harrop, et al. (40,43) that the pressure fall was due to a reduction of circulating blood volume resulting from excessive fluid loss because of impaired kidney function seems to be no longer tenable in the light of work already pointed out showing early pressure changes in adrenal insufficiency.

The sodium depletion hypothesis of Loeb (62) in which he postulated a specific ion effect upon the blood vessels following sodium loss is even less acceptable. It was shown conclusively by Gilman (30) that A C E is needed to correct some physiological defect not directly related to the level of serum sodium. Swingle et al. (114) showed that adrenalectomized dogs maintained on saline without hormone preparations appear normal in most respects, but have lowered blood pressure.

It is concluded that the present work confirms the findings of others that blood pressure changes in adrenalectomized dogs occur almost immediately after removal of the second adrenal gland. In addition, the present investigation provided more precise information concerning this magnitude of these pressure changes. The possible relationship of blood pressure to the output of the heart is discussed below.

Electrocardiogram

The early increase in amplitude of the T wave of the E C G was not noted in the present experiments. This can not be explained entirely on the basis of the less severe increase in plasma potassium values already noted, for Roberts (88) has reported that T wave elevation occurs even before electrolyte alterations are apparent. S-T segment

depression reported by others also was not observed. This alteration usually occurs at potassium levels somewhat higher than those obtained in the present experiments.

The rounding and widening of the P wave noted in almost every experiment when replacement therapy was withheld has not been reported before, and may or may not be a significant finding. The present experiments are not extensive enough to allow a positive evaluation of this finding.

The E C G recordings taken in the final experiments (when the dogs were off therapy) all indicated a severe decrease in R wave voltage.

The data certainly indicate that considerable alteration in blood pressure, cardiac index and stroke index in adrenalectomized dogs can occur before alteration in the E C G are apparent.

Heart Rate, Cardiac Index and Stroke Index

Intact Anesthetized Dogs. Table VII (page 62) shows the results obtained in the present work using the strain gage amplifier system described on page 23. A group of nine dogs anesthetized with 30 mg sodium pentabarbital per kg body weight was studied. The mean heart rate was 153 beats per minute with a range of 115 to 192. The mean value for the cardiac index was 2.55 liters per minute per square meter body surface with a range from 2.02 to 2.90. The mean stroke index was 16.8 with a range from 13.7 to 19.8. These figures are in excellent agreement with those obtained by others (67,84) using the same analytical method but employing optical systems for recording the central pulse.

The pressure pulse contour method has been checked by Hamilton and Remington (35) against the dye dilution method and found to have a correlation coefficient of 0.994. Numerous other workers have compared the pressure pulse method with the dye dilution, Fick and rotameter methods. Opdyke (77) has reviewed these comparisons and found the average differences for all investigators between the pressure pulse method and the others to be between $\pm$8.0 and $\pm$13.0 percent.

Normal Unanesthetized Dogs. Table VII shows the values obtained for nine unanesthetized dogs. The mean heart rate was 106 per minute with a range of 87 to 138. The average figure for the cardiac index was 3.78 liters per minute per square meter body surface with a range from 2.60 to 4.35. The average stroke index was 34.0 with a range from 30.2 to 41.6 cc per beat per square meter.

Figures for the cardiac index of the unnarcotized intact dog by the pressure pulse method are difficult, if not impossible, to find in the literature. Values obtained by the Fick method, by which the pressure pulse method has most often been standardized, are also not numerous for the unnarcotized dog. One of the classic studies on the cardiac output of the trained, conscious dog is that of Marshall (67), who studied the cardiac output of five dogs over a long period of time using the Fick method, and arrived at a figure of 3.10 liters per minute per square meter body surface area, with a range from 2.64 to 4.02. Harrison et al. (36) using a group of nine trained dogs and also using the Fick method arrived at a figure which when corrected for the surface area of the dogs was equal to 4.16 liters per minute per

square meter with a range from 3.03 to 5.81. The data of Harrison's group are probably more nearly representative since the study included almost twice as many dogs as that of Marshall (67). In addition, a much wider range of dogs with respect to body weight were used by Harrison.

In the present study the average cardiac index in nine dogs was 3.78. The range was 2.60 to 4.35 (Table VII). It is concluded that the values obtained in the present study using the pressure pulse contour method for computing the cardiac output in normal intact trained dogs are in good agreement with those obtained by others using the direct Fick method in the same type of experimental animal.

Bilaterally Adrenalectomized Dog. The data for heart rate, cardiac index and stroke index are included in protocol Tables II through VI and are summarized in Table VIII. The control figures are those obtained before removal of the second adrenal gland. In every case after removal of the second adrenal gland, the first series of measurements, taken when the dog was receiving replacement therapy, indicated a reduced pulse pressure. The reduction was due both to a decrease in systolic and an increase in diastolic pressures. The mean values for blood pressures illustrate this (Table VIII A and B). The decrease in pulse pressure resulted in a greatly decreased stroke index and cardiac index in four out of five animals in the group. The fifth dog (Brownie) was still on therapy when the first post-operative experiment was performed but had already undergone one acute circulatory collapse. This dog's

TABLE VIII

SUMMARY OF CARDIOVASCULAR MEASUREMENTS ON ADRENALECTOMIZED DOGS

	A. Control				B. Experiment I[1]			
Dog	Blood Pressure	Heart Rate	C. I.	S. I.	Blood Pressure	Heart Rate	C. I.	S. I.
Hound Dog	148/90	110	4.05	36.8	140/105	130	2.92	22.5
Smokey	170/110	130	4.29	31.5	150/125	156	2.06	13.0
Spot	150/98	138	4.28	31.0	150/115	150	2.80	18.6
Brownie	150/90	100	3.58	35.8	140/106	168	3.44	20.4
Champ	145/93	87	2.60	30.2	136/102	84	1.55	18.5
Mean	152/94	113	3.76	33.1	143/111	138	2.55	18.6

	C. Off Therapy					D. On Therapy[2]				
Dog	Blood Pressure	Heart Rate	C.I.	S.I.	Days[3]	Blood Pressure	Heart Rate	C.I.	S.I.	Days[3]
Hound Dog	76/55	130	2.40	17.0	4	100/63	100	2.95	29.5	11
	90/55	103	2.64	25.6	2					
Smokey	73/50	135	1.92	14.3	2	114/75	102	2.61	25.6	15
	105/80	156	2.33	14.9	4	138/105	156	3.25	20.8	12
Spot	80/50	110	1.88	17.3	2	105/72	125	2.78	22.2	9
	84/55	110	2.00	18.2	2					
Brownie	105/72	110	2.45	20.5	2	110/65	110	2.70	24.6	7
Champ	111/93	145	1.70	11.7	2	108/80	145	2.80	19.4	12
	72/55	156	1.77	11.3	2					
Mean	85/63	128	2.13	16.6		112/77	123	2.85	23.7	

[1] Performed 7 days after removal of second adrenal, except Spot which was 10 days and Brownie which was 15 days.

[2] Exact dosages of therapy have been indicated in detailed results for each dog.

[3] Days either on or off therapy before experiment was performed.

C.I.= Cardiac Index

S.I.= Stroke Index

heart rate was much greater than in the control experiment, but the cardiac index was almost equal to the control value.

These changes in all dogs were manifest even before significant alterations in E C G and blood chemistry had occurred.

Withdrawal of therapy for as little as two days in five out of seven instances (Table VIII C) resulted in a further reduction of the cardiac index and stroke index. When therapy was again administered, and measurements made while the dogs were still on therapy, improvement in the cardiac index was noted in each case. The improved cardiac output was associated with an increased pulse pressure with resulting increase in stroke output.

The increased pulse pressure upon re-administration of therapy was due to an increased systolic pressure (one exception, Champ, Table VIII D) with a relatively smaller increase in diastolic pressure. In the majority of cases the cardiac output was never returned to the normal control value, but this is believed to be due to the relatively short time on therapy between experiments, as well as less than optimum hormone replacement. However, there can be little doubt that restoration of the cardiac output to the control level could have been attained with more prolonged and vigorous replacement therapy. In one dog (Smokey, experiment IV) a deliberate attempt was made to restore the animal to an active and strong condition in a rather short time by use of a combination of D C A and A C E. The data taken at this time indicated the greatest improvement in the cardiac index achieved in any of the experiments (Table II, experiment IV).

The data show conclusively that removal of replacement therapy even for as little as two days causes a further depression in the output of the heart paralleling increased adrenal insufficiency as indicated by the hematocrit, blood N P N and plasma sodium and potassium levels. Re-administration of replacement therapy brings about improvement in heart function as indicated by an improved output.

This work is the first attempt to measure the output of the heart in a totally adrenalectomized dog without recourse to major surgery and without the use of general anesthetics. The first noted change is a marked decrease in the pulse pressure with a resulting decrease in stroke index and cardiac index. These two are depressed to a value about equal to that of an intact dog deeply anesthetized with sodium pentabarbital (compare Table VIII B and Table VII). Since this first depression takes place even before significant alterations in blood chemistry or E C G occur some factor other than these must be responsible.

The elevated diastolic pressure noted in the first post-operative experiment performed on each dog is probably indicative of an increased peripheral resistance. An increased peripheral resistance coupled with the observed decrease in cardiac index are congruent with the concept of a somewhat inadequate blood volume. No consistent indication of hemoconcentration is apparent in the data, however. Two dogs, Brownie and Hound Dog showed no significant changes in the hematocrit, whereas an elevated hematocrit was indicated in the other three animals.

The improved cardiac index obtained when the dogs were on therapy (Table VIII D) occurred in most cases even when marked improvement in blood pressure had not yet occurred. A lowered peripheral resistance could explain the improved cardiac flow and lagging blood pressure.

SUMMARY

A group of five bilaterally adrenalectomized dogs was studied in order to gain information on the output of the heart in adrenal insufficiency. Blood pressure, heart rate and cardiac index were followed simultaneously in the same animal in different states of mild insufficiency. The pressure pulse contour method of Hamilton and Remington was used to determine cardiac output. Pressure recordings of the central pulse were obtained with a strain gage pressure pick-up and recorded with a direct-writing galvanometer.

The finding by others that a fall in blood pressure is one of the earliest signs of adrenal insufficiency was confirmed. In addition, the present work involved measuring the central blood pressure and the data indicated that the first change in blood pressure measured at the carotid artery is a decrease in systolic and an elevation in diastolic pressures with an accompanying decrease in the pulse pressure. The decrease in pulse pressure was accompanied by a marked reduction in heart stroke index and cardiac index. Withdrawal of replacement therapy for two to four days in every case caused a further decrease in the cardiac index and stroke index. Readministration of therapy resulted in improvement of both indices, even when blood non-protein nitrogen, plasma sodium and potassium values were not greatly improved. Electrocardiographic records in the present work fail to confirm reported early alterations such as increased elevation of the T wave and S-T segment

depression reported by others, but a decreased R wave voltage in all leads occurred invariably in adrenal cortical deficiency.

It is concluded that the data indicate that a decrease in the cardiac output occurs early in adrenal insufficiency which is not dependent upon or necessarily associated with marked changes in the electrocardiogram, blood non-protein nitrogen or plasma sodium and potassium levels. Some direct beneficial influence of adrenal cortical hormones on heart muscle could be implicated. However, little, if any, investigation of this possibility has been done. One may recall here the work of Ingle showing the importance of the adrenal cortical hormones to skeletal muscle function.

The probable causes of altered cardiac output in adrenal cortical deficiency are discussed.

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APPENDIX

FIGURE 8.

STRAIN GAGE MANOMETER CALIBRATION CURVE

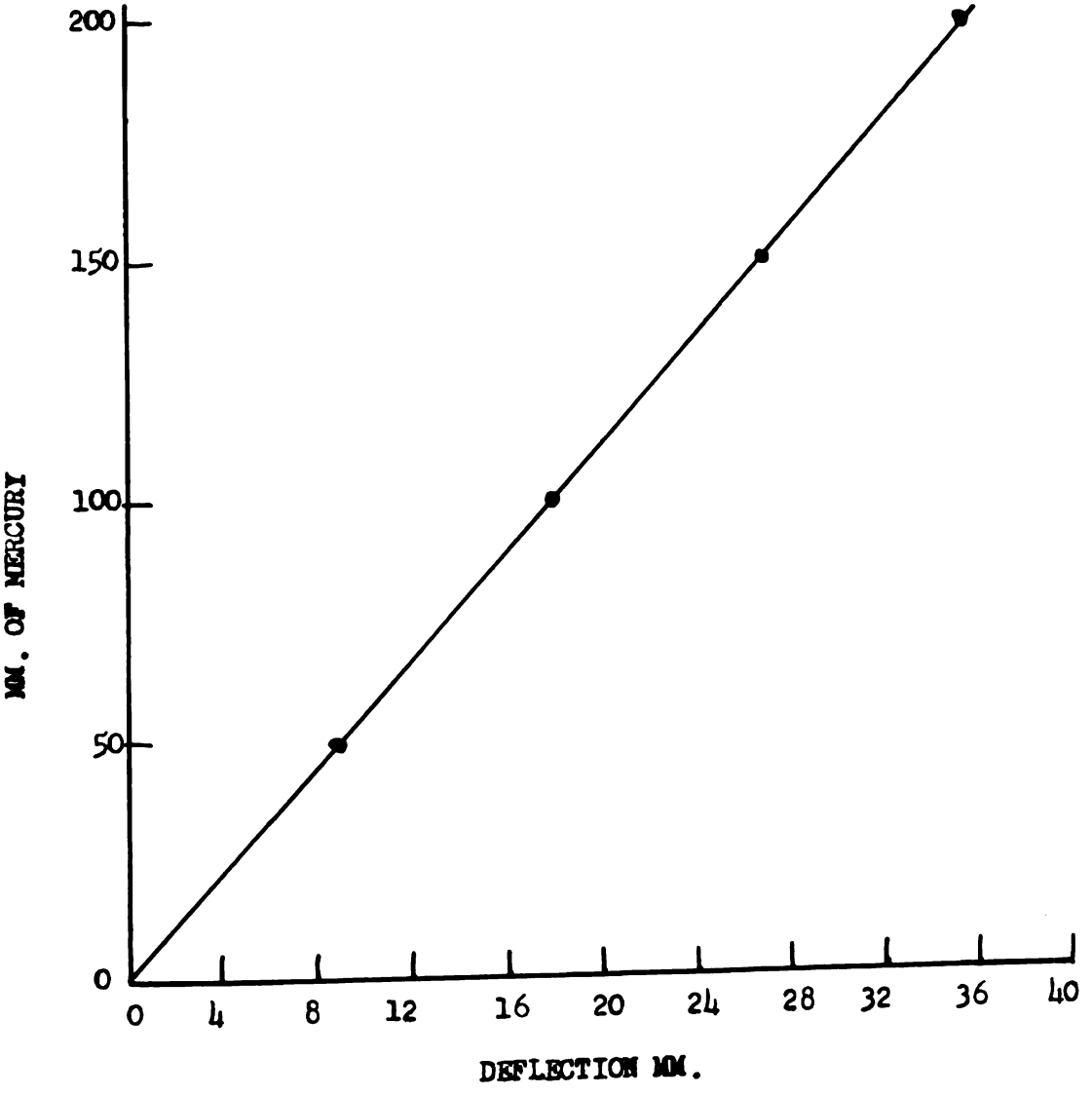

FIGURE 9.

OPTICAL MANOMETER CALIBRATION CURVE

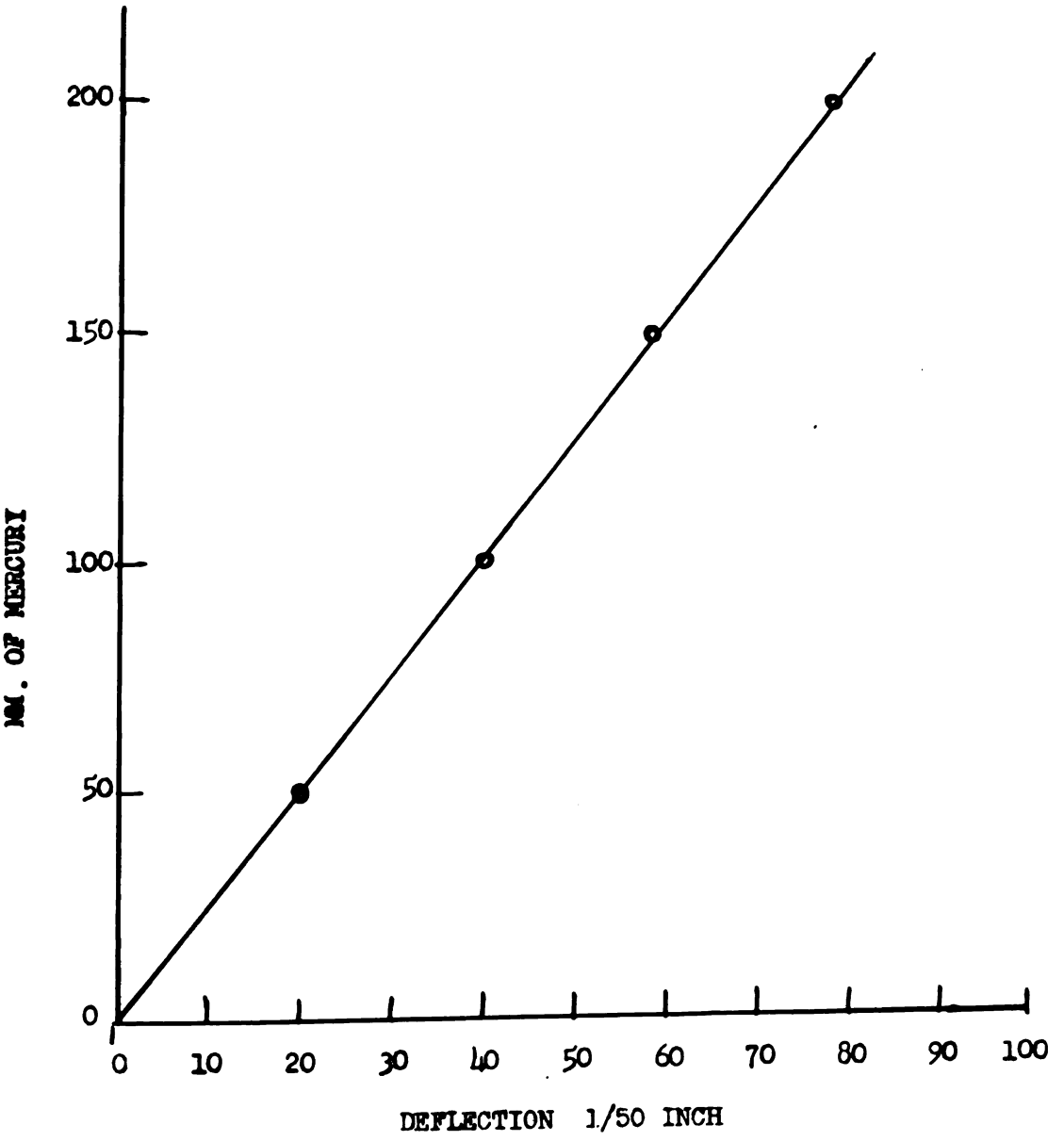

FIGURE 10.

STANDARD CURVE FOR DETERMINATION OF BLOOD NON PROTEIN NITROGEN

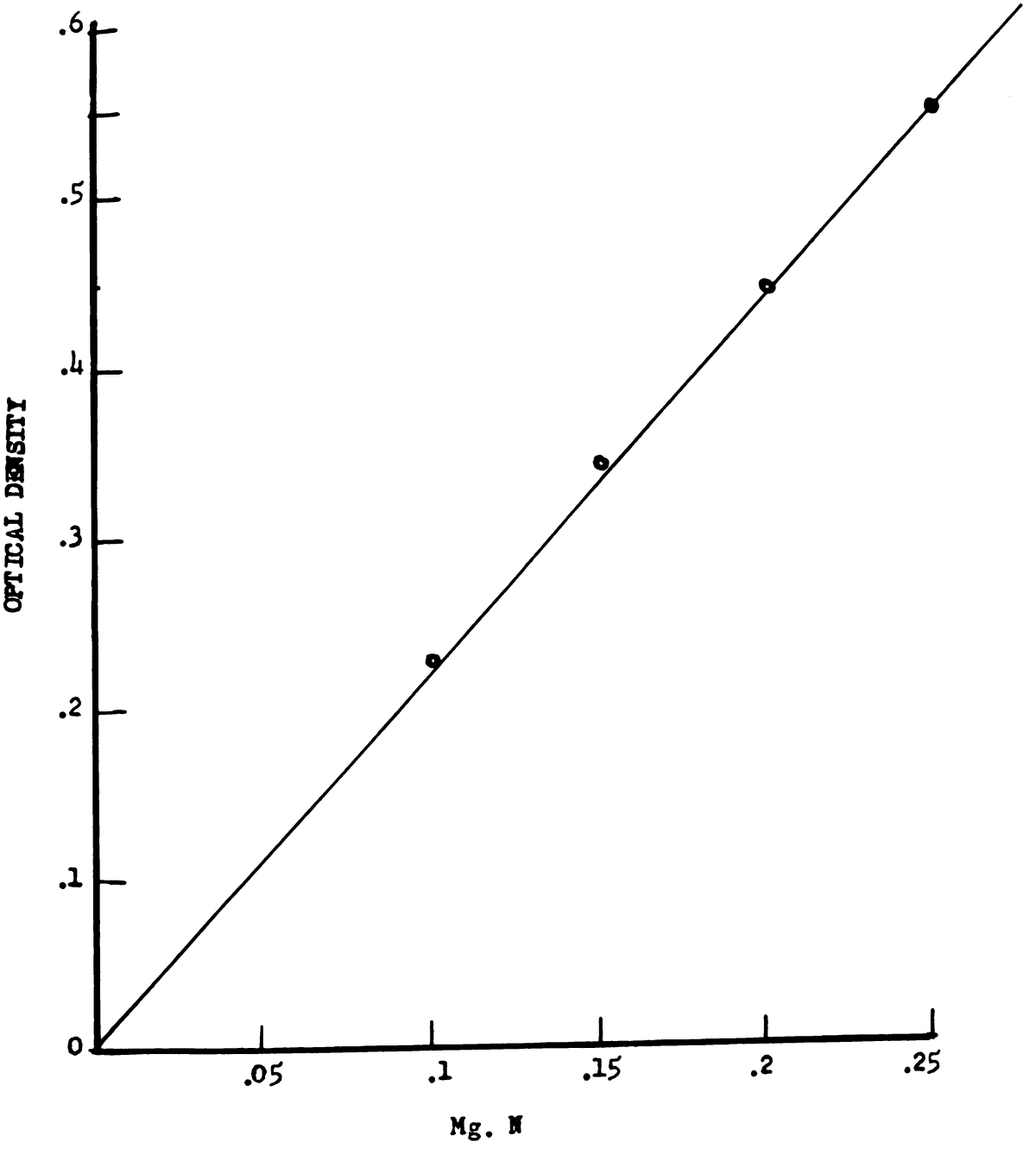

FIGURE 11.

CALIBRATION CURVE FOR SODIUM AND POTASSIUM ANALYSIS

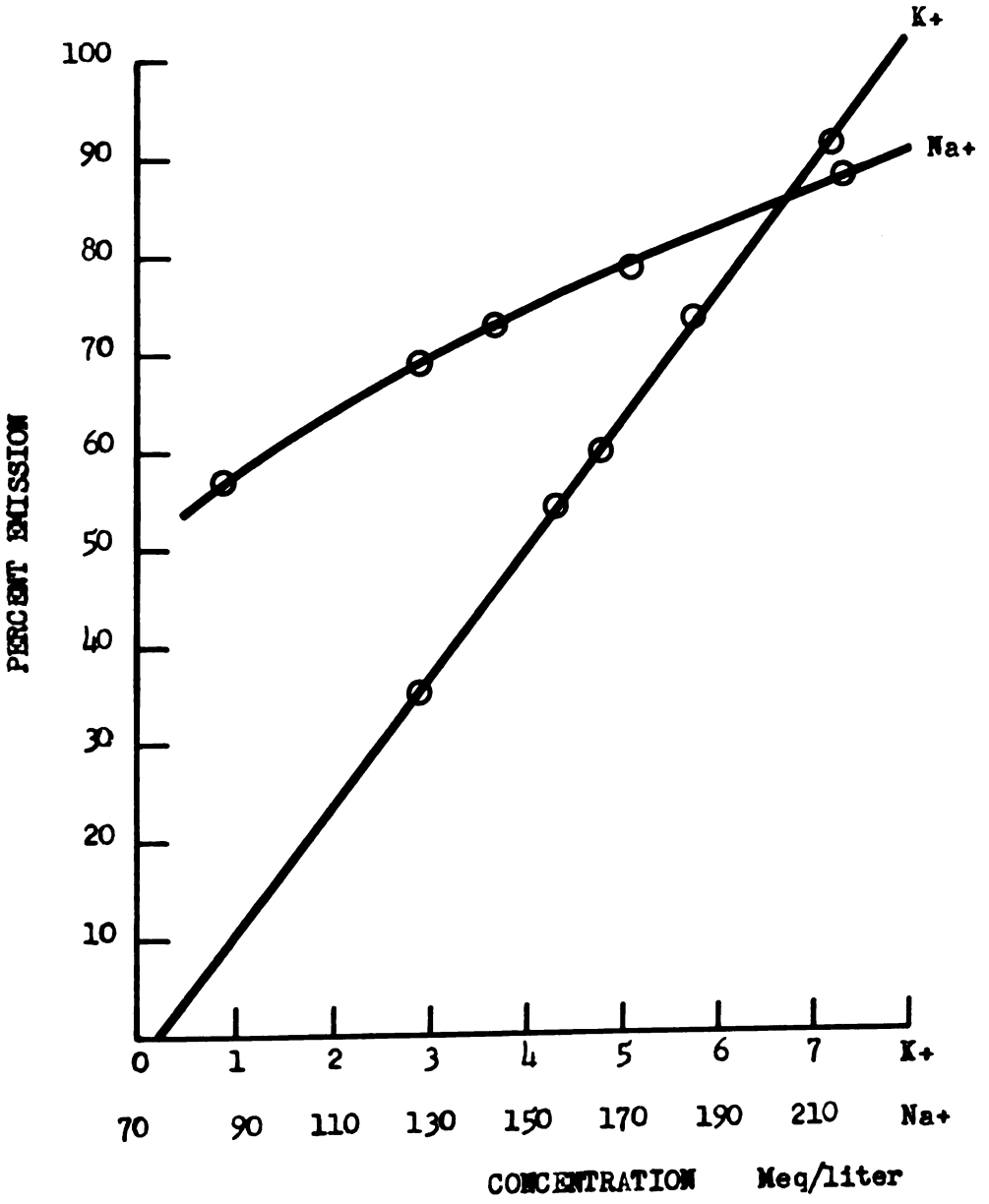

TABLE IX

[illegible] VALUES FOR A GROUP OF TRAINED DOGS

TABLE IX

PREOPERATIVE BLOOD PRESSURE, BLOOD NPN, AND PLASMA SODIUM AND POTASSIUM VALUES FOR A GROUP OF TRAINED DOGS
(Obtained at Various Dates During Control Studies)

Dog	Spot*	Smokey*	Queenie	2-C	Brownie*	C-6	Hound* Dog	Charcoal	Pinto	Collie	Brutus	Champ*	Group Mean
Body	16.0	9.8	8.5	9.4	10.2	10.0	11.7	11.2	14.0	16.0	14.0	15.4	
Weight	15.8	10.0	8.2	9.6	10.0	10.3	11.5	11.4	14.4	15.6	14.2	15.8	
Kg.	16.2	10.3	8.3	9.2	9.7	10.1	12.0	11.0	14.2	16.2	13.8	15.7	
Mean	16.0	10.0	8.3	9.4	10.0	10.1	11.7	11.0	14.2	16.0	14.0	15.6	12.2
Blood	165/105	180/100	160/100	160/100	155/85	130/80	180/110	130/75	150/80	140/100	168/105	160/90	
Pressure	175/110	160/100	160/100	155/95	145/85	145/85	180/95	140/85	140/80	140/95	160/100	165/90	
mm Hg	170/105	165/110	165/95	155/100	155/90	135/80	170/95	130/75	150/100	140/90	165/98		
	170/105	180/110	155/100		155/90	140/85	175/95	130/75	150/85	140/95			
Mean	170/105	173/106	162/100	157/98	153/88	138/82	176/98	133/78	148/86	145/95	164/101	162/90	157/106
N P N	35	40	37	37	37	34	38	37	35	35	33	34	
Mg %	32	37	40	37	32	32	36	35	32	35	37	36	
	38	37	35	36	37	37	38	34	34	33	34	35	
		35	36										
Mean	35	37	36	36	35	34	37	35	34	34	35	35	35
Plasma	143	143	135	136	136	145	146	141	145	143	141	142	
Sodium	145	145	136	140	137	141	144	141	142	142	140	144	
Meq/liter	142	143	136	139	139	142	146	144	143	144	142	141	
Mean	143	144	136	138	137	143	145	142	143	143	141	142	142
Plasma	5.05	5.02	4.78	4.25	5.05	4.34	4.34	4.92	4.65	4.85	4.70	4.85	
Potassium	4.86	4.88	4.75	4.42	4.83	4.38	4.47	5.01	5.14	4.90	4.85	4.76	
Meq/liter	4.65	4.75		4.47	4.65	4.12	5.05	4.74	4.80	4.75	4.75	5.15	
Mean	4.85	4.88	4.76	4.38	4.84	4.28	4.62	4.89	4.86	4.84	4.78	4.92	4.74

* Dog subsequently bilaterally adrenalectomized.

ABBREVIATIONS FOR TABLES X AND XI

P_s Systolic pressure in mm. Hg.

P_i Incisural pressure in mm. Hg.

P_d Diastolic pressure in mm. Hg.

T_h Transmission time to head in milliseconds.

T_v Transmission time to viscera in milliseconds.

T_l Transmission time to legs in milliseconds.

P_h Pressure in head system at incisural time, mm. Hg.

P_v Pressure in visceral system at incisural time, mm. Hg.

P_l Pressure in leg system at incisural time, mm. Hg.

U_a Uptake of aorta in cc.

U_h Uptake of head system in cc.

U_v Uptake of visceral system in cc.

U_l Uptake of legs in cc.

U Total arterial uptake during systole in cc.

T_c Cycle length in milliseconds.

T_s Length of systole in milliseconds.

T_w Average weighted transmission time in milliseconds.

S. I. Stroke index in cc/beat/sq. meter body surface.

C. I. Cardiac index in cc/min/sq. meter body surface.

TABLE X

DATA TABULATED FROM PRESSURE PULSE CURVES FOR CALCULATIONS OF CARDIAC INDEX OF ANESTHETIZED AND UNANESTHETIZED DOGS

Date	Dog	Wt. (Kg)	P_s	P_i	P_d	T_h	T_v	T_l	P_h
Anesthetized									
2/22/54	Blackie**	13.5	144	134	118	24	46	68	141
2/25/54	Black & White**	13.0	140	126	108	26	51	75	134
3/6/54	Black & White**	13.0	140	122	100	27	55	81	138
2/25/54	Police Dog**	25.0	146	140	118	24	47	69	144
3/12/54	Police Dog**	25.0	122	109	90	30	58	86	121
3/22/54	Chow**	11.8	148	132	116	27	54	72	146
3/22/54	Chow*	11.8	177	152	130	22	43	63	172
3/24/54	Spitz*	7.6	152	142	123	24	46	67	152
3/24/54	Spitz**	7.6	151	143	123	23	45	66	151
3/25/54	Jupiter**	10.6	148	140	115	25	49	72	148
3/25/54	Jupiter*	10.6	160	144	120	24	47	69	158
5/4/54	Exeter**	13.2	158	149	123	23	46	67	157
5/4/54	Exeter*	13.2	164	150	122	23	46	67	162
5/4/54	Julius**	14.2	150	140	123	23	46	67	150
5/4/54	Julius*	14.2	152	141	125	27	52	77	132
5/5/54	Spot - 2**	12.0	160	140	123	23	46	67	153
5/5/54	Spot - 2*	12.0	162	144	126	23	44	66	157
5/5/54	Champ - 1**	15.5	154	135	118	24	48	72	146
5/5/54	Champ - 1*	15.5	148	127	112	25	50	73	138
Unanesthetized									
	Collie**	14.5	140	126	80	35	68	100	135
	Collie*	14.5	145	129	85	34	68	100	140
	Brutus**	15.2	178	151	105	29	54	78	168
	Brutus*	15.2	180	153	104	27	53	77	170
	Pinto**	16.5	151	132	95	29	56	86	139
	Pinto*	16.5	165	140	98	28	56	84	158
	Pup**	10.6	156	146	102	27	54	79	154
	Pup*	10.6	158	147	100	26	53	78	155

* Record obtained using optical manometer.
** Record obtained using strain gage.

P_v	P_l	U_a	U_h	U_v	U_l	U	T_c	T_s	T_w	S. I	C. I
144	141	2.0	5.8	2.0	1.4	11.2	312	112	53	13.8	2.66
138	140	2.4	8.0	2.4	2.4	15.2	440	160	56	19.8	2.70
138	129	3.2	11.3	2.6	1.9	19.0	504	148	61	22.6	2.87
146	144	2.7	6.6	2.0	1.3	12.6	480	152	53	15.9	2.00
121	113	3.0	4.0	2.5	2.1	11.6	432	148	65	14.6	2.00
145	129	1.8	7.6	2.1	0.9	12.4	332	132	54	15.9	2.90
177	172	2.2	7.2	2.6	2.2	14.2	332	140	49	18.6	3.20
152	150	2.2	6.2	1.7	1.7	11.8	412	141	51	15.1	2.20
150	150	2.2	5.9	1.8	1.7	11.6	408	140	51	15.6	2.28
148	141	3.0	8.2	1.8	1.7	14.7	600	135	55	17.0	2.02
160	156	2.7	8.5	2.8	2.0	16.0	465	120	58	17.9	2.20
157	156	2.8	7.6	2.3	1.9	14.6	435	148	52	18.7	2.59
162	151	3.0	8.4	2.4	2.2	16.0	415	129	52	19.6	2.70
149	144	2.0	6.2	1.8	1.3	11.4	356	112	52	13.7	2.25
132	126	2.1	8.3	2.4	1.4	14.2	344	104	59	14.2	2.30
159	159	2.1	6.8	2.4	2.1	13.4	376	144	52	17.7	2.84
162	162	2.0	6.7	2.8	2.0	13.5	349	116	51	16.6	2.81
150	152	2.2	7.6	2.3	2.2	14.3	420	164	54	19.3	2.76
140	146	2.0	7.5	2.1	2.2	13.8	418	158	56	18.6	2.62
140	137	6.2	17.8	5.0	4.7	33.7	600	184	68	41.6	4.16
145	143	6.5	17.4	5.0	4.8	33.7	650	180	72	40.5	3.74
175	178	6.0	15.3	5.1	4.9	31.3	520	170	58	32.0	3.70
178	180	5.8	15.0	5.0	4.8	30.0	600	172	59	37.0	3.70
150	150	5.0	12.8	4.2	4.0	26.0	472	176	63	34.2	4.35
163	165	5.4	16.2	4.7	4.7	31.0	534	178	62	39.6	4.40
156	156	5.4	14.0	3.8	3.8	27.0	645	179	60	33.0	3.08
157	158	5.8	14.4	4.2	4.2	28.6	625	178	62	35.1	3.38

TABLE XI

DATA TABULATED FROM PRESSURE PULSE CURVES FOR CALCULATION OF CARDIAC INDEX OF BILATERALLY ADRENALECTOMIZED DOGS

Experiment	Dog	Weight Kg.	P_s	P_i	P_d	T_h	T_v	T_l	P_h
Control	Hound	12.0	148	128	90	30	59	87	140
Control*	Hound	12.0	159	137	100	27	55	81	150
I	Hound	11.5	140	128	105	27	52	77	136
II	Hound	10.5	76	63	55	43	81	123	80
III	Hound	10.8	100	85	63	38	73	109	100
IV	Hound	10.2	90	70	55	41	78	118	100
Control	Smokey	10.6	170	150	110	26	50	74	162
I	Smokey	10.5	150	145	125	28	45	61	150
II	Smokey	10.6	114	100	75	34	63	98	114
III	Smokey	9.2	73	60	50	42	81	124	73
IV	Smokey	9.2	138	124	105	27	52	77	136
V	Smokey	8.8	105	96	80	33	64	94	105
Control	Spot	15.6	150	135	98	28	55	82	145
I	Spot	15.5	150	140	115	25	48	72	150
II	Spot	14.7	80	63	50	43	81	123	80
III	Spot	14.0	105	93	72	35	68	100	105
IV	Spot	13.5	84	70	55	41	78	118	84
Control	Brownie	10.4	150	135	90	29	58	85	142
I	Brownie	9.4	140	128	106	28	52	78	140
II	Brownie	9.0	105	91	72	35	68	100	105
Control	Champ	15.5	145	120	93	28	58	85	130
I	Champ	15.4	136	121	102	27	54	79	132
II	Champ	14.5	111	105	93	29	58	85	111
III	Champ	14.0	108	98	80	32	64	94	108
IV	Champ	13.0	72	65	55	41	78	116	72

* Record obtained with optical manometer.

P_v	P_l	U_a	U_h	U_v	U_l	U	T_c	T_s	T_v	S. I.	C. I.
146	149	5.4	15.8	4.4	4.5	30.1	548	164	65	36.8	4.05
159	158	5.0	13.8	4.2	4.2	27.2	498	166	61	34.4	4.01
140	140	3.0	10.4	2.8	2.8	19.2	460	120	59	22.5	2.92
80	55	2.0	11.0	2.8	.8	16.6	460	120	91	17.0	2.40
100	100	3.6	13.0	4.2	3.6	24.4	623	160	82	29.5	2.95
90	90	2.5	12.6	4.0	3.8	22.9	584	148	87	25.6	2.64
168	168	4.7	12.2	3.9	3.6	24.4	440	156	57	31.5	4.29
145	140	2.2	5.9	2.4	1.0	11.5	376	96	51	13.0	2.05
113	93	4.2	14.0	3.1	1.8	23.1	585	128	74	25.6	2.61
73	55	1.8	8.6	2.2	0.8	13.4	448	120	91	14.3	1.92
136	135	2.6	9.4	2.4	2.3	16.7	352	128	59	20.8	3.25
96	85	2.8	9.0	1.6	0.4	13.8	336	96	72	14.9	2.33
145	147	4.6	13.0	3.4	3.6	24.6	424	148	62	31.0	4.28
150	140	3.0	8.8	2.4	1.8	16.0	400	104	48	18.6	2.80
80	55	2.0	11.0	2.8	0.8	16.6	560	112	91	17.3	1.88
100	91	3.4	12.2	3.0	1.8	20.4	480	116	76	22.2	2.78
80	62	2.6	10.6	3.2	0.8	17.2	544	120	88	18.2	2.00
145	150	5.8	15.0	3.9	4.4	29.1	600	176	64	35.8	3.58
137	130	3.0	10.1	2.5	2.2	17.8	352	104	59	20.4	3.44
100	89	3.0	12.0	3.0	1.9	19.9	530	120	76	20.5	2.45
138	145	4.1	12.2	3.8	4.1	24.2	696	204	57	30.2	2.60
136	131	2.9	8.6	2.8	2.5	16.8	713	176	60	18.5	1.55
110	105	2.0	5.6	1.6	1.1	10.3	392	120	64	11.7	1.70
100	98	2.9	10.0	2.1	1.8	16.8	408	128	73	19.4	2.86
65	58	1.8	6.2	2.2	0.4	10.6	384	112	87	11.3	1.77