STUDIES OF THE EFFECT OF CERTAIN CARDIOTONIC AGENTS ON THE CARDIAC OUTPUT IN DOGS AS DETERMINED BY THE DYE DILUTION METHOD

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Jack R. Schmid

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AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan
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THESIS ABSTRACT

The Stewart-Hamilton injection method for determining the cardiac output in the intact pentobarbitalized dog was explored as a possible method by which to study the effect of drugs e.g., Strophanthus or epinephrine, on the cardiac output.

Thirty healthy mongrel male and female dogs were used in this investigation. The dogs were deeply anesthetized with pentobarbital sodium; the common carotid artery was exposed and cannulated to facilitate the collection of blood samples needed to determine the cardiac output; the external jugular vein was made accessible for injection of the dye T-1824 and for the drugs used for experimentation. Three milligrams of the dye were used for each determination. A second determination was carried out thirty minutes after the first, and at this time a drug was introduced so that its effect could be measured and compared with the first. The samples of blood containing the dye were collected at two and three second intervals from the carotid artery. The plasma dye concentrations were determined using a Fisher electrophotometer and the values were plotted on a logarithmic ordinate against a linear time abscissa. The curve was then extrapolated to the base line and delineated. A linear replot was then made and the area inscribed by the curve was measured. The ordinate dividing the curve into two equal halves is the average concentration from which the cardiac output can be determined.

The results obtained from six dogs in which two dye injections were made thirty minutes apart were not significantly different and it was assumed that the method was feasible for showing the effect of a drug on the cardiac output. A commercial epinephrine solution was tested and found to give a significant increase in cardiac output in five dogs. Norepinephrine was next tested and it was shown that there was no significant difference in cardiac output, but that there was a trend toward a decrease in cardiac output. Sarveroside, a glycoside of Strophanthus, produced a profound decrease in cardiac output in some cases and a small but not significant decrease in others. The results depended on the time of the arterial sampling after injection of the drug.

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INTRODUCTION

During recent years there has been considerable investigation of the measurement of cardiac output by the dye dilution method. In 1932, Hamilton reported the use of this method in the study of mechanisms involved in the regulation of the circulation; in this study he reported the effects of epinephrine on the output of the heart using the dye Brilliant Vital Red as indicator. His procedure was to determine the cardiac output before and after injection of the drug using two successive injections of the dye. However, he presented no evidence that determining the cardiac output in two successive experiments on the same animal would give comparable results and thereby establish the validity of such a procedure. Surtshin (1950), using T-1824, found that the plasma dye concentration curves following two dye injections, given 49 to 241 minutes apart, were not significantly different.

These data suggest that if the validity of such a procedure could be established it would have the earmarks of a feasible pharmacological method for determining the effects of various cardiovascular agents on the output of the heart. With a few additional simultaneous measurements e.g., heart rate, hematocrit and electrocardiogram, it would be possible to obtain many other important data concerning the cardiovascular status of the subject under the influence of these agents. This technique presented an opportunity to gain insight to some of the actions and mechanisms involved in pharmacodynamics.

The selection of the cardiotonic agents to be employed in this study presented the usual problems encountered in most studies of this nature i.e., mode of administration, an effective dose and the time of onset of action. Epinephrine and norepinephrine,[1] because of their opposite effects on the total peripheral resistance, were chosen because it was thought that these two sympathomimetic amines would show some interesting effects on the cardiac output, which is dependent to some extent on the resistance to flow. Strophanthus is known to increase the cardiac output of the failing heart, but the normal heart responds to this glycoside in another fashion resulting in a decrease in cardiac output. It seemed worth-while to investigate the effects of Strophanthus on the cardiac output of the dog deeply anesthetized with pentobarbital sodium. The particular form of Strophanthus used in these experiments was sarveroside,[2] a short acting glycoside with one-half the activity and toxicity of ouabain.

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[1]Courtesy of Dr. A. M. Lands, Sterling-Winthrop Research Institute.

[2]Courtesy of Dr. M. J. Vander Brook, The Upjohn Company.

REVIEW OF THE LITERATURE

Dilution Principle

The importance of the dilution principle as a means of studying the composition of the body was reviewed by Edelman in 1952.

> The extent to which a substance is diluted in a solvent constitutes a measure of the volume of the solvent . . . . This simple relationship may be expressed mathematically by the following equation:
>
> $$V_2 = \frac{C_1V_1}{C_2}$$
>
> where C_1 and V_1 are, respectively, the concentration and volume of the solute before dilution, and C_2 and V_2 are the concentration and volume after dilution. . . . This equation is derived from the simple consideration that the product of the concentration and volume has the dimension of weight or mass, and within a closed system the mass of the solute is constant regardless of the extent of its dilution i.e.,
>
> $$C_1V_1 = C_2V_2 .$$
>
> The concentration and volume of the solute before dilution are known, and the concentration after dilution is experimentally measured. The only unknown in the equation then is V_2 which is easily computed. . . .

In applying the dilution principle to animal studies it is assumed that the cardiovascular system is a closed system, which of course is not the case at all. A very small amount of liquid is being added or removed all the time so that the dilution curve is not flat but is sloping constantly until the tracer is completely removed.

The Application of the Dilution Principle to the Measurement of Cardiac Output

In 1897, Stewart showed that it was possible to estimate the quantity of blood put out by the heart of a dog from the dilution of a known amount of injected foreign substance by the blood, which passes through the heart and lungs during a known period of time. Stewart devised two methods to obtain cardiac output which were both based on the assumption that none of the injected material had time to recirculate through the systemic cardiovascular bed before the sampling was completed. The first method is referred to as the "constant infusion method". In this method the indicator is injected into the blood stream at a constant rate; after a few seconds the indicator was said to have reached a constant concentration in the arterial blood which indicated that it had been diluted quantitatively by the aortic stream. This is said to occur prior to recirculation and reaches a "concentration plateau" from which cardiac output may be calculated. The following equation gives the relationship between the factors involved in the constant infusion method and the cardiac output:

$$1. \quad f = \frac{i}{c}$$

where f is the flow in L. per min., i is the rate of injection in mg. per min., and c is the concentration of the indicator at the height of the plateau in mg. per L. The second method is really a technical simplification of the first and is often referred to as the "rapid injection method". A known amount of indicator is rapidly injected.

The time required for collection of a sample of blood representing the average concentration of the injected substance in the arterial stream is necessary to determine the flow. If the rate of sampling is constant, the flow is equal to the amount of injected substance, divided by the product of the average concentration and the duration of sampling during the first circulation of the indicator:

$$2. \quad f = \frac{60\ I}{c\ t}$$

where f = flow (L./min.)
I = dye injected (mg.)
c = average concentration (mg./L.)
t = time of first circulation (sec.)

It is not clear from Stewart's description of these two methods if he had considered the possibility that the samples taken contained, in part, twice-circulated indicator. Hamilton and Remington (1947) observed while using the method that the indicator begins to recirculate before a "concentration plateau" has been established and that this invalidates a method of this type. Howard, et al. (1953), confirmed this viewpoint and further pointed out that spurious plateaus are frequently encountered that are unrelated to a valid estimate of flow, and that one cause of these plateaus is fluctuations in venous inflow as often observed in changes during the respiratory cycle. Rashkind and Morton (1949) and Wiggers (1944) found the constant infusion method entirely satisfactory in their hands. However, the basic soundness of the Stewart principles is recognized in their adoption for hydraulic measurements (Dow, et al., 1946), in which the flow of water in pipes and rivers is accurately determined. It may be noted that this was independently developed by engineers (Hamilton, 1945).

Development of the Hamilton Method

In 1928, Hamilton, et al., revived and extended the method by using non-diffusing and protein-binding dyes. They began a series of studies using Stewart's second method for the estimation of the output of the heart. Injection of the indicator was made rapidly into the jugular vein and serial samples were removed by cardiac puncture from the left ventricle under local anesthetic (novacaine) in dogs. Samples were collected into small tubes mounted on a revolving kymograph of known speed. The samples were analyzed for dye concentration, colorimetrically against standards, in the Bausch and Lomb microcolorimeter. The cardiac output was calculated in liters per minute using Stewart's second equation:

$$f = \frac{60\ I}{c\ t} .$$

In order to prove the validity of the method, the Hamilton group, under the direction of Kinsman (1929), proceeded to test it in artificial glass models in which recirculation was allowed to occur. They found that it was necessary to find some means of mathematically prolonging the primary curve so that all the dye during its first circulation could be accounted for. The fact that the time of recovery of all the dye approaches infinity suggested to them a logarithmic scale for concentration plotted against the time on a linear scale. Such a semilogarithmic plot made the descending limb of the curve a straight line to the point of recirculation and by extrapolation of this line to the base line the time for complete removal of the dye could be determined, and from this information an accurate measurement of the average concentration during this "wash out" period could be made by integration of the enclosed curve when replotted on a linear scale. From these recirculation experiments in glass models the average error was found to be +4.8 per cent.

This same group, under the leadership of Moore (1929), next proceeded to test the validity of the method by comparing it with an accepted method, the direct Fick, to show that in actual practice it gives output values that check. It was found that the average difference between the calculated values for cardiac output by the two methods was only 4.7 per cent in six dog experiments indicating that it was a valid method. When applied to human beings, the method gave values for the cardiac output which were much too high in comparison with those obtained by the Grollman acetylene technique which was then very popular; as a result the method fell into disrepute. When the direct Fick method of estimating blood flow became applicable for human experiments by venous catheterization of the heart, it was found that the values obtained for cardiac output by this method were considerably higher than those obtained by the acetylene method. Hamilton, _et al_.. (1948), once again investigated the validity of the dye method by comparing it with the direct Fick in man. The two methods showed satisfactory agreement in forty-two cases with no evidence of systematic differences in the determination of cardiac output. This was confirmed by Doyle, _et al_. (1953), Nahas, _et al_. (1953), and by Werko, _et al_.

(1949). Shore (1945) showed that samples taken from the right auricle or right ventricle may be in considerable error as a result of obtaining a non-representative sample of mixed venous blood. The dye method has been compared with the isotope dilution method by Dow, et al. (1946) and Lawson, et al. (1952b), and it was found that the curves for the dye and the tagged cells were practically identical, but there were indications of a more rapid transit of the cells in some part of the circuit. When the Hamilton dye method was compared with the cuvette and earpiece oximeter dye methods the average differences of cardiac output was of the order of 3.5 per cent (Ring, 1952).

Objections to the Dye Dilution Method

The Stewart-Hamilton method was criticized on the grounds that some of the dye was being retained in the lungs, and that this was responsible for the high values obtained for cardiac output. Hamilton, et al. (1930), affirmed that the dye he used in earlier studies, phenoltetraiod-pthalein sodium, was not a non-diffusible dye, and that it should not be used in this method; at the same time they showed, by perfusion of the lungs and the left heart, that the dye "brilliant vital red" did not diffuse from the pulmonary vascular bed during its first passage through; and that all the dye injected could be recovered. Gregersen and Rawson (1943) found that the dye T-1824 was so firmly bound to the albumin that its disappearance rate, during the first hour after injection, was a measure of the rate of escape of the circulating albumin. Dow and Hahn (1946), and later Lawson, et al. (1952), found no preferential retention of dye in or on the vessels of the lesser circulation. It was concluded by Dow and Hahn that the high cardiac outputs obtained by the dye injection method were not in error as the result of retention of dye in the lungs.

Hamilton, et al. (1948), defended the use of large vessel hematocrit, in preference to whole body hematocrit, on the grounds that the flow is measured from the great veins to the great arteries, each of which has the same hematocrit.

The trapping of plasma among the cells has been shown by Gregersen, Gibson and Stead (1935) to leave four per cent of T-1824 unaccounted for in the plasma above the cells. This cannot be dismissed as a possible source of error in cardiac output studies on the assumption that all the samples are likewise affected and therefore the error cancels. The areas inscribed in the linear time concentration curves, using samples which have and have not been corrected for trapped plasma, may not be the same and therefore not yield the same cardiac outputs. The factor used to correct for trapped plasma is considered by Reeve (1948) to be 0.95 while Gregersen (1951) maintains it is 0.96. This difference, although small in appearance, is one of an aggregate which could determine the accuracy of the method as Ring, et al. (1952), pointed out.

Hamilton and Remington (1947) observed that when injection of dye was made into the left ventricle it was practically cleared from the stream before recirculation occurred. In general, the dilution curves resulting from the more central injection sites describe a smaller area than do the more peripheral injection curves. The values obtained for the cardiac output from the more central curves were shown to be smaller, by Hetzel and Swan (1953), and larger by Coe, Best and Lawson (1950) and Lawson, et al. (1954), than the peripheral curves. It was pointed out by Werko, et al. (1949), that dilution curves derived from injection into the pulmonary artery nearly approach zero concentration and thus require extrapolation of a smaller part of the curve, which is most desirable. The variations in results obtained due to the different sites of injection of the dye could account for some of the inconsistencies reported.

While the basic principle for the dilution method is quite simple, its application to research on the cardiovascular system has proved to be more complicated. The accuracy of the dye method seems to rest upon the ability of the investigator to delineate the primary dilution curve (Lagerlof, et al., 1949; Dow and Hamilton, 1950; Nahas, et al., 1953; and Schreiner, et al., 1953). The part played by the state of the subject, e.g., anesthesia, voluntary movement, metabolism, extrinsic innervation of the heart, etc., must be considered. Stewart realized the influence of the many factors which control the output of the heart and discussed them in his treatise on the cardiac output back in 1921. Werko, et al. (1949), have further pointed out that the dye method covers the cardiac output during a short interval of time, and it is thus possible that the cardiac output determined by this method is more influenced by the phases of respiration.

Perhaps the most undesirable part of the Stewart-Hamilton method is the inconvenience of collecting the many blood samples required, and the many hours of tedious colorimetry, graphing, and calculating necessary to obtain a single cardiac output (White, 1947, and Lewis, 1953).

EXPERIMENTAL

General Procedure

Healthy male and female mongrel dogs in a post nutritive state were deeply anesthetized with pentobarbital sodium (approximately 30 mg./kg.) so that the respiratory rate was controlled between ten and fourteen ventilations per minute, and the palpebral reflex was abolished. A mid-line incision was made on the ventral surface of the neck and the external jugular vein on one side and common carotid artery on the other side were isolated by blunt dissection. The vago-sympathetic trunk was carefully separated from the carotid artery and the artery was cannulated with a piece of polyethylene tubing of the proper size, and approximately twenty centimeters long.

Electrocardiograms were taken in all the experiments using either a Sanborn "Poly-Viso" recorder or a Cardiotron. It was necessary to obtain the heart rates just prior to the cardiac output determination and at the time when the dilution curve was being formed. This was carried out in most experiments for both first and second determinations.

Approximately three mg. of T-1824[1] were carefully drawn into a calibrated syringe and the needle was inserted into the lumen of the exposed external jugular vein. The cannula was checked for flow and

[1]An amount necessary to insure adequate optical density in the plasma samples.

a sample of blood was drawn for the hematocrit and the blank. The signals for injections of the dye and drug, as well as the signals for the arterial sampling procedure, were accurately recorded on magnetic tape. The tape recorder and electrocardiograph were started and at the proper signal the dye was injected as quickly as possible into the jugular vein. Serial samples were collected in three ml. collecting tubes, each containing one drop of heparin sodium (10 mg./ml.), from the cannulated carotid artery at two second intervals up to twelve seconds, then every three seconds to twenty-seven seconds at which time the sampling was terminated.

Two Wintrobe[1] hematocrit tubes were filled, capped, and centrifuged for forty minutes at 2500 rpm. The samples were centrifuged for twenty minutes and set aside until the experiment was completed.

The second determination on each dog was made thirty minutes after the first injection of dye. It was carried out in much the same manner as the first determination, the difference being that a drug was generally injected, at a specified time, prior to the injection of the second sample of dye. In order to insure patency of the cannula during the relatively long interval of time between determinations, approximately three to five ml. of heparinized saline solution (10 mg./200 ml.) were flushed through the cannula and this solution was allowed to remain in the cannula until just prior to the beginning of the second determination. Samples for the plasma volume determinations were taken from

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[1]Capacity 1 ml., 3 mm. bore and graduated from 0-100, both up and down the hematocrit tube.

The carotid artery at an interval of eight minutes after the injection of the dye for each respective determination.

One ml. samples of plasma, containing the dye, were drawn from the tops of the sample tubes using clean pipettes. They were diluted to a total volume of three ml. with physiological saline solution in three ml. "Fisher" micro cells. Their optical densities were determined by a Fisher Electrophotometer using the 650 mμ red filter and subtracting the value obtained for the blank from each sample. The optical densities of the blank samples were generally values between 1.5-3.0 indicating a very small amount of lipoid material and hemoglobin present which was not significant enough to interfere with the colorimetry. Due to the fact that T-1824, at the concentration desired, did not strictly conform to Beer's Law a plotted calibration curve[1] was employed to obtain the individual sample concentrations. Strict analytical procedure was followed in preparing the series of standard solutions used in plotting the calibration curve.

The concentration values of the plasma samples were corrected for dilution and expressed in terms of mg. per ml.; and then further adjusted for any variations due to body weight by dividing by mg. dye injected per gram body weight. These values were then plotted on a logarithmic ordinate against a linear time abscissa and the dye concentration curve was drawn (Figure 1). There were generally three points in a straight

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[1]Optical density for the ordinate, and concentration in mg. per ml. for the abscicca. This curve was determined by plotting the optical densities of serial samples of T-1824 diluted to a final volume of three ml. i.e., one ml. of a standard dye solution, one ml. pooled dogs' plasma and one ml. physiological saline solution.

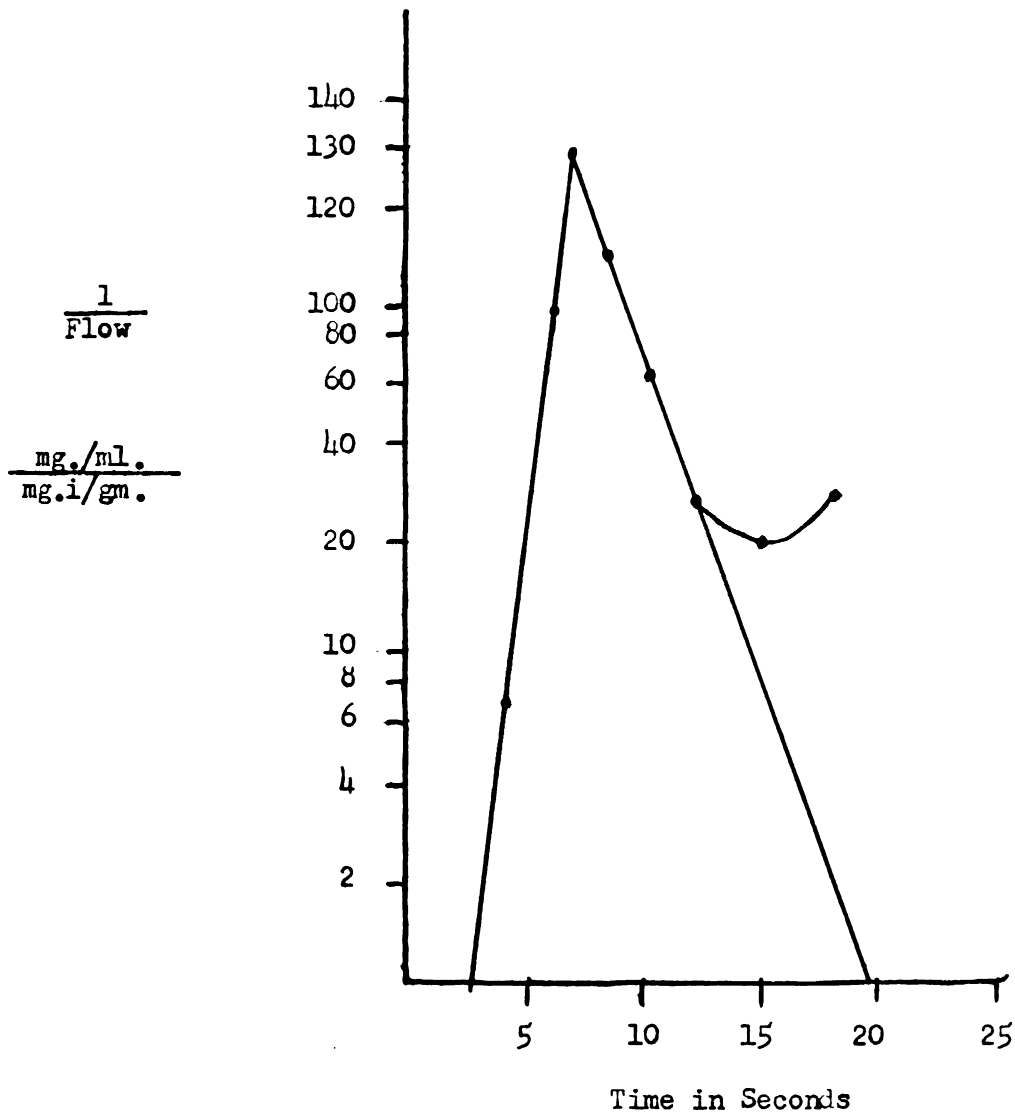

Fig. 1. Semi-logarithmic Plot of Time Concentration Curve. Experiment 6B.

line on the descending limb of the curve so that extrapolation to the base line was quite accurate in most cases. After delineation of the curve a linear plot of the curve was made using one second values from the semi-logarithmic curve (Figure 2). The area inscribed in the linear curve was determined using a Keuffel and Esser planimeter, and the ordinate that divides the inscribed curve into equal halves is the average value for reciprocal of flow which is used to determine the cardiac output.[1] The cardiac output, or flow, is calculated using the following equation:

$$(1)\quad F_p = \frac{60}{ct}$$

where F_p = plasma flow (ml./gm./min.)
c = reciprocal of flow (gms./ml.)
t = clearance time[2] of dye (seconds)

The plasma flow may be converted to a whole blood value using the equation:

$$(2)\quad Fb = \frac{F_p}{1.00 - Hct}$$

where F_p = plasma flow (ml./gm./min.)
F_b = whole blood flow (ml./gm.)
Hct = hematocrit

Stroke volumes were calculated from:

$$SV = \frac{F}{HR}$$

where SV = stroke volume (ml./beat)
F = whole blood flow (ml./dog/min.)
HR = heart rate (beats/min.)

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[1]In the process of correcting for any variation due to the body weight the average concentration is not obtained; instead a value is obtained which is the reciprocal of flow in terms of body weight.

[2]Theoretical time in which one central circulation would be cleared of dye if no recirculation occurred.

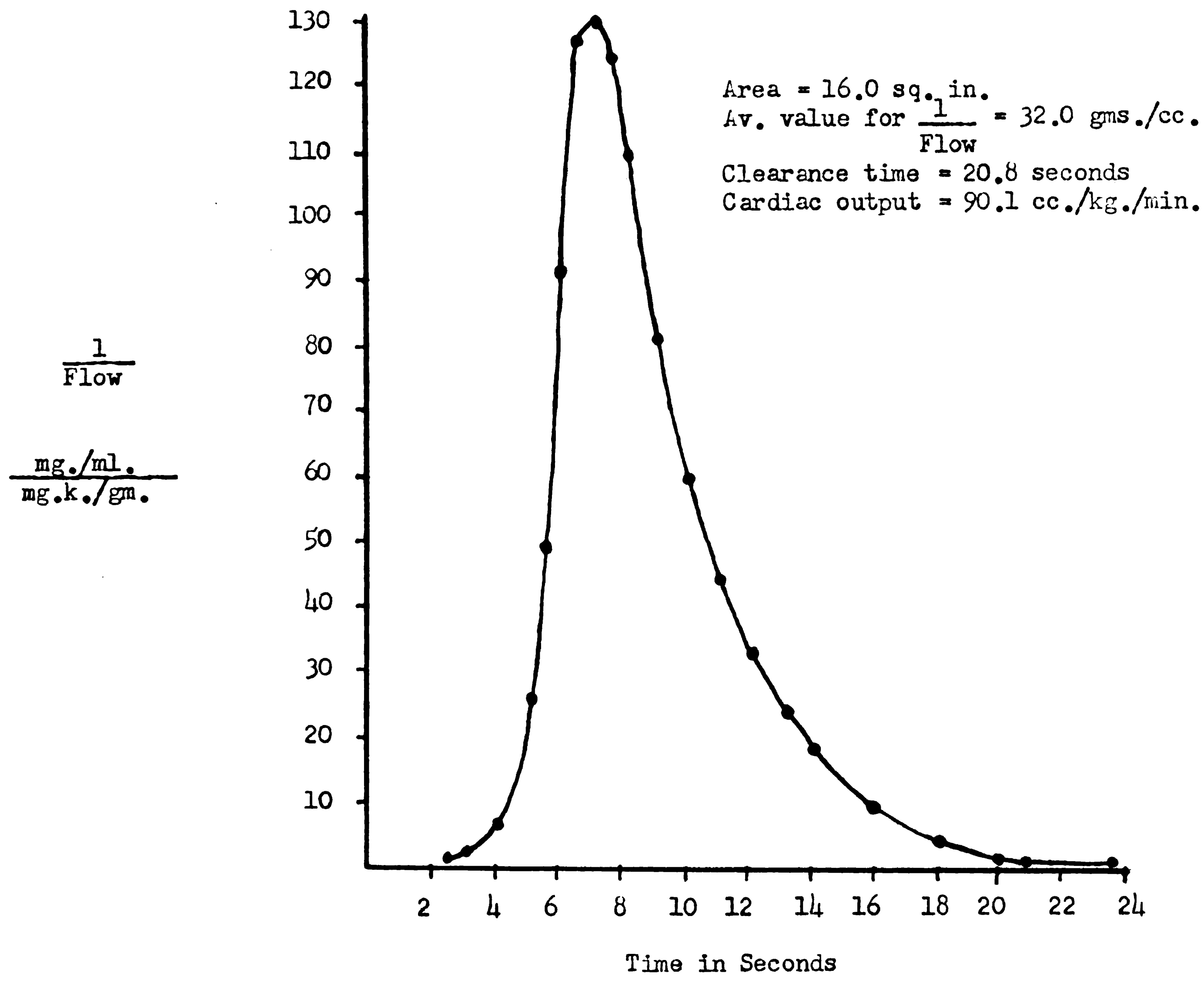

Fig. 2. Linear Replot of Time Concentration Curve. Experiment 6B.

Plasma volumes were calculated from the eight minute samples using the equation:

$$PV = \frac{c}{c'}$$

where PV = plasma volume (ml.)
c = dye injected (mg.)
c' = concentration of dye of eight minute sample (mg./ml.)

Blood volumes were obtained from the plasma volumes and the hematocrit as follows:

$$BV = \frac{PV}{1.00 - Hct}$$

where BV = blood volume (ml.)

Surface areas were calculated from the Rubner formula:

$$S\,A = 0.107 \times W^{2/3}$$

where S A = body surface area (sq. M.)
W = weight of dog (kg.)

Cardiac indexes were calculated so that the flow could be expressed in terms of the body surface area:

$$C\,I = \frac{F}{S\,A}$$

where C I = cardiac index
F = flow (L./min.)

Stroke indexes were also calculated to express the stroke volume in terms of surface area:

$$S\,I = \frac{S\,V}{S\,A}$$

where S I = stroke index (ml./beat/sq. M.)

Results

Group One

Before the dye method for determining the cardiac output could be accepted as a suitable one for testing the effect of a drug, it was necessary to compare the results obtained from a series of two successive determinations of cardiac output on the same dog, and to show that there was no significant difference in their values.

Six dogs weighing from eleven to nineteen kilograms were used in this group. The dogs received no drug treatment during the course of the experiments. They were anesthetized with pentobarbital sodium (30 mg./kg.). The operative procedure was carried out as quickly and carefully as possible to minimize any deterioration of the dog. A period of approximately thirty minutes were allowed for recovery from the surgery before the experiment was continued.[1]

Table I presents the data which were collected and computed for this series of experiments. Part A contains the values obtained from the first dye injection. This serves as the control for the values obtained from the second dye injection in part B. Values for the hematocrit remained unchanged from the first run to the second. Plasma volumes remained the same in dogs two, four and six. Experiment number five shows an increase in plasma volume from the first run of about fourteen per cent. Eight-minute samples were not taken for part B of

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[1]These preliminary steps and precautions were followed throughout the remaining experiments whenever possible.

experiments one and three in this group. The plasma volumes could not be computed for these dogs. The cardiac indexes varied from a difference of eighteen per cent in dog number two, to a difference of less than one per cent in dog number five; the difference between the means of parts A and B was only two per cent. There was little change in clearance time for dogs one, two and four, while dog number six showed a substantial change of 7.3 seconds, which is well over one-half the initial value for clearance of the dye in run A. Dogs three and five showed differences of comparable magnitude but opposite in direction. It should be noted that when the clearance time is either increased or decreased for a particular dog in this group, the cardiac index for this same dog is also changed but always opposite in direction. This point will be discussed in detail later. There was no significant difference shown for cardiac output ($t = 0.44$).

Group Two

A commercial epinephrine solution was injected rapidly into the external jugular vein of seven dogs, ranging in weight from 7.6 to 25.0 kgms., prior to the determination of cardiac output. Some of these dogs had been used in a previous experiment in which the stroke index was measured with a strain gauge and/or a Hamilton optical manometer system within two hours prior to this investigation, i.e., dogs three, four and five. The dose and the time from drug injection to blood sampling were varied in an effort to establish a suitable treatment for future experiments. The total volume of epinephrine injected was one milliliter. The dose varied from 1.5 to 2.6 micrograms per kilogram (Table IIb).

Table II shows the data collected and calculated for all experiments in which the commercial epinephrine was injected with, or just prior to, the injection of the dye. Dogs one and two should be considered in a separate group due to the mode of administration of the drug, i.e., mixed in the dye solution. It is not known if epinephrine is fully active when mixed with the dye. Furthermore, different time factors in drug action did not warrant including these two types of experiments in the same group. Epinephrine has a very short onset of action and duration which depends, in part, on the rate of its oxidation (Sollmann, 1950). Dogs one and two do not show any change worthy of discussion other than an increase in plasma volume over control of twenty-five per cent for dog one; also an increase of clearance time of 5.6 seconds or thirty three per cent over the control value for the same dog.

An examination of the data for the last five dogs in this group present some very interesting changes. Hematocrit values were increased slightly in dogs five and seven (7 and 8% respectively). In all five remaining experiments of the group the plasma volumes were increased substantially. Cardiac indexes were increased in all dogs with an average of thirty seven per cent. This was significant at the five per cent level (t = 2.49). Stroke indexes were also increased in every experiment with an average increase of twenty per cent. This was significant at the one per cent level (t = 4.28). When the clearance time decreased, i.e., dogs three and six, the cardiac index and stroke index increased more than when clearance time increased or remained unchanged. It appears that the heart rates were affected in these experiments. However, due to inadequate measurement of the pre-drug heart rate it is necessary to omit a comparison.

Group Three

Valuable information concerning dose and time of onset of action was obtained from the previous experiments using a commercial epinephrine preparation. It was shown that a dose of approximately two micrograms per kilogram body weight, given about five seconds prior to injection of the dye solution, altered cardiac output significantly. This dosage and time schedule was substantiated by Brown (1954) and supplemented by him with additional facts concerning the use of another sympathomimetic amine, l-norepinephrine.

Preliminary dose-response experiments showed that a dose of two micrograms per kilogram body weight of l-norepinephrine bitartrate monohydrate, administered rapidly into the external jugular vein, gave a substantial pressor response in the intact anesthetized dog. The time of onset of the pressor response varied in several experiments on two dogs between 7.5 and 9.5 seconds. It was the purpose of the following experiments to measure the cardiac output, after injecting this drug, and at a time just prior to and during the pressor response. The injection time for l-norepinephrine was set at 5.5 seconds prior to injection of the dye.

The results of seven experiments are shown in Table III in which the drug is compared to control values. In experiment five the hematocrit increased from 0.37 to 0.41, an increase of eleven per cent; the other six experiments showed no change. Plasma volume increased slightly in five out of seven cases. There is no significant difference in cardiac indexes at the five per cent level ($t = 1.38$) but attention should be called to the fact that in five of the seven dogs tested the cardiac index fell. Stroke indexes fell to a much lower level and are significant at the five per cent level ($t = 2.47$). Clearance time was prolonged in every experiment, and especially in experiment four where the difference is 29.3 seconds or a 141 per cent increase from the control value. Other experiments, i.e., two and seven, gave values for clearance time substantially increased.

Group Four

Sarveroside is a glycoside of Strophanthus with one-half the activity and toxicity of ouabain. The latency in the heart-lung preparation was very short (one to two minutes) and maximum effect was seen in ten minutes (Vander Brook, 1954). Meyers (1954) found in acute experiments, that two normal dogs responded similarly to intravenous ouabain (0.04 mg./kg.). The cardiac output was reduced thirty per cent after twenty minutes in these two experiments.

Preliminary experiments on four normal dogs showed that a dose of sarveroside (0.08 mg./kg.) injected at a constant rate for twenty seconds produced a mild bradycardia and a slight increase in blood pressure. The blood pressure returned to normal in approximately five minutes.

It was concluded from the results of these experiments that at an interval of one and one-half minutes after the beginning of injection of the drug, a substantial change had occurred in the cardiovascular system; and that the determination of the cardiac output at this time would probably show a significant change from the control value.

Table IV lists the data obtained on five dogs ranging in weight from 9.1 to 11.5 kgms. and treated using the procedure outlined above. The hematocrit fell slightly in dogs two, three and four. Changes in plasma volume were as follows; it decreased in dogs one, three and four, and increased in dogs two and five. The changes seen in dogs two and three were large (21 and 27% respectively), but in the opposite direction.

The cardiac indexes in all experiments were decreased from control values, with a mean decrease of forty-six per cent. This was significant at the one-tenth per cent level (t = 13.64). Stroke indexes also decreased in all experiments with a mean decrease of forty-two per cent. This was also significant at the one-tenth per cent level (t = 6.02). Clearance times were increased in all experiments. The magnitude and particularly the direction of the change in clearance time very obviously indicate a relationship between clearance time and cardiac output.

Group Five

The procedure was the same as for the previous group with the exception of the time of determination of cardiac output after injection of the drug. The preliminary dose-response experiments indicated that the effect of the drug had changed considerably at the end of five minutes in the direction of normalcy.

Sarveroside was injected five minutes before the determination of cardiac output in five dogs ranging in weight from 8.5 to 9.8 kgms. Table V presents the data for this group. The hematocrit values, with one exception, increased in this group in contrast to Group Four in which all decreased slightly. Dog one shows an increase of seventeen per cent over the control, and dog three also shows a definite increase. Plasma volume demonstrates the same variation as in Group Four. It appears that the trend is for a mild decrease in plasma volume after Sarveroside. Cardiac indexes were generally decreased, but dog one demonstrated a substantial increase. Although the average difference between the control and drug values was twenty-nine per cent, this was not significant at the five per cent level ($t = 1.23$). It appears that if more dogs would have been used there would have been a significant decrease in cardiac output. Stroke indexes decreased in four out of five cases. Dog one showed an increase which once again interfered with the possible significance of these values at the five per cent level ($t = 1.51$). As was expected, the clearance time increased for dogs demonstrating a decrease in cardiac output, and decreased for the one exception, dog number one.

TABLE I

CARDIAC OUTPUT* FROM TWO SUCCESSIVE INJECTIONS OF T-1824

Controls

Experiment No.	Weight (kg.)	Surface Area (M^2)	Hematocrit	Plasma Volume (cc.)	Cardiac Output (cc./min.)	Cardiac Index (L/min./M^2)	Clearance Time (sec.)
			A. First Dye Injection				
1A	19.3	0.77	0.46	827	4396	5.71	13.0
2A	12.1	0.56	0.51	596	3506	6.26	11.1
3A	14.0	0.62	0.37	689	2117	3.41	22.3
4A	11.0	0.52	0.42	649	1516	2.92	22.2
5A	13.6	0.61	0.46	632	2569	4.21	19.0
6A	11.0	0.52	0.43	545	2092	4.00	13.5
					Mean	4.42	
					S.E.	± 0.53	
			B. Second Dye Injection--Thirty Minutes Later				
1B	19.3	0.77	0.46	---	4969	6.45	12.3
2B	12.1	0.56	0.51	596	4124	7.36	12.2
3B	14.0	0.62	0.36	---	1823	2.94	18.3
4B	11.0	0.52	0.43	641	1467	2.82	21.8
5B	13.6	0.61	0.46	719	2581	4.23	23.9
6B	11.0	0.52	0.44	551	1770	3.40	20.8
					Mean	4.53	
					S.E.	± 0.95	
			t - differences			0.44	

* Derived from plasma dye concentration.

TABLE II

EFFECT OF COMMERCIAL EPINEPHRINE ON CARDIAC OUTPUT*

Experiment Number	Weight (kg)	Surface Area (M^2)	Hematocrit	Plasma Volume (cc)	Cardiac Output (cc/min)	Cardiac Index (L/min/M^2)	Stroke Volume (cc/beat)	Stroke Index (cc/beat/M^2)	Clearance Time (sec)	Heart Rate (beats/min)	Dose of Epinephrine (ug/kg)	Time Injection To Sampling (sec)
						A. First Dye Injection						
I-A-1**	10.5	0.51	0.43	515	2193	4.30	12.46	24.43	16.8	176	--	--
I-A-2**	13.6	0.61	0.42	561	1219	2.00	7.34	12.03	29.2	166	--	--
I-A-3	25.0	0.92	0.47	1021	5525	6.01	38.37	41.71	18.3	144	--	--
I-A-4	11.8	0.55	0.43	546	2139	3.89	11.88	21.60	14.7	180	--	--
I-A-5	7.6	0.37	0.42	307	1167	3.15	5.84	15.78	19.8	200	--	--
I-A-6	10.6	0.52	0.48	432	1787	3.44	12.16	23.38	16.3	147	--	--
I-A-7	13.7	0.61	0.37	561	2081	3.41	11.96	19.61	22.8	174	--	--
					Mean	3.98	Mean	24.42				
					S.E.	± 0.05	S.E.	± 4.49				
			B. Second Dye Injection--Thirty Minutes Later--Following Commercial Epinephrine									
I-B-1**	10.5	0.51	0.41	646	2169	4.25	11.92	23.37	22.4	182	1.9	With dye
I-B-2**	13.6	0.61	0.42	588	1236	2.03	6.94	11.38	33.0	178	1.5	With dye
I-B-3	25.0	0.92	0.47	1226	6821	9.59	43.67	47.47	13.4	202	2.0	5.0
I-B-4	11.8	0.55	0.43	594	2960	5.38	13.70	24.91	15.0	216	1.7	7.0
I-B-5	7.6	0.37	0.45	348	1355	3.66	7.40	20.00	24.5	183	2.6	4.5
I-B-6	10.6	0.52	0.47	484	2613	5.03	16.75	32.21	13.5	156	1.9	9.0
I-B-7	13.7	0.61	0.40	649	2177	3.57	13.36	21.90	19.5	163	1.5	9.5
					Mean	5.45	Mean	29.30				
					S.E.	± 1.22	S.E.	± 6.72				
			t - differences			2.49+		4.28++				

* Derived from plasma dye concentration.
** Not included in statistical analysis.
\+ Significant at 5% level.
++ Significant at 1% level.

TABLE III

EFFECT OF 1-NOREPINEPHRINE ON CARDIAC OUTPUT*

Dose 2ug./kg. Time from Injection to Sampling 5.5 Seconds.

Experiment Number	Weight (kg)	Surface Area (M^2)	Hematocrit	Plasma Volume (cc)	Cardiac Output (cc/min)	Cardiac Index (L/min/M^2)	Stroke Volume (cc/beat)	Stroke Index (cc/beat/M^2)	Clearance Time (sec)	Heart Rate (beats/min)
					A. First Dye Injection					
II-A-1	9.0	0.46	0.39	489	1239	2.69	11.16	24.26	25.5	111
II-A-2	10.0	0.50	0.37	372	2099	4.20	14.18	28.36	15.0	148
II-A-3	14.1	0.62	0.38	788	3018	4.87	15.72	25.35	13.5	192
II-A-4	11.5	0.55	0.42	462	1512	2.75	8.04	14.62	20.7	188
II-A-5	13.2	0.60	0.37	671	2542	4.24	17.53	29.22	17.8	145
II-A-6	12.0	0.56	0.37	709	2418	4.32	21.03	37.55	12.7	115
II-A-7	14.7	0.64	0.43	807	2165	3.38	15.25	23.83	25.3	142
					Mean	3.78	Mean	26.17		
					S.E.	± 0.32	S.E.	± 2.61		
		B. Second Dye Injection--Thirty Minutes Later--Following 1-Norepinephrine								
II-B-1	9.0	0.46	0.40	505	1010	2.20	6.60	14.35	28.3	153
II-B-2	10.0	0.50	0.37	422	1321	2.64	11.01	22.02	27.2	120
II-B-3	14.1	0.62	0.37	863	3378	5.45	17.32	27.94	15.9	195
II-B-4	11.5	0.55	0.41	514	946	1.72	5.70	10.36	50.0	166
II-B-5	13.2	0.60	0.41	621	2571	4.29	17.73	29.55	19.5	145
II-B-6	12.0	0.56	0.39	685	2384	4.26	18.63	33.27	15.0	128
II-B-7	14.7	0.64	0.42	879	1960	3.06	11.88	18.56	30.0	165
					Mean	3.37	Mean	22.29		
					S.E.	± 0.59	S.E.	± 4.11		
		t - differences				1.38		2.47+		

* Derived from plasma dye concentration.
\+ Significant at the 5% level.

TABLE IV

EFFECT OF SARVEROSIDE ON CARDIAC OUTPUT*

Dose 0.08 mg./kg. Time from Injection to Sampling 1.5 Minutes

Experiment Number	Weight (kg)	Surface Area (M^2)	Hematocrit	Plasma Volume (cc)	Cardiac Output (cc/min)	Cardiac Index ($L/min/M^2$)	Stroke Volume (cc/beat)	Stroke Index ($cc/beat/M^2$)	Clearance Time (sec)	Heart Rate (beats/min)
					A. First Dye Injection					
III-A-1	10.4	0.51	0.38	540	2229	4.37	18.58	36.43	12.8	120
III-A-2	11.5	0.55	0.42	617	2568	4.67	19.31	35.11	18.2	133
III-A-3	11.0	0.52	0.39	524	2275	4.38	15.80	30.38	15.0	144
III-A-4	11.0	0.52	0.48	564	1319	2.54	9.03	17.37	30.3	146
III-A-5	9.1	0.47	0.46	441	2218	4.72	12.32	26.21	12.2	180
					Mean	4.14	Mean	29.10		
					S.E. ±	0.41	S.E. ±	3.44		
					B. Second Dye Injection--Thirty Minutes Later--Following Sarveroside					
III-B-1	10.4	0.51	0.37	504	1281	2.51	10.09	19.76	26.3	127
III-B-2	11.5	0.55	0.40	746	1271	2.31	9.78	17.78	32.9	130
III-B-3	11.0	0.52	0.37	385	1319	2.54	11.57	22.25	28.0	114
III-B-4	11.0	0.52	0.45	504	533	1.03	4.44	8.54	84.2	120
III-B-5	9.1	0.47	0.45	473	1294	2.75	7.80	16.60	28.0	166
					Mean	2.23	Mean	16.99		
					S.E. ±	0.59	S.E. ±	4.11		
			t - differences			13.64**		6.02**		

* Derived from plasma dye concentration.
** Significant at the 0.1% level.

TABLE V

EFFECT OF SARVEROSIDE ON CARDIAC OUTPUT*

Dose 0.08 mg./kg. Time from Injection to Sampling 5.0 Minutes

Experiment Number	Weight (kg)	Surface Area (M^2)	Hematocrit	Plasma Volume (cc)	Cardiac Output (cc/min)	Cardiac Index (L/min/M^2)	Stroke Volume (cc/beat)	Stroke Index (cc/beat/M^2)	Clearance Time (sec)	Heart Rate (beats/min)
					A. First Dye Injection					
IV-A-1	9.2	0.47	0.42	509	1697	3.61	9.81	20.87	16.2	173
IV-A-2	9.8	0.49	0.43	473	1284	2.62	11.67	23.82	23.9	110
IV-A-3	8.6	0.45	0.45	435	1787	3.97	10.83	24.07	15.7	165
IV-A-4	8.5	0.45	0.45	392	898	2.00	6.91	15.36	31.6	130
IV-A-5	8.9	0.46	0.37	564	2313	5.03	13.69	29.76	17.1	169
					Mean	3.45	Mean	22.78		
					S.E. ±	0.53	S.E. ±	2.34		
		B. Second Dye Injection--Thirty Minutes Later--Following Sarveroside								
IV-B-1	9.2	0.47	0.49	462	2187	4.65	11.22	23.87	15.2	195
IV-B-2	9.8	0.49	0.45	435	905	1.85	10.28	20.98	41.0	88
IV-B-3	8.6	0.45	0.48	504	949	2.11	5.45	12.11	32.0	174
IV-B-4	8.5	0.45	0.45	376	981	2.18	6.86	15.24	37.4	143
IV-B-5	8.9	0.46	0.38	564	638	1.39	4.31	9.37	59.5	148
					Mean	2.44	Mean	16.31		
					S.E. ±	0.78	S.E. ±	3.58		
		t - differences				1.23		1.51		

* Derived from plasma dye concentration.

DISCUSSION

The reproducibility of values for cardiac output was of paramount importance in this investigation due to lack of evidence in the literature to substantiate that two successive injections of the dye T-1824, thirty minutes apart, would give similar values for cardiac output. The results of the experiments in Group I strongly indicate that the Stewart-Hamilton dye method for determining cardiac output gives consistently similar results under the conditions in which these experiments were carried out. The hematocrit values obtained from successive determinations were practically unchanged. Plasma volumes and clearance times generally showed little variation between the first and second dye injections. A comparison of heart rates during the first dye injection with those just prior to drug administration show no consistent pattern. These findings have been taken to indicate that no major changes occurred in cardiovascular status of these dogs during the time of collection of the arterial samples used in the determinations.

The increase in hematocrit seen in Group II after injection of epinephrine is explained by the action of this agent in contracting the spleen and therefore increasing the cell-plasma ratio (Sollmann, 1950; Kaltreider, Meneely and Allen, 1942; and Ahlquist, et al., 1954). Large doses of epinephrine generally cause a decrease in plasma volume (Kaltreider, Meneely and Allen, 1942; and Sollmann, 1950). Small intravenous doses of epinephrine, of the order employed in these experiments, generally enhance the cardiac output (Goodman and Gilman, 1941; and Hamilton, 1932a), and this in turn would increase the effective plasma volume. All the dogs in this group showed an increase in plasma volume while under the influence of this dose of epinephrine. With an increase in cardiac output of the magnitude shown in some of these experiments, it is not surprising to see a substantial decrease in the clearance time because the velocity of flow is also increased at this time. It has been shown that epinephrine stimulates the myocardium, and that this results in a more forceful cardiac systole which increases the stroke volume of the heart (Goodman and Gilman, 1944). Stroke volumes were increased in every experiment in Group II which indicates that the force of contraction was increased along with the increase in output. It would have been desirable to have determined the heart rate immediately prior to the epinephrine injection for comparison with the drug effects on rate. The magnitude of the stroke volume increase in some instances accounted for the increased cardiac output, because rate changes of the heart were insufficient. In other cases the only explanation for the change could be an increase or decrease in rate.

In six out of seven experiments following the injection of two micrograms per kilogram of l-norepinephrine, it was shown that there was little if any change in the hematocrit. This is in contrast to the results seen in the epinephrine experiments. A possible explanation for the absence of an increase in hematocrit in these experiments is that this dose of l-norepinephrine is evidently not effective in causing severe contraction of areas in which the red corpuscles are sequestered under pentobarbital anesthesia. Alquist (1954) found that epinephrine is significantly more effective in contracting the spleen than l-norepinephrine. The literature on the effects of circulating epinephrine and norepinephrine in the dog reveals a variety of findings. The pressor response which follows shortly after the injection of epinephrine or norepinephrine is greater for norepinephrine (Tainter and Lands, 1953; Ahlquist, 1950; and Ahlquist, et al., 1954). This difference has been found to be due to a greater vasodilator action of epinephrine which tends to reduce the total peripheral resistance (Ahlquist, 1950). Wakim and Essex (1952) found no significant difference in arterial blood pressure, heart rate or blood flow with identical doses of these two amines (dose varied from 0.01 to 10 ug./kg.). They also showed that immediately before the maximum increase in arterial blood pressure after intravenous injection of both norepinephrine and epinephrine, there was a transient increase in blood flow of several hundred per cent accompanying the augmented force of heart contraction and cardiac output. This was attributed to the direct stimulation of the myocardium by these agents. Grant and Lands (1950) believe the difference in effect demonstrated by epinephrine and norepinephrine are of a quantitative rather than a qualitative nature. This opinion is substantiated by Jochim (1952), Wakim and Essex (1952), and Zanetti and Opdyke (1953). In view of the opinions given above it seems likely that if the total peripheral resistance is augmented for norepinephrine after the period of extreme cardiac stimulation, then the cardiac output should not increase as much as for epinephrine which causes more vasodilation. The cardiac indexes in Group III are not significantly decreased as a whole but do show a trend in this direction. This is interpreted to mean that at this dose level and time of arterial sampling after injection of l-norepinephrine, the values for cardiac output were determined at a time when the peripheral resistance was relatively high and/or there was a change in heart rate. The data collected are insufficient to make any definite conclusions as to the cause of the fall in cardiac indexes and stroke volumes in this series of experiments. It should be pointed out that neither blood pressure nor peripheral resistance were measured, and that these measurements are absolutely necessary in order to determine the mechanisms involved in cardiac output and stroke volume during drug action. It is believed by Zanetti and Opdyke (1953) that the flow response to a pressor amine depends on the initial cardiac and vasomotor status of the dog prior to injection. It has been shown by Tainter and Lands (1953) that nor-epinephrine injected intramuscularly in humans consistently elicits a bradycardia. This is also generally the case for the dog although it varies greatly and is probably due to less vagal control of the heart, or greater sensitivity to stimulatory action of norepinephrine. In the experiments reported here this variation in heart rate is also demonstrated (see appendix).

The failing heart responds to intravenous administration of Strophanthus with an increase in cardiac output due to direct stimulation of the myocardium producing longer and more powerful contractions. This longer contraction is responsible for greater filling of the heart which aids in reducing a high venous pressure (if initially present). The heart rate before administration of Strophanthus is usually very rapid due to the increase in venous and intra-auricular pressure which is often present in failure. This increase in pressure excites the Bainbridge reflex which lowers vagal tone, and increases the heart rate in an attempt to eliminate the back-log of venous blood. An increase in heart rate further weakens the failing heart and a vicious circle results. Strophanthus, by increasing the cardiac output, causes a fall in venous pressure which slows the heart and the circulation is restored to a more normal level (Sollmann, 1950). Cattell and Gold (1938) by removing all extraneous factors controlling the papillary muscle of the cat showed that ouabain or digitoxin produced a variable but marked increase in force of contraction indicating that these extraneous factors were non-essential in explaining the increase in myocardial contraction of the failing cat heart muscle. This is substantiated by the work of Lee (1953) who further pointed out that ouabain increased the force of contraction of cat papillary muscle without a concurrent increase in oxygen consumption. This evidence for direct action of the cardiac glycosides on the myocardium helps us to understand the over-all effect of these agents on the entire cardiovascular system including influences upon heart rate, diastolic size, peripheral resistance, venous return and coronary flow.

The effects of cardiac glycosides (e.g., sarveroside, which was used in this experiment) on the intact dog heart is entirely different from those on the failing heart or isolated cat papillary muscle.

Page, et al. (1951), showed that 0.037 milligrams per kilogram of ouabain in the intact anesthetized dog decreased the coronary flow, pulse rate and cardiac output, and increased the arterial blood pressure, total peripheral resistance and stroke work. The increase in blood pressure was transient and leveled off in approximately fifteen minutes. This group used the Fick method to determine cardiac output. Successive determinations prior to and after thirty minutes from the time of injection of ouabain gave no significant results ($t = 0.68$) for cardiac output. However, when the dose was reduced to 0.026 milligrams per kilogram the values for cardiac output were increased significantly over the controls. Previous to this work many other investigators (Harrison and Leonard, 1926; Dock and Tainter, 1930; Tainter and Dock, 1930; Bing, et al. 1950; and McMichael and Sharpey-Schafer, 1944), using full therapeutic doses of various glycosides, showed similar results marked by a fall in cardiac output in the normal intact anesthetized dog.

The results in Group IV and V of this research with sarveroside are in agreement with the results obtained by the above investigators for changes in cardiac output following the injection of other glycosides. The present experiments are the first of this kind ever to be performed with sarveroside. All dogs in Group IV demonstrated a decrease in heart rate which agrees with the findings of Page, et al. (1951), while those in Group V varied considerably. Dock and Tainter (1930) account for the fall in cardiac output in their experiments by the fact that the spleen and liver both increased in volume. Since this would tend to reduce the effective plasma volume, this may account for low values for plasma volume obtained in some of the experiments reported here. The heart, when working against a higher pressure which is probably the case in these experiments, finds it more difficult to maintain a respectable cardiac output. This is particularly true if the blood volume decreases and thus reduces venous return as Dock and Tainter (1930) suggest. During cardiac decompensation the circulation time often increases to levels of thirty-one to fifty-four seconds (McMichael, 1948). When the cardiac output decreases to the extent that it has in some of the experiments reported here, then it seems logical to assume that this may really be a temporary cardiac decompensation. This is indicated by the fact that in the cases showing the largest fall in cardiac output, the clearance time for the dye was increased. This is the expected direction of change of the circulation time during cardiac decompensation.

SUMMARY AND CONCLUSIONS

Evidence has been presented which suggests that the Stewart-Hamilton dye method for determining cardiac output is a feasible pharmacological laboratory procedure for studying the effects of a cardiotonic agent on the output of the heart in the dog. The application of the dye-dilution method to the study of three cardiotonic agents i.e., epinephrine, norepinephrine and sarveroside, has given comparable measurements for cardiac output and plasma volume compared to data in the literature.

The following conclusions have been reached:

1) There is essentially no difference in the values obtained for cardiac output in parts A and B of Group I. This indicates that two successive runs can be made on the same animal with minimal variation.

2) When approximately two micrograms per kilogram body weight of a commercial epinephrine solution were administered intravenously from 4.5 to 9.5 seconds prior to collection of arterial blood samples for cardiac output, there was generally a substantial increase in cardiac output in the intact anesthetized dog.

3) On intravenous administration of two micrograms per kilogram body weight of norepinephrine, 5.5 seconds prior to cardiac output measurement, there was generally a slight decrease in cardiac output but this was not significant.

4) The dose of sarveroside (0.08 mg./kg.), used in these experiments and given 1.5 minutes prior to collection of the arterial blood for cardiac output, was found to decrease heart output profoundly in five dogs tested. When the heart output was measured five minutes after the drug was given, Group V, the results showed some variation but were not significantly different from the control values.

5) The average plasma volume calculated from a single eight minute sample for thirty dogs was found to be 48.83 cc. per kg. body weight. This figure agrees nicely with the accepted value of 50.0 cc. per kg. body weight (Gregersen, 1954).

6) The average cardiac index for the thirty dogs used in these experiments was 3.9 liters per minute per square meter of body surface. This value is slightly high compared to the average value in the literature.

7) The dye-dilution method is a feasible technique for studying certain cardiac effects of drugs in the intact anesthetized dog.

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APPENDIX

A. Statistics Formulae

1. Standard Deviation of The Differences

$$\sigma_d = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{n - 1}}$$

2. Standard Error of The Differences

$$\sigma_{\bar{d}} = \frac{\sigma}{\sqrt{n}}$$

3. t - Test of The Differences

$$t = \frac{\bar{d} - o}{\sigma_{\bar{d}}}$$

CHANGES IN HEART RATE DURING EXPERIMENTS

Dog Number	Heart Rates (Beats Per Minute)			
	Control	During First Dye Injection	Pre-Drug	During Second Dye Injection
		Group III l-Norepinephrine		
1	120	111	168	153
2	160	148	120	120
3	192	192	180	195
4	168	188	160	166
5	140	145	140	145
6	111	115	150	128
7	144	142	144	165
		Group IV Sarveroside		
1	120	120	140	127
2	136	133	135	130
3	144	144	136	114
4	140	146	140	120
5	180	180	173	166
		Group V Sarveroside		
1	168	173	200	195
2	111	110	88	88
3	167	165	174	174
4	128	130	136	143
5	172	169	156	148

RAW DATA FOR TIME CONCENTRATION CURVES

A. Controls

Dog No. 1A		Dog No. 1B		Dog No. 2A		Dog No. 2B	
Wt. 19.3 kg.				Wt. 12.1 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0	2	0.00740	2	0.00935
4	0.02691	4	0.01285	4	0.03050	4	0.02740
6	0.02445	6	0.03010	6	0.01590	6	0.00885
8	0.00531	8	0.01265	8	0.00310	8	0.00280
10	0.00219	10	0.00215	10	0.00390	10	0.00415
12	0.00630	12	0.00315	12	0.00675	12	0.00675
15	0.00999	15	0.00740	15	0.00705	15	0.00795
18	0.00789	18	0.01075	18	0.00615	18	0.00660
21	0.00660	21	0.00860	21	0.00640	21	0.00640
24	0.00645	24	0.00705	24	--	24	--

Dog No. 3A		Dog No. 3B		Dog No. 4A		Dog No. 4B	
Wt. 14.0 kg.				Wt. 11.0 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0	2	0	2	0
4	0.00309	4	0.01320	4	0.00570	4	0.00225
6	0.01815	6	0.02740	6	0.02964	6	0.02840
8	0.07301	8	0.02065	8	0.03276	8	0.03485
10	0.01290	10	0.00860	10	0.01815	10	0.01875
12	0.00675	12	0.00355	12	0.00747	12	0.00835
15	0.00330	15	0.00300	15	0.00336	15	0.00480
18	0.00438	18	0.00465	18	0.00423	18	0.00338
21	0.00504	21	0.00535	21	0.00546	21	0.00513
24	0.00504	24	0.00500	24	0.00576	24	0.00588

Dog No. 5A		Dog No. 5B		Dog No. 6A		Dog No. 6B	
Wt. 13.6 kg.				Wt. 11.0 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0	2	0	2	0
4	0.01560	4	0.00444	4	0.02901	4	0.00195
6	0.03855	6	0.02940	6	0.03087	6	0.02703
8	0.02601	8	0.02979	8	0.00900	8	0.02940
10	0.01224	10	0.02025	10	0.00249	10	0.01659
12	0.00720	12	0.01191	12	0.00324	12	0.00753
15	0.01101	15	0.00639	15	0.00723	15	0.00567
18	0.01101	18	0.00794	18	0.00744	18	0.00801
21	0.01101	21	0.01059	21	--	21	--
24	0.01125	24	0.01059	24	--	24	--

B. Commercial Epinephrine

Dog No. I-A-1		Dog No. I-B-1		Dog No. I-A-2		Dog No. I-B-2	
Wt. 10.5 kg. Dose 1.9 ug/kg.				Wt. 13.6 kg. Dose 1.5 ug/kg.			
Duration with Dye				Duration with Dye			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0.00060	2	0	2	0	2	0
4	0.02250	4	0.00750	4	0.00504	4	0.00528
6	0.03645	6	0.03102	6	0.03105	6	0.02706
8	0.02751	8	0.02664	8	0.04155	8	0.03420
10	0.01089	10	0.01506	10	0.03525	10	0.03261
12	0.00576	12	0.00504	12	0.02415	12	0.02706
15	0.00660	15	0.00639	15	0.01164	15	0.01452
18	0.00699	18	0.00519	18	0.01164	18	0.01038
21	0.00831	21	0.00546	21	0.01203	21	0.00807
24	0.00999	24	0.00546	24	0.01191	24	0.00591

Dog No. I-A-3		Dog No. I-B-3		Dog No. I-A-4		Dog No. I-B-4	
Wt. 25.0 kg. Dose 2 ug/kg.				Wt. 11.8 kg. Dose 1.7 ug/kg.			
Duration 5.0 Sec.				Duration 7.0 Sec.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0	2	0	2	0
4	0.00816	4	0.00669	4	0.02100	4	0.01731
6	0.01530	6	0.01800	6	0.03066	6	0.02289
8	0.01914	8	0.00609	8	0.01056	8	0.00570
10	0.00746	10	0.00171	10	0.00315	10	0.00354
12	0.00336	12	0.00645	12	0.00279	12	0.00432
15	0.00240	15	0.00759	15	0.00516	15	0.00786
18	0.00528	18	0.00576	18	0.00531	18	0.00759
21	0.00675	21	0.00528	21	0.00579	21	0.00699
24	0.00621	24	0.00570	24	0.00624	24	0.00594

Dog No. I-A-5		Dog No. I-B-5		Dog No. I-A-6		Dog No. I-B-6	
Wt. 7.6 kg. Dose 2.6 ug/kg.				Wt. 10.6 kg. Dose 1.9 ug/kg.			
Duration 4.5 Sec.				Duration 9.0 Sec.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0	2	0.00099	2	0.00129
4	0.02466	4	0.01839	4	0.01587	4	0.03225
6	0.04560	6	0.03420	6	0.03990	6	0.02589
8	0.03795	8	0.02976	8	0.02529	8	0.00639
10	0.01815	10	0.01590	10	0.00837	10	0.00408
12	0.00783	12	0.01155	12	0.00426	12	0.00600
15	0.00981	15	0.01086	15	0.00729	15	0.01061
18	0.01359	18	0.01014	18	0.00654	18	0.00963
21	0.01197	21	0.01206	21	0.00591	21	0.00774
24	0.01041	24	0.01170	24	0.00714	24	0.00900

Continued

Dog No. I-A-7		Dog No. I-B-7	
Wt. 13.7 kg. Dose 1.5 ug/kg. Duration 9.5 Sec.			
Seconds	Mg./ml.	Seconds	Mg./ml.
2	0.00294	2	0
4	0.00720	4	0.03063
6	0.02091	6	0.01965
8	0.01674	8	0.01107
10	0.00954	10	0.00498
12	0.00615	12	0.00567
15	0.00537	15	0.00894
18	0.00639	18	0.00837
21	0.00639	21	0.00846
24	--	24	--

C. l-Norepinephrine.

Dose 2.0 ug. /kg. Duration 5.5 Sec.

Dog No. II-A-1		Dog No. II-B-1		Dog No. II-A-2		Dog No. II-B-2	
Wt. 9.0 kg.				Wt. 10.0 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0	2	0	2	0
4	0	4	0.00159	4	0.00423	4	0
6	0.00069	6	0.01905	6	0.03039	6	0.00309
8	0.02115	8	0.02364	8	0.02478	8	0.02478
10	0.03270	10	0.02265	10	0.00792	10	0.02877
12	0.02889	12	0.01428	12	0.00315	12	0.01599
15	0.01506	15	0.00894	15	0.00540	15	0.00894
18	0.00693	18	0.00816	18	0.00774	18	0.00591
21	0.00597	21	0.00909	21	0.00876	21	0.00645
24	--	24	--	24	--	24	--

Dog No. II-A-3		Dog No. II-B-3		Dog No. II-A-4		Dog No. II-B-4	
Wt. 14.1 kg.				Wt. 11.5 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0	2	0	2	0
4	0.01611	4	0.01653	4	0.00045	4	0
6	0.02151	6	0.01584	6	0.02250	6	0.00189
8	0.00585	8	0.00513	8	0.03315	8	0.01542
10	0.00195	10	0.00345	10	0.02403	10	0.02502
12	0.00279	12	0.00393	12	0.01155	12	0.02454
15	0.00444	15	0.00540	15	0.00546	15	0.01776
18	0.00486	18	0.00498	18	0.00546	18	0.01242
21	0.00468	21	--	21	0.00816	21	0.00963
24	--	24	--	24	--	25	--

Dog No. II-A-5		Dog No. II-B-5		Dog No. II-A-6		Dog No. II-B-6	
Wt. 13.2 kg.				Wt. 12.0 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0	2	0	2	0
4	0	4	0	4	0.00045	4	0.00228
6	0.01335	6	0.01233	6	0.01551	6	0.02766
8	0.02214	8	0.02325	8	0.02790	8	0.02190
10	0.01224	10	0.01233	10	0.01428	10	0.00708
12	0.00435	12	0.00528	12	0.00414	12	0.00399
15	0.00213	15	0.00378	15	0.00201	15	0.00336
18	0.00372	18	0.00591	18	0.00420	18	0.00315
21	0.00459	21	0.00708	21	0.00489	21	0.00498
24	--	24	--	24	--	24	--

Continued

Dog No. II-A-7		Dog No. II-B-7	
Wt. 14.7 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0
4	0	4	0
6	0.00219	6	0.00729
8	0.01521	8	0.02310
10	0.02190	10	0.01752
12	0.01512	12	0.01056
15	0.00714	15	0.00597
18	0.00321	18	0.00294
21	0.00378	21	0.00234
24	--	24	--

D. Sarveroside

Dose 0.08 mg./kg. Duration 1.5 Min.

Dog No. III-A-1		Dog No. III-B-1		Dog No. III-A-2		Dog No. III-B-2	
Wt. 10.4 kg.				Wt. 11.5 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0	2	0	2	0
4	0.00075	4	0	4	0.00015	4	0
6	0.02640	6	0.00540	6	0.01929	6	0.00381
8	0.02925	8	0.02664	8	0.02190	8	0.01674
10	0.01155	10	0.03075	10	0.00894	10	0.02427
12	0.00351	12	0.02100	12	0.00378	12	0.02340
15	0.00384	15	0.00969	15	0.00225	15	0.01542
18	0.00546	18	0.00546	18	0.00408	18	0.00837
21	--	21	0.00852	21	0.00507	21	0.00549
24	--	24	--	24	--	24	0.00561

Dog No. III-A-3		Dog No. III-B-3		Dog No. III-A-4		Dog No. III-B-4	
Wt. 11.0 kg.				Wt. 11.0 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0	2	0	2	0
4	0.00489	4	0	4	0	4	0
6	0.03150	6	0.00111	6	0.00498	6	0
8	0.01914	8	0.01281	8	0.02151	8	0
10	0.00552	10	0.02379	10	0.02889	10	0.00051
12	0.00249	12	0.02502	12	0.02379	12	0.00534
15	0.00336	15	0.01713	15	0.01218	15	0.01890
18	0.00597	18	0.00939	18	0.00639	18	0.02739
21	--	21	0.00624	21	0.00561	21	0.02841
24	--	24	0.00690	24	0.00639	24	0.02364
				27	0.00615	27	0.01953

Dog No. III-A-5		Dog No. III-B-5	
Wt. 9.1 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0
4	0.03546	4	0
6	0.02928	6	0.00909
8	0.00684	8	0.03345
10	0.00345	10	0.03099
12	0.00528	12	0.02076
15	--	15	0.00984
18	--	18	0.00690
21	--	21	--
24	--	24	--

E. Sarveroside

Dose 0.08 Mg./kg. Duration 5.0 Min.

Dog No. IV-A-1		Dog No. IV-B-1		Dog. No. IV-A-2		Dog No. IV-B-2	
Wt. 9.2 kg.				Wt. 9.8 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0	2	0	2	0
4	0.01107	4	0.02553	4	0	4	0
6	0.04005	6	0.03321	6	0.00075	6	0
8	0.02703	8	0.01605	8	0.01464	8	0.00069
10	0.00909	10	0.00534	10	0.03408	10	0.01326
12	0.00429	12	0.00498	12	0.03099	12	0.02952
15	0.00759	15	0.00894	15	0.01722	15	0.03099
18	0.00852	18	0.00969	18	0.00744	18	0.02226
21	--	21	--	21	0.00582	21	0.01437
24	--	24	--	24	--	24	0.00954

Dog No. IV-A-3		Dog No. IV-B-3		Dog No. IV-A-4		Dog No. IV-B-4	
Wt. 8.6 kg.				Wt. 8.5 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.	Seconds	Mg./ml	Seconds	Mg./ml
2	0	2	0	2	0	2	0
4	0.01899	4	0	4	0	4	0
6	0.03990	6	0.00213	6	0.00165	6	0.01188
8	0.02238	8	0.01404	8	0.02337	8	0.03075
10	0.00684	10	0.03126	10	0.03918	10	0.03345
12	0.00279	12	0.03705	12	0.03630	12	0.02700
15	0.00537	15	0.03186	15	0.02565	15	0.01629
18	--	18	0.01596	18	0.01404	18	0.00969
21	--	21	0.00900	21	0.01209	21	0.01059
24	--	24	0.00846	24	--	24	--

Dog No. IV-A-5		Dog No. IV-B-5	
Wt. 8.9 kg.			
Seconds	Mg./ml.	Seconds	Mg./ml.
2	0	2	0
4	0.00636	4	0
6	0.02901	6	0.00183
8	0.01785	8	0.01605
10	0.00699	10	0.02802
12	0.00321	12	0.02862
15	0.00507	15	0.0249
18	--	18	0.01914
21	--	21	0.01497
24	--	24	0.01272