THE DYNAMICS OF EPINEPHRINE CIRCULATION AND RENAL EXCRETION

by

Richard T. Jones, B.S.

A THESIS

Presented to the Department of Physiology
and the Graduate Division of the University of Oregon Medical School
in partial fulfillment
of the requirements for the degree of
Master of Science

June 1956

APPROVED:

[redacted]

(Professor in Charge of Thesis)

[redacted]

(Chairman, Graduate Council)

ACKNOWLEDGEMENT

The author wishes to express his appreciation to Dr. William Blake for his constant help and advice during the investigation and writing of this thesis. Advice and suggestions from Drs. John Brookhart, Carl Hopkins, Benjamin Ross, and Agnar Straumfjord and technical assistance from Miss Gladys Skoch are also gratefully acknowledged.

The epinephrine and norepinephrine bitartrate standards were contributed by Winthrop-Stearns, Inc. of New York City. The U.S.P. thyroid extract was donated by Naack Laboratories, Inc., of Portland, Oregon.

The work of this thesis was supported financially by the Life Insurance Medical Research Fund.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The sympatho-adrenal system has been a much-discussed and studied subject in physiology. Its popularity, in part, may be due to the multiplicity of its actions and effects and its wide distribution throughout the animal kingdom. Not only does it modify the activity of smooth muscle, but it also influences the metabolism of carbohydrates, fats and pigments, to mention only a few examples. (1-7) The role of this system in homeostatic mechanisms was one of Walter Cannon's most important contributions. His work and that of others not only helped to correlate and unify much of the previous knowledge but have also given impetus to further study of this system.

Investigation in the past ten years with a background of earlier experimentation has clarified, almost beyond all doubt, the nature of the chemical and mediators of this system. Extensive studies of the chemical and biological properties of these mediators demonstrate them to be two varieties of B-catechol-ethanolamines:(8,9) epinephrine and norepinephrine.

Though epinephrine was discovered, isolated and chemically characterized over 50 years ago, there still remains today many unanswered questions regarding its hormonal properties. Prior to the past ten years most of the studies relevant to the role of the sympatho-adrenal system and its mediators were of a qualitative nature based upon interpretations of various biological responses. In more recent years, interest has developed in the quantitative measurement of these mediators under normal and altered conditions. Because of this interest, techniques for quantitatively estimating these substances have been developed.

The classical definition of hormones according to Oscar Hechter (10) states that "hormones are discrete chemical substances produced in an organ or tissue, which are discharged into the circulating fluids and in minute concentrations markedly influence the function of other organs or tissues". This definition provides several interesting problems regarding the presence and fate of epinephrine in these "circulating fluids". The manner in which this hormone is dispersed throughout the fluids, as well as the relationship of the hormone concentration to its input and disappearance from these fluids and the mechanisms of its removal from circulation, are of fundamental importance to the understanding of its biological activity and function.

Hecter's definition, however, points to a complicating problem, namely that epinephrine, like other hormones, is active in minute quantities. As a reflection of this, the best estimates of normal plasma epinephrine and norepinephrine concentrations are in the range of 0.01 to 0.5 micrograms of hormone per 100 ml. of plasma. If samples are taken at normal or even 100 times elevated epinephrine concentrations, the assay methods must be sensitive to fractions of micrograms of material in order to be useful. Furthermore, there are several other naturally occurring catechol amines and closely related compounds which have certain biological and chemical properties similar to the mediators of the sympatho-adrenal system. Therefore, any assay method to be useful must also be very specific for epinephrine and norepinephrine.

These stringent requirements for sensitivity and specificity have limited quantitative study of the problems regarding the mechanisms influencing the circulation and excretion of epinephrine. The development of a useful assay method and its application to the study of these

problems of the dynamics of epinephrine circulation and renal excretion under normal and hyperthyroid conditions have been the objectives of this thesis work.

REVIEW OF THE LITERATURE

The review of the literature has been separated into two divisions. The first part deals with methods for the quantitative estimation of epinephrine and norepinephrine. In the remaining portion the literature pertaining to questions regarding the dynamics of epinephrine circulation and its renal excretion has been reviewed. In addition, the relation of the sympatho-adrenal system and hyperthyroidism has also been discussed.

I. Epinephrine and Norepinephrine Assay Methods.

The quantitative measurement of any substance present in a biological system encompasses two important considerations. The first consideration deals with the isolation of the substance under study from the biological system in a form suitable for measurement. The second consideration concerns the quantitative measurement of a graded response of the measuring system to certain relatively unique properties of the substance under study. Detection procedures have been discussed first since, in the case of epinephrine and norepinephrine, they are of primary importance to the overall methods.

Presently there are two groups of techniques available for the detection of epinephrine and norepinephrine, namely the biological and the chemical assays. Bioassays depend upon the graded biological responses of certain animal tissues or systems to relatively specific properties of the assayed substance. The responses of several different systems reflecting the biological activity of epinephrine and norepinephrine have been used including: blood pressure alterations in the dog, cat, and rabbit (12-14), inhibition of gastrointestinal mobility (14-16),

response of the nictitating membrane (17-19), contraction of uterine muscle (17-18), and activity of the isolated frog's heart (20). No attempt has been made here to survey all of these biological methods; rather a procedure currently popularized by U.S. von Euler has been discussed (16,20). This bio-assay exemplifies many of the important limitations common to the other biological methods.

Euler's method is applicable to the estimation of both epinephrine and norepinephrine either alone or together. The assay utilizes changes in the blood pressure of cats and relaxation of the hen's rectal caecum produced by these compounds. A differential analysis can be obtained, since mechanisms regulating the cat's blood pressure are five times more sensitive to norepinephrine than to epinephrine, while epinephrine is sixty times more active in relaxing the hen's rectal caecum. Absolute quantitative expressions of the hormone content of extracts of samples are obtained by comparing the responses of these systems to the extracts and to standard solutions of epinephrine and norepinephrine. The exact mathematical expressions used for the calculation of hormone concentrations have not been listed here but may be found in several references (16,20). Suffice it to say, this differential analysis depends upon at least six separate response measurements: the response of the intestinal motility and blood pressure each to the extract and to the standard solutions of both epinephrine and norepinephrine. Each of these measurements probably varies at least ± 10%. The relative activities of the test systems vary with different preparations and are not even constant in a given preparation. Therefore, the estimation of hormone concentrations is subject to several sources of error.

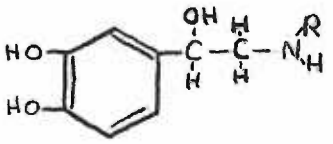
The lower limits of the sensitivity of the cat's blood pressure are in the range of 0.2 to 0.4 micrograms of norepinephrine, while the gut is sensitive to as little as 0.3 micrograms of epinephrine. Such amounts of these catechol amines can be obtained from concentrated extracts of large volumes of urine and certain tissues; however, a liter or more of plasma would be required to obtain enough material for analysis by these bio-assay techniques. Such sample sizes are not possible in most physiological studies. Furthermore these biological responses of the test tissues are not entirely specific for epinephrine and norepinephrine. Other naturally occurring substances like histamine, serotonin, and epinephrine-like compounds including hydroxytyramine, may produce significant responses in certain circumstances (20-22). Therefore, the specificity of these methods becomes dependent upon the isolation or extraction techniques. These limitations are also common to the other bio-assay techniques. Thus it becomes clear that the applicability of these bio-assay methods to the study of plasma epinephrine and norepinephrine becomes difficult because of their relatively low sensitivity and specificity.

Chemical detection methods are dependent upon the measurement of certain physical or chemical properties of the substances under study. The specificity of these chemical procedures is related to the uniqueness of the properties of the substance relative to other materials present in the test system. The sensitivity of these methods is a function of the relative magnitude of the physical or chemical property and the power of the measuring technique.

Several chemical and physical chemical methods have been developed for the quantitative estimation of epinephrine and/or norepinephrine

including: differential solubilities, (23) colorimetric estimation of compounds formed by reactions with iodine, (24,25) arsenomolybdic acid, (26,27) 3-naphthoquinone-4-sulfonic acid and benzalkonium chloride, (28), and several fluorimetric methods (29-38). Many of these colorimetric and fluorimetric techniques are more than 1000 times as sensitive as the best bio-assay procedure. A comprehensive review of these chemical methods has been given by Persky (21). The fluorimetric techniques are the most sensitive of all the procedures available, being powerful enough to detect the normal concentration of epinephrine and norepinephrine in plasma. Presently there are two essentially different fluorimetric methods which are useful. The first method depends upon the fluorescence of the compounds formed by the condensation of ethylenediamine with epinephrine and norepinephrine (32,35). These two products have different fluorescent spectra; therefore, a differential analysis can be obtained by comparing the measurement of the sample at the peak fluorescence of each compound with the measurements of standard epinephrine and norepinephrine solutions. This detection technique is sensitive to as little as 2 to 20 millimicrograms of material; however it lacks specificity. Many other catechol derivatives including hydroxytyramine, dopa, and catechol acetic acid also form fluorescing condensation products. Chr. von Euler et al have demonstrated that very large quantities of catechol acetic acid appear in the urine and therefore make this technique unsuitable for estimating epinephrine and norepinephrine in urine (39). Recently Price et al (40) have demonstrated that this condensation technique is not suitable for plasma estimations either because of the presence of significant amounts of interfering catechol derivatives.

The second fluorimetric detection technique depends upon the oxidation of epinephrine and norepinephrine to adrenochrome and noradrenochrome. These adrenochromes fluoresce strongly in alkaline solutions when irradiated with ultraviolet light. The fluorescence of oxidised epinephrine was noted as early as 1918 by Loewi (41); however, it was not until the 1940's that this fluorescent property was utilised for assay techniques. Lund (31, 42-44) was one of the first to effectively utilise these fluorescent properties to develop a relatively simple and rapid procedure for the measurement of these substances in plasma as well as in other fluids and tissue extracts. His method is sensitive to as little as 2 to 200 millimicrograms of epinephrine or norepinephrine. It is specific for B-catechol ethanolamines with the structural variations shown below according to the findings of Lund (44) which are consistent



where R may be hydrogen as in norepinephrine
or CH_3 as in epinephrine
or $CH(CH_3)_2$ as in isopropylarterenol

with theoretical considerations (45). The fluorescent spectra of these alkalinized adrenochromes are very similar, with fluorescent maxima in the range of 500 to 550 (43,46). Differentiation between primary and secondary amines is possible because of varying rates of oxidative ring closure at different pH's. At present norepinephrine and epinephrine are the only B-catechol ethanolamines which have been found to occur naturally in mammals, with the exception of very small amounts of isopropylnorepinephrine recently reported to be present in adrenal extracts (47).

In brief summary, Lund's method for the simultaneous determination of epinephrine and norepinephrine is as follows. Both catechol amines are oxidized to their respective adrenochromes with manganese dioxide. Epinephrine is easily oxidized at neutral and acid pH's, while norepinephrine is only oxidized appreciably at neutral pH's. The adrenochromes are converted to the fluorescent adrenolutins by the addition of strong alkali, and further oxidation is prevented by the simultaneous addition of ascorbic acid. The fluorescent intensities of two samples oxidized at a neutral and an acid pH are measured. The background fluorescence is determined in similar oxidized samples in which ascorbic acid is added several minutes after alkalinizing the adrenochromes. In the absence of ascorbic acid the alkalinized adrenochromes are irreversibly oxidized further to non-fluorescing compounds thus leaving only background fluorescence in these blanks. The amount of epinephrine and norepinephrine in the samples can be determined by comparing their fluorescence measured with a photo tube with standard solutions of epinephrine and norepinephrine.

The isolation of epinephrine and norepinephrine in suitable concentrations for their measurement by the detection methods has been accomplished in several ways. In general, untreated plasma, urine or crude tissue extracts cannot be used directly in any of the assay methods because of the presence of many interfering substances. Protein free extracts have been used; however, protein precipitation methods appear to bind irreversibly significant amounts of the catechol amines (34). In 1938 Shaw introduced a useful technique for the adsorption of the catechol amines onto aluminum hydroxide (48). Lund found that epinephrine and norepinephrine could be adsorbed onto a column of aluminum oxide at

neutral pH's, contaminants washed off with neutral buffer and the catechols then removed by an acid elution (44). This isolation technique appears to be quite specific for catechols and is employed for both biological and chemical methods (44,49).

II. Literature Regarding Dynamics of Epinephrine Circulation and Renal Excretion in Normal and Hyperthyroid Conditions.

A. Dynamics of Epinephrine Circulation

The dynamics of epinephrine circulation deal with the forces and factors which influence and control the presence and shifts in epinephrine molecules throughout the circulating fluids. These forces and factors include the concentration of epinephrine, the rates of addition and the rates and mechanism of removal of epinephrine from these circulating fluids.

Many groups have attempted the estimation of circulating epinephrine and norepinephrine under normal conditions in a variety of animals (11,31,34,37). The normal concentrations reported vary greatly depending upon the method of assay employed. The most reliable estimates appear to be at the limit of the sensitivities of the methods. The newer fluorimetric chemical methods, however, appear to be reliable for quantitatively estimating plasma epinephrine and norepinephrine at concentrations which are two or more times greater than the normal resting level.

Several aspects regarding the dynamics of circulation of epinephrine and its removal from circulation have been studied (34,51,52). In all these cases large amounts of exogenous epinephrine were added in order to obtain plasma concentrations which could be detected. The plasma concentrations achieved were 50 to 1000 times the best estimates of resting

levels. These were unquestionably greater than the highest concentration which animals can achieve under the most stressful conditions (53). Another complicating feature of these previous studies was that the epinephrine was introduced as single rapid intravenous injections. Under such conditions the cardiovascular system of the test organisms were undoubtedly in a state of rapid alteration as a result of the physiological activity of the injected material. The simple dynamics of epinephrine circulation may well be altered by these rapid changes in the circulatory system.

These earlier techniques, however, did yield several interesting observations. Cohen (52) noted that intravenously injected epinephrine disappeared from the blood rapidly, following a hyperbolic curve. Pekkarinen (3h) and Miller and Elliott (51) confirmed these findings, but the dynamics of circulating epinephrine have not been carried further than these observations.

Many studies have been carried out to determine the sites at which epinephrine is removed from the circulation. Pekkarinen (3h) has reviewed the literature up to 1948. There is ample direct and indirect evidence that epinephrine is removed from circulation in the liver, spleen, kidney, and hind limb circulation. The mechanisms by which this epinephrine is removed are not clear, nor are the subsequent metabolism of the hormone completely understood (50,54,55).

B. Renal Excretion of Epinephrine

Epinephrine has been detected and quantitatively measured by both biological and chemical methods in the urine from a variety of mammals subjected to conditions of rest and stress (22,49,56,57,58,59). The

resting excretion rates again vary depending upon the detection methods employed. However, the average resting excretions of free hormones are in the range of 5 to 10 micrograms of epinephrine per 24 hours. According to Mann (60) free adrenaline is more stable in urine than in water even at body temperatures and at an alkaline pH. This stabilisation is dependent upon the natural presence of ascorbic acid and phosphates.

Epinephrine appears in the urine not only as the free alcohol but also chemically combined as ethereal sulfates and glucosides (24,49,56,61, 62,63). These conjugated compounds of epinephrine are neither biologically active nor can they be isolated by the aluminum oxide technique. Free hormone can be obtained from the conjugated forms by acid hydrolysis of the urine (49,56,64).

Under conditions of constant intravenous infusions of epinephrine into humans 1.5 to 3.3% of the infusion was recovered quantitatively in the urine as free hormone (65-67). The renal mechanisms by which this epinephrine is excreted have not yet been elaborated.

C. Hyperthyroidism and the Dynamics of Epinephrine Circulation and Renal Excretion

There are many clinical and experimental observations which suggest that the activity of the sympatho-adrenal system and its mediators and thyroid hormone activity may be interrelated (68). Many of the metabolic and hemodynamic alterations in experimental and spontaneous hyperthyroidism are similar to those associated with an increase in sympatho-adrenal activity (69). Recently Brewster et al (70) have observed that the hemodynamic and metabolic effects of thyrotoxicosis in dogs could be abolished if reflex release of epinephrine and norepinephrine were prevented by means of total epidural preganglionic sympathetic blocks. Furthermore, the

physiological activities of parenterally administered epinephrine were progressively increased by increases in thyroid hormone administration. The exquisite sensitivity of hyperthyroid humans had suggested earlier that the destruction of epinephrine may be decreased in hyperthyroidism (71). However, other interpretations of these observations would include the possibility of increased epinephrine and norepinephrine synthesis and release or an increased sensitivity of tissues to the sympatho-adrenal mediators. Ragoff and Cortell (72) were unable to detect any change in epinephrine output from the suprarenal glands in hyperthyroid dogs compared to normal animals. This observation appears incompatable with the thesis of increased epinephrine production in hyperthyroidism. Urinary catechol amine excretion in hyperthyroid human patients have been found not to deviate significantly from excretion in euthyroid humans (73). If renal excretion of epinephrine and norepinephrine is dependent upon blood concentrations of these hormones, then these excretion observations would not appear compatable with either the thesis of increased epinephrine production or decreased rates of epinephrine destruction.

Direct measurement of in vivo epinephrine destruction have not yet been made. Plasma epinephrine disappearance studies might be an index of these destruction rates and yield information regarding epinephrine activity in hyperthyroidism.

STATEMENT OF THESIS PROBLEMS AND THE GENERAL EXPERIMENTAL APPROACH

The problems studied in this thesis are several in variety though interrelated in many ways. A statement of these problems and the general experimental approaches chosen have been given in this section.

The first problem was that of the applicability of epinephrine and norepinephrine assay methods to the direct measurement of these compounds in small samples of plasma and urine of animals under conditions which approached or simulated physiological states. Lund's fluorimetric method was chosen as the basic technique to apply to these problems.

The second problem was the study of the dynamic of epinephrine circulation. That is, the questions of the interrelationship between the input of epinephrine into the vascular system, the plasma epinephrine concentration, and the rate of removal or disappearance of the hormone from circulating plasma. Since the endogenous production of epinephrine is small, variable, and difficult to measure accurately, its utilization as the input source was felt to be unsuitable for these studies. Constant intravenous infusions of known amounts of exogenous epinephrine were, therefore, used as the input source because of the ease of measurement and control of such a technique. The plasma epinephrine concentration was measured directly during the constant infusions and the rate of disappearance noted after stopping the infusions. Other related problems concerning the relative activity for the removal of epinephrine from circulation of several areas of the body including noncirculating plasma, and pulmonary, hind limb, liver, and portal circulation, were also studied at different rates of epinephrine infusion.

The third major problem concerned the study of the mechanism of renal excretion of circulating epinephrine. Again, in order to obtain measurable plasma concentrations of epinephrine, the technique of constant intravenous infusion was chosen. The comparison of the excretion of epinephrine to simultaneous excretions of creatinine and para amino hippurate (PAH) were selected as the method for studying renal elimination of epinephrine.

The fourth problem concerned the study of the previously mentioned parameters of epinephrine circulation and renal excretion under conditions of induced hyperthyroidism. By this study it was hoped that a clue to the relationship of the sympatho-adrenal system to thyrotoxicosis might be detected. Specifically it was wished to determine if the relation between plasma epinephrine concentrations and constant rates of epinephrine infusion might be altered in thyroid-fed animals. Determinations of the rate of disappearance of epinephrine from circulation and the renal excretion of epinephrine were also felt to be important.

METHODOLOGY

This section has been divided into two parts, the first dealing with the development of the epinephrine and norepinephrine assay procedure and the second concerning the experimental preparations and related procedures.

I. Fluorimetric Method of Epinephrine Estimation

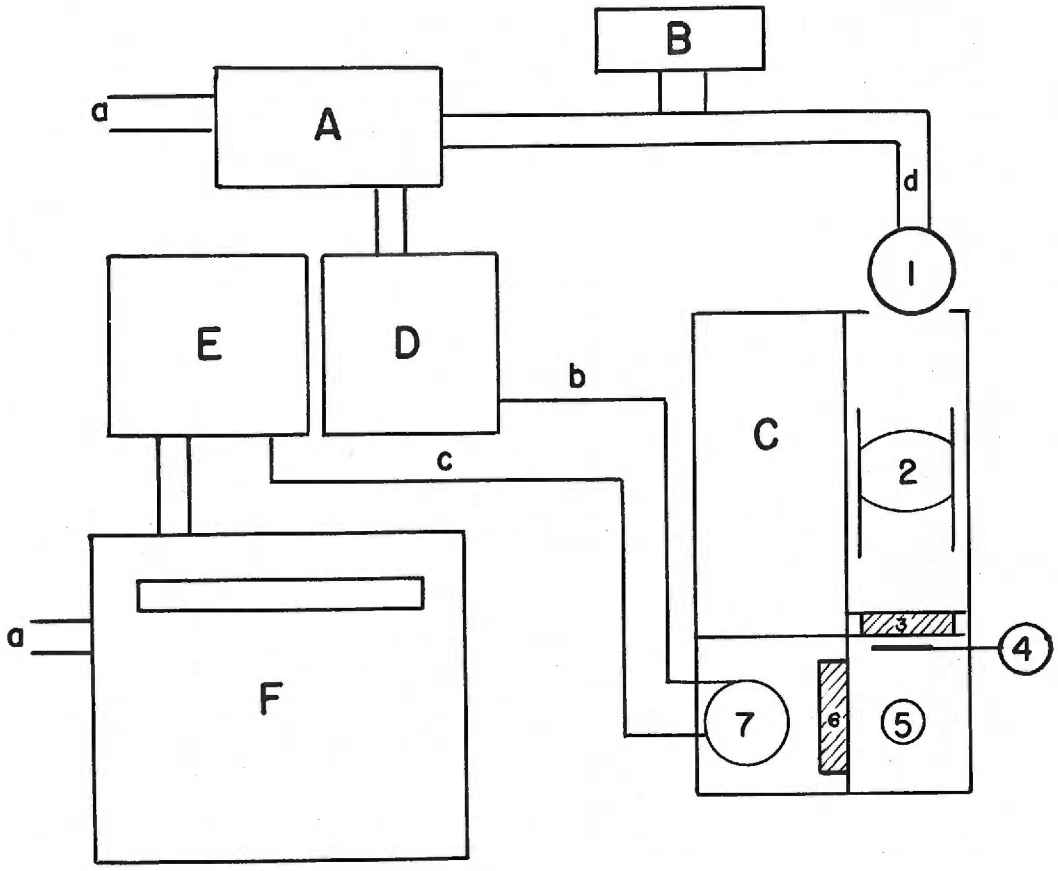
The procedures for the quantitative estimation of epinephrine and norepinephrine were designed after the methods of Alf Lund (31,42-44) which have already been outlined and reviewed. The basic components of the final fluorimeter assembly are shown in Figure 1. The sample chamber was irradiated by ultraviolet light from a General Electric H 100 A4 lamp. The 365 millimicron wave length of light was selectively transmitted into the sample chamber through a primary filter (Corning Color Specificity # 7-37). Fluorescent light in the yellow-green wave length was transmitted out of the chamber through a secondary filter (Corning 4-102) placed at right angles to the incident light. This fluorescent light was quantitated by means of a multiplier photo tube (931-A). The high voltage supply circuit for the photo tube was designed and built at Yale University after a fluorimeter of Heller et al (46). For the preliminary studies, an amplifying and measuring circuit similar to that described by Heller was used. Because of the instability of this amplifying system, it was replaced by a Leeds Northrup Co. Speedomax Recorder. The diagram of the photo cell and sensitivity selection circuit connecting the tube to the recorder was designed and built by Dr. Agnar Straumfjord and has been shown in Figure 1.

Figure 1

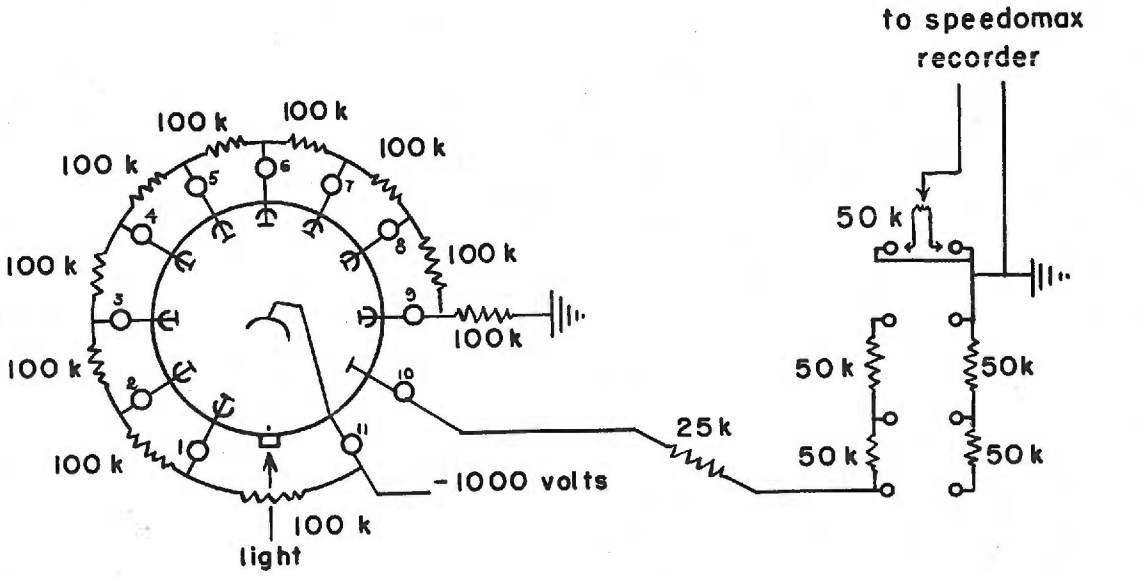
Part I. Fluorimeter Assembly

A: Constant Voltage Transformer; B: Ultra-Violet Ballast; C: Optical System and Photo-tube 1) Ultra-violet lamp, 2) Condensing Lens, 3) Primary Filter, 4) Shutter, 5) Cuvette Support in Sample Chamber, 6) Secondary filter, 7) Multiplier Photo-tube and Photo-tube Circuit; D: Photo-tube High Voltage Supply Circuit; E: Sensitivity Circuit; F: Leeds Northrup Speedomax Recorder; a. Line Voltage; b. Photo-tube high voltage; c. Photo-tube Output; d. Ultra-violet lamp Supply.

Part II. Photo-tube and Sensitivity Selection Circuits



I. FLUORIMETER ASSEMBLY



PHOTOTUBE CIRCUIT

SENSITIVITY SELECTION CIRCUIT

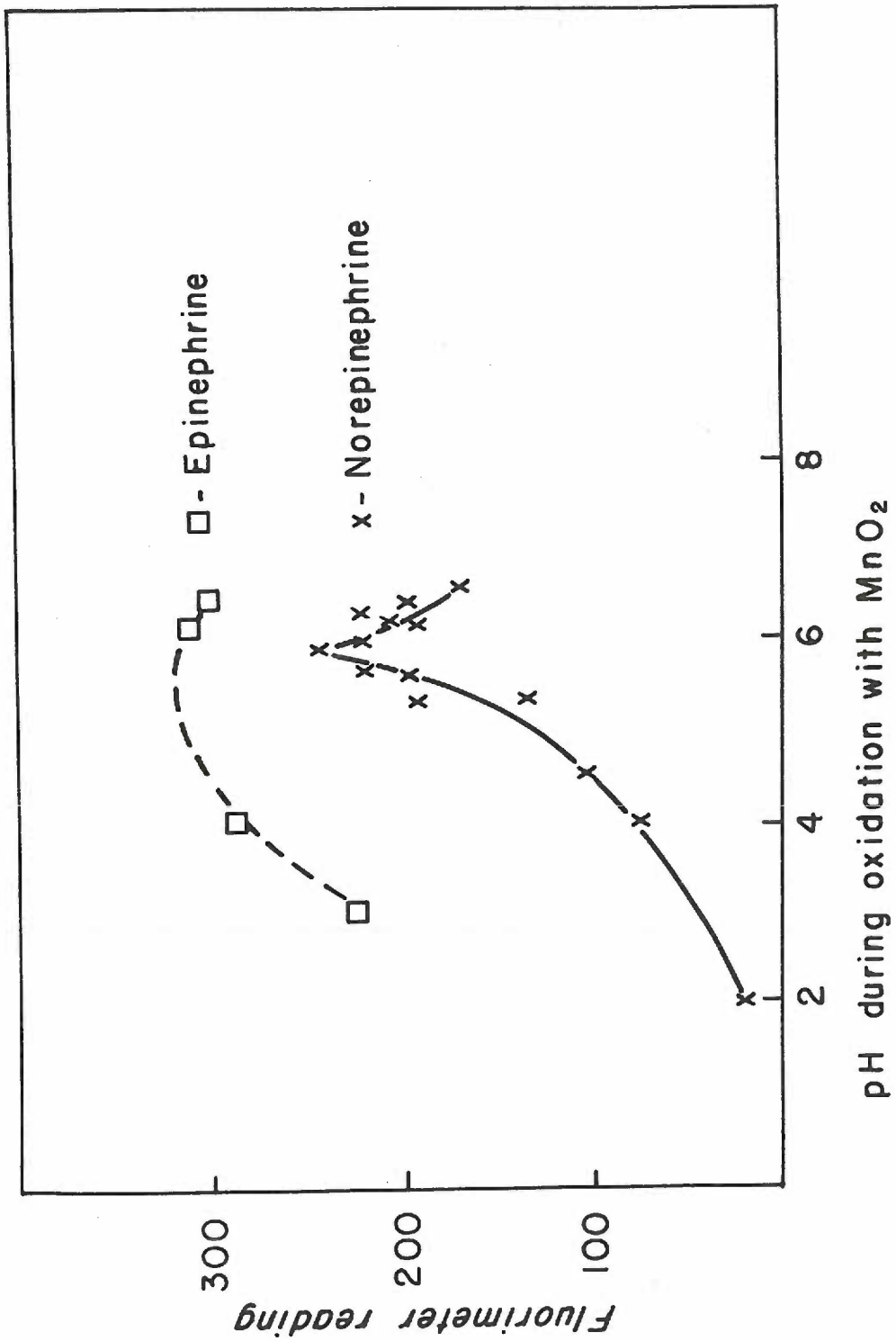
II. PHOTOTUBE TO RECORDER CIRCUIT

A means for standardization of the fluorimeter assembly was necessary because of "drift" and other variation in the electronic apparatus. Commonly used standards such as fluorescein, quinine sulfate, thiochromes, and fluorescent glass often utilized in less sensitive fluorimeters were not applicable in this instrument either because their fluorescence was too intense or their fluorescent intensities were variable with storage and irradiation (74). Fading was found to be quite appreciable in the case of quinine sulfate which was first employed for the standardization. The reference standard chosen was a Pyrex glass rod 1 cm. in diameter. The response of the apparatus to the irradiation of this glass was found to be constant, provided the photo tube high voltage supply was not altered. An arbitrary value for this glass rod was selected and all measurements were then expressed relative to this standard.

Various steps in Lund's procedure were difficult to reproduce. Precision was possible only by carefully controlling many factors in the procedure including the type of manganese dioxide, the pH of oxidation, duration of oxidation, filtration of MnO_2, order of addition and mixing of the reagents, and time of fluorescence measurement. The particle size of MnO_2 was found to be important as was its purity. Interfering precipitates and colored solutions were encountered when using most preparations of MnO_2. Unwashed Baker's Analyzed 94.6% Manganese dioxide was found to give the best results. Three minutes of constant mixing with this MnO_2 were found necessary for consistent oxidation of both epinephrine and norepinephrine. The Ph of oxidation was also found to be very important. The effect of pH of oxidation on the amount of fluorescence of these compounds has been illustrated in Figure 2. Not

Figure 2

Fluorescent activity of epinephrine and norepinephrine solutions after oxidation with MnO_2 at different pH's (adjusted with 0.8 M dibasic sodium phosphate buffer).


□- Epinephrine
x- Norepinephrine
Fluorimeter reading
300
200
100
2
4
6
8
pH during oxidation with MnO_2

only was there a marked difference in fluorescent activity of norepinephrine, as Lund (31) has described, but a significant variation in epinephrine fluorescence with varying pH was also demonstrated. This difference in fluorescence with change in pH of oxidation was marked when assaying epinephrine isolated from aqueous solutions but less prominent with hormone recovered from plasma or urine.

Standard solutions of epinephrine and norepinephrine bitartrate were prepared by dissolving accurately weighed quantities of these materials in N/50 hydrochloric acid and storing under refrigeration. Such solutions remained stable, relative to the assay method, over a period of several months. Several dilutions of these standard solutions were subjected to the analytical procedure to obtain standard curves relating the intensity of fluorescence to concentration. These standard curves are illustrated in Figure 3. It is apparent from these curves that the fluorescence of norepinephrine produced when oxidized at pH 3.0 is very small relative to the fluorescence of epinephrine and will not interfere unless present in relatively large quantities. On the other hand, both substances fluoresce appreciably when oxidized at a pH of 6.5. Thus by measuring the fluorescence of samples at both pH's (3.0 and 6.5) the absolute amount of each hormone can be determined.

Isuprel^{R} (isopropylarterenol hydrochloride) a secondary B-catechol ethanolamine and an epinephrine analogue, was also found to possess very similar oxidation and fluorescent properties to epinephrine.

Since the applications of these assay methods were mainly relevant to the measurement of epinephrine, the limits of relative error of the chemical and fluorescence portion of the procedure have been calculated and shown in Table 1 on the following page.

Figure 3

Standard curves of epinephrine and norepinephrine in aqueous solution when oxidized with MnO_2 at pH 3.0 and pH 6.5 (buffered with 0.8 M dibasic sodium phosphate at pH 6.5).

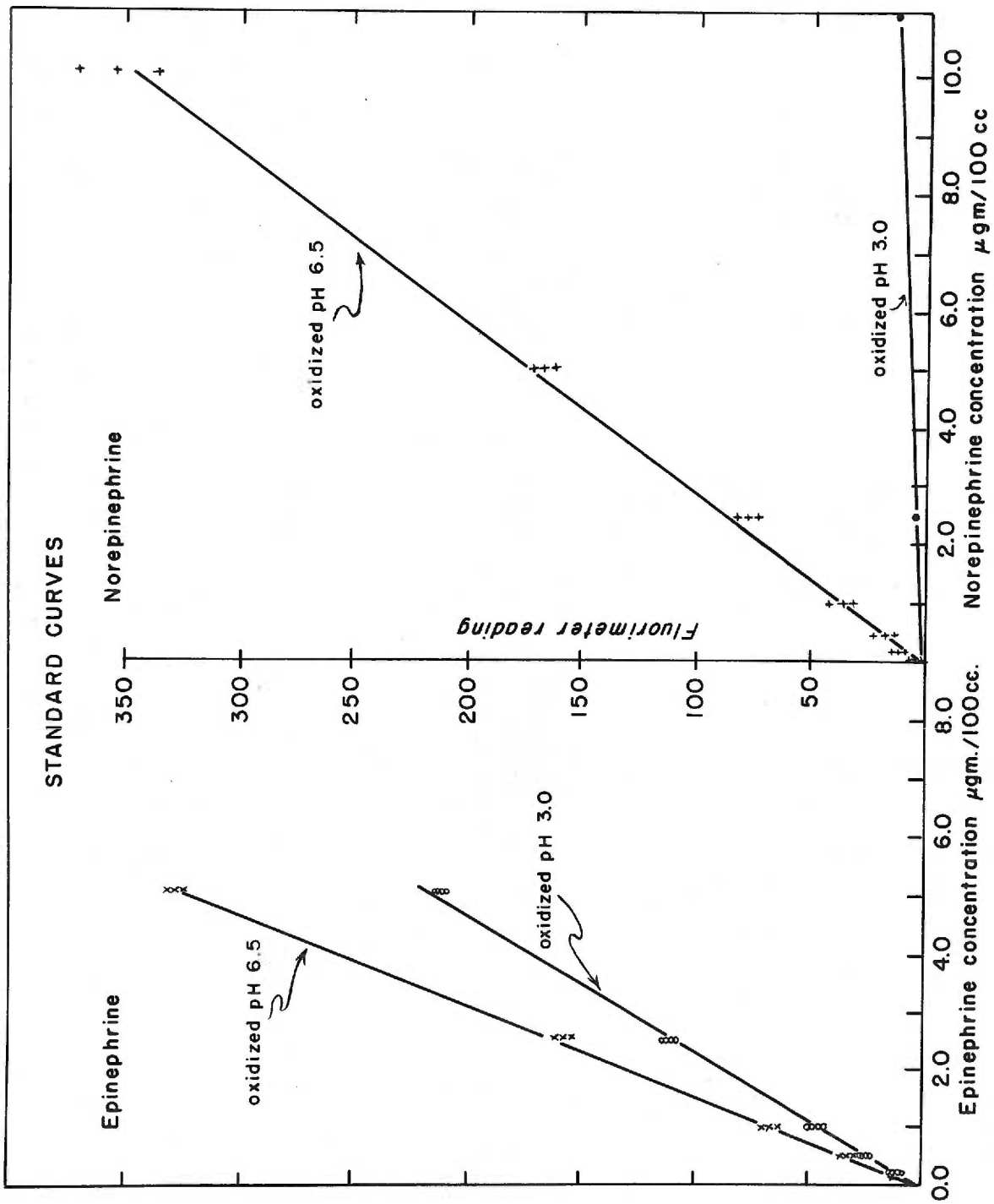

STANDARD CURVES
Epinephrine
oxidized pH 6.5
oxidized pH 3.0
Norepinephrine
oxidized pH 6.5
oxidized pH 3.0
Fluorimeter reading
350
300
250
200
150
100
50
0.0
2.0
4.0
6.0
8.0
10.0
Epinephrine concentration µgm./100cc.
Norepinephrine concentration µgm/100 cc

TABLE 1

90 Percentile Ranges of Estimates of Epinephrine Standard Curve and Percent Error of Range Extremes Oxidized at pH 3.0

Concentration µgm./100 ml.	N	Average Reading	Best Linear Estimate of	90% Range 1.65	% Error of Range Extremes
5.0	4	210.6	4.5	210.6 ±7.4	±3.5%
2.5	4	114.2	1.7	114.2 ±2.8	±2.5%
1.0	4	47.5	2.7	47.5 ±4.5	±9.5%
0.5	4	24.5	2.9	24.5 ±4.8	±19.5%
0.2	4	10.7	1.6	10.7 ±2.6	±24.3%

Lund's aluminum oxide isolation technique was modified for the isolation and purification of the epinephrine from the crude samples. Lund employed a 1 gm. column of alumina for the adsorption of these hormones. Following a suggestion of Peldarinen (75), it was found that the alumina might be added directly to a neutrally buffered sample solution. If this combination was well mixed and then centrifuged, the epinephrine and norepinephrine would adsorb to the alumina and the sample supernatant could be poured off and several neutral washing of the Al_2O_3 could then be made. Finally the hormones could be eluted from the Al_2O_3 in the test tube with 0.2 N acetic acid.

The adsorption characteristics of aluminum oxide was found to be variable depending upon the brand employed and the method of preparation. The best results were obtained when using untreated Woelm non-alkaline aluminum oxide (76). The recovery characteristics of this and Merck alumina are illustrated in Figure 4.

Table 2 on the next page indicates the percent recovery of epinephrine added to dog plasma for different epinephrine concentrations and oxidation pH's.

Figure 4

Percent recovery of epinephrine from aqueous solutions with aluminum oxide with variation of pH of adsorption.

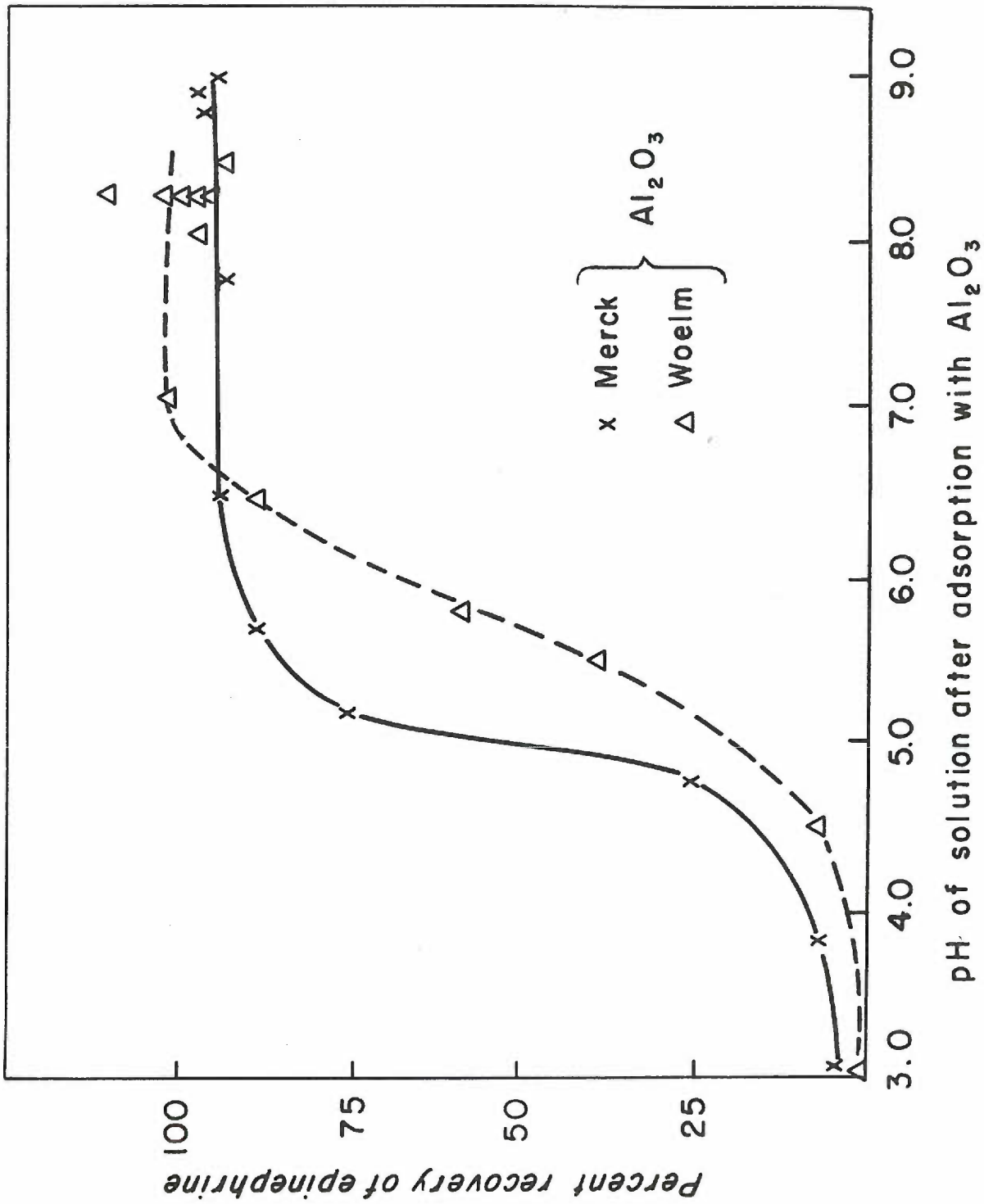

Percent recovery of epinephrine
100
75
50
25
3.0
4.0
5.0
6.0
7.0
8.0
9.0
pH of solution after adsorption with Al_2O_3
x Merck
Δ Woelm
Al_2O_3

TABLE 2

Percent Recovery of Added Epinephrine
From Dog Plasma

Concentration ugm./100 ml.	Number of Recoveries	Recoveries at Different Oxidation pH's in %	
		pH 3.0	pH 6.5
5.0	3	112.5%	
2.5	3	100.2%	82.9%
1.0	3	89.3%	75.0%
0.5	3	101.7%	79.6%
0.25	3	90.7%	74.1%
0.1	3	98.7%	
	Average % recovery	99.0%	77.9%

Recoveries from water and urine appeared to be comparable to plasma recoveries. Recoveries of norepinephrine were about 80% of that added.

It is known that other catechol amines, like hydroxytyramine, can be isolated by this alumina technique. However, it was found that adrenochrome, formed from oxidized epinephrine, could not be adsorbed onto the aluminum oxide under the previously mentioned conditions, and therefore did not interfere with the assay of epinephrine.

The exact details and steps taken in the fluorescent procedure have been listed completely in Section I of the Appendix.

II. Experimental Preparations and Related Procedures

Mongrel dogs, 11.8 to 24.1 kg. in weight were utilized as experimental animals. The blood and urine samples were obtained under conditions of anesthesia. Intravenous or intraperitoneal nembutal in the dose of 30 mg./kg. of dog weight was employed as the anesthetic agent. The experimental preparation varied in several ways. The first studies were made in dogs following acute surgery necessary for renal function studies being carried on by others in the laboratory. Most of the experimentation, including all of the renal excretion studies, were made using

healthy, previously untreated animals.

Blood samples were collected either through indwelling arterial needles or from plastic catheters placed in certain venous areas either by direct vision or fluoroscopy. The blood samples were centrifuged at 2500 r.p.m. for 15 to 20 minutes immediately after collection. Coagulation was prevented by the addition of two or four drops of heparin solution (10 mg./ml.). The plasma was removed from the cells after centrifugation and stored at room temperature. Femoral arterial blood samples were taken as representative of systemic arterial blood.

Female dogs were used exclusively for the collection of urine samples. The urine was collected either directly in the acute preparation through a polyethylene catheter from the cannulated ureters or in the chronic preparations through a rubber urethral catheter from the bladder. These urine samples were collected and measured in graduated cylinders and likewise stored at room temperature until assayed for epinephrine or norepinephrine content.

The studies of the dynamics of circulating epinephrine and renal excretion of epinephrine were made in dogs infused intravenously at constant rates with either epinephrine bitartrate (Suprarenin^{R}, Winthrop Stearns) or adrenaline (Eastman Kodak Co.) solutions. These infusions were made using either a Martin-Hubbard Constant Rate Injection Apparatus or a "pulse-flow" injection apparatus made by Process & Instruments (Brooklyn, N. Y.).

Hind limb blood and plasma flows were measured in two different ways. In Dog B samples of blood were collected by free flow from the distal end of a sectioned femoral vein. These collections were timed and the volumes measured. From these measurements the hind limb blood flows were estimated.

The cut ends of the femoral vein were joined together by a polyethylene tube except when making the collections. In Dog L a bubble flow meter in series with the femoral vein was employed to estimate hind limb blood flow. The flow meter was made from a two-foot section of polyethylene tubing (inside diameter 1/8") into which a hypodermic needle was placed for the introduction of air. Simultaneous hematocrits were measured in graduated centrifuge tubes in order to calculate plasma flow.

The measurements of renal function in both the acute and chronic animal preparations were carried out as follows. Urine flow in ml./min. as well as plasma and urine concentrations of creatinine, para amino hippurate (PAH), and epinephrine were measured during periods of control, intravenous epinephrine infusion and recovery. Creatinine concentrations were measured colorimetrically by the picric acid method (77). PAH was quantitated colorimetrically by diazotizing with NNO_2 and coupling with N-(1-naphyl)ethylenediamine (78). Sufficient urine flow was achieved by intravenous pre-infusion of 500 ml. of 1.1% sodium chloride. Adequate plasma concentrations of creatinine and PAH were produced by the intravenous injection of a priming solution containing 1.2 gm. creatinine and 0.3 ml. of a 20% solution of PAH. The urine flow and plasma concentrations of creatinine and PAH were maintained by the continuous infusion of 1.1% saline containing 2.0 ml. of PAH solution (20% by weight) and 2.0 gm. creatinine per 500 ml. infused at a constant rate of about 3.0 ml. per minute.

Several special renal excretion studies were made after injections of 2,4, dinitrophenol and Hydergine[3] (equiproportional mixture of dihydroergocornine, dihydroergokrynine and dihydroergocristine as methylsulfonates 0.1 mg. of each / cc. These compounds were injected slowly

intravenously into nembutal anesthetized dogs. The renal function measurements were made before and after these injections in the same way as in the other renal excretion experiments.

In an attempt to measure the effect of hyperthyroidism on the parameters under investigation, USP thyroid extract (79) was administered in daily oral doses to three dogs. The specific doses have been listed in the Appendix with the measurement data. Oxygen consumption measured by a spirometer (Collins Respirometer) connected to an endotracheal tube were made in an attempt to measure the hypermetabolic effects of both thyroid extract and intravenous epinephrine. These were not corrected to standard conditions because such corrections did not alter their relative relationship.

Intra-arterial blood pressures were measured and recorded by means of a Lilly Manometer from an indwelling Cournand needle.

EXPERIMENTAL RESULTS

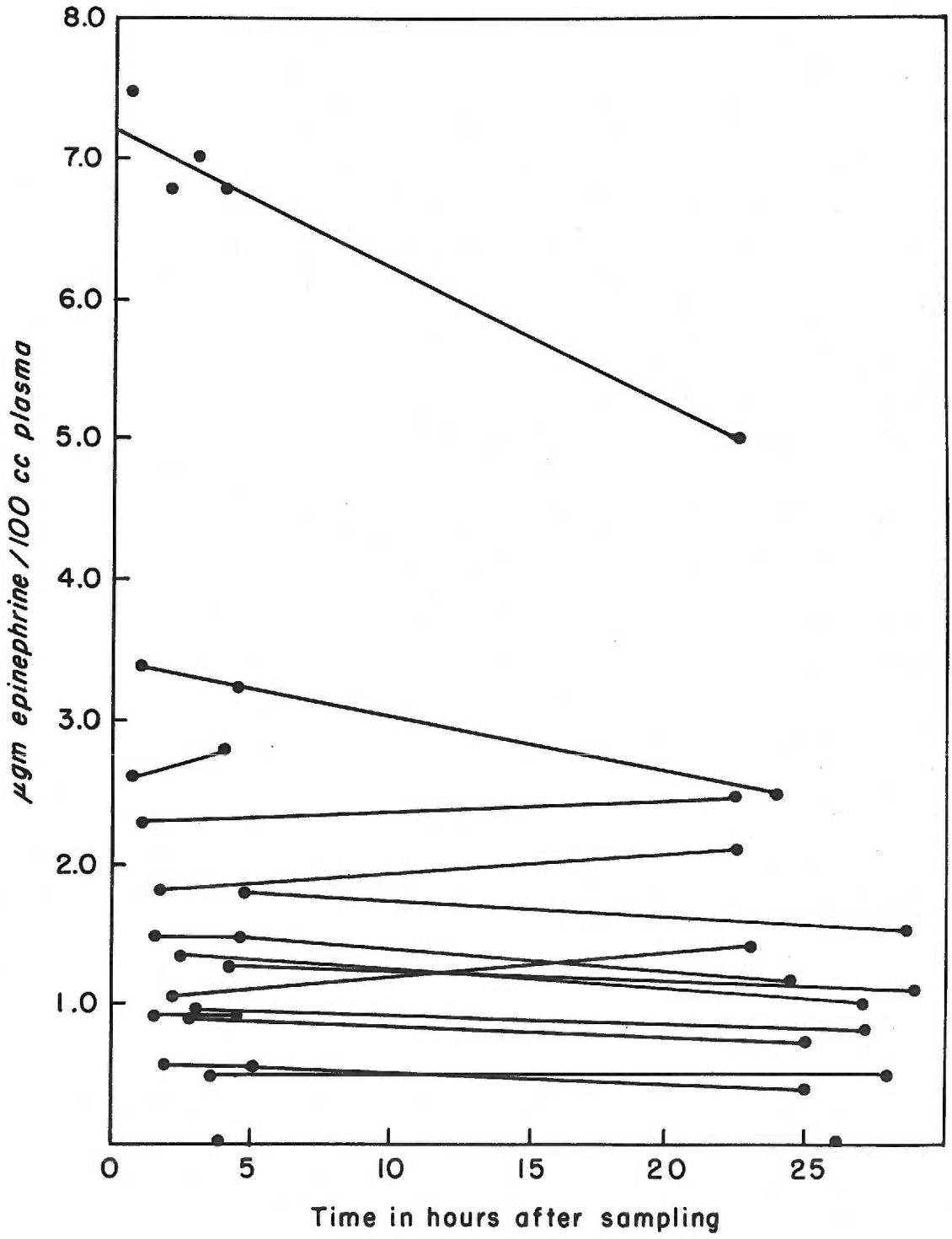
The results from the modification of Lund's fluorimetric method have been given in the section on methodology. These will be reviewed in the Discussion and Conclusion section.

Results of the animal experimentation have been divided into several parts, although data for different parts may have been obtained from the same experimental preparation. These parts in the order presented are: a) stability of epinephrine in plasma and urine stored at room temperature, b) the relation of arterial plasma epinephrine concentration to duration of constant infusion, c) the relation of arterial plasma epinephrine concentration to different rates of constant epinephrine infusion, d) the rate of disappearance of arterial plasma epinephrine after stopping the infusions, e) extraction ratios of epinephrine in the pulmonary, hepatic, portal and hind limb circulations at different arterial plasma concentrations, f) the simultaneous measurement of hind limb blood flow and epinephrine extraction ratios at different arterial plasma concentrations, g) renal excretion of epinephrine, and h) the influence of thyroid feeding on the parameters of epinephrine circulation and renal excretion.

a) Plasma was isolated from blood samples removed from dogs receiving constant infusions of l-epinephrine bitartrate at various intravenous rates. Repeat epinephrine assays were made on these plasma samples over a period of several hours. The results are graphically represented in Figure 5. From this it is apparent that epinephrine, assayed by this method, is relatively stable at room temperature

Figure 5

Stability of epinephrine in dog plasma stored at room temperatures.


8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
µgm epinephrine/100 cc plasma
0
5
10
15
20
25
Time in hours after sampling

in their epinephrine content.

b) Repeated samplings of arterial plasma were made at various times after starting intravenous epinephrine infusion. The infusion rates were constant for a given experiment, although varied between experiments. Figure 6 illustrates the relationship between femoral arterial plasma epinephrine concentration and duration of the infusion. The rates of change in epinephrine concentration per unit time have been calculated and listed in Table 3. An application of the sign test (80) to the data indicates that the slopes of the curves observed after about 8 minutes of infusion, are not significantly different from zero at the 95 percentile range of significance.

c) On 52 separate occasions, femoral arterial plasma samples were obtained 8 or more minutes after beginning intravenous epinephrine infusions in anesthetized dogs. In 16 dogs samples were also taken before the infusions were started. Epinephrine assays of the pre-infusion samples indicated their epinephrine content to be, in general, 0.1 microgram per 100 ml. or less. The elevations in plasma concentrations during different rates of infusion have been indicated in Figure 7. In this figure the log of concentration has been plotted against the rate of infusion. It is observed that samples from a single dog made the same day followed a straight line log function. Although the variation was greater for all observations in all the dogs, statistically they are close to a straight log concentration versus rate of infusion line. For infusion rates less than 2.5 micrograms/kg. the observed concentrations were close to a straight line. All of these observations have been included in Table I of the Appendix.

Figure 6

Epinephrine concentration in femoral arterial plasma at various times after starting the constant intravenous infusions of epinephrine. (The infusion was constant for any given line though varied with different lines.)

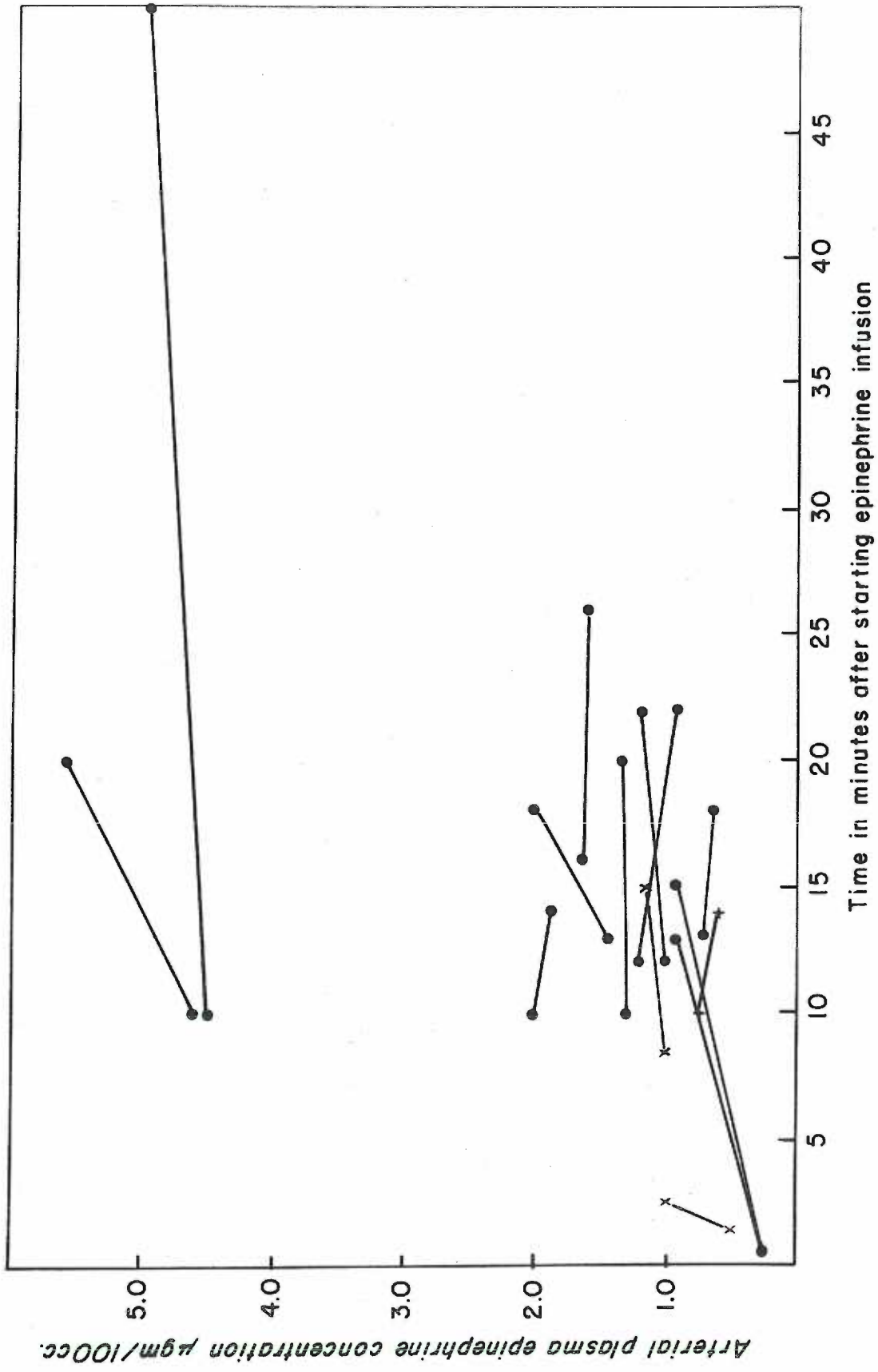

Arterial plasma epinephrine concentration µgm/100cc.
5.0
4.0
3.0
2.0
1.0
5
10
15
20
25
30
35
40
45
Time in minutes after starting epinephrine infusion

TABLE 3

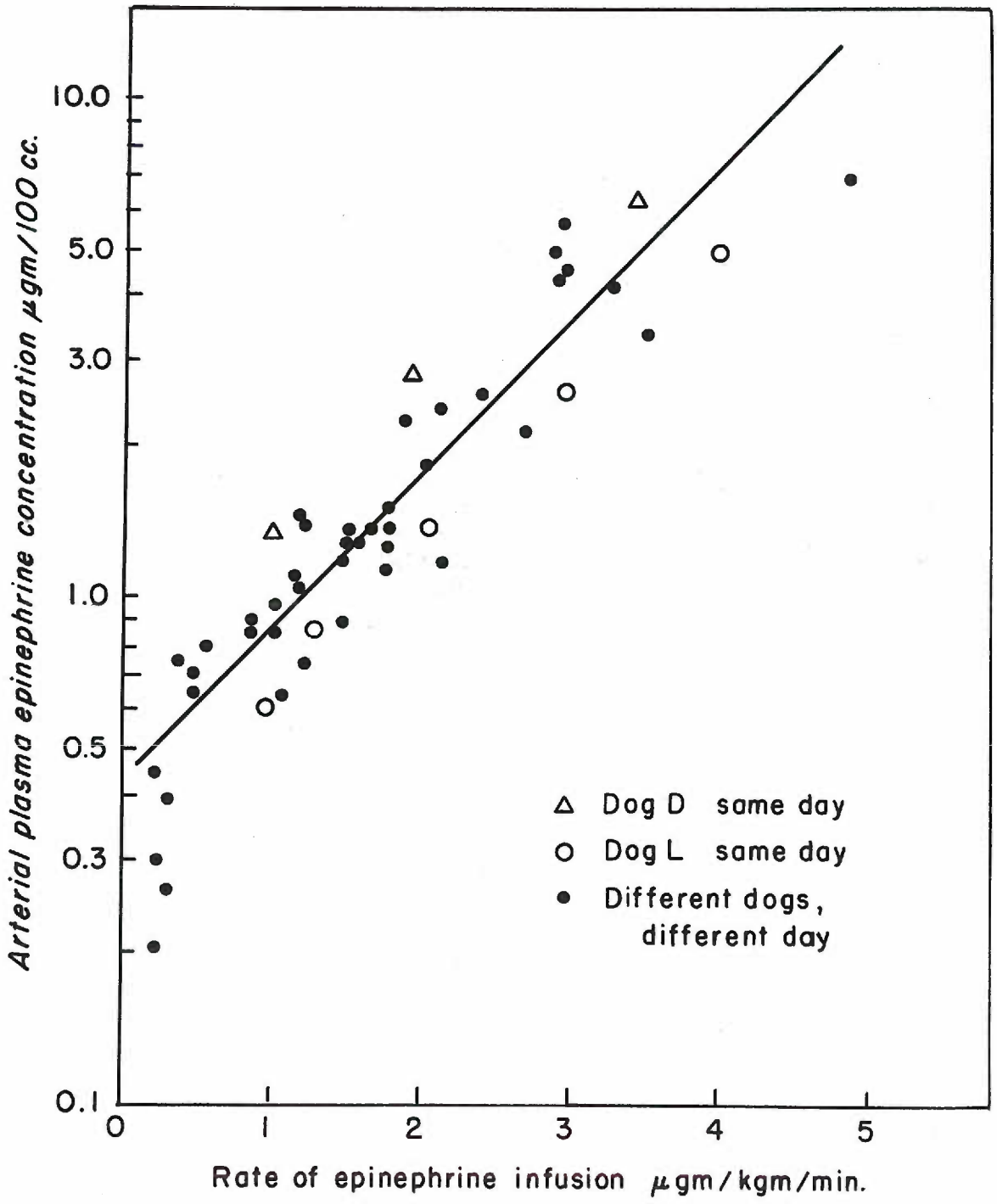
Rate of Change in Epinephrine Concentration
During Constant Infusion

Animal	Epinephrine Concentration in ugm./100 ml at Time in Minutes after Starting Infusion					
Dog	C_1	t_1	C_2	t_2	Δt	$\frac{C_2-C_1}{\Delta t\ C_2} \times 100$
17	4.5	10	5.0	55	45	0.25
18	4.6	10	5.6	20	10	2.3
19	1.3	10	1.35	20	10	0.38
DNP-1	1.0	12	1.2	20	8	2.5
C.	1.0	2.25	1.0	8.5	6.25	0.0
C.	1.0	8.5	1.15	15	6.5	2.3
DNP-2	1.2	12	0.9	22	10	-2.5
D.	0.75	13	1.4	28	15	5.7
D.	2.0	15	1.3	40	25	-1.4
H.	0.77	10	0.6	14	4	-5.5
H.	2.0	10	1.85	14	4	-1.9
B.	0.7	13	0.65	18	5	-1.2
B.	1.45	13	2.05	18	5	8.5

Table 4. The epinephrine concentration of arterial plasma during continuous constant infusion of epinephrine has been listed with the times the samples were taken after starting the infusion of epinephrine. The percent change in concentration of epinephrine per unit time during the period studied has been represented by: $\frac{C_2-C_1}{\Delta t\ C_2} \times 100$, where Δt represents the change in time from t_1 to t_2 in minutes.

Figure 7

The concentration of epinephrine in the femoral arterial plasma compared with the rate of constant infusion (after eight or more minutes of infusion). The epinephrine concentration has been plotted on a logarithmic scale.


Arterial plasma epinephrine concentration μgm/100 cc.
10.0
5.0
3.0
1.0
0.5
0.3
0.1
0
1
2
3
4
5
Rate of epinephrine infusion μgm/kgm/min.
Dog D same day
Dog L same day
Different dogs, different day

d) The rate of disappearance of epinephrine from arterial plasma was determined by assaying samples removed shortly after stopping the infusions. These concentrations decreased rapidly following a logarithmic curve, one half of the epinephrine having disappeared within three-fourths of a minute. Figure 8 indicates the relation between the log of the concentration and the time the sample was taken after stopping the infusion. All of the data of these disappearance rates have been listed in Table II of the Appendix.

e) Because the overall removal or disappearance of epinephrine is a sum of the various mechanisms operating, several areas of possible removal were studied. Epinephrine concentrations in arterial plasma were measured and compared with samples obtained from the hepatic vein, portal vein, femoral vein, and pulmonary artery. These comparisons were made by calculating the percent extraction ratio of epinephrine by each area drained or supplied by the previously mentioned vessels. The extraction ratio is the amount removed divided by the amount brought to the area. If the inflow is equal to the outflow (not really true of the liver or lungs) then the difference in concentration divided by the inflow concentration equals the extraction ratio. These extraction ratios have been plotted relative to the inflow concentration in Figures 9 and 10. From these figures it appears that after 8 minutes of infusion there is, on the average, no epinephrine removed by the lungs. The liver removed on the average about 60% of the inflowing epinephrine. The portal circulation also removes a considerable amount of epinephrine. On the basis of three observations these portal system extraction ratios appeared to decrease with increase in arterial epinephrine concentration. The hind limb extraction ratios varied directly with changes in arterial

Figure 8

The epinephrine concentration in femoral arterial plasma samples has been compared with the time of sampling during and after stopping the intravenous infusion of epinephrine. The epinephrine concentrations have been plotted on a logarithmic scale. The slope of the natural logarithmic lines has been denoted by $-k$ which is equal to $\frac{\ln x - \ln x_o}{t - t_o}$ where x represents the epinephrine concentration and t, the time after stopping the infusion. x_o represents the concentration just before stopping the infusion where t_o is taken as zero.

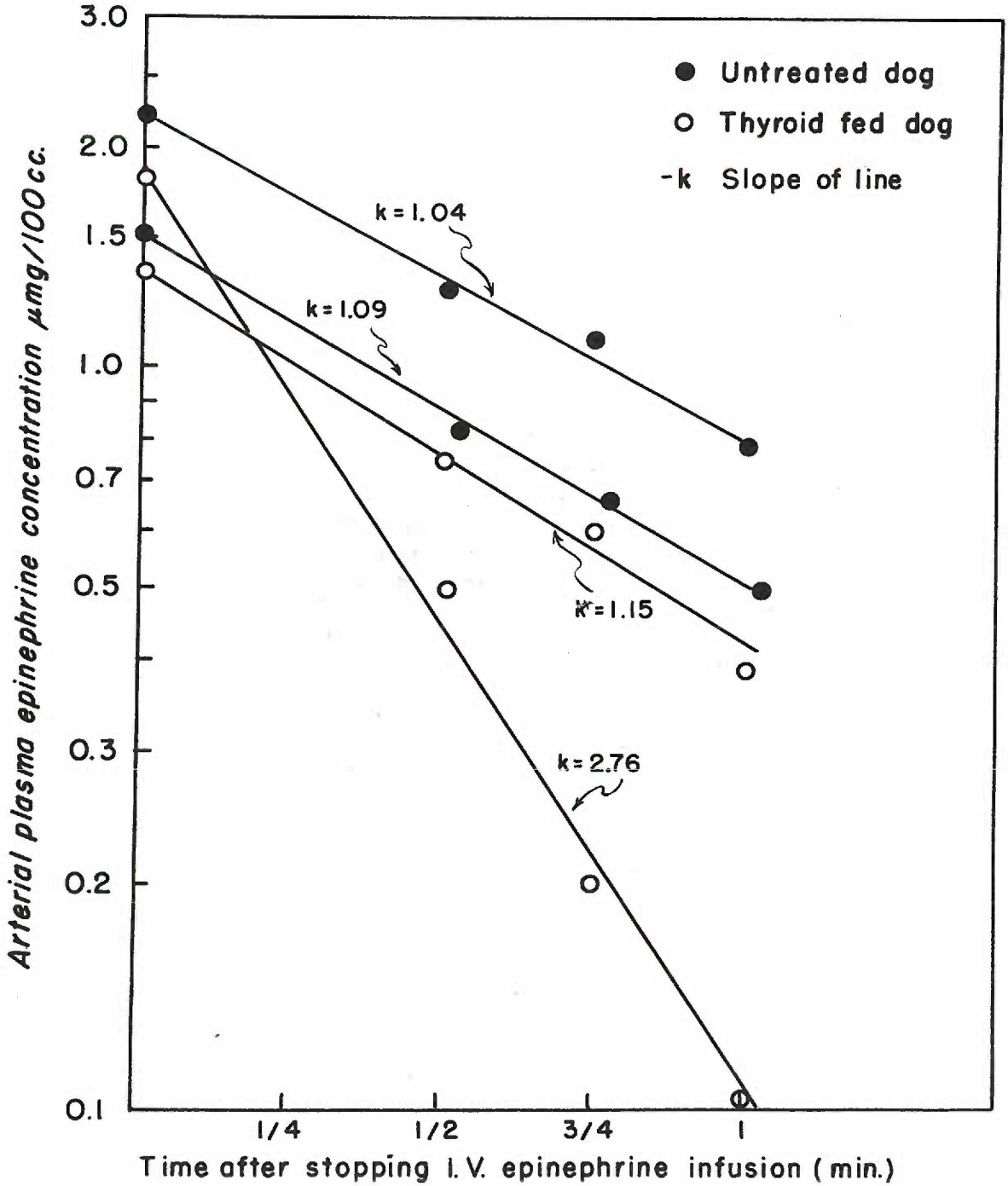
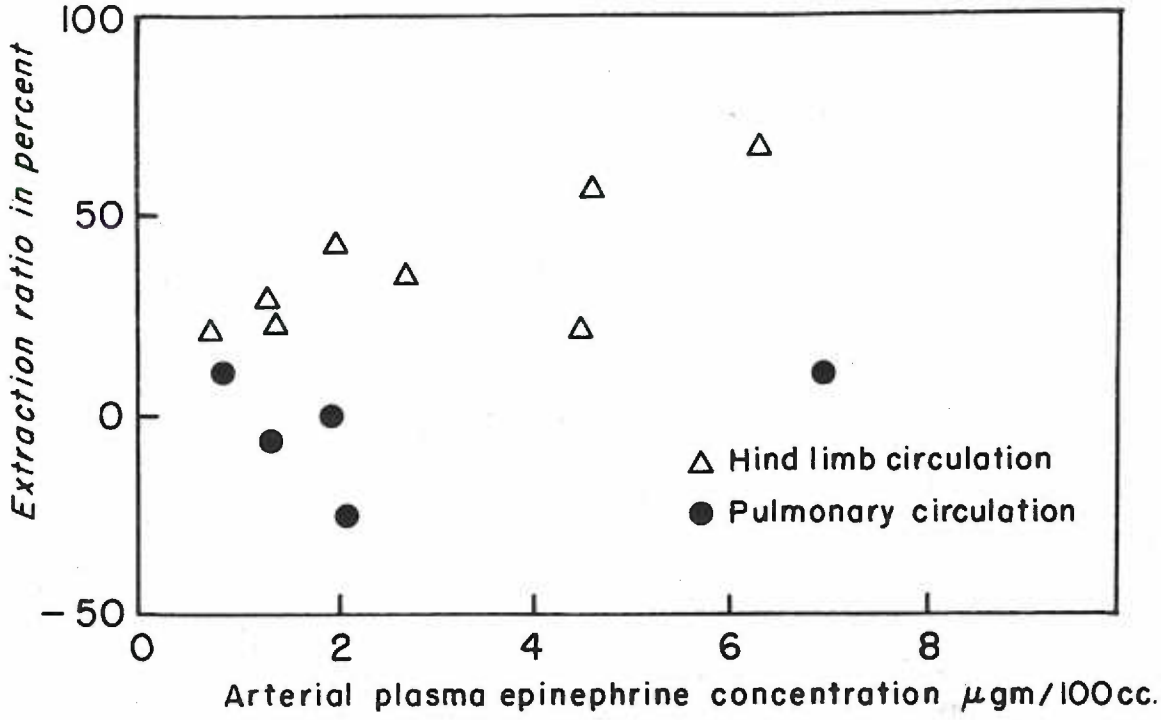

3.0
2.0
1.5
1.0
0.7
0.5
0.3
0.2
0.1
Arterial plasma epinephrine concentration µmg/100cc.
Untreated dog
Thyroid fed dog
-k Slope of line
k = 1.04
k = 1.09
k = 1.15
k = 2.76
1/4
1/2
3/4
1
Time after stopping I.V. epinephrine infusion (min.)

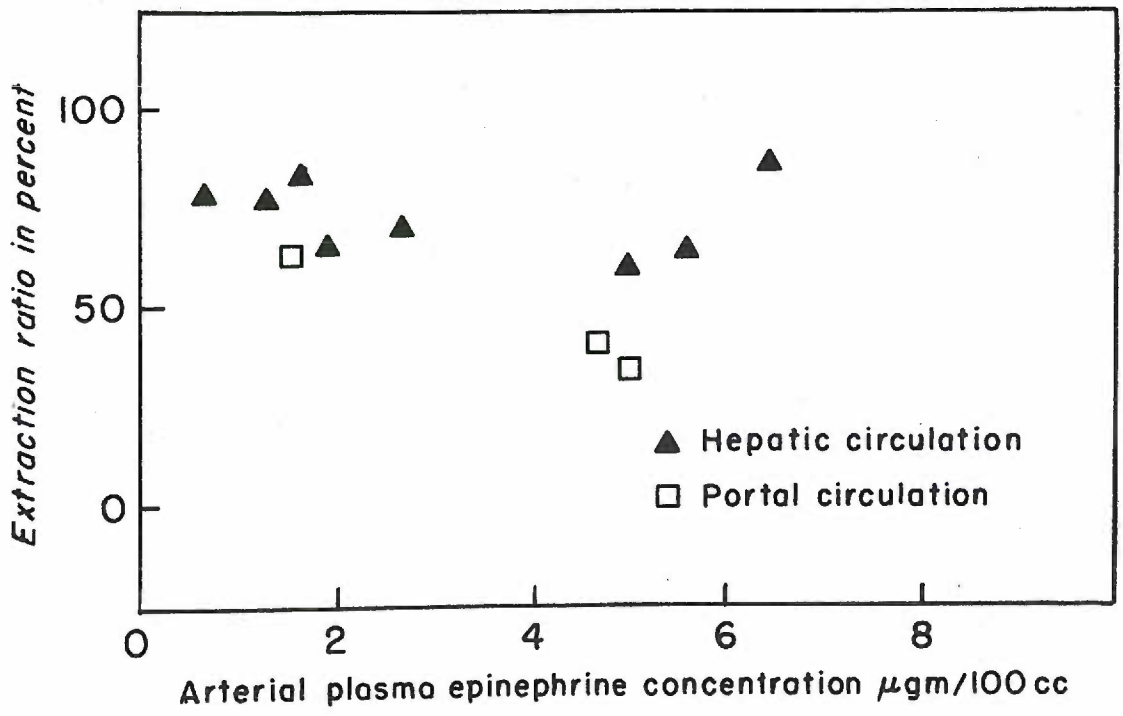
Figure 9

Epinephrine extraction ratios (in percent) from hind limb and pulmonary artery circulations have been compared at various arterial plasma epinephrine concentrations. The extraction ratio was calculated as $\frac{X_i - X_o}{X_i} 100$ where X_i and X_o represent the epinephrine concentration in the plasma flowing into and out of the areas, respectively.

Figure 10

Epinephrine extraction ratios from the hepatic and portal circulations at various concentrations of epinephrine in femoral arterial plasma. The extraction ratios have been calculated as in Figure 9.


100
50
0
−50
Extraction ratio in percent
0
2
4
6
8
Arterial plasma epinephrine concentration μgm/100cc.
Hind limb circulation
Pulmonary circulation


100
50
0
Extraction ratio in percent
0
2
4
6
8
Arterial plasma epinephrine concentration μgm/100 cc
Hepatic circulation
Portal circulation

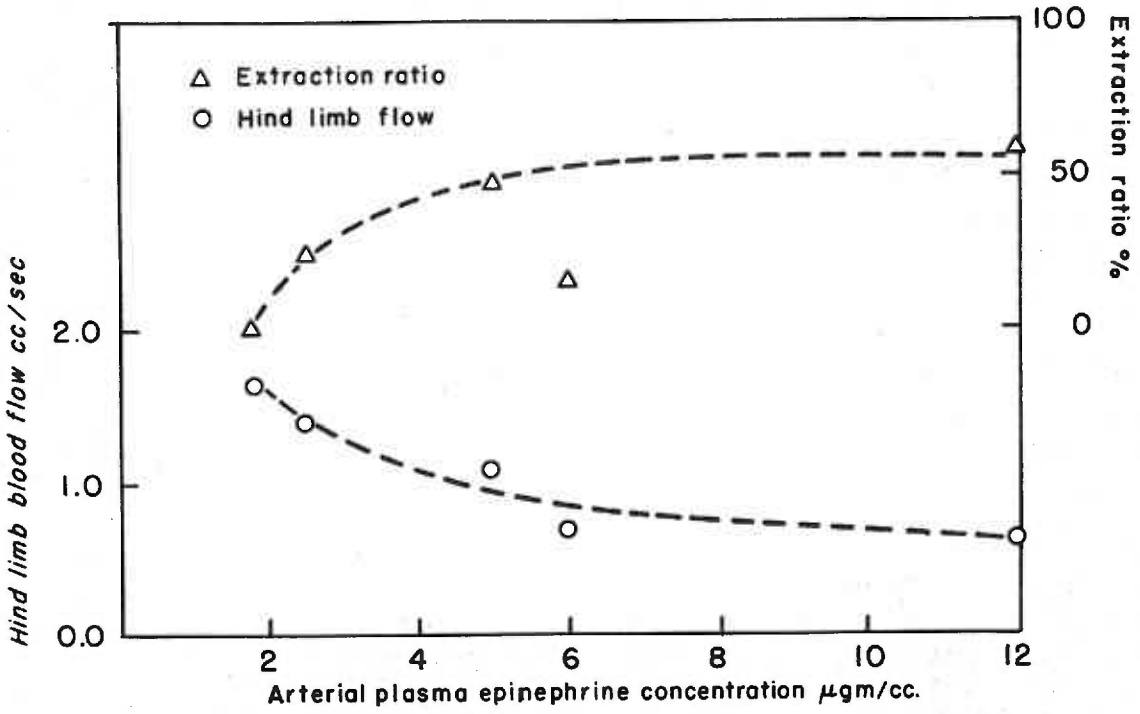
epinephrine concentration. Hind limb extraction ratios from experiments with five different dogs are shown in Figure 9.

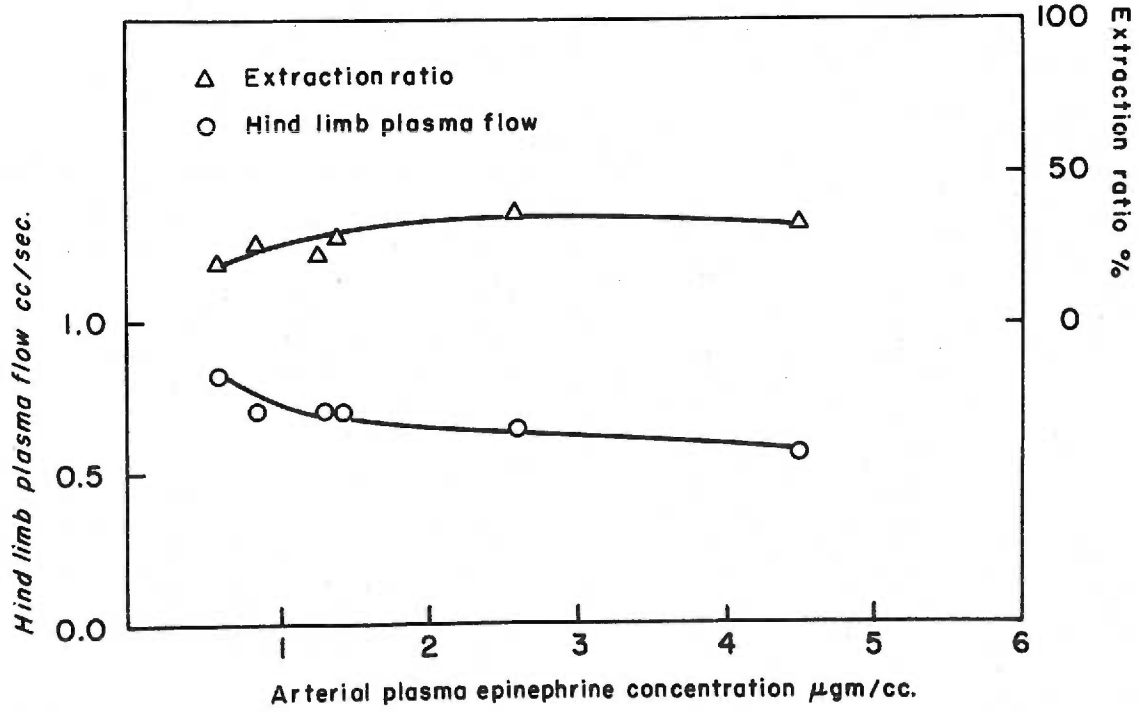
f) Because the hind limb epinephrine extraction ratios tended to increase with increased arterial concentrations of epinephrine other factors which might be influencing the extraction mechanisms were investigated. Since vascular smooth muscle is very sensitive to epinephrine, hind limb blood flows and arterial and venous epinephrine concentrations were measured simultaneously in two different animals during different rates of constant infusion. The observations from these preparations have been graphically represented in Figures 11a and 11b. From the first figure it was again noted that the epinephrine extraction ratio tended to increase with increased arterial concentrations, while the hind limb blood flow tended to decrease at infusion rates greater than the first infusion. In the second experiment only small changes in both hind limb plasma flow and extraction ratio were noted, although again the changes were in opposite directions.

g) The mechanisms of renal excretion of epinephrine were studied by comparing the elimination of this hormone with the excretion of known substances. The excretion of any substance can be quantitatively expressed by comparing the amount of this substance in the urine to the concentration in the plasma. This expression is the renal clearance and is equal to $\frac{\text{urine concentration} \times \text{urine volume}}{\text{plasma concentration}}$. Since creatinine is excreted only by glomerular filtration in the dog (81), the creatinine clearance is an index of glomerular filtration. PAH, on the other hand, is both filtered by the glomerulus and actively secreted by tubular cells (81). The PAH clearance, at the concentrations used in these

Figures 11a and 11b

The epinephrine extraction ratio and hind limb blood flow (Figure 11a) or plasma flow (Figure 11b) have been plotted at different epinephrine concentrations in femoral arterial plasma.


Extraction ratio
Hind limb flow
Hind limb blood flow cc/sec
Extraction ratio %
0.0
1.0
2.0
0
50
100
2
4
6
8
10
12
Arterial plasma epinephrine concentration μgm/cc.


Extraction ratio
Hind limb plasma flow
Hind limb plasma flow cc/sec.
Extraction ratio %
0.0
0.5
1.0
0
50
100
1
2
3
4
5
6
Arterial plasma epinephrine concentration μgm/cc.

experiments, was an index of renal plasma flow. That portion of PAH which was actively transported by tubular cells was calculated by subtracting the amount of PAH filtered at the glomerulus from the total amount in the urine. The amount filtered was estimated from the product of plasma PAH concentration and glomerular filtration rate (creatinine clearance). This PAH transport was an expression of active tubular secretion, a mechanism which is dependent upon intracellular chemical processes (82).

Creatinine, PAH, and epinephrine clearances were measured a total of 10 different times in six different normal dogs. The results of these experiments are listed in Tables III, IV, V, and VI in the Appendix. In general, two epinephrine infusion rates were studied each time in each animal. These infusion periods were preceded by a control series and followed by a recovery series of measurements. Several things were observed from these measurements. First, the renal output of epinephrine increased during the constant intravenous infusion of epinephrine. The percent of infusion appearing in the urine was, on the average, 4.6% with a standard error of the mean estimate of 0.2%. The percent excreted did not change significantly with different rates of infusion. Secondly, the clearances observed for epinephrine were in all cases greater than the creatinine clearances though less than the PAH clearances. The average of 21 epinephrine clearances was 1.64 times the magnitude of their corresponding creatinine clearance. The standard error for the mean of these ratios of the corresponding clearances was about 0.076. By applying the sign test to the difference in the corresponding clearances and to their ratio minus 1, it was shown that the epinephrine clearances were statistically greater than the

creatinine clearances at the 99 percentile level of significance.

Tubular transport of epinephrine was also calculated in each case and the estimates have been listed in the Appendix. Since 2,4-dinitrophenol has been used to inhibit tubular transport mechanisms (82,83), the effect of this chemical on epinephrine and PAH transport were studied in two different anesthetized animals. These experiments have been listed in Table VI of the Appendix. The clearance and transport of PAH were observed to decrease very appreciably in these two dogs; however no decrease in epinephrine clearance or transport was noted. No significant changes in creatinine clearances were noted either. Similar measurements of epinephrine and PAH transports and clearances were also made after the infusion of 4.5 ml. of Hydergine® (Table VI, Appendix); however, no significant changes were noted which were not felt to be due to the decrease in arterial blood pressure also observed.

In Table VII of the Appendix, results have been listed from an acute experiment in which creatinine, PAH, and epinephrine were measured in the right renal venous plasma as well as in the arterial plasma and urine. In addition to clearance and transport information this data also yielded a more direct measurement of renal plasma flow (RPF). The amount of epinephrine removed by the kidney was calculated from these measurements of RPF and the arterial-venous epinephrine concentration difference. It was observed that more epinephrine was removed from the plasma by the kidney than was eliminated in the urine.

On four occasions the amount of norepinephrine found in the urine during the infusion with epinephrine was estimated to be less than 10% of the total catechol-ethanolamine output. On two other occasions urine specimens collected during epinephrine infusions were hydrolyzed

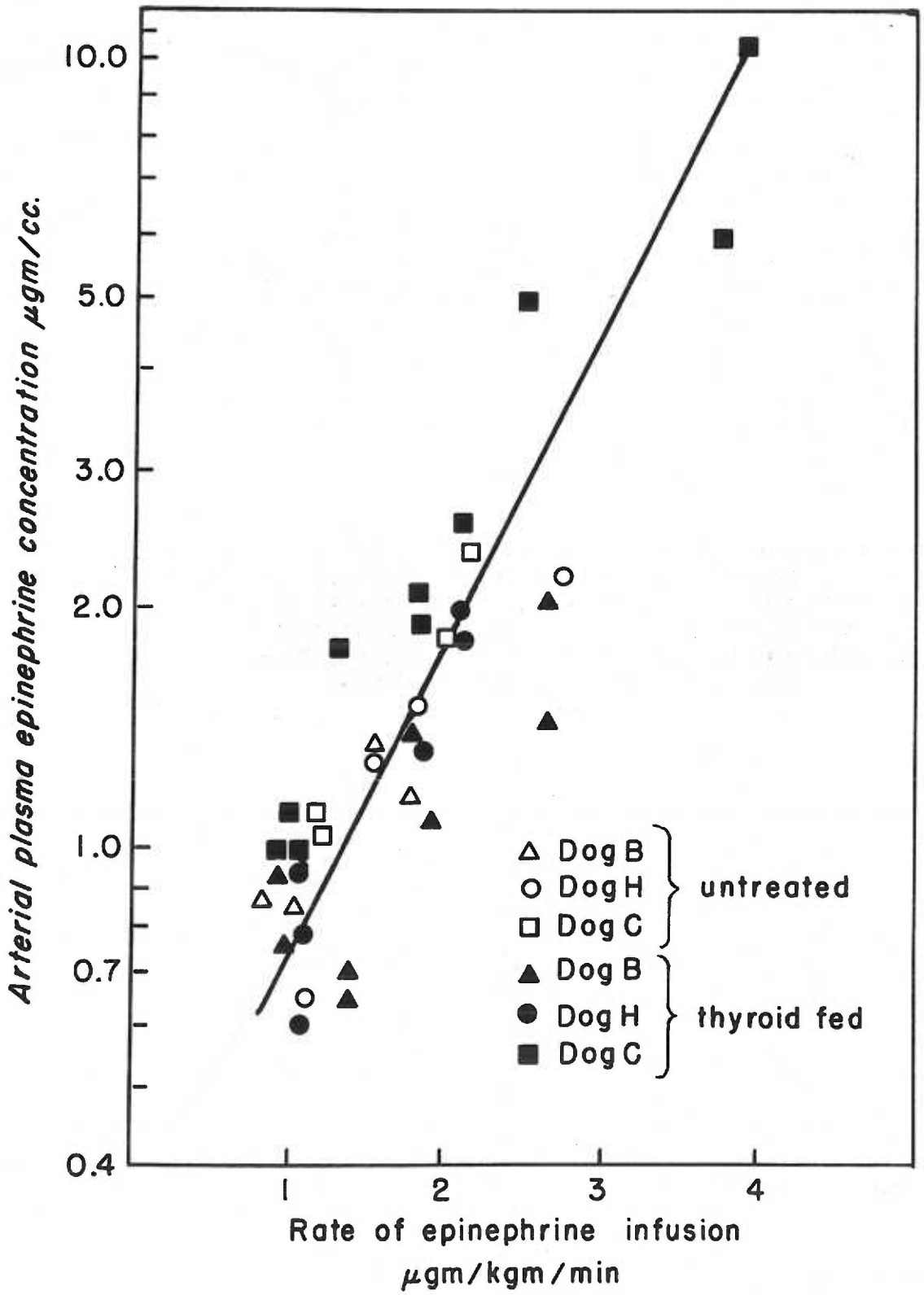
at a pH of 1.5 in boiling water for 20 minutes. However, there was no increase in total epinephrine excretion in these cases.

h) Thyroid extract (USP) was administered in various doses to three different animals. The dynamics of epinephrine circulation and renal excretion were studied prior to and during the feeding of thyroid extract to these dogs. The data regarding these experiments have also been included in the tables of the Appendix for dogs B, H, and C. In Figure 12 the log of the arterial plasma epinephrine concentration and the rate of constant infusion have been plotted. No significant change was observed in this relationship after the feeding of thyroid. The disappearance constants likewise were not altered significantly from the pre-thyroid periods. There was an increase in the PAH clearance in the control period after feeding thyroid, especially in dig B. The average percent excretion of infused epinephrine was also significantly increased (t calculated from data equalled 2.26; t from tables with 19 d.f. at 95% level of significance equals 2.09 (80)).

The measurements of oxygen consumption during the control periods before and during thyroid feeding were not significantly different.

Figure 12

The concentration of epinephrine in the femoral arterial plasma has been compared with the rate of constant intravenous infusion of epinephrine before and during thyroid feeding. The epinephrine concentration has been plotted on a logarithmic scale.


10.0
5.0
3.0
2.0
1.0
0.7
0.4
Arterial plasma epinephrine concentration µgm/cc.
1
2
3
4
Rate of epinephrine infusion
µgm/kgm/min
Dog B
Dog H
Dog C
untreated
Dog B
Dog H
Dog C
thyroid fed

DISCUSSION AND CONCLUSIONS

I. Epinephrine and Norepinephrine Assay Procedure

The data presented and the findings of others (30,31) indicate that epinephrine and norepinephrine can be quantitatively estimated in plasma and urine. Specificity of the estimation method employed was dependent upon three factors. First, the isolation technique with aluminum oxide yielded mainly substances which have properties the same or similar to 3,4-dihydroxyphenols. In particular, oxidized epinephrine, presumably adrenochrome, could not be isolated by this technique. Second, specificity was enhanced by the ease of oxidative ring closure of the 3,4-dihydroxyphenol-ethanolamines obtained from the alumina isolation technique. The differentiation of primary (norepinephrine) from secondary (epinephrine and n-isopropylarterenol) amines was achieved because of different rates of oxidative ring closure varying with pH of oxidation with MnO_2. The third factor imparting specificity was the requirement for the presence of the alpha hydrogen and the beta alcoholic groups in the oxidized catechol ethylamines. These groups were necessary for the development of fluorescence of the oxidized compounds in alkaline solutions.

The sensitivity of the estimation on procedure was sufficient to detect 0.2 micrograms of epinephrine per 100 ml. of sample. The percent error of the extremes of the 90 percentile range for the estimates at 1.0 ugm./100 ml. was of the order of ±10%.

XI. The Dynamics of Epinephrine Circulation and Renal Excretion in Normal and Thyroid Fed Animals.

A. Application of the Epinephrine Assay Procedure to Plasma and Urine Samples

The substances measured in the plasma, urine, and infusion samples were b-catechol-ethanolamine according to the specificity conditions discussed above. In general, the analysis was made for secondary amines only. These estimates of secondary amines were assumed to be epinephrine entirely, because of the negligible amounts of other secondary amines in plasma and urine (47). Conjugated epinephrine was probably not estimated because such chemical combination prevents the isolation by the procedure (49). Irreversible binding of the assayed epinephrine with other plasma or urine constituents appears unlikely since all of the chemically active groups (amine, alcohol, and phenols) in this molecule are required for the estimation procedure. Furthermore, the recoveries of epinephrine added to plasma, urine, and water were comparable and in the order of 100%. It therefore appears unlikely that the added epinephrine was bound irreversibly to such plasma constituents as protein. This is not in support of the hypothesis that epinephrine is carried by plasma proteins (84).

B. Dynamics of Epinephrine Circulation

1. Experimental Conditions: The studies of the dynamics of epinephrine circulation were made by infusing epinephrine at a constant rate into a systemic vein of a dog. Estimation of the epinephrine concentration of plasma samples were made from various vessels of the

circulatory system. Relationships between the epinephrine concentrations and infusions were noted as given in the Results section.

2. Equilibrium: The concentration of epinephrine in femoral arterial plasma was observed to increase rapidly immediately after starting intravenous infusion of epinephrine. With eight or more minutes of infusion these concentrations remained relatively constant with continued infusion. These observations suggest that there was a re-circulation of infused epinephrine. Further, it appears that all factors influencing epinephrine concentration in arterial plasma were in a state of dynamic equilibrium. That is, factors which tended to increase the concentration were equally offset by factors which tended to lower the concentration. The simplest factors which may operate to establish such an equilibrium include: the rate of infusion, the rate of removal, and the volume of epinephrine distribution. This volume of distribution is defined as the volume of fluid into which the epinephrine is dispersed at an uniform concentration equal to that of the femoral arterial plasma.

Assuming this simple case and definition of volume of distribution, the concentration of epinephrine in arterial plasma appeared to be a function of the infusion rate, the rate of removal or disappearance, and the volume of distribution. Mathematically this relation can be expressed as

$$(1) \qquad X = f(I,D,V)$$

where X equals the epinephrine concentration; f ( ) represents a function; I, the infusion rate; D, the disappearance rate; and V, the volume of distribution. If the volume of distribution of epinephrine was assumed to be constant after equilibrium was reached, the rate of

of infusion must have just equalled the rate of disappearance at equilibrium. Mathematically this can be represented as

$$(2) \qquad D_e = I_e$$

where the subscript "e" denotes equilibrium conditions.

3. The Relationship of Epinephrine Concentration to Infusion Rate at Equilibrium: At infusion rates less than about 3.0 ugm./kg./min. the concentration of epinephrine in femoral arterial plasma was linearly proportional to the rate of infusion as a first approximation. At higher infusion rates the logarithm of the concentration appeared to be more closely correlated with a linear change in infusion rate. The logarithmic relationship at higher infusion rates may have been due either to a relatively decreased rate of removal or to a decreased volume of epinephrine distribution. At equilibrium with the lower infusions therefore, the rate of disappearance appeared to be directly related to the epinephrine concentration since the disappearance at equilibrium equalled the rate of infusion. These relations may be represented symbolically as

$$(3) \qquad I_e = C \cdot X_e \quad \text{and} \quad (4) \qquad \therefore D_e = C \cdot X_e$$

wher C is a proportionality constant and in the case of equation 3 represents the slope of the infusion-concentration line.

4. Estimation of Epinephrine Disappearance Rates: Epinephrine concentration of femoral arterial plasma were estimated during epinephrine infusions at equilibrium and at intervals immediately after stopping the infusion. These concentrations diminished exponentially with the time after stopping the infusion. Such disappearance curves

are typical of mechanisms in which the rate of disappearance at any moment is proportional to the concentration at that moment. The differential equation expressing this relationship is

$$(5) \qquad \frac{dX}{dt} = -k \cdot X$$

where $\frac{dX}{dt}$ is the rate of change in epinephrine concentration with time and k is the proportionality constant. When this equation is integrated the equation of the disappearance lines in Figure 8 are obtained (also see Table XI in the Appendix.). The integrated formula of equation 5 is

$$(6) \qquad \ln\left(\frac{X}{X_o}\right) = -kt$$

where X_o represents the epinephrine concentration in the femoral artery just as the epinephrine infusion was stopped and X represents the concentration at time t after stopping the infusion. From this equation and the disappearance studies, the proportionality constants k can be estimated for each separate experiment.

5. Volume of Epinephrine Distrubution: The total rate of epinephrine disappearance immediately after the cessation of the infusion was equal to the concentration of epinephrine times its volume of distribution. The equation of this relationship is

$$(7) \qquad D = V \frac{dX}{dt}$$

From equation 5 $\frac{dX}{dt}$ was shown equal to -kX; therefore combining 5 and 7 the following is obtained:

$$(8) \qquad D = V(-kX) \quad \text{or} \quad D = -kVX$$

If the volume of distribution at equilibrium is assumed to be equal to the volume immediately after cessation of the infusion, equation 8

may be combined with equation 2 to obtain

$$(9)\quad I_e = -kV_eX_e \quad \text{or} \quad (10)\quad V_e = \frac{I_e}{-kX_e}$$

Equation 10 is the simplest approximation of the functional interrelationship of the rate of infusion, rate of disappearance, and volume of distribution in the observed experimental animals at equilibrium. The volume of distribution at equilibrium can therefore be estimated since the other factors in equation 10 were either estimated directly or calculated from the disappearance data. The volumes of epinephrine distribution have been calculated from formula 10 and the data from 23 different experiments and have been listed in Table II of the Appendix.

The average volume of distribution, measured 13 times in normal anesthetized dogs, was 107 ml./kg. with a standard error of the mean equal to 8.6 ml./kg. Plasma space in dogs has been estimated as about 40 to 50 ml./kg. It is reasonable to assume that the average plasma volume in the dogs studied with epinephrine was not greater than 80 ml./kg. even though the animal had received as much as 500 ml. of 1.1% saline intravenously during the experiment. From the experimental data it was found that the average epinephrine space was statistically greater than 80 ml./kg. at the 95 percentile level of significance. (The t from table for 12 d.f. with error of 0.05 equals 2.18 while the t calculated from the data was 3.2.) Furthermore, the concentration of epinephrine in venous plasma was found to be significantly lower than in arterial plasma. Therefore, the actual fluid volume with which the infused epinephrine equilibrated must have been greater than this theoretical volume of distribution which was calculated.

Hence, it is concluded that the volume of fluid with which the exogenous epinephrine equilibrates under conditions of constant intravenous infusion included and was larger than the total intravascular volume. That is, the epinephrine appeared to pass reversibly from the vascular system into other fluids. This again would indicate that the circulating epinephrine assayed was not bound to plasma constituents.

6. Mechanisms of Removal: It is apparent from the preceding discussion that the rate limiting mechanism regulating epinephrine disappearance was one in which the rate of disappearance was proportional to the concentration. Such rates are typical of first order reactions in which the rates are directly proportional to the concentration of the reacting substance (85). The overall disappearance rate, however, appears to have been a reflection of several different mechanisms at various sites of the circulation. The concentration of epinephrine was not altered appreciably by passing through the lungs; however, passage through the liver, kidney, gut, and hind limb resulted in substantial decreases in the arterial epinephrine concentration. The kidney removed a portion of the epinephrine by excretion through mechanisms which will be discussed. It is suggested that the rate limiting mechanism operating in the other sites of disappearance may have been movement of epinephrine into the cell. This would be a first order reaction if the concentration with in the cell were very small compared to that in the extracellular fluids.

A significant increase in the epinephrine extraction ratio of the hind limb with increased infusion rates was observed as mentioned in the section on Results. These increases in extraction ratio appeared to be correlated with a proportional decrease in hind limb blood flow.

The decrease in blood flow after the initial infusion rate was probably a result of vasoconstriction produced by higher concentrations of epinephrine. It is suggested that the increase in extraction ratios may have been related to this decrease flow rate. Assuming the volume of the blood or plasma in the hind limb was relatively stable, the mean transit time or average time blood was in the leg would have increased as the flow rate decreased. If such were the case in these experimental animals, the average time the plasma epinephrine was exposed to the removal mechanisms would also increase. The increase in exposure time might have led to an increase in the amount of epinephrine extracted relative to the amount entering the limb. Such an interpretation is consistent with first order reaction mechanisms.

The chief mechanisms regulating the disappearance of epinephrine from the circulation appear to lay outside of the normal plasma constituents. This was apparent from the observations that the epinephrine assayed only slowly disappeared from plasma stored at room temperatures.

C. Renal Excretion of Epinephrine

The clearances of epinephrine in the animals studied were calculated from estimates of urine flow and epinephrine concentrations in plasma and urine. As previously mentioned in the discussion, the recoveries of epinephrine added to urine and plasma appeared comparable and in the order of 100%. The epinephrine concentrations in plasma and urine immediately before and after cessation of the infusion were very small relative to the concentrations during the infusion. Therefore, epinephrine was not being synthesized from plasma constituents by the kidneys and excreted into the urine. Thus, these clearance values for

epinephrine are believed to be valid estimates of epinephrine clearance by the kidneys. As noted earlier, the epinephrine clearances were significantly greater than their corresponding creatinine clearances in all of the renal experiments. Therefore, the excretion of epinephrine could not have been a result of glomerular filtration alone.

The other mechanism of renal excretion of plasma constituents is active tubular secretion. In the experiments the apparent excretion of epinephrine by active tubular secretion was appreciable, in the order of 0.3 to 0.5 of the total amount in the urine.

Substances known to be actively secreted by tubular transport include phenol red, diodrast^{R}, para aminohipparate, penicillin, and n-methyl nicotinamide (81). All of these substances, except the last, were foreign to the animal in which they were investigated. The tubular transport mechanism requires chemical energy to operate, apparently derived from ATP (82). 2,4-Dinitrophenol (DNP) has become a useful tool for the study of active tubular secretion since it inhibits the transport of these substances, presumably by depletion of the renal ATP (82). In the experiments on epinephrine secretion in which DNP was administered, no changes were observed in epinephrine clearance or transport. However, marked diminution of PAH transport was noted as originally observed by Mudge and Taggart (83). The conclusion that epinephrine was not actively transported by tubular mechanisms dependent upon ATP is not necessarily true. The amount of epinephrine transported after DNP was still only about 1/1000 as much as the PAH transported. Therefore, though the ATP may have been depleted to a point where PAH transport was reduced, there may still have been enough to maintain the transport of the fraction of microgram amounts of epinephrine.

Because Hydergine® is known to block many of the biological effects of epinephrine (86,87), it was used in one of the renal experiments in order to determine, if possible, any inhibition of epinephrine transport. As previously mentioned, no significant changes were noted.

It is interesting to note that the amount of epinephrine in the urine was less than the amount extracted by the kidney (as estimated from renal plasma flow and arterio-venous concentration differences). This would indicate that part of the epinephrine extracted by the kidney was removed from circulation by mechanisms other than the previously discussed excretion processes.

Of interest also was the finding that epinephrine did not appear in the urine in a hydrolysable, conjugated form. Furthermore, the excretion of norepinephrine was not elevated during the infusion indicating that the infused epinephrine was not appreciably demethylated to the primary amine. These two observations suggest that demethylation to norepinephrine and conjugation with subsequent excretion were not important mechanisms in the disappearance of epinephrine during the infusion period.

D. Dynamics of Epinephrine Circulation and Renal Excretion after Thyroid Feeding

The parameters of epinephrine circulation and renal excretion previously discussed were estimated in 3 dogs before and during the feeding of U.S.P. thyroid extract.

The estimation of oxygen consumption was originally chosen as an index of hypermetabolism expected to develop with thyroid feeding. The explanation for failing to note any significant increase in oxygen

consumption may have been either insufficient thyroid treatment or interference of deep nembutal anesthesia. The considerations which favor the attainment of a degree of hyperthyroidism were: the dose and duration of treatment, the concomitant weight loss of two dogs and the increase in PAH clearance. Others have observed hyperthyroidism in dogs on similar dose schedules (72,88). Smith reported that PAH clearances have been noted to increase with hyperthyroidism (81).

The amount of the infusion excreted in the urine was the only significant change noted in epinephrine circulation and excretion after feeding thyroid. This change may have been due, in part, to an increase in renal plasma flow as reflected by the elevation in PAH clearance.

The overall parameters of the dynamics of epinephrine circulation were not significantly altered from the pre-thyroid state. These findings are therefore, consistent with the hypothesis that hyperthyroidism is not associated with an alteration in the release or overall rate of disappearance of epinephrine.

E. General Conclusions

From the experimental data and the foregoing discussion the conclusion is made that the concentration of epinephrine in arterial plasma was determined by at least three factors: the rate of epinephrine input, the volume of distribution, and the rate of disappearance.

The input rate was controlled by constant intravenous infusions of exogenous epinephrine.

The volume of distribution of epinephrine was greater than the intravascular fluid space, indicating that the arterial epinephrine was exchanging with extravascular fluids.

The overall rate of disappearance of arterial epinephrine was of the first order reaction variety. The disappearance process was, in part, a summation of various removal mechanisms located in the liver, gut, hind limb, and kidneys. It is suggested that the common rate limiting process in these areas may be that of simple diffusion of epinephrine into the cells. The removal of epinephrine in the various areas may be markedly influenced by the rate of blood flow, as demonstrated in the hind limb.

Removal of circulating epinephrine by the kidneys was effected by both intrarenal destruction and excretion. However, renal excretion accounted for only 4 to 5% of the amount of epinephrine infused per unit time. One mechanism of epinephrine excretion was that of active tubular secretion. Excretion by glomerular filtration was also probable since the epinephrine assayed was not bound to plasma constituents and passed reversibly into the extravascular fluid space.

Although these studies of the dynamics of epinephrine circulation and renal excretion were made with infusions of exogenous epinephrine, they are offered as first approximations of the circulation and excretion of endogenous epinephrine.

SUMMARY

A fluorimetric procedure for the estimation of epinephrine and norepinephrine has been modified and applied to the quantitative detection of these substances in plasma and urine.

The dynamics of epinephrine circulation and excretion have been studied by infusing epinephrine intravenously into normal and thyroid-fed dogs. These problems were approached by estimating the epinephrine concentration in the urine and plasma sampled from various sites of the circulatory system during and after the infusions.

The studies indicate that the epinephrine estimated by the method was not bound to plasma constituents and that it passed reversibly into a fluid space which exceeded the size of the intravascular volume. The epinephrine disappeared rapidly from arterial plasma after cessation of the infusion, being removed, in part, by the liver, gut, kidney, and hind limb. The lungs, however, did not remove appreciable amounts of the hormone. The rate of disappearance of epinephrine in the plasma stored at room temperature was very small compared to the rate of disappearance in circulating plasma.

From 4 to 5% of the infusion per unit time was excreted in the urine by the mechanisms of active tubular secretion and probable glomerular filtration. A portion of the epinephrine extracted by the kidney was also destroyed in the organ.

The dynamics of epinephrine circulation were not altered during the feeding of thyroid extract. However, the amount of infused epinephrine excreted in the urine did increase during the thyroid feeding.

BIBLIOGRAPHY

1. Holmo-Lundholm, K. The Mechanism of Relaxing Effect of Adrenaline on Smooth Muscle. Acta physiol. Scandinav. 29: Suppl. 108; 1-63, 1953

2. Celander, O. V. The Range of Control Exercised by the Sympathico-Adrenal System. Acta physiol. Scandinav. 32: Suppl. 116; 1-132, 1954

3. Euler, U. S. von. A Functional Differentiation of Adrenaline and Noradrenaline as a Reflected in Their Metabolic Actions. Estratto da Medicina (Parina), 2: 41-52, 1952

4. Cori, C. F.; Cori, G. T.; and Buchwald, K. W. The Mechanism of Epinephrine Action VI. Changes in Blood Sugar, Lactic Acid, and Blood Pressure During Constant Intravenous Injection of Epinephrine. Am. J. Physiol. 93: 273-283, 1930

5. Sutherland, E. W. The Effect of the Hyperglycemic Factor and Epinephrine on Enzyme Systems of the Liver and Muscle. Ann. New York Acad. Sc., 54: 693-706, 1951

6. Wool, I. G.; Goldstein, M. S.; Ramey, E. R.; and Levine, R. Role of Epinephrine in the Physiology of Fat Mobilization. Am. J. Physiol., 178: 427-432, 1954

7. Lerner, A. B. Melanin Pigmentation. Am. J. Med., 19: 902-924, 1955

8. Euler, U. S. von. The Nature of Adrenergic Mediators. Pharmacol. Rev., 3: 247-277, 1951

9. Euler, U. S. von. Epinephrine and Norepinephrine: Distribution and Action. Pharmacol. Rev., 6: 15-22, 1954

10. Hechter, O. Concerning Possible Mechanisms of Hormone Action. Vitamins and Hormones, 13: 293-342, 1955

11. Weil-Malherbe, h. and Bone, A. F. The Adrenergic Amines of Human Blood. Lancet, v.1: 974-977, 1953

12. Noel, R. H. The Biological Assay of Adrenaline. J. Pharmacol. & Exper. Therap., 84: 278-283, 1945

13. Elliot, T. R. Nerve Control of Suprarenals, J. Physiol., 44: 374-409, 1912

14. Burn, J. H. Biological Standardisation, Oxford Univ. Press, London, England, 1937

15. Barcoum, G. S. and Gaddum, J. H. The Pharmacological Estimation of Adenosine and Histamine in Blood. J. Physiol., 85: 1-14, 1935

16. Euler, U. S. von. The Distribution of Sympathin N and Sympathin a in Spleen and Splenic Nerves of Cattle. Acta Physiol. Scandinav., 19: 207-214, 1949

17. Cannon, W. B. and Rosenblueth, A. Autonomic Neuro-Effector Systems. 1937, The MacMillan Company, New York

18. Bülbring, E. and Burn, J. H. Liberation of Noradrenaline from the Suprarenal Gland. Brit. J. Pharmacol., 4: 202-208, 1949

19. Hedgate, E. S. and Gellhorn, E. Nature of Sympathetico-Adrenal Discharge Under Conditions of Excitation of Central Autonomic Structures. Am. J. Physiol., 174: 475-480, 1953

20. Gaddum, J. H. Assay of Neurohumors, Section II. Methods in Medical Research, Vol. 3, Edited by R. W. Gerand. Year Book Pub., Inc., Chicago, Ill.

21. Persky, H. Chemical Determination of Adrenaline and Noradrenaline in Body Fluids and Tissues. Methods of Biochemical Analysis, Vol. II: 57-82, 1955, Edited by David Glick, Interscience Pub., Inc., New York

22. Euler, U. S. von, and Hellner, S. Excretion of Noradrenaline, Adrenaline, and Hydroxytyramine in Urine. Acta physiol. Scandinav., 22: 161-167, 1951

23. Tullar, B. F. The Separation of l-Arterenol from Natural U. S. P. Epinephrine. Science, 109: 536-537, 1949

24. Schild, H. Adrenaline in the Suprarenal Medulla. J. Physiol. 79: 455-469, 1933

25. Euler, U. S. von and Hamberg, U. Colorimetric Determination of Noradrenaline and Adrenaline. Acta physiol. Scandinav., 19: 74-84, 1949

26. Shaw, F. H. The Estimation of Adrenaline. Australian J. Exp. Biol. and M. Sc., 24: 53-55, 1946

27. Raab, W. Adrenaline and Related Substances in Blood and Tissues. Biochem. J., 37: 470-473, 1943

28. Auerbach, M. E. and Angell, E. The Determination of Arterenol in Epinephrine. Science, 109: 537-538, 1949

29. Ehrlen, I. Farm. Revy., 47: 321, 1948. Sited from Euler and Floding; see reference #30

30. Euler, U. S. von and Floding, I. A Fluorimetric Micromethod for Differential Estimation of Adrenaline and Noradrenaline. Acta physiol. Scandinav., 33; Suppl. 118: 45-56, 1955

31. Lund, A. Simultaneous Fluorimetric Determinations of Adrenaline and Noradrenaline in Blood. Acta pharmacol. et toxicol., 6: 137-146, 1950

32. Weil-Malherbe, H. and Bone, A. D. The Chemical Estimation of Adrenaline-like Substances in Blood. Biochem. J., 51: 311-318, 1952

33. Gaddum, J. H. and Schild, H. J. Physiol., 80: 9, 1934, as sited from reference #20

34. Peikarinen, A. Studies on the Chemical Determination, Occurrence, and Metabolism of Adrenaline in the Animal Organism. Acta physiol. Scandinav., 16; Suppl. 54: 1-111, 1948

35. Natelson, S.; Lugovoy, J. K.; and Pincus, J. B. A New Fluorimetric Method for the Determination of Epinephrine. Arch. Biochem., 23: 157-158, 1949

36. Aronow, Lewis and Howard, F. A. Improved Fluorimetric Technique to Measure Changes in Adrenal Epinephrine and Norepinephrine Output Caused by Veratrum Alkaloids. Fed. Proc., Vol. 14: 315, 1955

37. Ludemann, H. H.; Filbert, M. G.; and Cornblath, M. Application of a Fluorimetric Method for Adrenaline-like Substances in Peripheral Plasma. J. Appl. Physiol., 8: 59-66, 1955

38. Euler, U. S. von and Floding, I. Fluorimetric Estimation of Noradrenaline and Adrenaline in Urine. Acta physiol. Scandinav., 33; Suppl. 118: 57-63, 1955

39. Euler, Chr. von; Euler, U. S. von; and Floding, I. Biologically Inactive Catechol Derivatives in Urine. Acta physiol. Scandinav., 33; Suppl. 118: 32-38, 1955

40. Price, H. L.; Valk, A.; and Valk, De. Chemical Assay of Epinephrine and Norepinephrine in Plasma. Fed. Proc., Vol. 15: 469, 1956

41. Loew (Loewi), O. Über die Natur der Giftwirkung des Suprarenins. Biochem. Ztschr., 85: 295-306, 1918

42. Lund, A. Fluorimetric Determination of Adrenaline in Blood. I. Isolation of Fluorescent Oxidation Product of Adrenaline. Acta pharmacol. et toxicol., 5: 75-94, 1949

43. Lund, A. II. The Chemical Constitution of Adrenolutine (The Fluorescent Oxidation Product of Adrenaline). Acta pharmacol. et toxicol., 5: 121-128, 1949

44. Lund, A. III. A New Sensitive Specific Method. Acta pharmacol. et toxicol., 5: 251-247, 1949

45. Bulock, J. and Harley-Mason, J. The Chemistry of Adrenochrome, Part II, J. Chem. Soc. 1951: 712-716

46. Heller, J. H.; Setlow, R. B.; and Nylon, E. Fluorimetric Studies of Epinephrine and Arterenol. Am. J. Physiol., 161: 266-277, 1950

47. Lockett, M. F. A Compound Very Closely Resembling N-isopropyl-noradrenaline in Saline Extracts of Cat Adrenal Gland. J. Physiol., 124: 20 P, 1954

48. Shaw, F. H. The Estimation of Adrenaline. Biochem. J., 32: 19-25, 1938

49. Euler, U. S. von and Orwen, I. Preparation of Urine and Organs for Estimation of Free and Conjugated Noradrenaline and Adrenaline. Acta physiol. Scandinav., 33; Suppl. 118: 1-9, 1955

50. Lund, A. Elimination of Adrenaline & Noradrenaline from the Organism. Acta pharmacol. et toxicol., 7: 297-308, 1951

51. Miller, J. W. and Elliott, H. W. Plasma Sympathin Concentration of Dogs. Proc. Soc. Exper. Biol. & Med., 87: 487-488, 1954

52. Cahen, A. Vitesse de Disparition de l'Adrenaline Dans le Sang. Compt. rend. Soc. biol., 139: 22-24, 1945

53. Weil-Malherbe, H. The Effect of Convulsive Therapy on Plasma Adrenaline and Noradrenaline. J. Ment. Sc., 101: 156-162, 1955

54. Euler, U. S. von and Hellner-Bjorkman, S. Effect of Amine Oxidase Inhibitors on the Noradrenaline and Adrenaline Content of Cat Organs. Acta physiol. Scandinav., 33; Suppl. 118: 21-25, 1955

55. Euler, U. S. von and Hetterstrom, B. The Role of Amine Oxidase in the Inactivation of Catechol Amines Injected in Man. Acta physiol. Scandinav., 33; Suppl. 118: 26-31, 1955

56. Richter, D. The Inactivation of Adrenaline in vivo in Man. J. Physiol., 98: 361-374, 1940

57. Euler, U. S. von. Excretion of Noradrenaline and Adrenaline in Muscular Work. Acta physiol. Scandinav., 26: 185-191, 1952

58. Peikarinen, A. and Pitkanen, M. E. Noradrenaline and Adrenaline in Urine. Part II, Their Excretion in Certain Normal and Pathological Conditions. Scandinav. J. Clin. and Lab. Invest., 7: 8-14, 1955

59. Frankson, C.; Gemzell, C. A. and Euler, U. S. von. Cortical and Medullary Adrenal Activity in Surgical and Allied Conditions. J. Clin. Endocrinol., 14: 606-621, 1954

60. Mann, M. The Stability of Adrenaline and Noradrenaline in Human Urine. J. Pharm. and Pharmacol., 5: 1024-1030, 1953

61. Beyer, K. H. and Shapiro, S. H. The Excretion of Conjugated Epinephrine Related Compounds. Am. J. Physiol., 144: 521-530, 1945

62. Clark, W. G.; Akawie, R. I.; Pegrund, R. S. and Geissman, T. A. Abstr. Comm. XVIII Intn. Physiol. Congr. 159

63. Holtz, P.; Credner, K.; and Kronsberg, G. Uber das sympathicomimetsche pressorische Prinzip des Harns. Arch. exp. Path. Pharm., 204: 228-245, 1947

64. Goldenberg, M.; Serlin, I.; Edwards, T.; and Rapport, M. M. Chemical Screening Methodsfor the Diagnosis of Phaeochromocytoma. Am. J. Med., 16: 510-527, 1954

65. Euler, U. S. von and Luft, R. Noradrenaline Output in Urine after Infusion in Man. Brit. J. Pharmacol., 6: 286-288, 1951

66. Goldenberg, M. Adrenal Medullary Function. Am. J. Med., 10: 627-641, 1951

67. Euler, U. S. von; Luft, R.; and Sundin, T. Excretion of Urinary Adrenaline in Normals Following I. V. Infusion. Acta physiol. Scandinav., 30: 249-257, 1954

68. Leduc, J.; Dubreuil, R.; and D'Iorio, A. Distribution of Adrenaline and Noradrenaline in the Normal and Hyperthyroid Rat Following Adrenaline Administration. Canad. J. Biochem. and Physiol., 35: 203-208, 1955

69. Gellhorn, E. and Feldman, J. The Influence of the Thyroid on the Vago-insulin and Sympathetico-adrenal System. Endocrinology, 29: 467-474, 1941

70. Brewster, W. R.; Isaacs, J. P.; Osgood, P. F.; and King, T. L. The Hemodynamic and Metabolic Interrelationships in the Activity of Epinephrine, Norepinephrine and the Thyroid Hormones. Circulation, 13: 1-20, 1956

71. Smith, D. Immediate Sensitization of Isolated Swine Arteries and their Vasa Vasorum to Epinephrine, Acetylcholine, and Histamine by Thyroxine. Am. J. Physiol., 177: 7-12, 1954

72. Ragoff, J. M. and Cortell, R. Effect of Hyperthyroid on Epinephrine Secretion by the Adrenal Gland. J. Lab. and Clin. Med., 36: 981-987, 1941

73. Euler, U. S. von. Some Aspects of Clinical Physiology of Noradrenaline. Scan. Jr. Clin. Lab. Invest., 4: 254-262, 1952

74. Loewenstein, E. Glass Standards for Fluorimetric Determinations. Ind. Eng. Chem. Anal. Ed., 15: 658, 1943

75. Pekkarinen, A. Adrenaline and Noradrenaline in Blood and Urine. Pharmacol. Rev., 6: 35-37, 1954

76. Aranow, L. Personal communication

77. Bonsnes, R. W. and Taussky, H. H. On the Colorimetric Determination of Creatinine by the Jaffe Reaction. J. Biol. Chem., 158: 581-591, 1945

78. Smith, H. W.; Finkleestein, N.; Aliminosa, L.; Crawford, B.; and Graber, M. The Renal Clearances of Substituted Hippuric Acid Derivatives and Other Aromatic Acids in Dog and Man. J. Clin. Invest., 24: 388-404, 1945

79. U. S. P. Thyroid Extract donated by Mr. Baucher of Hasck Laboratories, Inc.

80. Dixon, W. J. and Massey, F. J. Introduction to Statistical Analysis. 1951; McGraw-Hill Book Co., Inc. New York

81. Smith, H. The Kidney. 1951; Oxford University Press, New York

82. Taggart, J. V. Some Biochemical Features of Tubular Transport Mechanisms. The Kidney, pp 65-68. Ciba Fdn. Symp. Little, Brown and Co., 1953

83. Mudge, G. H. and Taggart, J. V. Effect of 2,4-dinitrophenol on Renal Transport Mechanisms in the Dog. Am. J. Physiol., 161: 175-180, 1950

84. Raab, W. and Gigee, W. Norepinephrine and Epinephrine Content of Normal and Diseased Human Hearts. Circulation, 11: 595-603, 1955

85. Glasstone, S. The Elements of Physical Chemistry, pp 585-600. D. Van Nostrand Co., Inc. New York, 1946

86. Ellis, S; Anderson, H.; and Collins, W. Pharmacological Differentiation Between Epinephrine- and NGF-Hyperglycemia. Proc. Soc. Exper. Biol. and Med., 84: 383-386, 1953

87. Goodman, L. S. and Gilman, A. The Pharmacological Basis of Therapeutics. 1955, New York pp 886-896

88. Danowski, T. S.; Man, E. B.; and Winkler, A. W. Tolerance of Normal, of Thyroidectomized, and of Thiourea or Thiouracil Treated Dogs to Oral Desiccated Thyroid and to Intravenous Thyroxin. Endocrinology, 38: 230-237, 1946

APPENDIX

Section I. Modification of Lund's Procedure for the Estimation of Epinephrine and Norepinephrine

The final procedure after the modifications mentioned in the text of the thesis was as follows: A sample of 5.0 ml was added to 5.0 ml of 0.2 M sodium acetate and the pH adjusted to 6.7 to 7.0 with 0.2 to 1.0 N sodium hydroxide. This combination was mixed for 2.0 minutes with 0.5 gm. "Woelm" non-alkaline aluminum oxide in a centrifuge tube. The mixture was centrifuged and the supernatant discarded. The remaining alumina was washed 2 to 4 times with 0.2 M sodium acetate (pH 6.75) by mixing, centrifuging, and discarding the supernatants. The catechols were eluted from the alumina with 10.0 ml of 0.2 N acetic acid and 1.0 ml of 0.2 N hydrochloric acid. The resulting supernatant and a subsequent wash with 4 to 9 ml of distilled water were combined and the pH adjusted to either 3.0 or 6.5 (with dilute hydrochloric acid or 0.8 M dibasic sodium phosphate, respectively). The buffered eluate was then diluted to exactly 20.0 ml and oxidized for 3.0 minutes by mixing continuously with 0.1 gm. of Baker's Analyzed 94.0% manganese dioxide. The manganese dioxide was separated from the fluid phase by centrifugation and the supernatant filtered through a funnel with a fritted disc. Two portions of 8.0 ml of the filtrate were placed in two matched cuvettes. A 1.0 ml volume of a mixture of 2.0 ml of 20% sodium hydroxide and 0.5 ml of 1.0% ascorbic acid was added to the first cuvette. After inverting the cuvette 3 times for mixing the maximum

fluorescent intensity relative to the glass reference standard was measured with the fluorimeter. A 0.8 ml volume of 20% sodium hydroxide was mixed with the contents of the second cuvette and the mixture was allowed to stand for 10 or more minutes. After standing, 0.2 ml of a 1% ascorbic acid solution was added and the maximum fluorescent intensity relative to the reference standard was measured.

The fluorescence of the first tube was considered to be due to background fluorescence and the fluorescence of oxidized epinephrine (at pH 3.0) or oxidized norepinephrine and epinephrine (at pH 6.5). The fluorescence of the second cuvette was considered due to background fluorescence only. Therefore, the net fluorescence of the oxidized catechol ethanolamines was obtained by subtracting the reading of the second cuvette from that of the first. The concentrations of the two hormones were estimated from the standard curves.

Section II. Tabulation of Experimental Data

The experimental results have been tabulated in Tables I to VII which follow.

TABLE I

Epinephrine Concentration in Arterial Plasma
During Constant Intravenous Infusion of Epinephrine

Animal, Weight, and Treatment*			Rate of Infusion	Epinephrine Concentration in ugm./100 ml at Time in Minutes After Starting**				
Dog	Kg.	Treatment	ugm./kg./min.	C_o	C_1	t_1	C_2	t_2
1	22.3	A.R.E.	0.264	0.1	0.4	13		
2	12.7	A.R.E.	0.47	0.1	0.8	-		
3	12.7	A.R.E.	0.47	0.0	0.65	14		
4	11.8	A.R.E.	0.85	0.3	1.2	-		
5	17.3	A.R.E.	0.58	0.0	0.8	10		
6	24.1	A.R.E.	0.21	0.0	0.45	12		
7	24.1	A.R.E.	0.37	0.0	0.75	12		
8	16.5	A.R.E.	0.23	0.05	0.35	10		
9	17.3	C.R.E.	0.29	0.0	0.4	10		
10	14.5	A.R.E.	4.83	0.1	7.0	19		
11	20.0	A.R.E.	1.13	0.0	1.45	12		
12	15.0	A.R.E.	2.4	-	2.6	-		
13	15.9	A.R.E.	0.25	0.0	0.2	-		
14	17.3	A.R.E.	1.63	0.0	1.4	-		
"	"	"	3.24	0.0	4.3	-		
15	19.3	A.R.E.	4.14	-	3.4	-		
16	19.5	A.R.E.	1.9	-	2.3	-		
17	16.0	A.R.E.	2.9	-	4.5	10	5.0	55
18	17.5	A.R.E.	2.96	-	4.6	10	5.6	20
19	17.6	A.R.E.	1.99	-	1.3	10	1.35	20
B.	18.6	B.T.F.	0.87	-	0.87	9		
"	"	"	1.50	-	1.35	10		
H.	13.2	B.T.F.	1.5	-	1.3	10		
"	"	"	2.7	-	2.2	11		
C.	13.6	B.T.F.	1.14	-	1.1	15		
"	"	"	2.10	-	2.4	14		
B.	18.6	B.T.F.	1.0	-	0.85	15		
"	"	"	1.77	-	1.14	15		
H.	13.6	B.T.F.	1.06	-	0.66	13		
"	"	"	1.78	-	1.50	12		
C.	13.6	B.T.F.	1.19	0.05	1.05	13		
"	"	"	2.0	-	1.85	14		
DNP1	12.7	D-DNP	2.1	-	1.0	12	1.2	22
"	"	A-DNP	2.2	-	1.65	16	1.6	26
S.	23.6	N.P.T.	1.65	-	2.1	16		
B.	16.2	T-1/dx 7	1.0	-	0.96	13		
"	"	"	1.75	-	1.37	10		
H.	14.3	T-1/d x 7	1.0	-	0.96	15		
"	"	"	1.81	-	1.35	13		
C.	13.2	T-16/7d	0.98	-	1.0	4½	1.0	8½
"	"	"	1.8	-	2.06	15		
"	"	"	1.8	-	1.92	15		

TABLE I
(Continued)

Epinephrine Concentration in Arterial Plasma
During Constant Intravenous Infusion of Epinephrine

Animal, Weight, and Treatment*			Rate of Infusion	Epinephrine Concentration in ugm./100 ml at Time in Minutes After Starting**				
Dog	Kg.	Treatment	ugm./kg./min.	C_o	C_1	t_1	C_2	t_2
DNP2	22.8	B-DNP	1.45	-	1.2	12	0.9	22
"	"	A-DNP	1.42	-	1.17	12	0.65	32
B.	16.2	T-1/d x 22	0.94	-	0.77	15		
"	"	then 5/d x 6	1.85	-	1.1	14		
D.	15.9	B.H.	1.21	-	0.75	13	1.4	28
"	"	A.H.	1.16	-	2.0	15	1.3	40
H.	13.6	T-1/d x 22	1.01	-	0.77	10	0.6	14
"	"	then 5/d x 10	2.02	-	2.0	10	1.85	14
D.	16.1	N.P.T.	0.96	-	1.35	-		
"	"	"	1.83	-	2.72	-		
"	"	"	3.4	-	6.3	-		
D.	15.4	N.P.T.	1.46	-	0.9	13		
B.	16.2	A.T.F.	1.37	-	0.7	13	0.65	18
"	"	"	2.62	-	1.45	13	2.05	18
C.	13.0	A.T.F.	1.24	-	1.8	-		
"	"	"	2.07	-	2.55	-		
"	"	"	2.48	-	5.0	-		
"	"	"	3.72	-	6.0	-		
"	"	"	3.82	-	12.0	-		
L.	12.7	N.P.T.	0.97	-	0.6	10		
"	"	"	1.26	-	0.88	10		
"	"	"	1.56	-	1.3	10		
"	"	"	2.06	-	1.4	10		
"	"	"	2.97	-	2.62	10		
"	"	"	3.94	-	4.5	10		

Notes to Table I.:

* Treatment code for Tables I and II.: N.P.T., no previous treatment; A.R.E., acute renal experiment; C.R.E., chronic renal experiment; B.T.F., before thyroid feeding; A.T.F., one week or more after thyroid feeding; T-#/d x #, after feeding # grams thyroid extract per day for # days; B-DNP and A-DNP, before and after 2,4-dinitrophenol injection, respectively; B.H. and A.H., before and after receiving 4.5 ml of Hydergine®.

** C represents the epinephrine concentration in arterial plasma at time t after starting the constant infusion at rate listed. C_o represents the epinephrine concentration before starting the infusion.

TABLE II

Disappearance of Arterial Plasma Epinephrine

Animal	Treatment*	Wt. Kg.	Epinephrine Infusion µgm./kg/min.	Duration of Infusion - min.	Epinephrine concentrations at times after stopping infusions								Epinephrine "Space" ml./kgm.
					Co. µgm./100 ml.	C_1 µgm./100 ml.	t_1 min.	C_2 µgm./100 ml.	t_2 min.	C_3 µgm./100 ml.	t_3 min.	$\frac{\log Co}{t-to}$ **	$\frac{\ln Co}{t-to}$	
Dog 9.	C.R.E.	17.3	0.29	11	0.4	0.2	1	0.00	1½			0.3	0.7	104
Dog 11.	A.R.E.	20.0	1.13	12	1.45	0.65	1	0.50	2	0.40	3	0.35	0.8	97
Dog 12.	A.R.E.	15.2	2.40		2.6	0.9	½	0.35	1	0.10	2	0.87	2.0	180
Dog 14.	A.R.E.	17.3	3.24		4.3	2.1	½	1.00	1	0.55	1½	0.62	1.43	108
Dog 15.	A.R.E.	19.3	4.10		3.4	1.45	½	0.55	1	0.32	1½	0.76	1.75	70
Dog 16.	A.R.E.	19.5	1.9		2.3	1.80	1/4	1.08	½	0.90	3/4	0.60	1.38	60
Dog B.	B.T.F.	18.6	1.35	10	1.35	0.70	½-3/4	0.60	5/6	0.50	1 1/3	0.40	0.92	109
Dog H.	B.T.F.	13.2	2.70	11	2.70	1.30	½	1.10	3/4	0.78	1	0.45	1.04	118
Dog C.	B.T.F.	13.6	2.10	14	2.40	1.55	½	1.00	5/6	0.75	1 1/12	0.45	1.04	84
Dog B.	B.T.F.	18.6	1.77	15	1.14	0.50	½	0.30	5/6	0.1	1 1/8	0.70	1.61	97
Dog H.	B.T.F.	13.6	1.78	12	1.5	0.82	½	0.66	3/4	0.5	1	0.47	1.09	109
Dog C.	B.T.F.	13.6	2.00	14	1.85	1.25	½	0.82	3/4	0.65	1	0.44	1.01	107
Dog DNP1 (125mg DNP)	B-DNP	12.7	2.10	12	1.20	0.60	½	0.50	5/6			0.50	1.15	150
	A-DNP		2.0	16	1.60	0.78	½	0.78	5/6			0.45	1.04	120
Dog S.	M.P.T.	23.2	1.65	16	2.1	1.0	2/3	0.67	5/6	0.5	1 1/8	0.65	1.43	55
Dog B.	T-1/d x 7	18.2	1.75	10	1.37	1.1	½	0.60	3/4	0.44	1 1/12	0.45	1.04	122

TABLE II

Disappearance of Arterial Plasma Epinephrine

Animal	Treatment*	Wt. Kg.	mgm./kg./min. Epinephrine Infusion	Duration of Infusion-min.	Epinephrine concentrations at time after stopping infusions: Co. ugm./100 ml.	C_1 ugm./100 ml.	t_1 min.	C_2 ugm./100 ml.	t_2 min.	C_3 ugm./100 ml.	t_3 min.	$\frac{\log Co}{\log C}$ t-to**	$\frac{\ln Co}{\ln C}$ t-to	Epinephrine "Space" ml./kgm.
Dog H.	T-1/d. x 7	14.3	1.61	13	1.35	0.75	1/2	0.60	5/6	0.38	1 1/12	0.50	1.15	117
Dog C.	T-16/7d	13.2	1.85	15	1.92	0.86	1/2	0.74	3/4	0.40	1 1/12	0.60	1.38	60
Dog DNP2 (170mg DNP)	A-DNP	22.8	1.42	12	0.65	0.44	1/2	0.42	3/4	0.24	1	0.34	0.78	280
Dog B.	T-1/d. x 22 then 5/d. x 6	16.2	1.85	14	1.1	0.85	1/2	0.32	3/4	0.22	1	0.70	1.61	105
Dog H.	T-1/d. x 22 then 5/d. x 10	13.6	2.02	14	1.85	0.50	1/2	0.20	3/4	0.10	1	1.2	2.76	40
Dog D.	A. H.	15.9	1.16	40	1.3	0.48	1/2	0.30	3/4	0.10	1	0.86	1.98	33.5
Dog C.	N.P.T.	15.9	1.02		2.72	1.45	1/2	0.73	3/4	0.74	1	0.60	1.38	48.0

*Treatment code. See Notes of Table I

**C equals plasma epinephrine concentration at time t after stopping infusion. Co represents concentration before stopping infusion.

TABLE III

Animal, weight and Treatment	Time in min.	No. of collection periods averaged	Epinephrine Infusion Rate γ/Kg./min.	Epinephrine Concentration gm./100 ml. Plasma	Epinephrine Concentration gm./100 ml. Urine	Urine Flow ml./min.	% Infusion excreted in urine		Clearances Epinephrine ml./min.	Clearances Creatinine ml./min.	Clearances PAH ml./min.	Epin. clearance Creat. clearance	Epinephrine Transport gm./min.	*Oxygen Consumption ml./min.
Dog. B. 18.6 Kg.	0-10	(1)	Control		0.3	2.0				69.7	150.4			110
First control	20-25	(1)	0.87	0.75	35.6	2.88	6.4	137		96.8	172.0	1.41	0.305	123
experiment	35-40	(1)	1.50	1.50	67.7	2.34	4.6	122		79.5	149.0	1.53	0.552	125
	50-60	(1)	Recovery		4.7	2.01				77.0	129.0			129
Dog B. 18.6 Kg.	0-15	(1)	Control		0.4	1.58				60.0				131
Second control	25-35	(1)	1.00	0.85	32.8	2.52	4.4	97.2		68.8		1.41	0.241	121
experiment	45-55	(1)	1.77	1.14	54.7	2.09	3.5	100.0		66.9		1.35	0.383	128
	65-95	(3)	Recovery		1.6	1.78				67.5				121
Dog B. 18.6 Kg.	0-20	(2)	Control		0.5	1.29				84.7	333.1			64
Thyroid Extract	40-50	(1)	1.00	0.96	65.5	1.30	4.7	88.8		79.5	198.0	1.12	0.089	63
per os, 1 gm./day	60-70	(1)	1.75	1.37	144.4	1.12	5.1	118.2		79.6	262.3	1.49	0.528	129
x 8 days	80-120	(4)	Recovery		1.3	2.02				78.9	245.3			50
Dog B. 16.2 Kg.	0-30	(3)	Control		1.3	0.48				76.0	248.0			111
Thyroid Extract	40-50	(1)	0.94	0.77	170.3	0.58	6.5	128.3		86.2	211.0	1.49	0.316	180
per os, 1 gm./day	60-70	(1)	1.85	1.10	309.0	0.635	6.5	178.2		81.2	205.5	2.19	1.080	299
x 22 days then	80-110	(3)	Recovery		3.5	0.57				92.0	291.0			134
5 gm./day x 6 days														

*No epinephrine during control and recovery periods

**Oxygen consumption not corrected to S.T.P. Temperature range 27 to 32°C. Barometric pressure range from 745 to 765 mm. Hg.

TABLE IV

Animal, Weight and Treatment	Time in min.	No. of collection periods averaged	Epinephrine Infusion Rate mcg./kg./min.	Epinephrine Concentration mgm./100 ml. Plasma	Epinephrine Concentration mgm./100 ml. Urine	Blood Flow ml./min.	% Infusion excreted in urine	Clearances Epinephrine	Clearances Creatinine ml./min.	Clearances PAH ml./min.	Endin. clearance Creat. clearance	Epinephrine Transport mgm./min.	Oxygen Consumption ml./min.
Dog H. 13.2 Kg.	0-10	(1)	Control		0.12	6.28			53.6	158.3			74
First control	20-25	(1)	1.51	1.3	53.4	1.4	3.8	57.5	46.8	120.8	1.23	0.139	253
experiment	35-40	(1)	2.69	2.2	157.0	0.88	3.9	62.8	54.2	154.0	1.16	0.190	138
	50-70	(2)	Recovery		1.2	4.19			73.6	133.4			52
Dog H. 13.6 Kg.	0-20	(2)	Control		1.7	1.15			45.0				54
Second control	30-40	(1)	1.06	0.66	33.6	2.61	6.1	133	67.6		1.97	0.423	70
experiment	50-60	(1)	1.78	1.50	30.5	3.08	3.9	62.5	41.4		1.51	0.317	94
	70-100	(3)	Recovery		1.3	4.71			52.1				71
Dog H. 14.3 Kg.	0-20	(2)	Control		0.18	5.05			50.9	165.0			53
Thyroid Extract	30-40	(1)	1.01	0.96	18.6	3.05	3.9	59.0	41.7	111.2	1.41	0.167	36
per os, 1 gm./day	50-60	(1)	1.81	1.35	39.0	2.40	3.6	69.4	42.0	90.0	1.65	0.370	51
x 8 days	70-90	(2)	Recovery		0.6	4.50			50.0	170.1			41

TABLE V

Animal, weight and Treatment	Time in min.	No. of collection periods averaged	Epinephrine Infusion Rate mgm./kg./min.	Epinephrine Concentration µgm./100 ml. Plasma	Epinephrine Concentration µgm./100 ml. Urine	Urine Flow ml./min.	% Infusion excreted in urine	Clearances Epinephrine ml./min.	Clearances Creatinine ml./min.	Clearances PAH ml./min.	Epin. clearance / Creat. clearance	Epinephrine Transport ugm./min.	Oxygen Consumption ml./min.
Dog C. 13.6 Kg.	0-10	(1)	Control		0.3	2.28			48.0	212			56
First control	20-25	(1)	1.07	1.1	45.8	1.80	5.3	75.0	48.8	118	1.31	0.287	65
experiment	35-40	(1)	2.10	2.4	157.8	0.80	4.3	52.5	33.2	87.0	2.62	0.463	39
	50-80	(3)	Recovery		1.0	1.59			44.5	171.0			36
Dog C. 13.6 Kg.	0-20	(2)	Control		0.53	1.31			48.2				48
Second control	30-40	(1)	1.19	1.05	67.6	1.04	4.5	67.0	51.0		1.31	0.167	82
experiment	50-60	(1)	2.00	1.85	153.0	0.76	4.2	62.8	40.2		1.56	0.416	79
	70-100	(3)	Recovery		1.1	2.20			53.8				70
Dog C. 13.2 Kg.	0-20	(2)	Control		0.15	3.87			59.5	237.3			
Thyroid Extract	30-40	(1)	0.98	1.15	85.5	1.25	8.2	93.0	57.7	177.1	1.61	0.407	apparatus
per os, 16 gm.	50-60	(1)	1.85	2.06	436.0	0.38	6.8	84.4	42.8	106.3	1.97	0.774	failure
in one week ca.	70-80	(1)	1.85	1.92	232.5	0.69	6.7	83.6	51.0	124.8	1.64	0.622	
3 gm./day	90-120	(3)	Recovery		2.7	1.13			53.1	180.2			

TABLE VI

Animal, Weight and Treatment	Time in min.	No. of collection periods averaged	Epinephrine Infusion Rate mg./kg./min.	Epinephrine Concentration mgm./100 ml. Plasma	Epinephrine Concentration mgm./100 ml. Urine	Urine Flow ml./min.	% Infusion excreted in urine	Clearances Epinephrine ml./min.	Clearances Creatinine ml./min.	Clearances PAH ml./min.	Epin. clearance/Creat. clearance	Epinephrine Transport	PAH Transport µgm./min.	Oxygen Consumption ml./min.
Dog DNP #1. 12.7 kg.	0-20	(2)	Control			1.29			51.2	111.7			1.113	57
125 mg. 2.4 Dinitro-	30-40	(1)	2.08	1.0	42.2	3.15	5.1	134.0	50.9	119.5	2.28	0.751	1.082	33
phenol infused during	40-50	(1)	2.08	(1.1)	29.8	4.15	4.7	109.0	57.2	158.8	1.91	0.612	2.878	35
first 10 min. of the	50-55	(1)	2.08	1.2	29.2	4.38	4.8	106.0	55.6	194.3	1.91	0.611	2.613	51
fifth period	55-70	(1)	DNP injec.			2.61			44.7	85.4			0.885	ca60
	70-90	(2)	after DNP			0.75			59.2	72.5			0.279	242
	110-118	(1)	2.0	1.65	121.0	1.38	5.3	100.0	55.3	68.3	1.81	0.757	0.352	252
	118-128	(1)	2.0	1.15	133.5	1.00	6.6	116.0	49.0	60.5	2.37	0.747	0.310	259
	128-138	(1)	DNP recov.			0.30			22.2	23.8			0.017	285
Dog DNP #2. 22.75	0-20	(2)	Control			2.11			82.1	273.7			1.728	65
kg., 170 mg. 2.4	30-40	(1)	1.45	1.20	83.0	2.05	5.4	142.0	89.2	199.0	1.89	0.633	1.049	180
Dinitrophenol in-	40-50	(1)	1.45	(1.03)	88.9	2.02	5.7	173.0	99.9	205.5	1.75	0.767	1.026	250
fused i.v. 60 min.	50-60	(1)	1.45	0.87	71.2	2.06	4.7	170.0	101.5	195.2	1.68	0.583	0.921	160
after start of first	130-140	(1)	DNP+1.42	1.17	254.0	0.83	6.8	180.0	109.2	169.5	1.65	0.825	0.570	1200
collection period	140-150	(1)	DNP+1.42	(0.91)	135.0	1.16	5.1	172.5	198.9	148.5	1.75	0.670	0.489	1200
	150-160	(1)	DNP+1.42	0.65	175.0	0.75	4.3	202.0	76.7	122.2	2.64	0.812	0.435	1200

TABLE VI

Animal, Weight and Treatment	Time in min.	No. of collection periods averaged	Epinephrine Infusion Rate ugm./Kg./min.	Epinephrine Concentration ugm./100 ml. Plasma	Epinephrine Concentration ugm./100 ml. Urine	Urine Flow ml./min.	% Infusion excreted in urine	Clearances ml./min. Epinephrine	Clearances ml./min. Creatinine	Clearances ml./min. PAH	Epin. clearance / Creat. clearance	Epinephrine Transport µgm./min.	PAH Transport	Oxygen Consumption ml./min.
Dog B. 15.9 Kg.	0-20	(1)	Control											not measured
4.5 cc. of Hydergine	20-40	(1)	1.21	0.8	41.7	5.11	4.4	77.5	54.8	169.5	1.47	0.273	1.394	
i.v. in 10 min. 1 hr.	40-47½	(1)	1.21	1.1	42.7	2.05	5.0	95.6	52.6	130.4	1.82	0.397	0.947	
after start of first	47½-55	(1)	1.21	1.1	41.6	2.24	4.3	24.4	50.7	108.6	1.88	0.220	0.713	
collection period	75-90	(1)	Hyd.+1.16	2.0	230.7	1.97	6.5	72.9	38.5	108.5	1.36	0.282	0.679	
	90-105	(1)	Hyd.+1.16	1.3	302.2	0.60	7.1	83.0	38.9	76.6	1.87	0.696	0.710	
	120-140	(2)		1.6		0.45			40.8	85.8	2.02		0.880	
									48.9	108.5			1.214	

TABLE VII

Animal, Weight, Treatment	Period number	Time in min.	No. of collection periods averaged	Epinephrine Infusion Rate ugm./Kg./min.	Plasma Epinephrine Concentration ugm./100 ml. Arterial	Plasma Epinephrine Concentration ugm./100 ml. Venous	Urine: Epinephrine Concentration ugm./100 ml.	Urine: Flow ml./min.	Urine: % Infusion Excreted in Urine	Clearances: Epinephrine	Clearances: Creatinine	Clearances: PAH
Dog B. 16.2 Kg. 28 days after last dose of thyroid extract Adrenaline (Eastman Organic) used in infusion - Right ureter, right renal vein	I	1-15	(3)	Control	0.7	0.67	0.9	0.930	1.8	57.1	27.3	70.9
	II	25-30	(1)	1.37								
	III	30-35	(1)	1.37	0.65	0.00	68.2	0.710	2.1	71.4	27.1	59.6
	IV	45-50	(1)	2.62	1.45	0.28	235.0	0.600	2.4	70.6	29.1	59.2
	V	50-55	(1)	2.62	2.05	0.30	215.0	0.437	2.4	48.3	28.1	54.7
								0.460	2.3		26.6	54.6

TABLE VII

Period numbers	Plasma Creatinine Concentration mgm./100 cc. Arterial	Plasma Creatinine Concentration mgm./100 cc. Venous	Plasma PAH Concentration mgm./100 cc. Arterial	Plasma PAH Concentration mgm./100 cc. Venous	Renal Plasma Flow ml./min. Epinephrine	Renal Plasma Flow ml./min. Creatinine	Renal Plasma Flow ml./min. PAH	Epinephrine Transported ugm./min.	Epinephrine removed by kidney ugm./min.	Total urine Epinephrine ugm./min.
I	18.57	13.31	1.442	0.480	62	91.3	99.4	0.204	0.599	0.429
II	17.18	11.37	1.600	0.569	67	81.6	92.6	0.275		
III					71			0.550		
IV	17.18	10.80	1.770	0.503	88	74.7	76.5	0.445	1.127	1.008
V					57					
					72					