STUDIES ON THE MECHANISM OF VASOPRESSOR DRUG RESPONSE IN EXPERIMENTALLY INDUCED SHOCK

by

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A THESIS
Presented to the Department of Pharmacology
and the Graduate Division of the University of Oregon Medical School
in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

June 1964

APPROVED:

[redacted]

Professor in Charge of Thesis

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ACKNOWLEDGMENT

I would like to express my sincere gratitude to Dr. Elton L. McCawley for his encouragement and guidance throughout my graduate studies. Thanks go also to Dr. Norman A. David who, along with the rest of the Pharmacology Staff, have been very helpful and encouraging.

Special appreciation goes to Dr. Melvin M. Reeves who conducted the clinical trial of the steroid treatment of which which was indicated by this research. Sterling M. Sorenson has been most helpful in the flame photometry determination of the sodium and potassium which was so necessary to this study.

I would also like to thank my wife, Naomi, for her help in typing rough drafts of the thesis, and for much needed assistance.

For the excellent secretarial work in typing the final manuscript, appreciation is extended to Mrs. Helen MacLean and Mrs. Marion Bergstrom.

This work was done while I was a Fellow of the American Foundation of Pharmaceutical Education, and the research was supported by Grant OHA 7-2811 from the Oregon Heart Association.

Special thanks go to Frank J. Zaccaria of the CIBA Pharmaceutical Company for the generous supply of aldosterone and hypertensin used in this study.

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INTRODUCTION

The word "shock" is used to designate clinical conditions which include various signs and symptoms but usually include pallor, weakness, sweating, a rapid thready pulse, oliguria and hypotension. The one condition essential to all forms of shock is a significant fall of arterial blood pressure. Therefore, the cause of shock must be sought among factors that regulate or limit pressure in the systemic arteries.

The importance of shock is immediately apparent if one considers that a patient with myocardial infarct without shock has an 80 per cent chance of survival, while if there is shock along with the infarct there is only about a 20 per cent chance of recovery. Shock accompanying any disease or injury can place the patient on the critical list (50). Shock is an especially ominous condition in battlefield injuries. In a study of battlefield casualties conducted during the Korean War, 12 per cent of 105 patients wounded and not in shock died and 21 per cent of 33 who were in shock died (2).

The etiology of shock varies widely and the condition can result from exsanguination, dehydration, coronary insufficiency, severe allergic reactions, traumatic injury, severe stress and infection with release of endotoxins. Thus, it has proven difficult for everyone to have the same understanding of shock and its treatment. It would appear very useful to abandon the use of the term "shock" and to

substitute more definitive words to identify the causative factors involved. Such a classification of shock, as proposed by Rushmer (5), is shown in Figure 1.

Even though one of the most pronounced facets of shock is the marked hypotension, a lowered blood pressure does not always indicate shock. Hypotension occuring as syncope is of a short duration, and is usually neurogenic or reflex in nature. This condition is usually self-limiting and the blood pressure will return to normal without medical attention. This must be distinguished from the prolonged hypotension, which eventually leads to an irreversible stage and death. It is hoped that with future research this may not be the case.

At the present time, therapeutic management of shock with drugs cannot be aimed at correcting the underlying pathology. Rather, the drugs used in the management of shock merely attempt to maintain adequate circulatory function. This, then, is merely symptomatic treatment. The various forms of treatment are essentially based upon maintaining a systolic blood pressure between 80 and 120 mm Hg. Even with exsanguination hypotension, this is not always accomplished by transfusions of blood, plasma or saline to replace the estimated amount of fluid lost. In cases where there has been no appreciable blood lost, the use of pressor amines is commonly employed to raise the blood pressure. The rationale of pressor amine therapy is to cause a generalized vasoconstriction that will greatly reduce the size of the essential vascular system and reduce the tendency of the blood to pool in large areas of the peripheral vasculature.

FIGURE 1

CLASSIFICATION OF VARIOUS TYPES

OF SHOCK (RUSHMER)[5]

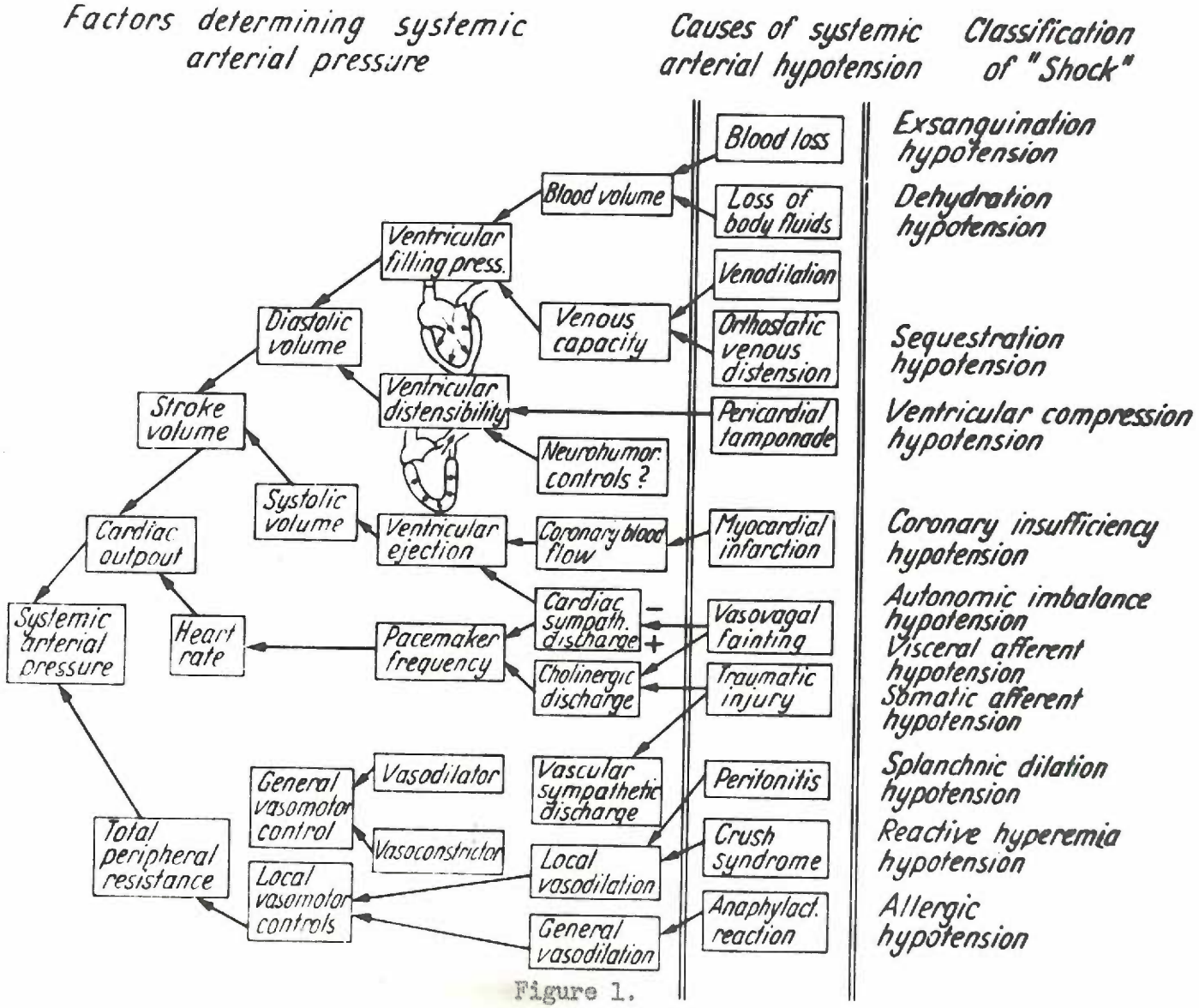

Factors determining systemic arterial pressure
Causes of systemic arterial hypotension
Classification of "Shock"
Systemic arterial pressure
Cardiac output
Heart rate
Stroke volume
Diastolic volume
Systolic volume
Ventricular filling press.
Ventricular distensibility
Ventricular ejection
Pacemaker frequency
Total peripheral resistance
General vasomotor control
Local vasomotor controls
Vasodilator
Vasoconstrictor
Blood volume
Venous capacity
Neurohumor. controls ?
Coronary blood flow
Cardiac sympath. discharge
Cholinergic discharge
Vascular sympathetic discharge
Local vasodilation
General vasodilation
Blood loss
Loss of body fluids
Venodilation
Orthostatic venous distension
Pericardial tamponade
Myocardial infarction
Vasovagal fainting
Traumatic injury
Peritonitis
Crush syndrome
Anaphylact. reaction
Exsanguination hypotension
Dehydration hypotension
Sequestration hypotension
Ventricular compression hypotension
Coronary insufficiency hypotension
Autonomic imbalance hypotension
Visceral afferent hypotension
Somatic afferent hypotension
Splanchnic dilation hypotension
Reactive hyperemia hypotension
Allergic hypotension
−
+

Figure 1.

The original assumptions that pressor agents should be the drugs of choice in combating the hypotension of shock are based on the testing of these compounds in normal animals. It is well established that agents such as norepinephrine*, phenylephrine, mephenteramine and angiotensin amide will raise the blood pressure in normal animals. It has also been demonstrated that these drugs raise blood pressure in animals in experimentally induced shock but it is not universally successful in preventing death. Recently some clinical investigators indicate that the use of pressor drugs may be, indeed, harmful (5) (55).

The idea of testing drugs in animals with an experimentally-induced disease state is not new. The concept of testing drugs in animals having a model disease was promulgated by Paul Ehrlich in 1905. Ehrlich began to administer arsenical drugs to mice infected with trypanosomes, a technique described by Laveran and Mesnil only in 1902. However, most of the classical pharmacological experimentation has been done on normal animals. When studying a new drug in normal animals it would be easy to reject such a drug as penicillin as one having no activity, or digitalis on the basis that its toxicity was excessive. If penicillin were to be injected into a normal animal, no effect could be observed or measured. But, if the animal had an infection due to an organism susceptible to penicillin, a dramatic

*Generic or public names for drugs will be used throughout the text of this thesis. For the convenience of the reader, a list of trade-mark names synonymous with these generic names will be found in the Appendix.

drug-induced effect would be seen. If digitalis were given to a normal animal, it would be apparent that the drug was too toxic for any practical use. But, if it were tried in animals with congestive heart failure, the results would be immediately evident. This then leads one to experiment on animals who have had an experimentally-produced disease when studying new drugs or re-evaluating existing drugs currently used in therapy. The testing of drugs on normal animals is, however, useful in determining what may be expected as side effects when they are introduced into animals with experimental disease.

Despite its importance and decades of extensive research, the treatment of shock remains a controversy. Blood transfusions, norepinephrine and angiotensin amide all have been administered to sustain an adequate blood pressure, but deaths still occur. This would lead to speculation that the reason for the failures of these pressor agents to maintain an adequate vasoconstriction may be due to the failure of receptor materials in the vascular walls themselves, or merely due to physical exhaustion of the musculature.

It has recently been shown that the accumulation of sodium and/or potassium ions in the arterial walls may be an etiologic factor in the development of some forms of hypertension (32)(33)(91). This accumulated sodium and potassium is decreased by the use of the thiazide diuretics and, at the same time, the blood pressure is lowered. This fact would implicate either the potassium or sodium ions in the vascular wall as an important factor in the ability of the arterial wall to contract.

THE PURPOSE OF THIS STUDY WAS TO TEST THE HYPOTHESIS THAT DURING THE PRODUCTION OF THE SHOCK-LIKE STATE, AND DURING THE FAILURE OF THE PRESSOR AMINES TO COMBAT HYPOTENSION, THERE IS ACTUALLY A CHANGE IN THE ARTERIAL WALL SODIUM AND POTASSIUM CONTENT THAT MAY MAKE THE VASCULATURE UNABLE TO CONTRACT.

History of Shock

As early as the sixteenth century, Ambrose Pare recognized the clinical picture of acute peripheral circulatory failure. The term "shock" was first used however by the French surgeon, Ledran, in 1743 to describe "a deathlike state seen after trauma". Larrey, a surgeon in Napoleon's army, then used the term shock to describe the hypotensive condition which may develop after surgical operations or severe injuries (20).

In England, shock was prominent in the notes of Guthrie in 1827 in his "On Gunshot Wounds of the Extremities". The physiological nature of surgical shock and methods for combating it were especially studied by the American surgeon, George W. Crile, in the years following the Civil War (18)(19)(20).

Crile later described the usefulness of blood transfusions in cases of shock and pointed out the importance of maintaining an adequate volume of circulating blood to allow for proper oxygenation of the tissues. In 1914 Crile and Lower published a book (19) in which a broader approach was described using various techniques for the prevention of shock by employing local anesthesia of the organs and areas involved in surgery. They ascribed the detrimental effects of

the surgery to reflex overstimulation from the site of injury that would then lead to a post-stimulatory depression. This initial over-activity was thought to utilize all of the energy stores in special areas of the body. The group of organs considered by them to be the most concerned with the process of converting potential energy into kinetic energy includes the brain, the thyroid, the adrenals, the muscles and the liver. If the stimuli for the conversion of this energy are very intense, then this "kinetic system", especially the brain, is exhausted or even permanently injured. This condition is described as acute shock.

These observations of Crile and Lower point out the damaging effects of the stress reaction in the ultimate production of the shock-like state. These studies were carried out by microscopic examination of sections of the brain, adrenals and thyroid. They found in these tissues of animals subjected to traumatic shock: chromatolysis, rupture of the nuclear and cell membranes, alterations of nucleus-plasma relation, and cellular disintegration. They were able to produce the same histological lesions from such diverse stimuli as strychnine convulsions, experimental insomnia in various laboratory animals, and fright in rabbits.

It is very interesting that this same form of shock treatment is being employed in Russia by B. K. Ossipov et al (5). They used a 0.25 per cent solution of procaine to produce a nerve block that is supposed to prevent excess reflex stimulation, which can cause damage to the organ(s) so innervated. They believe that whatever the cause of the shock (trauma, burns, surgical operation, transfusion), the mechanism

of its development begins from a primary nervous reaction. Under these conditions the treatment of shock, like that of any other disease, must be causal, pathogenetic and symptomatic. The basis for their treatment of shock is designed chiefly to combat the pathogenetic factors.

Interest was greatly renewed in the problem of shock during the First World War. Walter B. Cannon (13) relates in his book the experiences of himself and his colleagues who were involved in a carefully controlled approach to the evaluation and treatment of battlefield shock. He also was aware of the inadequacy of the term "shock". He observed that shock-like conditions were caused by different stimuli and also, there seemed to be two types of shock: one, a rapidly occurring condition and the other occurring only after several hours. He classified the two conditions by the terms "early" and "late" shock, although these terms have been abandoned today. Cannon advocated the use of blood transfusions, or if blood were not available, some of the plasma substitutes such as gum acacia to replace the blood lost from wounds. Another main point in the treatment was keeping the patient warm to avoid the loss of body heat. This was based on the observations that the body temperature of a person in shock would fall 2° to 3° C. and that the skin was invariably cold and clammy. The theory of using the vasoconstrictor drugs employed at that time was questioned because of the belief that this arteriolar constriction would add to the anoxia of the tissues by restricting

capillary flow, a recurring nagging doubt even today. The agents then used were epinephrine and pituitrin.

In 1940 Scudder (79) presented results obtained with the clinical employment of a crude adrenal cortical extract (Eschatin^{R}) in the treatment of shock. In his series of 28 patients so treated, 14 were cured, 9 died with no benefit, and 5 were temporarily benefited but later relapsed and died. It is somewhat doubtful if all of these persons died only of shock as they also had extensive burns or were inoperable acute emergency situations. The treatment advocated at that time was a dosage of 10 to 20 cc of the adrenal cortical extract usually given intravenously. The material contained not less than 25 dog units per cc. This would correspond to the activity of 1 to 2.5 milligrams of hydrocortisone (72). It is doubtful that the dosage used was anything but a small fraction of that required. He also advocated the use of sodium chloride injections which were thought to counteract or aid in the excretion of the high blood levels of potassium. The potassium was believed to come from the injured tissues.

Problems in the Treatment of Shock

The etiology of shock is so poorly understood that as yet no standard procedure is generally recognized as proper therapy (Table 1). The most striking feature of shock is the depressed blood pressure which will vary considerably depending upon the individual case. Patients with shock have had blood pressures reported as ranging from 90/70 mm Hg to 0.0 mm Hg, i.e., unobtainable by sphygmomanometer (2)(26)(50). If the patient has suffered a loss of blood through hemorrhage, or from

TABLE I.

SUBSTANCES AND PROCEDURES

THAT HAVE BEEN EMPLOYED TO REVERSE SHOCK

Pressor Amines

Adrenal extracts

Adrenalcortical hormones

Adrenocorticotrophic hormone (ACTH)

Blood

Plasma

Saline

Plasma expanders (substitutes)

Local Anesthetics

Hypothermia

Angiotensin amide

Vasopressin

Adrenergic blocking agents

Ganglionic blocking agents

plasma loss following extensive burns, the first object of therapy is to replace the estimated loss by giving the proper fluid. In patients who have had no fluid loss and in cases where the replaced fluid is ineffective, the next procedure is the intravenous administration of pressor drugs such as norepinephrine or angiotensin amide. This procedure will at times raise the blood pressure to physiological levels (25). At other times, one pressor agent may be ineffective where a somewhat different compound will work. This seems to be especially true in the cases of norepinephrine and angiotensin amide. One of the main problems encountered, even when the pressor agents prove to be effective when first employed, is that the dose required for maintenance of elevated pressure will rise. After a time, the agent becomes completely ineffective and the patient will die.

To explain the effect of tolerance* and ineffectiveness of these amines, many theories have been proposed. These include acidosis, adrenal exhaustion, tachyphylaxis,* ferritin and endotoxin. (Figure 2).

Acidosis

The observation that the presser response to epinephrine is lessened by acidosis has been reported many times (9)(12)(48). It must be remembered that epinephrine's action on blood pressure is biphasic, i.e., after injection there is an initial slight fall (depressor) followed by a gross rise (presser) and a secondary fall. As proposed by Wood et al this altered reactivity could be explained by three different mechanisms (93).

*See Appendix for definition of these terms.

FIGURE 2

PROPOSED MECHANISM FOR DIMINISHED CATECHOLAMINE RESPONSE IN SHOCK.

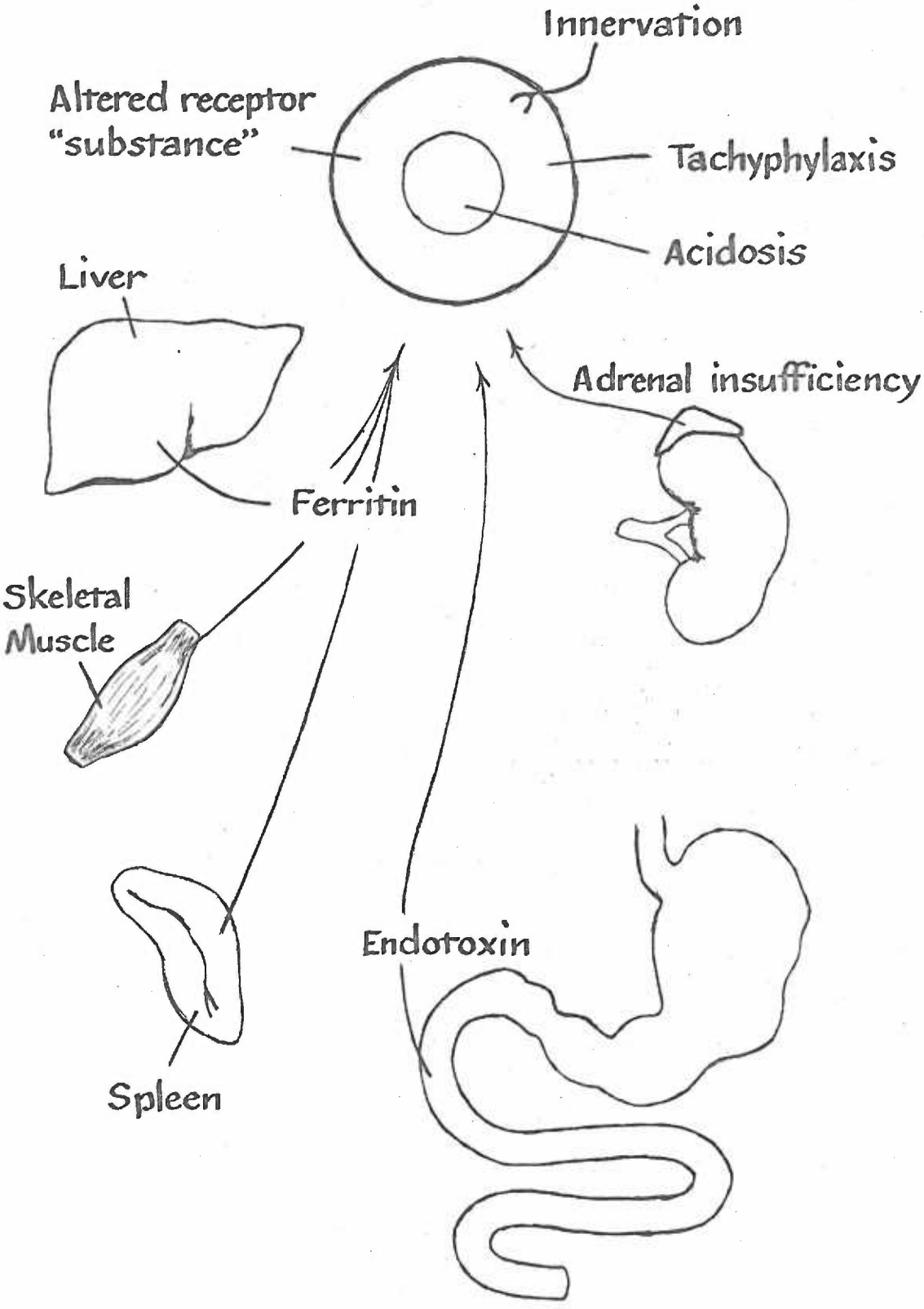

Innervation
Altered receptor
"substance"
Tachyphylaxis
Acidosis
Liver
Adrenal insufficiency
Ferritin
Skeletal
Muscle
Endotoxin
Spleen

FIGURE 2

1. Acidosis increases the reactivity of the inhibitory receptors (depressor mechanism).

2. Acidosis decreases the reactivity of the excitatory receptors (pressor mechanism).

3. Acidosis produces concurrent changes in the reactivities of both receptors, the algebraic sum of which could explain the reduction in the amplitude of the hypertensive response to epinephrine.

To resolve this problem they employed adrenergic blocking agents which would selectively block either the pressor response (phentolamine, phenoxybenzamine and dihydroergotamine) or the depressor response (dichloroisoproteronal), thus allowing the opposite response revealed for study. It was found that both the pressor and depressor responses were both markedly reduced by acidosis. The depressor effect being somewhat more susceptible than the pressor response. This data then favors the proposal that the diminished response is due to simultaneous changes in both receptors which result in an overall lessened response to epinephrine.

As to whether the acidosis produced during the course of the development of shock accounts for the diminished blood pressure response to pressor amines is still not settled. It has been shown by Rosenthale and DiPalma (77) that even though the lowered blood pH was restored to normal in dogs made tolerant to norepinephrine, there was no restoration of its effectiveness. Broder and Weil (8) have recently used a correlation of the increase in blood lactate during shock as a prognostic measure of the lethality of the condition. It was found that if this excess lactate during shock is above 4 m mole/

liter the prognosis is grave and will probably lead to death. The same outcome occurred even if the excess lactate and acidosis was promptly reduced. This would indicate then that acidosis seems to follow the damaging effects of shock and depressed response to pressor compounds, but is not necessarily responsible for the alterations.

Adrenal Exhaustion

Adrenal exhaustion is another possible explanation for the failure of pressor amine activity. This aspect has received considerable attention in the last ten years. Both augmentation and inhibition of responses to catecholamines have been suggested as mechanisms of the steroid protection in shock (5)(40)(82). The latter possibility receives some support from the observation that large doses of hydrocortisone provide considerable protection against the lethal effect of epinephrine infusion. In addition there is accumulated evidence that there is an excessive adrenergic vasoconstriction in the genesis of lethal shock. However, a general pharmacological antagonism between the steroids and catecholamines has not been demonstrated.

The importance of the pituitary-adrenal axis as described by Selye has been recognized since 1947 (78). He described a general adaptation syndrome in animals which results from continuous nonspecific nervous stimuli or stress. This general adaptation syndrome is subdivided into three stages. The first stage is the alarm reaction, which is characterized by the immediate response to the stress. If the stress is too severe, the individual dies. If not excessive, certain symptoms occur, including hypotension, hemoconcentration,

increased capillary permeability, decreased body temperature, and depression of the central nervous system. These symptoms very accurately describe the syndrome we know as shock. If this shock is not fatal the second stage comes into play. This is characterized by resistance to the stress stimuli, believed to be brought about by the release from the adrenal cortex of steroids that are from the trophic influence of adrenocorticotrophic hormone. Stage three is described as the state of exhaustion, where the resistance to stress is lost and the original state of shock is re-instituted. This concept has not been entirely adopted because some of the details cannot subetantiated experimentally, although it serves as a useful working hypothesis (3).

It has further been demonstrated in dogs by Papacostas (69) that during adrenocortical suppression by the administration of 50 mg of prednisolone phosphate daily for 14 days there was no alteration in their response to test doses of catecholamines or to their survival after the stress of experimental laparotomy. A time lapse of 48 hours was used between the last dose of the steroid and the beginning of the test procedure to allow for the removal of the exogenously supplied steroid.

There seems to be no question that the administration of a relatively small dose of certain adrenal steroids can increase dramatically the survival of adrenal deficient animals, but it is not equally clear that adrenal deficiency exists during the development of shock in either animals or man, unless the adrenals are specifically compromised by disease, surgery, or the prior administration of exogenous adrenal steroids (5)(15)(89).

Measurements of adrenal steroids under conditions of protracted stress, including lethal shock of varying etiology, both in experimental animals and in man, have demonstrated increased plasma levels, increased rates of secretion, and a good response to ACTH (31)(51)(63). Output may decrease somewhat when adrenal blood flow becomes very low, but the gland is still capable of responding when the blood flow is again increased.

Tachyphylaxis

The tachyphylaxis which results from repeated injections of ephedrine is well known (21)(42)(43)(44). Most present-day text books of pharmacology also state that there is no tachyphylaxis from repeated doses of epinephrine (42)(52).

Rosenthale and DiPalma (1962) reported on the development of the acute tolerance or tachyphylaxis to norepinephrine in dogs (77). Hemoconcentration, acidosis and decreased blood volume were proposed to explain this observed tolerance. It was also noted that the administration of plasma expanders at the height of tolerance temporarily restored normal sensitivity, but the adjustment of the blood pH to normal with THAM buffer (tris hydroxymethylaminomethane) or sodium bicarbonate did not affect the tolerance. Histamine has also been proposed as a factor in the tolerance to norepinephrine in dogs by Coppola & DiPalma, 1962 (16). They noted a simultaneous increase in the blood histamine content as the tolerance to norepinephrine develops. They were also able to show that in histamine depleted dogs, the tolerance to norepinephrine was still exhibited, although

it did develop more slowly. Tachyphylaxis to epinephrine in dogs has also been shown by Reyes & Lipton in 1963 (70). In this study, it was demonstrated that as the size of the dose increased, tachyphylaxis developed much more quickly. They demonstrated a potentiation of the tachyphylaxis effect by cocaine although no attempts to explain this interaction were made.

Before the pressor amines can become useful in raising the blood pressure by either causing generalized or restricted peripheral vascular resistance or by increasing the rate and strength of the heart, they must combine with some material or group of materials that will mediate this activity. This theoretical receptor substance has received much attention in the past five years. It has been established by Ahlquist et al that at least two different receptors exist for sympathomimetic agents, i.e., Alpha and Beta receptors (53). The Alpha receptors are primarily designated as stimulating to smooth muscle and the Beta receptors are generally regarded as being inhibitory in nature. This is not altogether satisfactory, as a Beta receptor seems to be involved in the chronotropic effect on the heart and the increase in blood sugar seen after injections of pressor amines (88).

The most recent report as to the chemical nature of these receptors has come from Belleau (10)(88). Most of the information regarding the chemical nature of the receptor substance has come for research employing blocking agents that are rather specific from either the Alpha or Beta system. It has thus been shown that the potent Alpha blocking drug, dibenamine, assumes an ethyleniminum form in vitro

before it is effective. Theoretically, even though direct evidence does not exist, the catecholamine can also assume a similar structure as shown in Figure 3.

It would then be assumed that the receptor materials in the tissue would have to be of such a nature that the positively charged ion would be free to react with it. It has been proposed by Belleau that this anionic site in the receptor is a phosphate moiety. It has previously been shown by Boln that tissue storage granules of catecholamines are in the form of complexes associated with adenosine phosphates, mainly as the triphosphate. The catecholamines were incorporated to a ratio of 3.9 moles of catecholamine to 1 mole of adenosine phosphates. Similar granules are found in adrenal medulla and sympathetic nerves. It has been postulated that the receptor for both the Alpha and Beta responses may have the following configuration. (Figure 4.)

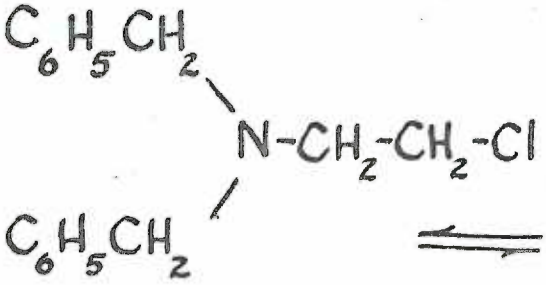
Ferritin

Ferritin released from the skeletal muscle, liver and spleen during anaerobic conditions has been implicated in the production of irreversible shock (3). Ferritin, a storage form of iron in the tissues, has not been shown to have vasodepressor activity in normal animals. Its presence has, however, been demonstrated in the circulation during various forms of shock. When ferritin was infused into normal animals they did not develop shock. However, failure of ferritin to produce shock may be due to rapid inactivation by transferring the sulfhydryl radical of ferritin to its inactive disulfide

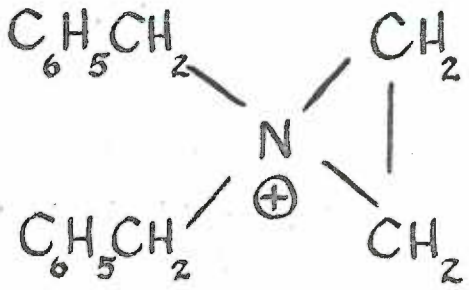
FIGURE 3

ACTIVE FORM OF DIBENZOLINE AND EPINEPHRINE

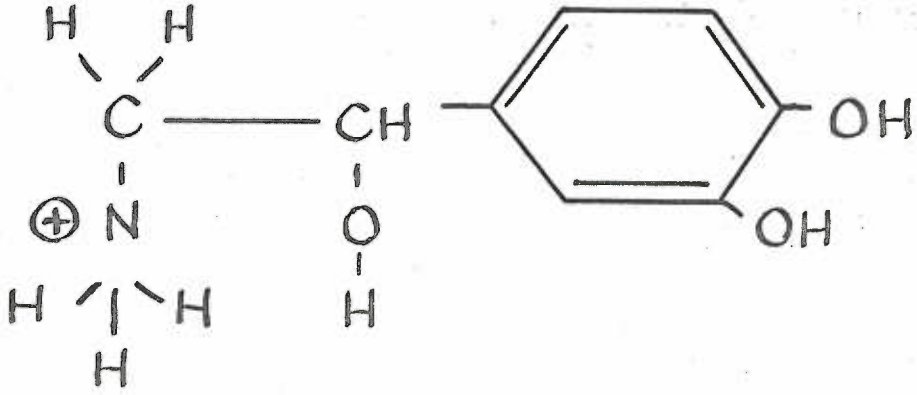
AS PROPOSED BY BELLEAU



Dibenamine



Ethyleniminium ion form



Norepinephrine

FIGURE 3

FIGURE 4

ADRENERGIC RECEPTOR AS PROPOSED

BY BELLEAU

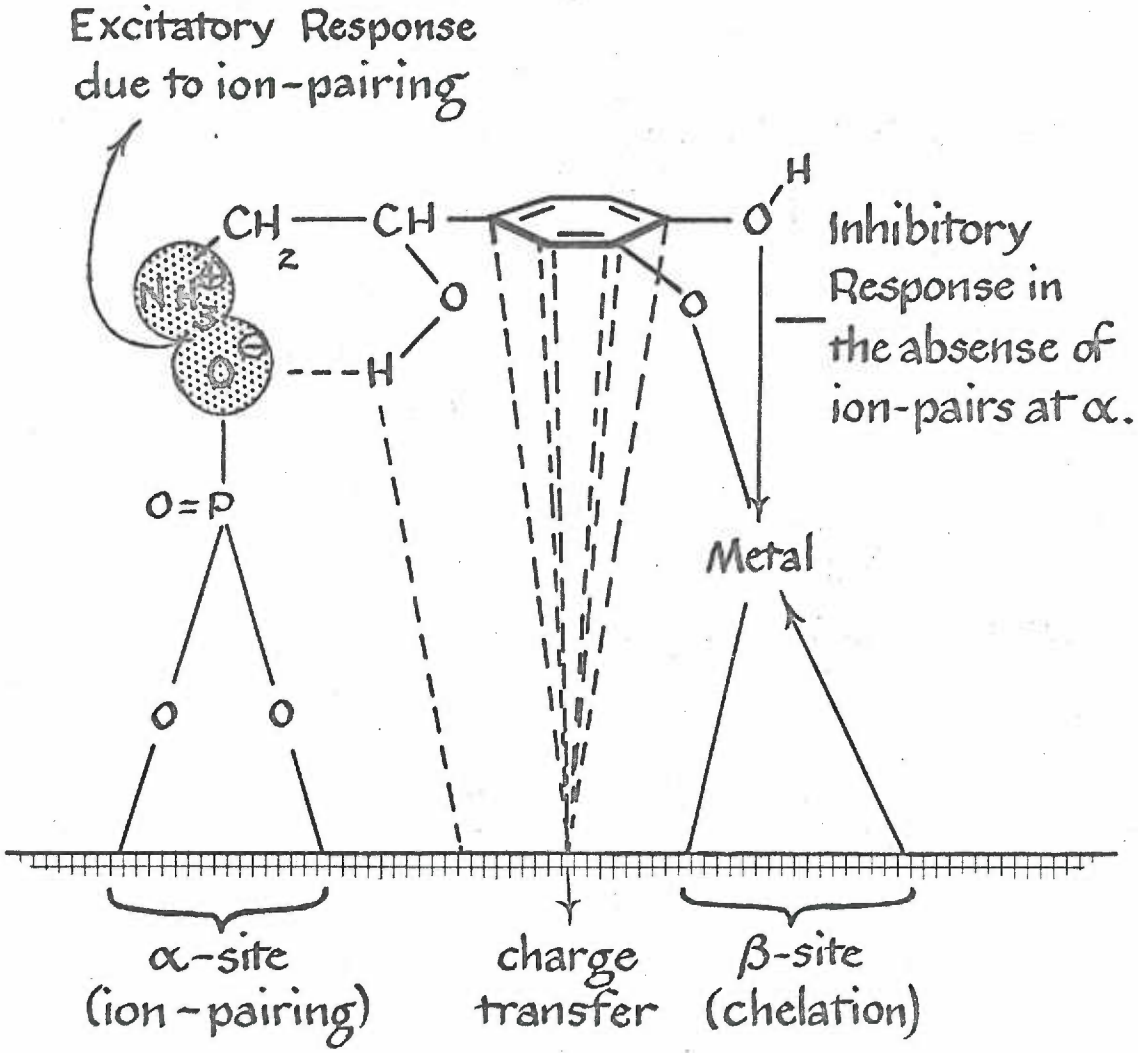

Excitatory Response
due to ion-pairing
CH
2
CH
O
H
O
H
O
H
Inhibitory
Response in
the absense of
ion-pairs at α.
O=P
O
O
Metal
α-site
(ion-pairing)
charge
transfer
β-site
(chelation)

FIGURE 4

form in the normal liver. It is possible that in animals with shock this hepatic destruction is so impaired that the conversion cannot take place and the vasoactive form of ferritin then acts as a vasodepressor substance and is a mediating factor in the irreversible stage of shock. It has also been proposed by Reissman and Dietrich (1956) that the ferritin may be released in its inactive disulfide form for they found no vasoactive response from large amounts of ferritin released into the circulation in patients with hepatocellular disease (73). This influence of ferritin has not been adequately demonstrated, however, and the release of ferritin does not always accompany irreversible shock conditions.

Vasoconstriction

One of the most significant observations on the hypotension that accompanies shock is that there is a pronounced decrease in the cardiac output (3)(4)(92). The cardiac output is defined as the product of the stroke volume and the heart rate (3).

In a normal animal the vascular response to a fall in the mean systemic blood pressure will evoke a compensatory vasoconstriction. This generalized vasoconstriction leads to a reduced blood flow through the capillary bed as the precapillary sphincters are constricted and the peripheral flow is predominately directed through the thoroughfare channels, the metarterials. A further fall in the systemic arterial pressure would not be corrected by increasing the peripheral resistance after it reaches its maximum. This would then cause a marked decrease in the cardiac return and a further lowering of the blood pressure. To

break this vicious cycle, the original vasoconstriction must be abolished, allowing increased oxygenation of the tissues and a better cardiac return with a resulting increased cardiac output and a satisfactory blood pressure. It has been shown repeatedly that in the early stages of shock, there is still an elevated peripheral resistance (74)(75)(76)(92)(96). Gregg has shown that the pressure-flow curves during shock due to hemorrhagic hypotension revealed a mild increase in total peripheral resistance and in resistance in the mesenteric, renal and iliac arterial beds as well (5). Trank and Visscher (86) also report as unchanged or only slightly increased total peripheral resistance during endotoxin shock. Wong et al have demonstrated a calculated increase in peripheral resistance in dogs subjected to traumatic shock (90)(92).

This increase in peripheral resistance in the systemic circulation is thought to be caused by the generalized or localized contraction of the precapillary sphincters. That the prolongation of this vasoconstriction effect by the use of pressor amines may be harmful in the treatment of shock has been of concern to some of the clinical personnel involved in the study of this perplexing problem (5)(41).

In fact, this increase in total peripheral resistance may be mediated through existing high blood levels of pressor amines. Manger (60) has demonstrated an increase in the plasma concentration of epinephrine from 1.0 to 7.8 microgram/liter of plasma and an increase of norepinephrine from 2.5 to 3.6 microgram/liter of plasma in dogs subjected to hemorrhagic shock. Dogs that had been induced into anaphylactic shock by repeated injections of egg albumin also showed an

increase of from 0.7 to 7.7 micrograms/liter in the plasma level of epinephrine. The norepinephrine concentrations increased in some of the animals thus treated, but decreased in others. This difference in response was not elucidated, but it demonstrates that animals in shock do, in fact, have a pre-existing high plasma level of pressor amines, but still exhibit a lowered blood pressure.

Zweifach (1962) mentions increases in plasma levels of epinephrine and norepinephrine as well as 5-hydroxytryptamine (serotonin) in animals that have been subjected to experimental shock (96). He proposed that epinephrine has been implicated as a mediator of the shock reaction both directly and indirectly. It has been shown that the pattern of increased vascular reactivity and subsequent decreased reactivity is manifest primarily on the basis of an altered response to epinephrine.

Longerbeam (1962) also noted that in dogs with endotoxin shock, an increasing concentration of catecholamines in the peripheral blood, and a decreasing visceral blood flow, were characteristic of the irreversible state of shock (57). In these dogs, the mesenteric circulation suffered the greatest relative decrease in blood flow regardless of the etiology of shock, and at autopsy, ischemic changes were invariably most pronounced in the small bowel.

Young and Gray (1956) have demonstrated that rats subjected to tumbling stress in the Noble-Collip drum also develop increased plasma levels of both epinephrine and norepinephrine (94). It is very interesting to note that the increase of pressor amines, especially that of epinephrine, parallels the increase in mortality from increasing

degrees of stress. This then allows a correlation of plasma level of amines with the mortality of a given stress procedure.

If this release of large amounts of pressor amines in the genesis of shock is what leads to the irreversible state, then it would follow that the adrenergic blocking agents should prevent the irreversible state of shock.

Adrenergic and Ganglionic-Blocking Agents

It has been shown that the adrenergic and ganglionic blocking agents have been beneficial in preventing irreversible shock (5)(46)(55)(58). The beneficial effect however is exerted only if the agents are given prior to the stress that induces the shock. They have been totally ineffective when given after the stress situation (5). It is believed that these agents exert their effect by preventing the constriction of the vascular system that would normally occur in shock. This allows a better oxygenation of the tissues and prevents the pooling of blood in the capillaries.

Dichloroisoproterenol, a beta receptor adrenergic-blocking agent, has been shown recently to be effective in preventing the lethal effects of endotoxin on mice (62). This agent is effective only if given five hours prior to the administration of the endotoxin. The dichloroisoproterenol was, however, not as effective as hydrocortisone.

Chlorpromazine has been used by Horn et al (1963) to induce a protective effect in rats subjected to high temperature skin injury

(47). At autopsy it was noted that in the control animals the spleens were small and dark, while those of the chlorpromazine group were normal in size and color. It was also noted that the splanchnic vascular bed in the mesentery and intestinal tract appeared more dilated in treated rats than in the controls; the skin did not have as blanched an appearance as the other groups.

This data would suggest that the adrenergic-blocking agents and chlorpromazine, which also has an adrenergic-blocking component, as well as a central nervous system calming effect, were instrumental in inhibiting the endogenously released pressor amines which may lead to irreversible shock.

Sodium and Potassium Fluxes in Vascular Walls

It is currently believed (3) that during muscular contraction or during the transmission of a neurone, there is a cellular redistribution of sodium and potassium ions. During the resting state of cells, there is more potassium inside, and at the same time more sodium outside the cell. During activity sodium moves into the cell and potassium leaves. Then during the recovery of the normal resting cellular potential, the excess intracellular sodium is removed by an energy-requiring enzyme system, the so-called "sodium pump". The potassium also returns and the normal resting situation is restored.

It has been shown by Friedman et al (36) that in nephrectomized rats or dogs there is a loss of sodium from the extracellular fluid and an increase of potassium in this space during the blood pressure elevation induced by norepinephrine, vasopressin or angiotensin.

These ions move in opposite directions during the depressor response from such drugs as isoproterenol and carbachol. It has also been concluded by Daniel et al (22)(23)(24) that during aortic contraction in the rat, the increase in blood pressure is accompanied by an increase in extracellular sodium concentration and also by a depletion of potassium. These results would fit the well-known observation of electrolyte shifts during tissue activity.

Muirhead (1954) demonstrated that in dogs the intravenous infusion of norepinephrine for periods of 20 to 50 minutes resulted in a gross elevation of the blood pressure, a fall in plasma sodium concentration, and a rise in plasma potassium level without significant changes in chloride concentration (64). Goffert et al (1951), using an isolated rat diaphragm which was irrigated with potassium-free Tyrodes solution, showed that when epinephrine, norepinephrine, and isoproterenol were added to the bath that each of these compounds caused a decrease and then an increase in the rate of loss of potassium from the muscle (39).

Born et al (1956) found the opposite result following the addition of epinephrine on the isolated intestinal smooth muscle of the guinea pig (6). However, here histamine and acetylcholine produce a contraction of this smooth muscle which is manifest by an outward flux of K^{42}. This same response occurs when the muscle is stretched mechanically. Epinephrine on the other hand causes a relaxation of the intestinal smooth muscle and at the same time increases the rate of inward movement of K^{42}. This would then suggest that the potassium

fluxes in smooth muscle will be either inward or outward depending upon whether the muscle is contracting or relaxing.

These same observations of a variable increase in sodium content with a definite decrease in potassium content in the arterial wall have been noted numerous times (23)(36)(39)(85). Daniel et al in 1957 (24) did, however, show that depletion of potassium definitely precedes any change in the sodium ion content. They employed rat aorta and induced the effect with a single pressor response elicited by epinephrine. In their very short-duration experiment, no change in the content of sodium was observed. This is in contradiction to the changes observed by the others in the pressor infusion experiment.

The observation that hypertensive agents caused changes in electrolyte composition of arterial wall prompted research into this area in an attempt to elucidate the antihypertensive action of the thiazide diuretics.

Weller and Haight in 1963 found that when rats were made hypertensive by kidney ligation they developed an increase in the sodium content of the aorta wall (91). They were also able to show that when the thiazides were administered, the sodium content declined with the blood pressure. It was concluded that this lowering of the blood pressure along with a simultaneous decrease in artery sodium content suggested a decrease in sensitivity to the pressor amines (34)(54)(59).

Freed et al (1963), using a new non-diuretic, thizide, diazoxide, which will also lower the blood pressure in rats made hypertensive by renal ligation, found a lowering of the blood pressure of

the renal hypertensive rats to be associated only with a decrease in the aorta wall potassium content (33). At the same time, sodium and water concentrations and their distributions in the tissue compartments of the aortas of such rats remains unchanged. They suggest that the antihypertensive effects of the thiazides is mediated only through the reduction in potassium concentration of the arterial smooth muscle.

The purpose of this research, then, is to determine if some similar shift or alteration of the electrolyte content of arterial wall occurs in animals subjected to shock-like conditions.

METHODS AND MATERIALS

When studying the effects of various agents in the shock-like states as seen in human patients, it is imperative that similar conditions be induced in the animal. A study of drugs in all forms of shock is almost prohibitive because of the wide variety of experimental methods that are available. The most common form of shock studied seems to be that which results from exsanguination of the dog. Here the dog can be bled until the blood pressure reaches and maintains any desired level for a designated length of time. Drugs can be applied before, during, or after the blood pressure fall and the animal can be returned to his normal blood volume by re-infusion of the drawn blood. Probably the second most popular method is induction of shock by the injection of a standard preparation of endotoxin. The endotoxin is usually prepared from Gram-negative entero-organisms. Here the severity of the shock can be controlled by the dose of endotoxin given.

A recent method of considerable interest for provoking shock in rats involves tumbling them in the Noble-Collip drum (66). Here the degree of shock is controlled by varying the number of rotations to which the animal is subjected. As a fourth method, cardiac shock can be induced by ligation of one of the branches of the coronary arteries. This method, especially in the dog, is not too reliable

in that all dogs do not develop a shock condition and some of the dogs will die prematurely of ventricular fibrillation.

Shock-like states can also be produced by burning the animal in a controlled manner by dipping it in water of a specific temperature for a given time, or by controlled exposure to a heat lamp. This method is only satisfactory when studying the shock produced by burns. Anaphylactic shock can be easily produced in experimental animals by sensitizing them to foreign proteins and then inducing the shock by a second injection of the same protein. This method is very satisfactory when studying the allergic type of shock situation.

Even though all of the methods of experimental shock have certain limitations and advantages, the Noble-Collip drum was selected for screening studies of the pressor agents. It produces a traumatic type of shock that is not dependent upon blood loss. The reproduction of the "irreversible state" is quite good at a rather narrow range in the duration of the tumbling. Tumbling allows for a rather rapid screening of agents as the time required for the stress procedure is less than 30 minutes. One possible disadvantage is that the animals usually die within three hours after the stress, which is faster than the normal clinical condition seen in humans. This method does not readily lend itself to the study of parameters which are taking place in the animal during the production of shock, such as blood pressure and blood flow measurements.

The production of epinephrine shock in dogs overcomes this latest objective in that the dog lends itself to multiple measurements, but the dog is usually anesthetized while the rats are not. This may

be a serious handicap, but experiments performed on non-anesthetized dogs show a similarity to those of the anesthetized animals.

Experimental Traumatic Shock in Rats

A total of 325 female white rats of the Sprague-Dawley strain were used in the rat-tumbling experiments. The rats were tumbled in a Noble-Collip rotating drum which was fifteen inches in diameter, eight inches deep and equipped with two baffles 3 inches high on opposite sides of the drum (66). The apparatus was so constructed that two identical drums were attached to the same axle and would rotate together. The drum assembly was then connected to a motor-drive pulley system that would give approximately 40 turns per minute (Figure 5). Prior to placing the rats in the drum, both the hind legs and front legs were wrapped with adhesive tape to prevent the animals from running and jumping over the baffles. This procedure would also reduce the handicap or bias of animals that may be drowsy from injections of drugs given before the stressing in the drum.

The animals were rotated in the drum for a total of either 400 or 500 rotations, depending upon the drugs to be tested. At 400 turns, 0 to 40 per cent of the control animals died, while at 500 turns, 90 to 100 per cent died. This procedure then allowed the testing of drugs which were thought able to "protect" the animal and of drugs that might "potentiate" the shock-like state produced by this tumbling.

To obtain a control series during each run in the stressing drum, an animal to be treated was placed in one side of the cage and an untreated animal was placed on the other side, and both animals

FIGURE 5.

NOBLE-COLLIP DRUM

(Note the identical drums on the same axle.)

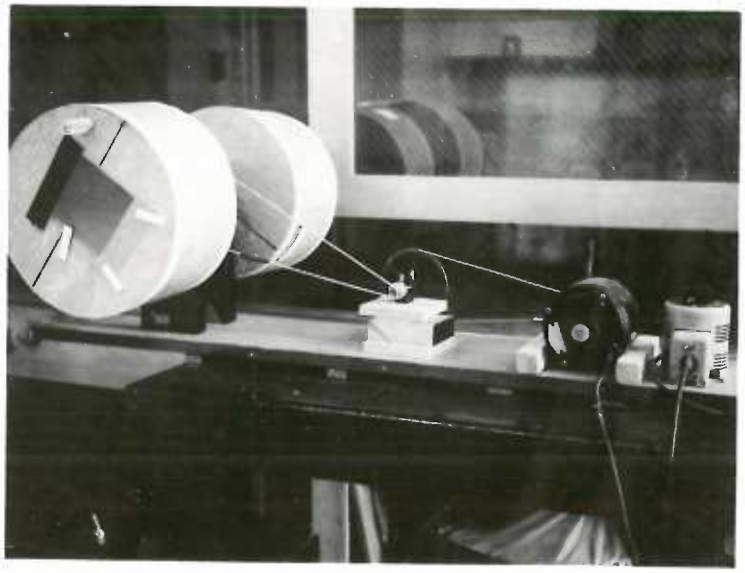


FIGURE 5

stressed simultaneously. To reduce the possibility of there being differences in the two ends of the drum, the treated and control animals were placed in alternate sides of the cage. After the predetermined amount of stress, the animals were removed from their cages and the tape binding their legs was removed within two minutes. The animals then received either the test drug or saline, depending upon the experimental design. They were then placed in individual cages and observed until they either died or recovered. After an eight-hour observation period the animals were placed in their maintenance cages and observed for an additional 48 hours. There were no deaths in any of the groups after 24 hours. The bulk of the animals died within the first three hours. Prior to, and after, the eight-hour observation period, the animals were allowed food and water ad libitum.

In order to demonstrate that the animals were actually in a hypotensive state after removal from the tumbling cage, the blood pressure of a group of ten rats was taken before and after the stress in the Noble-Collip drum. The procedure employed was the indirect microphonic method as described by Friedman and Freud (37). This method depends on occluding the caudal artery in the rat's tail and detecting the sound produced at the instant the pressure in the cuff becomes less than that of the arterial pressure (Figure 6). There is one limitation to this procedure in that only the systolic pressure is measured. The average pressure of the rat before the stress was 126 mm Hg. After 500 turns in the Noble-Collip drum the rats had an average blood pressure of 22 mm Hg (Figure 7). The accuracy of the blood

FIGURE 6

MICROPHONIC BLOOD PRESSURE DETERMINATION

EQUIPMENT AS DESCRIBED BY FRIEDMAN

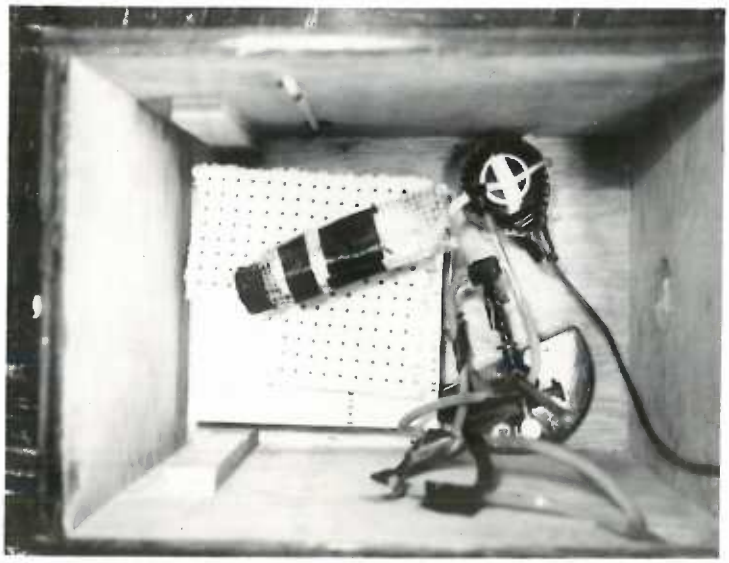


FIGURE 6

FIGURE 7.

SYSTOLIC BLOOD PRESSURE OF RATS

BEFORE AND AFTER 500 TURNS

IN NOBLE-COLLIP DRUM

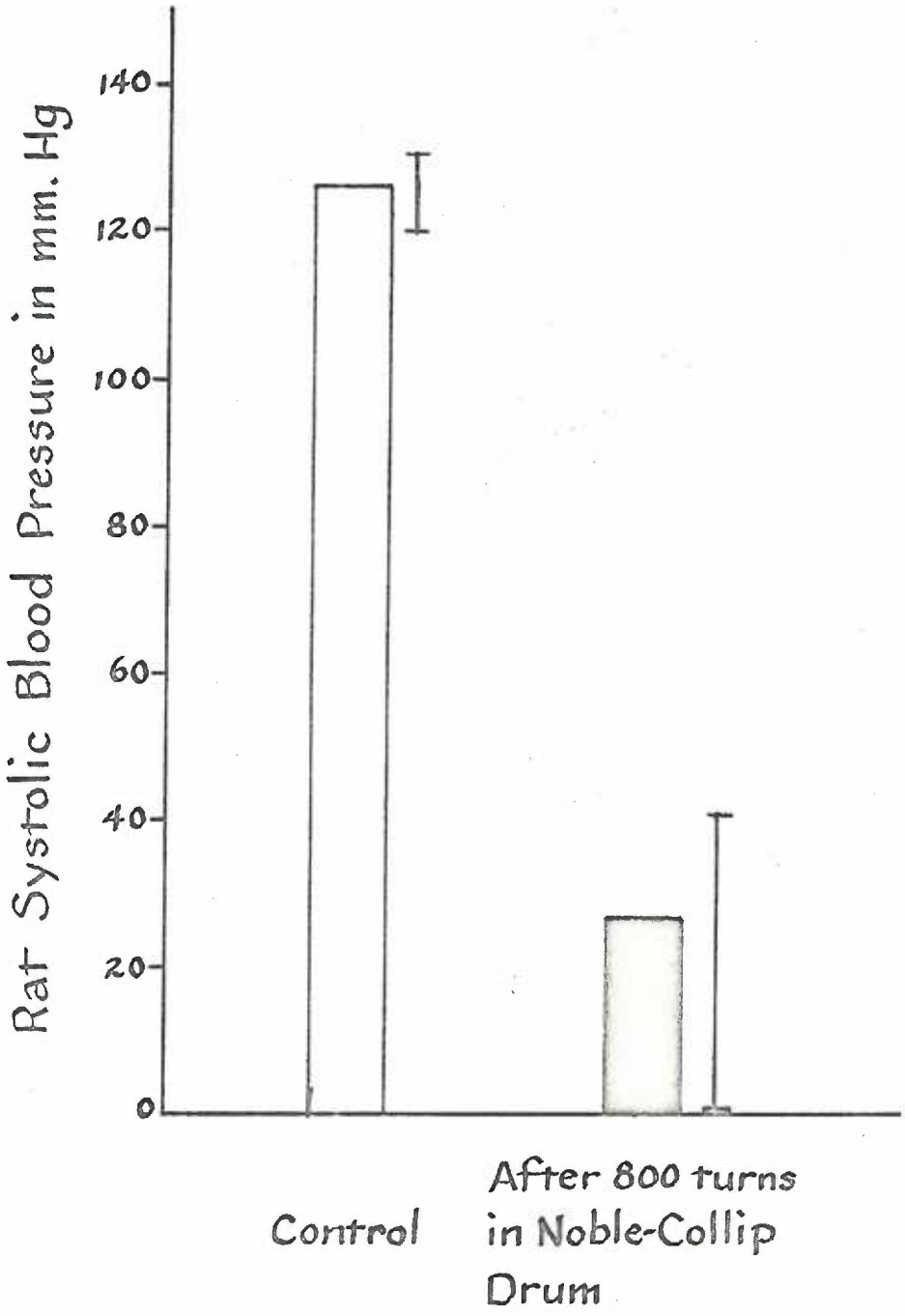

Rat Systolic Blood Pressure in mm. Hg
140
120
100
80
60
40
20
0
Control
After 800 turns in Noble-Collip Drum

FIGURE 7.

pressure determinations after shock however is very poor as it is very difficult to hear the weak sound below about 15 mm Hg so that some of the values that were recorded as 0 were probably between 15 and 0. It is quite apparent however that definite drops in systolic arterial pressure would be compatible with a shock-like state in the rats.

Methods of Administration and Dosage of Drugs

The same dosages of the pressor drugs of the tumbling-shock experiments were also given to control, non-stressed rats in groups of five each, and the animals were observed for 48 hours. No deaths occurred from these dosages of drugs. The animals that had received the ephedrine and mephenteramine however did show some increase in overall activity.

To show that the drugs given were capable of raising the blood pressure, rats were anesthetized with urethane (1 cc of 50 per cent solution per pound) and the carotid artery was cannulated for recording blood pressure on a Grass polygraph. The drugs were then injected either subcutaneously or intraperitoneally as indicated, in such doses as would raise the blood pressure for about one-half hour (Table 2) (Figure 8).

Sodium and Potassium Determinations

In order to determine the arterial content of sodium and potassium, groups of six rats were subjected to 500 turns in the Noble-Collip drum. They were removed from the cage, quickly anesthetized with 0.5 cc of a 50 per cent urethane solution given intraperitoneally, and were unconscious within two minutes. The chest was then opened and the thoracic aorta quickly removed by blunt dissection, blotted free of blood and stripped free of adhering tissue. The samples were then weighed to the nearest 0.1 mg on a Chain-o-matic balance, placed in small thick-walled glass test tubes, and dried in an oven at 100° C. for 24 hours. After attaining room temperature in a desiccator over

TABLE II.

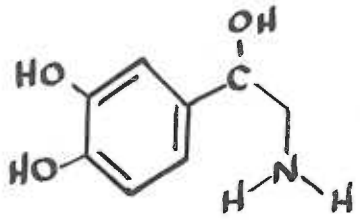
METHODS OF ADMINISTRATION AND DOSAGE OF DRUGS

Morphine	subcutaneously	2 mg/100 gm
Ephedrine	intraperitoneally	25 mg/animal
Epinephrine	subcutaneously	1 mg/animal
Norepinephrine	intraperitoneally	1 mg/animal
Hydrocortisone	intravenously (tail vein cut down)	5 mg/animal
Heparin	intravenously (tail vein cut down)	1000 units/animal
Angiotensin amide	intraperitoneally	1.25 mg/animal
Mephenteramine	intraperitoneally	5 mg/animal
Chlorthiazide	intraperitoneally	10 mg/animal for 10 days prior to stress

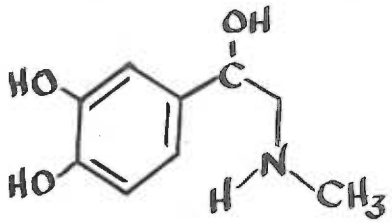
FIGURE 8.

STRUCTURES OF DRUGS

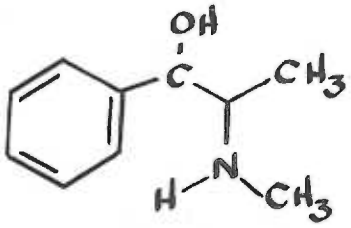
EMPLOYED IN THIS STUDY



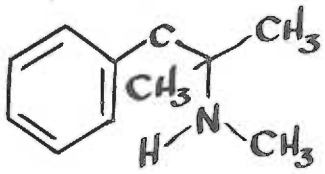
Norepinephrine



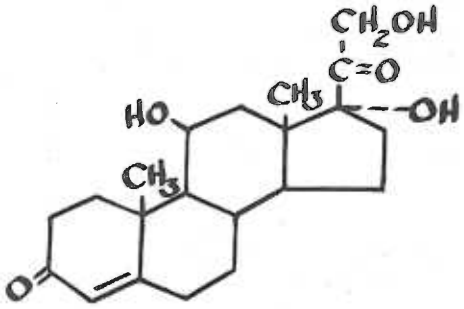
Epinephrine



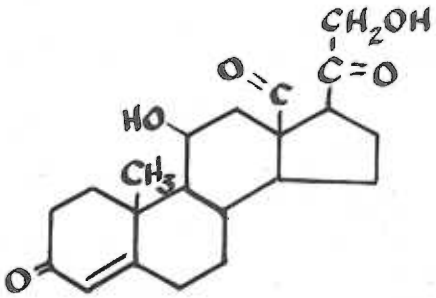
Ephedrine



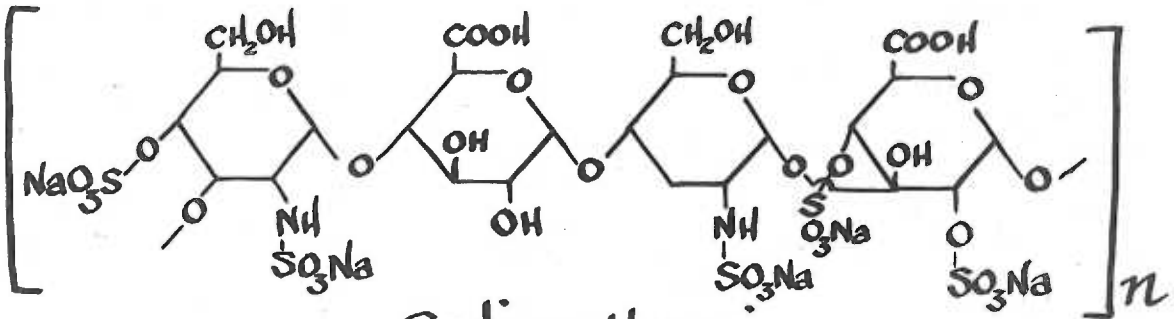
Mephenteramine



Hydrocortisone



Aldosterone



Sodium Heparin

FIGURE 8.

phosphorous pentoxide, the samples were weighed to obtain the dry weight of the artery sample. The percentage of water loss was then calculated from the difference in weight.* For the determination of the sodium and potassium, the tissue was placed in a 25 ml Kjeldahl flask and digested in 5 ml of concentrated nitric acid with 5 drops of perchloric acid added. This solution was then boiled to dryness and the remaining residue was dissolved in 5 cc of distilled water containing 1 drop of concentrated hydrochloric acid. This is an adaptation of the digestion procedure described by Stewart and Stolman (63).

The final determination of the sodium and potassium was carried out employing a flame spectrophotometer attachment on the Beckman D.U. spectrophotometer. Standards containing 1:100 dilutions of 145, 290, and 72.5 milliequivalents per liter of sodium or 4.5, 9, and 2.25 milliequivalents per liter of potassium as the chlorides were used. The final calculation of the sodium and potassium content is expressed in milliequivalents per 100 grams of fresh weight tissue. The determinations were made comparing each unknown sample to a sample of known concentration at 50 per cent transmittance. This is an adaptation of the method described by Telott (84). The formula used for the calculations was:

$$\frac{T_u\ 50\%}{T_s\ 50\%} \times C_s = C_u$$

C_s = concentration standard

C_u = concentration unknown

T_s = transmittance standard

T_u = transmittance unknown

*See Appendix for data.

Experimental Shock in Dogs

Catecholamine tachyphylaxis shock

Fifteen male and female mongrel dogs weighing between 10 and 11 kg were used for this part of the study. The dogs were anesthetized with pentobarbital sodium, 35 mg/kg intravenously. Supplemental doses were administered when needed. After induction of anesthesia, a Magill endotracheal tube was inserted for attachment to a Takioka respirator. The animals were then placed in a supine position on a surgical table and respiration was maintained by use of a 2.5% CO_2-97.5% O_2 mixture throughout the experiment. This was needed because the animals would otherwise develop respiratory arrest and die following the first dose of epinephrine. The area over the femoral artery was shaved and washed, the skin over the artery and vein was incised, and both were exposed using blunt dissection. The femoral vein was then catheterized with a 10 cm length of polyethylene tubing for the administration of drugs. The femoral artery was catheterized with polyethylene tubing connected to a Statham transducer for recording the femoral arterial pressure (Figure 9). Both the transducer and tubing were filled with heparinized saline. The blood pressure was recorded on a Grass Polygraph or Sanborn Model 500 recorder depending on the availability of the instruments. Both instruments were calibrated employing a mercury manometer before and after each experiment.

Drugs Utilized in This Study

Epinephrine - 0.05 mg/kg

Norepinephrine - 0.05 mg/kg

FIGURE 9.

DOG IN POSITION FOR ADMINISTERING

PRESSOR AMINES AND RECORDING

ARTERIAL BLOOD PRESSURE

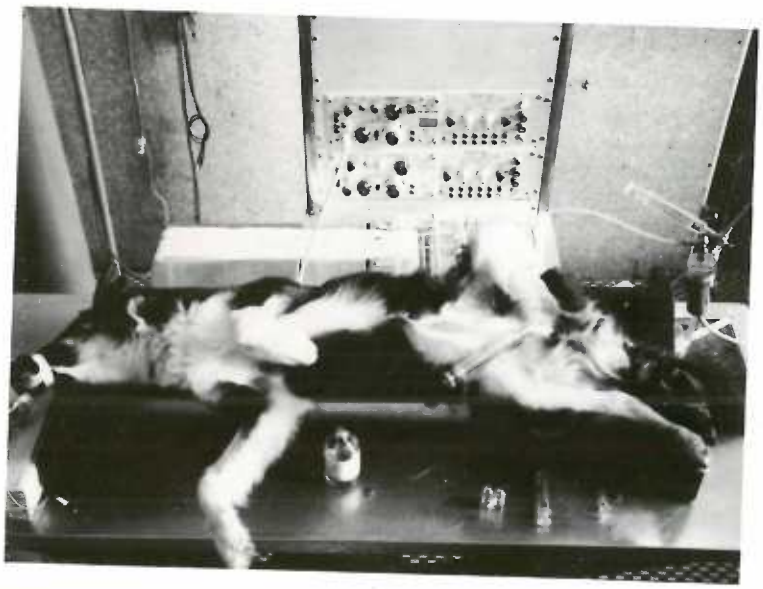


FIGURE 9

The drugs were administered intravenously via the indwelling polyethylene catheter in the left femoral vein of the dog. The doses were repeated at five minute intervals until the animal failed to respond to the last dose given. The corresponding segment of the right femoral vein was then removed, blotted free of blood and stripped of adhering tissue, and weighed to the nearest 0.1 mg. The sodium and potassium determinations were then carried out as described previously for the rat aorta. The data was calculated as milliequivalents per 100 gm of arterial wet weight.

Myocardial Infarct Shock in Dogs

For the production of cardiac shock in dogs, ten animals of approximately 10 kg weight were anesthetized with either pentobarbital or thiopental. The animal was placed on its right side and a thoracotomy was performed through the fourth rib interspace. The left descending coronary was ligated within 5 mm of the circumflex artery. In some of the animals there were up to four such branches from the circumflex artery which were all tied in an attempt to keep the amount of cardiac damage constant.

During the thoracotomy, respiration was maintained by a Takioka respirator using a gas mixture of 97.5 per cent oxygen and 2.5 per cent carbon dioxide. Respiration was maintained until the chest was closed and the dog tried to breathe against the respirator. The spleen of each animal was removed prior to the ligation as this has been shown to increase the incidence of shock in dogs subjected to experimental coronary occlusion (14).

The arterial blood pressure was measured from the right femoral artery using the same procedure as previously described. At the time of cannulation, a segment of the right femoral artery was removed and treated as the other artery samples for the determination of arterial sodium and potassium.

The animals were then placed in recovery cages and allowed to recover from the surgery. The animals became hypotensive during the first 48 hours after the ligation. At this time, the animal was cannulated and the blood pressure recorded via the left femoral artery. At the time of cannulation another segment of artery was removed for sodium and potassium analysis.

The animal was then given, by intravenous drip, either norepinephrine (4 mg in 500 cc) or angiotensin amide (2.5 mg in 500 cc of normal saline solution). This was infused at a rate sufficient to maintain the dog's control blood pressure. It was necessary to increase rate of infusion until the dog failed to respond any longer and died.

RESULTS

Morphine Sulfate Experiments in Rats

Preliminary experiments were done on rats employing two different dosage sequences of morphine sulfate. One group was given 2 mg/100 gm body weight as a single dose one hour prior to the stress, while the other group received the same dose daily for five days. The rats were then subjected to stress in the Noble-Collip drum. At 400 turns in the drum the animals which had received the single dose of morphine were more susceptible to the stress than control rats receiving only saline. However, the animals which had been given morphine daily for five days were more resistant to the stress produced by 500 turns in the drum than their corresponding controls. In the single dose experiment 70 per cent of the morphine-treated animals died, while only 14 per cent of the controls were killed (Figure 10). For the multiple-dose experiment, where the animals were subjected to 500 turns in the drum, 100 per cent of the control animals died while only 40 per cent of the treated group were killed (Figure 11).

Pressor Amine Experiments in Rats

To test the hypothesis that the release of pressor substances into the general circulation during the stress situation was detrimental to survival of animals in irreversible shock, animals were treated with pressor amines immediately after tumbling 400 turns in the Noble-Collip drum. Epinephrine, ephedrine, norepinephrine, or mephenteramine were administered to rats in this experiment. The

FIGURE 10.

SURVIVAL OF RATS GIVEN A SINGLE DOSE
OF MORPHINE SULFATE (2 MG/100 GM BODY WEIGHT)
ONE HOUR PRIOR TO STRESSING 400 TURNS
IN THE NOBLE-COLLIP DRUM

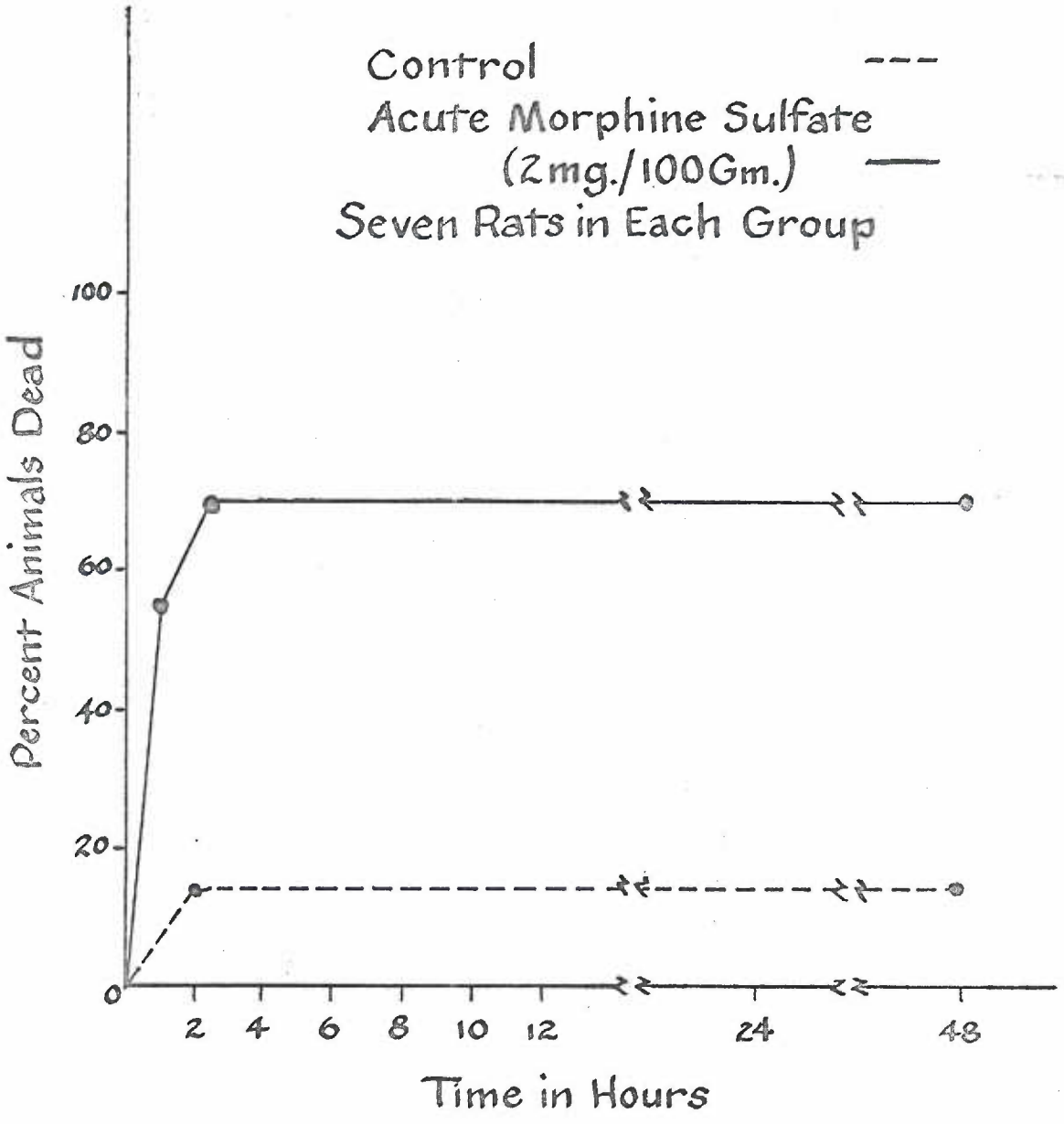

Control ---
Acute Morphine Sulfate
(2mg./100Gm.) ——
Seven Rats in Each Group
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
48
Time in Hours

FIGURE 10.

FIGURE 11.

SURVIVAL OF RATS GIVEN MORPHINE SULFATE (2 mg/100 GM BODY WEIGHT) DAILY FOR FIVE DAYS PRIOR TO STRESSING 500 TURNS IN THE NOBLE-COLLIP DRUM

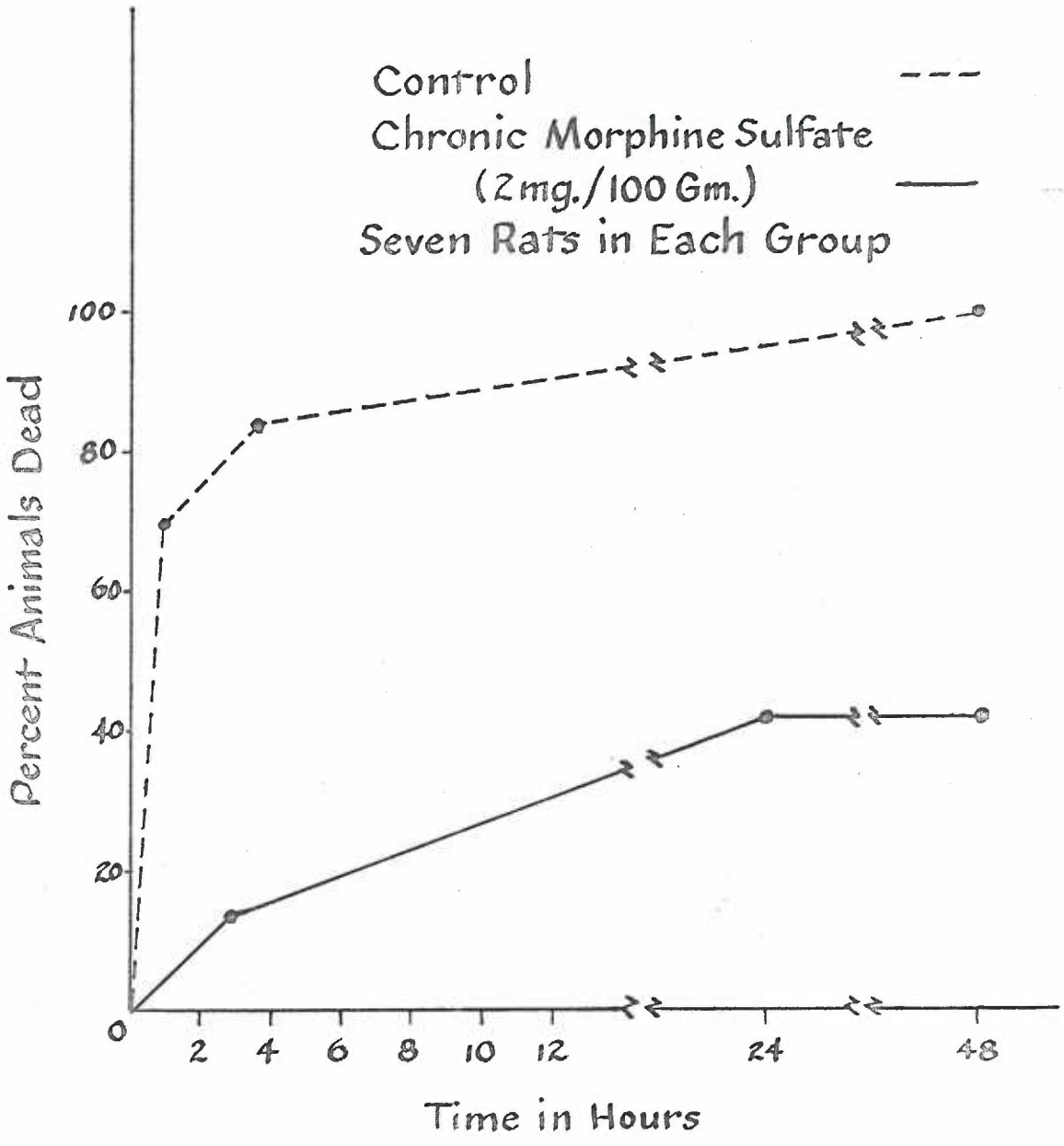

Control
Chronic Morphine Sulfate
(2 mg./100 Gm.)
Seven Rats in Each Group
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
48
Time in Hours

FIGURE 11.

results are shown in Figures 12, 13, 14 and 15 respectively. In all four experiments with these different pressor drugs, the results were, for all practical purposes, identical. A composite graph (Figure 16) shows the combined data from these four experiments. The rats which had been treated with the pressor agents all died in less than four hours after removal from the stress drum. The animals which had been given saline had a mortality rate of 27 per cent at four hours and 40 per cent at the end of the 48 hour observation period. These results are then indicative that pressor amines do contribute to irreversible states of shock, and in these experiments the doses employed seemed to be equivalent in stress to 100 added revolutions in the drum, as the curve for the animals receiving pressor amines at 400 turns is very similar to the curve for control animals at 500 turns in the drum.

This conclusion is substantiated by results of Lum and Calvert (50) and others who have reported that both adrenergic and ganglionic blocking agents will protect rats and dogs from the lethal effects of stress (5)(6). These agents which block sympathetic nervous system activity must, however, be given prior to the stress before they are effective. This fact is added evidence that the high blood levels of the catechol pressor amine compounds may cause some deleterious effect which leads to irreversibility of hypotensive shock.

The previous observations that pressor amines administered to stressed rats resulted in decreased survival led to a study of some vasopressor compound that was not of this class. For this experiment angiotensin amide was administered intraperitoneally to a group of rats (1.25 mg per animal) immediately after removal of the animals

FIGURE 12

SURVIVAL OF RATS GIVEN EPINEPHRINE
(1 MG PER ANIMAL) AFTER 400 TURNS
IN THE NOBLE-COLLIP DRUM

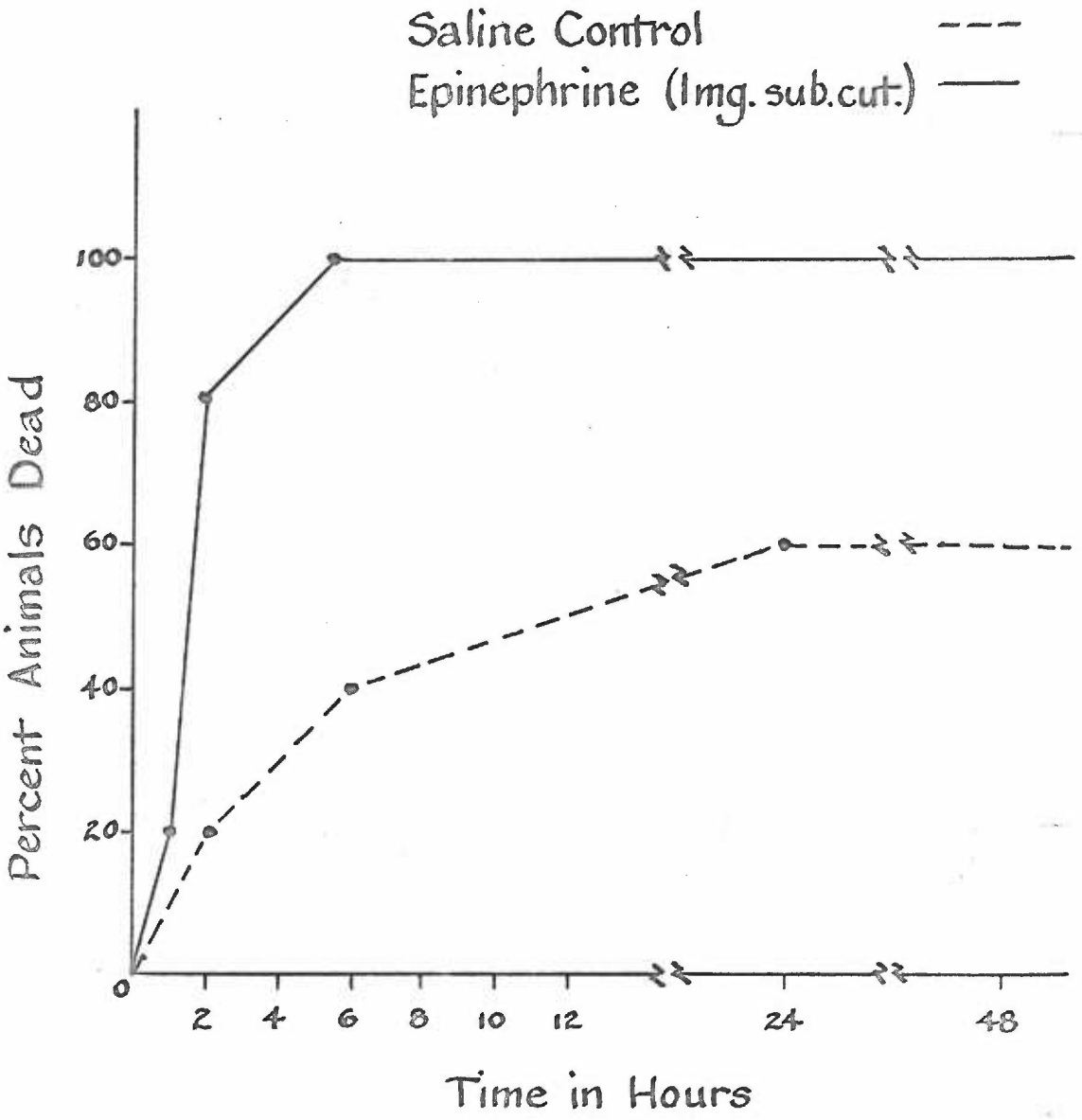

Saline Control
Epinephrine (1mg. sub.cut.)
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
48
Time in Hours

FIGURE 12

FIGURE 13

SURVIVAL OF RATS GIVEN EPHEDRINE
(25 MG PER ANIMAL) AFTER 400
TURNS IN THE NOBLE-COLLIP DRUM

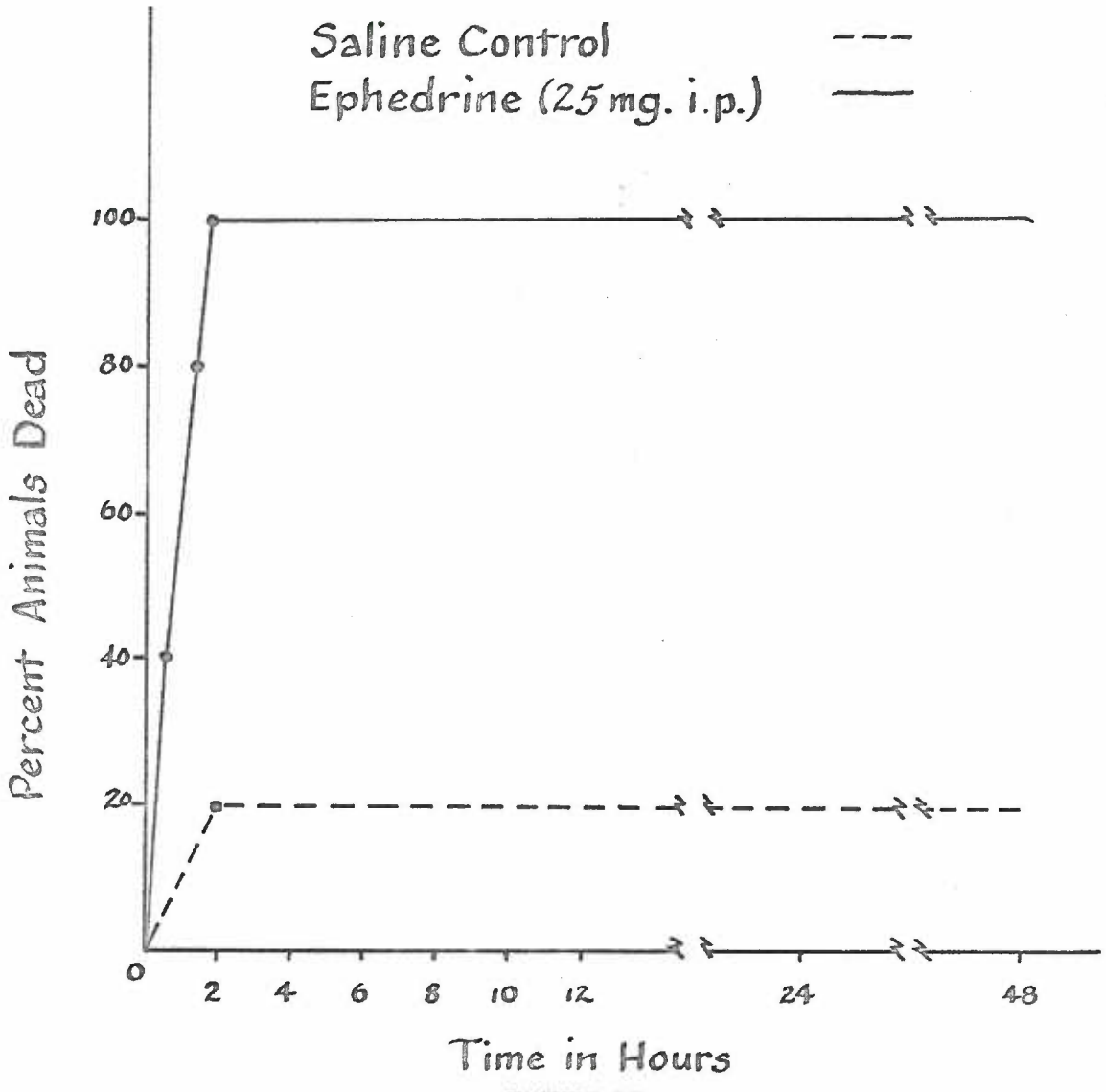

Saline Control
Ephedrine (25 mg. i.p.)
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
48
Time in Hours

FIGURE 13

FIGURE 14

SURVIVAL OF RATS GIVEN NOREPINEPHRINE

(1 MG PER ANIMAL) AFTER 400 TURNS

IN THE NOBLE-COLLIP DRUM

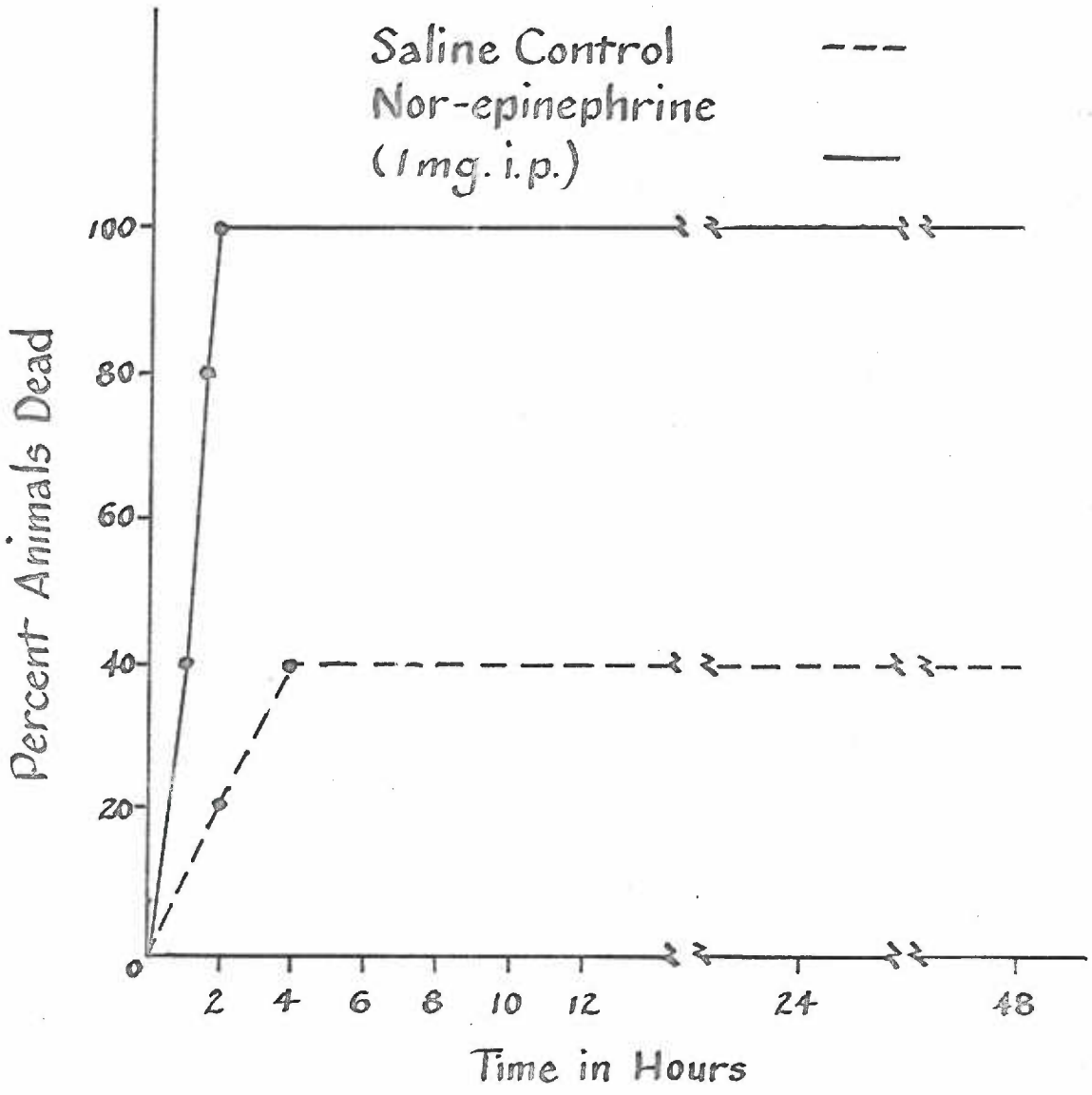

Saline Control
Nor-epinephrine
(1 mg. i.p.)
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
48
Time in Hours

FIGURE 14

FIGURE 15

SURVIVAL OF RATS GIVEN MEPHENTERAMINE
(5 MG PER ANIMAL) AFTER 400 TURNS
IN THE NOBLE-COLLIP DRUM

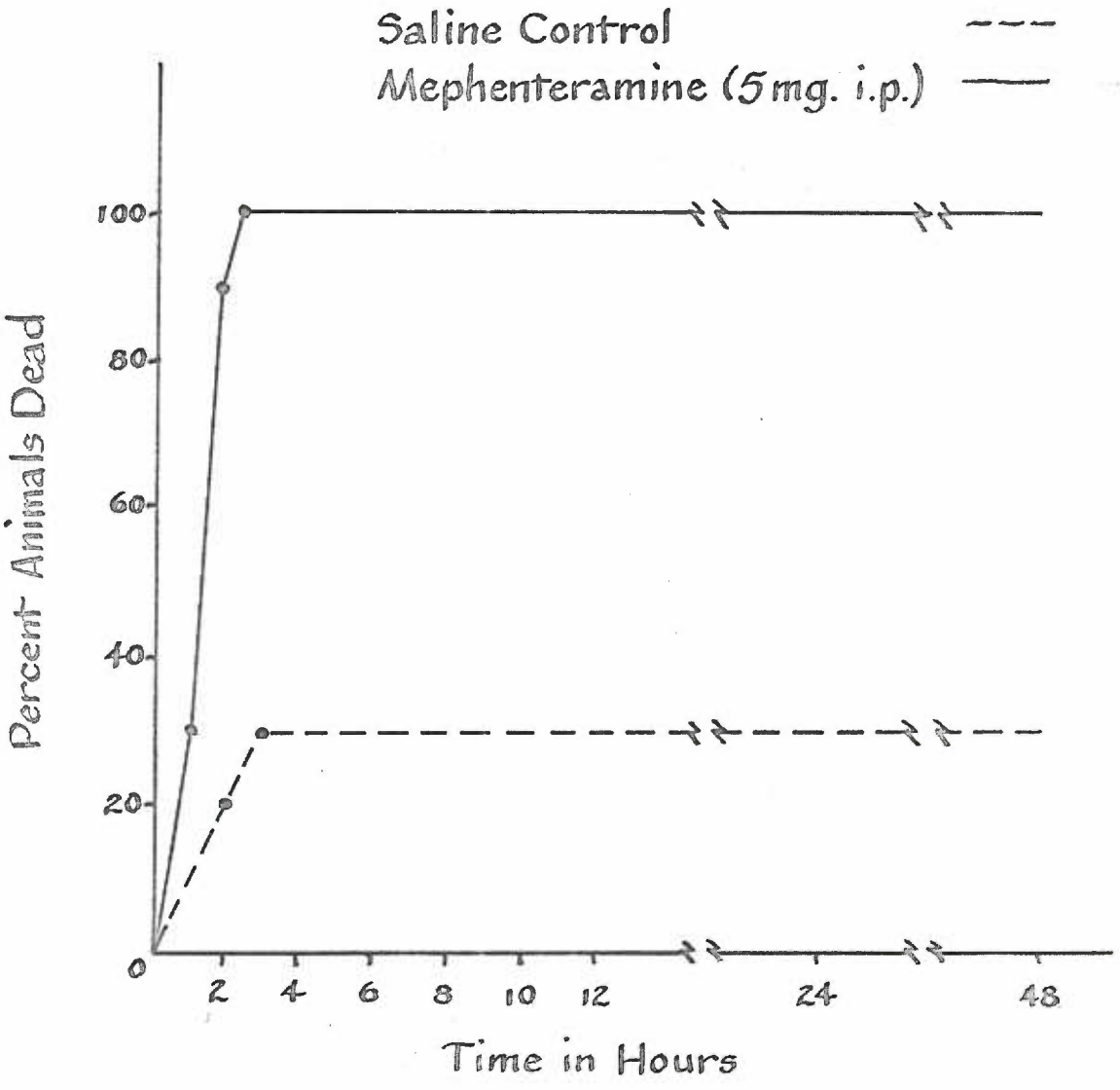

Saline Control
Mephenteramine (5 mg. i.p.)
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
48
Time in Hours

FIGURE 15

FIGURE 16.

SURVIVAL OF RATS FROM COMBINED DATA

OF ANIMALS RECEIVING PRESSOR AMINES

AFTER 400 TURNS IN NOBLE-COLLIP DRUM

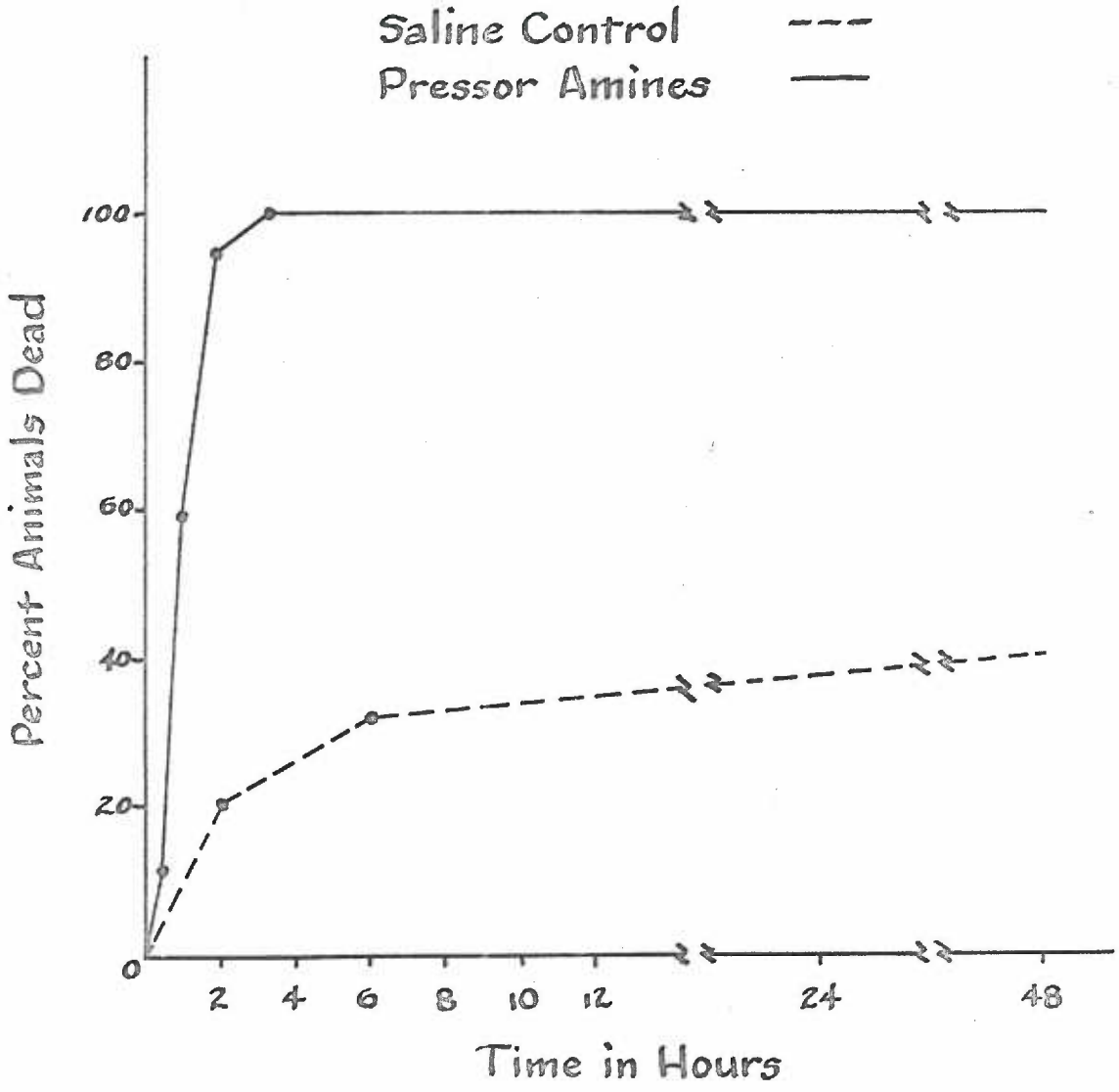

Saline Control
Pressor Amines
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
48
Time in Hours

FIGURE 16

from the Noble-Collip drum. These rats had been stressed 400 times in the drum, as were the animals that had been treated with pressor amines. The rats receiving the angiotensin amide had an ultimate death rate of 50 per cent, while that of the controls was 30 per cent (Figure 17). This showed a somewhat higher death rate in the angiotensin amide treated animals, but it was much less than that of the animals which were given pressor amines.

Sodium and Potassium Changes in Rat Aorta Tissue

The repeated observations mentioned above in relation to changes in arterial sodium and potassium ions in response to chronic hypertension and subsequent lowering by the thiazide diuretics and the acute experiments with pressor amines led us to speculate that some effect may occur in the sodium and potassium concentration of the arterial wall in animals which are in shock due to stressful stimuli.

The normal sodium and potassium of our control untreated rats was 16.92 mEq/100 gm wet weight tissue for sodium and 3.87 mEq/100 gm wet weight tissue for potassium (average of 6 rats). After the rat had been subjected to 600 turns in the Noble-Collip drum, the content of sodium was 8.27 mEq/100 gm and 2.59 mEq/100 gm potassium (average of 6 rats). This then represented a reduction of 51 per cent of the sodium content and a reduction of 33 per cent of the potassium content in the thoracic aorta of the stressed rat. Statistical evaluation of the data using the student T-test showed that both of these changes are significant with $p = 0.001$ for sodium and $p = 0.01$ for potassium (Tables 3 and 4).

FIGURE 17

SURVIVAL OF RATS GIVEN ANGIOTENSIN AMIDE (1.25 MG PER ANIMAL) AFTER 400 TURNS IN NOBLE-COLLIP DRUM

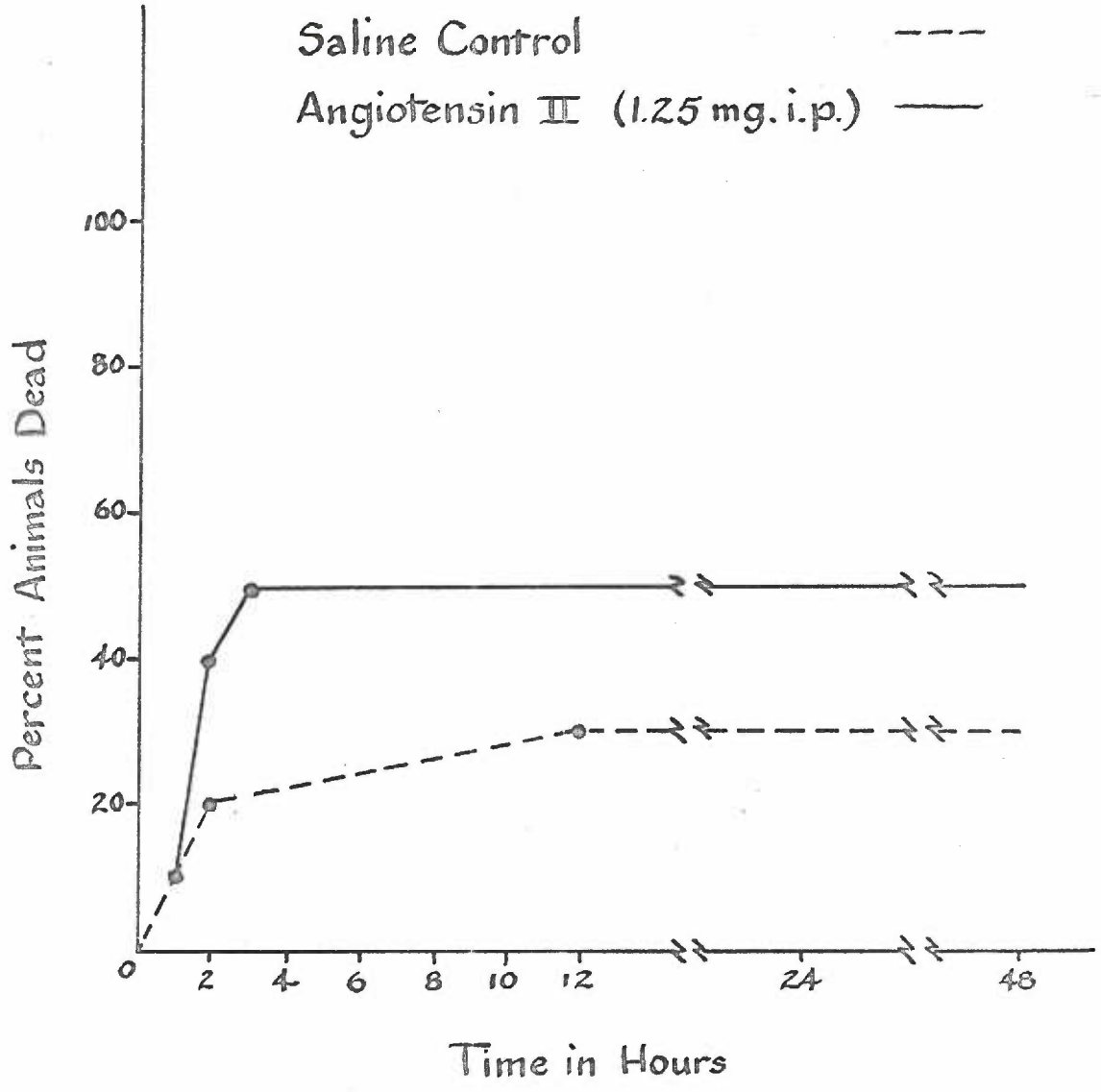

Saline Control
Angiotensin II (1.25 mg. i.p.)
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
48
Time in Hours

FIGURE 17

TABLE III.

POTASSIUM CONTENT IN RAT AORTA

WITH STATISTICAL ANALYSIS

Control Rats*	Shock Rats*
4.39	2.15
3.82	2.43
4.37	2.62
3.39	2.92
3.66	2.98
3.58	2.44

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\Sigma X_1^2 - (\Sigma X_1)^2/N + \Sigma X_2^2 - (\Sigma X_2)^2/N}{N(N-1)}}}$$

$$t = \frac{3.54 - 2.29}{\sqrt{\frac{90.67 - 89.78 + 40.75 - 40.25}{30}}}$$

t = 4.52 with (2N - 2) or 10 degrees of freedom

p = 0.01

*Milliequivalents per 100 grams fresh weight aorta wall tissue.

TABLE IV.

SODIUM CONTENT IN RAT AORTA

WITH STATISTICAL ANALYSIS

Control Rats*	Shock Rats*
19.31	7.34
14.64	8.39
20.65	8.63
14.70	8.62
14.90	8.71
17.33	7.94

$$\frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\Sigma X_1^2 - (\Sigma X_1)^2/N + \Sigma X_2^2 - (\Sigma X_2)^2/N}{N(N-1)}}}$$

$$\frac{16.91 - 8.27}{\sqrt{\frac{1748.60 - 1714.60 + 411.96 - 410.52}{30}}}$$

t = 8.00 with (2N - 2) or 10 degrees of freedom

p = 0.001

*Milliequivalents per 100 grams fresh weight aorta wall tissue.

To see if these changes in the sodium and potassium content of the rat aorta would be altered by the pressor amines, ephedrine was administered to a group of eight rats immediately after removal from the stress cage and allowed to recover for one hour. The animals remaining alive after this hour's observation period were sacrificed and their aortic sodium and potassium determinations made. The animals that had received the ephedrine still had the low sodium and potassium values, but the control saline group had values that indicated a return toward normal (Figure 18). This evidence suggests that the pressor amines tend to prolong this lowered electrolyte content of the vascular wall and in some way initiates or potentiates the irreversible stage of shock.

It was then desirable to ascertain if a "graded dose" of stress would progressively alter the electrolyte content of the aortic wall. The progressive lethal nature of the stress procedure has been studied in some detail by Noble and Collip (66) and also repeated by others (94). It has been demonstrated by Young and Graf that increasing numbers of turns in the drum causes increasing plasma levels of both epinephrine and norepinephrine (94). If the animals did have such a correlation it may be indicative that the degree of stress which alters the blood levels of pressor amines might also be related to the observed changes in aorta electrolytes. Rats were then subjected to 0, 100, 400 and 800 turns in the drum, promptly sacrificed, and sodium and potassium determinations performed on the aorta sample.

FIGURE 18

SODIUM AND POTASSIUM CONTENT OF RAT AORTA ONE HOUR AFTER 400 TURNS IN THE NOBLE-COLLIP DRUM COMPARING EPHEDRINE-TREATED AND CONTROL ANIMALS

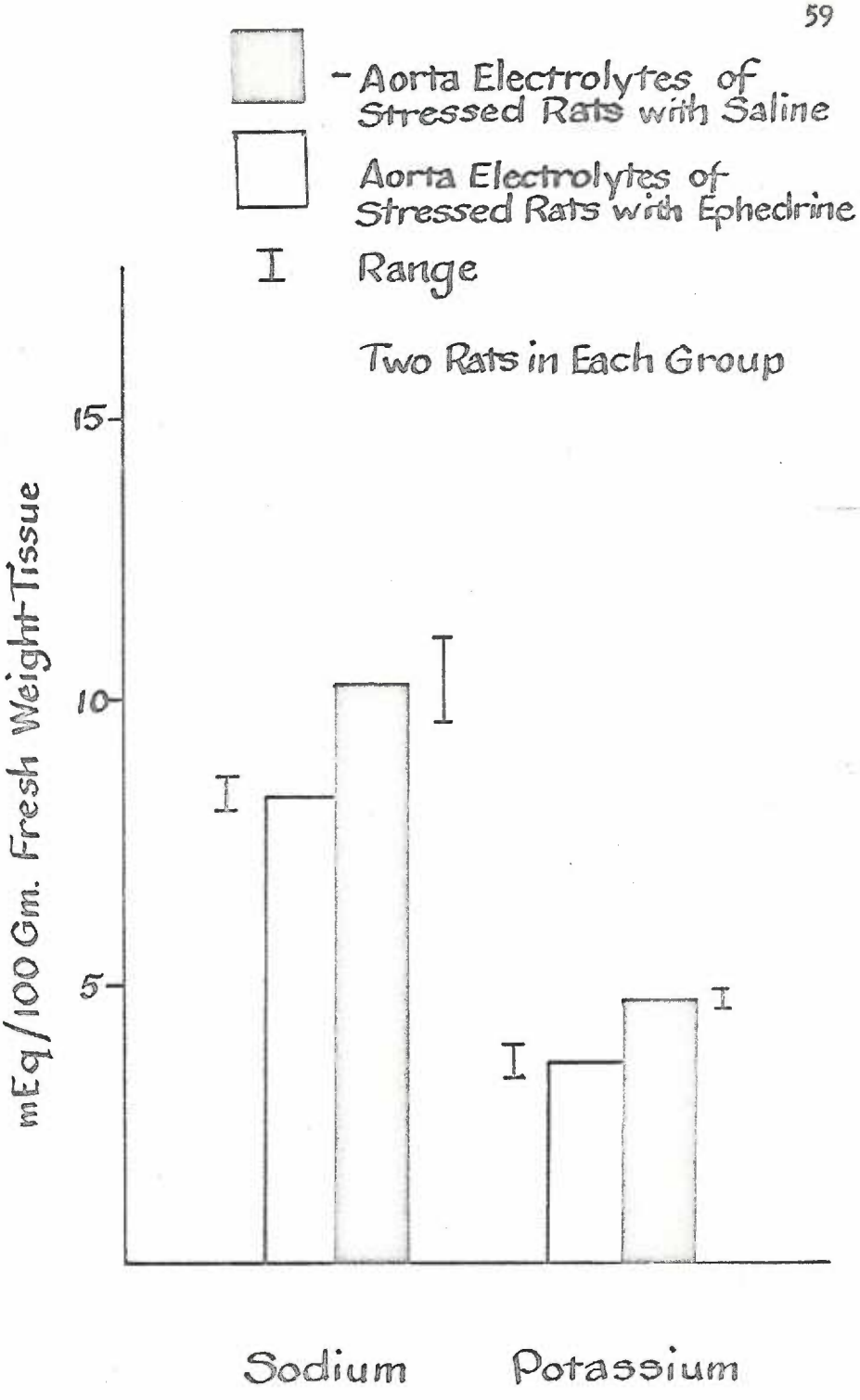

Aorta Electrolytes of Stressed Rats with Saline
Aorta Electrolytes of Stressed Rats with Ephedrine
Range
Two Rats in Each Group
mEq/100 Gm. Fresh Weight Tissue
15
10
5
Sodium
Potassium

FIGURE 18

This data indicates a progressive fall in the sodium content of the aorta which was postulated from the previous data, however the potassium content falls at first and then begins to return toward normal. The difference between 2.75 and 3.06 is not a significant change in this small group of animals with P = 0.1. However, the initial fall in aorta potassium from a control value of 3.49 to 2.75 mg is significant with P = 0.02 calculated with the student T-test (Figure 19).

As the thiazide diuretics have the ability to depress the sodium and potassium content of the body, it was decided to employ this agent to test the hypothesis that the lowered sodium and potassium content of the rat aorta may have a bearing on the lethality of the stress. The results of this experiment are shown in Figure 20. The rats were treated with chlorothiazide (10 mg per animal) daily for ten days. One hour after their last injection on the tenth day of the experiment, the rats were subjected to 400 turns in the Noble-Collip drum. The animals that were treated with the chlorothiazide prior to the stress were all killed by this amount of stress. However, only 30 per cent of the animals receiving saline as a control died. This experiment seemed to indicate then that the depression of the electrolytes of the rat aorta had some significance in the survival of rats subjected to traumatic shock. To see if the release of sympathomimetic amines may have had some influence in the depression of these electrolytes, it was decided to attempt a block of the normal adrenergic mechanisms.

This hypothesis was then tested in the rats by injecting intraperitoneally 0.5 mg/kg of phentolamine methansulfonate, an adrenergic

FIGURE 19

SERIAL CHANGES IN RAT AORTA SODIUM AND

POTASSIUM CONTENT AFTER

INCREASING AMOUNTS

OF STRESS IN THE NOBLE-COLLIP DRUM

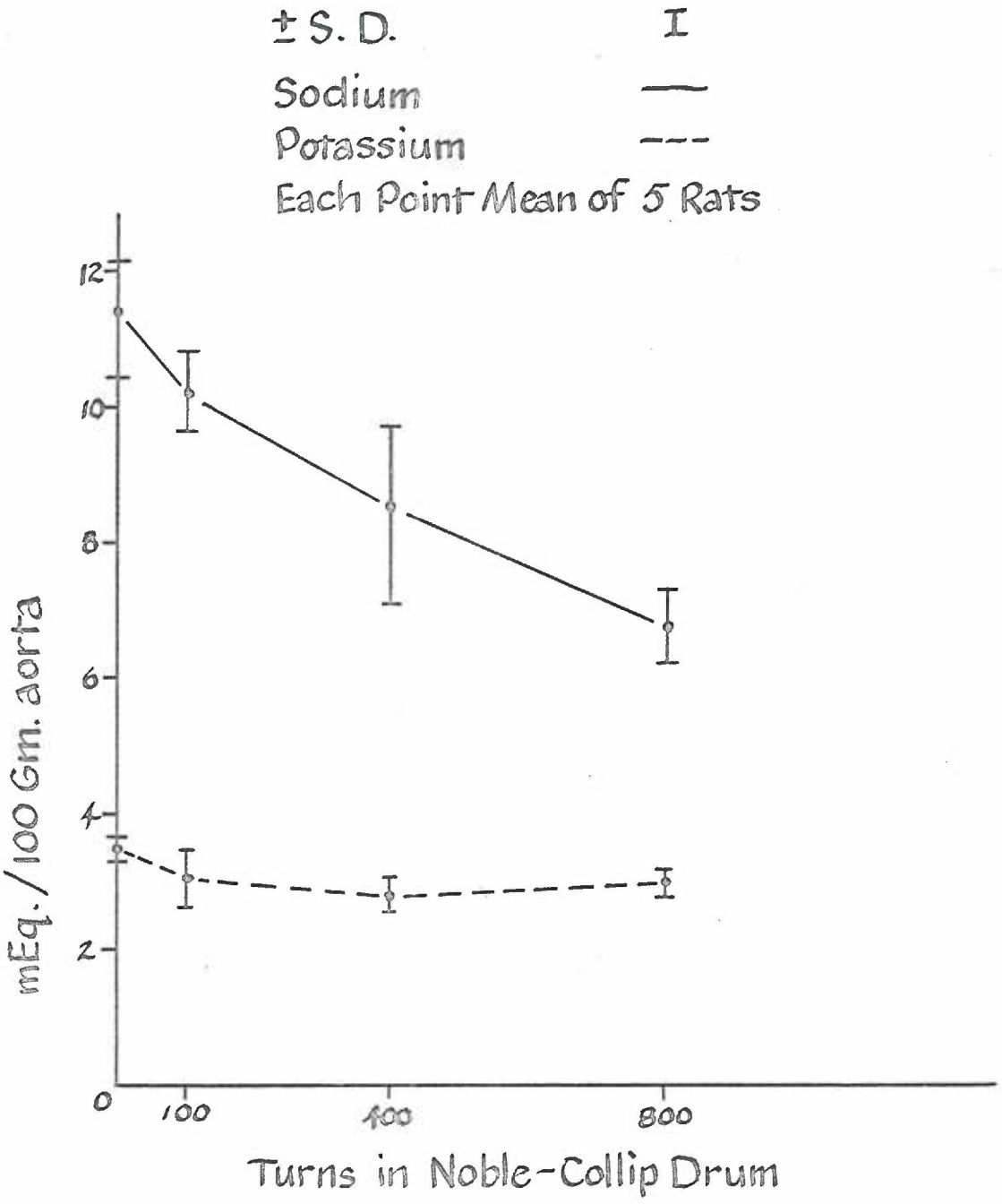

± S. D.
I
Sodium
Potassium
Each Point Mean of 5 Rats
12
10
8
6
4
2
0
mEq./100 Gm. aorta
100
400
800
Turns in Noble-Collip Drum

FIGURE 19

FIGURE 20

SURVIVAL OF RATS PRETREATED WITH CHLOROTHIAZIDE (10 MG PER ANIMAL) FOR TEN DAYS PRIOR TO 400 TURNS IN THE NOBLE-COLLIP DRUM

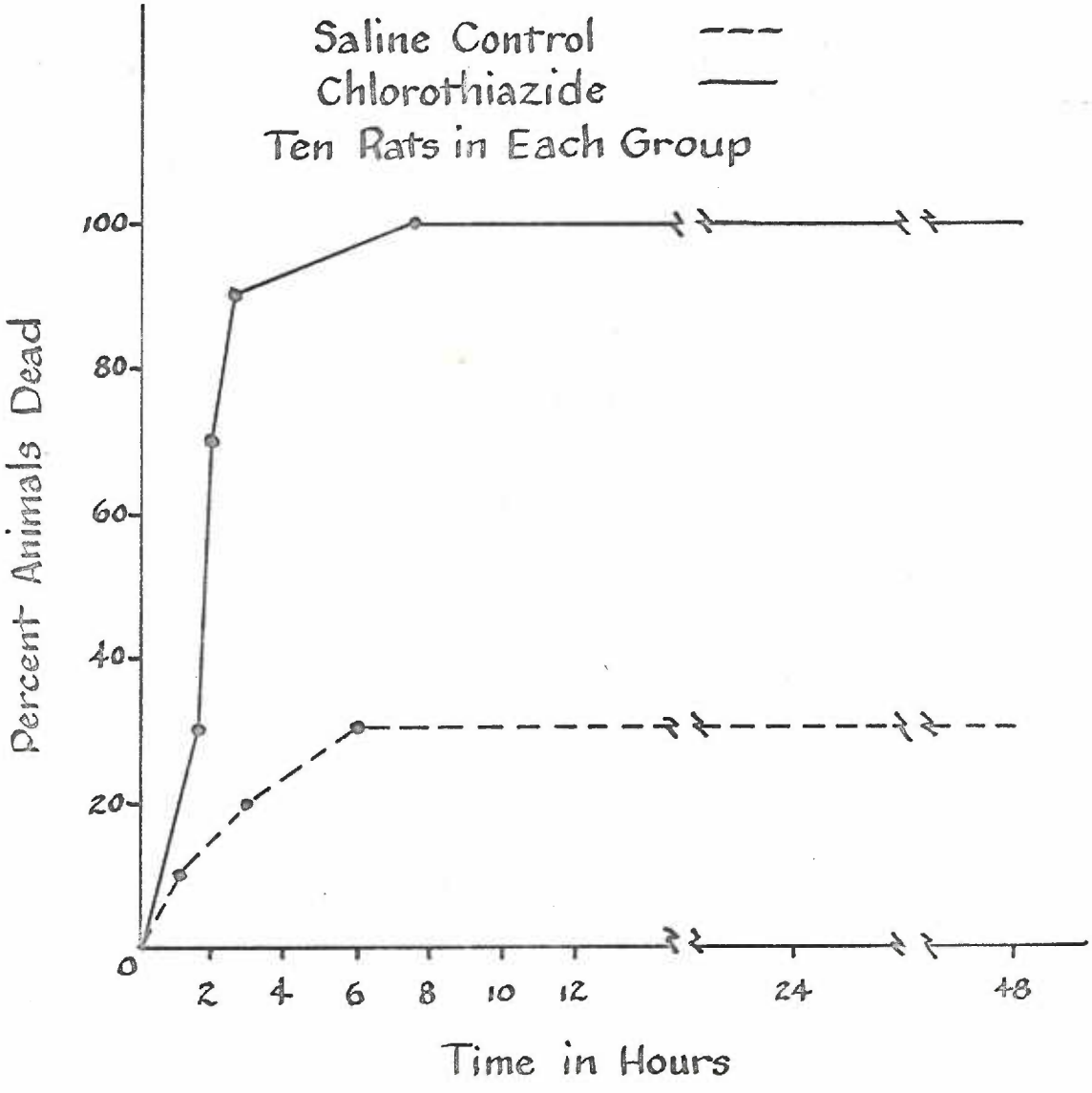

Saline Control
Chlorothiazide
Ten Rats in Each Group
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
48
Time in Hours

FIGURE 20

blocking agent, one hour prior to stressing the animals. The rats were stressed 400 turns in the Noble-Collip drum. After the stress procedure the aortic sodium was 11.51 mEq/100 gm and the potassium was 3.67 mEq/100 gm of fresh weight tissue. These figures are not significantly different from the data of the control rats of 11.28 mEq/100 gm of sodium and 3.49 mEq/100 gm of potassium. This data would then support the hypothesis that the release of large amounts of pressor amines in the animal subjected to stressful situations leads in some way to a decrease in sodium and potassium ions in the arterial wall and possibly to irreversibility of the shock.

Catechol amine tachyphylaxis in dogs

If it is, in fact, the increased levels of pressor amines in the circulation that lead to irreversibility of the shock-like state, it should be possible to produce shock-like conditions by continuous high doses of these amines. The animals in this state should have the same alterations in arterial electrolytes that were observed in the rats subjected to traumatic stress in the Noble-Collip drum. The animal chosen for this study was the dog because its blood pressure can be recorded with ease and the arteries allow removal for repeated measurements. Doses of pressor amines (0.05 mg/kg of both epinephrine and norepinephrine) were administered to these animals every five minutes until they died in shock. The animals subjected to this procedure showed an increase in hematocrit up until the animal died, which is in agreement with the work of Rosenthale and DiPalma (77). This is usually seen in shock conditions and has been ascribed to the leakage

of fluid into the interstitial spaces. It is usually thought of as being the result of increased capillary fragility, or the result of continually maintained high blood pressure. The animals subjected to this treatment of repeated doses of 0.05 mg/kg of either epinephrine and norepinephrine showed a rapid tolerance or tachyphylaxis to the pressor response of these amines (Figures 21 and 22). The pressure rise after the first dose was approximately 210 mm Hg and after the 30th dose was about 80 mm Hg. The initial pressor response to norepinephrine was usually 20 to 30 mm Hg higher than the initial response to epinephrine (Figure 23). This difference is to be expected as norepinephrine has primarily a pressor component, the alpha receptor of Ahlquist, and only a small, if any, depressor response, the beta receptor of Ahlquist. Epinephrine however has both, and some of the beta receptor effect usually offsets some of the raise in blood pressure that is otherwise seen. After the initial dose, however, the pattern of decline seems to be identical for both drugs.

Sodium and Potassium Changes in Dog Artery

At the instant when the last dose of the drug was given and no further increase in blood pressure resulted, the opposite femoral artery was quickly dissected free, the adhering tissue removed, and the sodium and potassium content determined by flame photometry as described previously. The sodium content in the control arteries had an average of 21.83 mEq/100 gm of fresh weight tissue (Figure 24). After the animals were in the shock-like state they had an average sodium content of 10.78 mEq/100 gm of fresh weight tissue (P=0.01).

FIGURE 21

TYPICAL ARTERIAL BLOOD PRESSURE RESPONSE

SHOWING TACHYPHYLAXIS FROM

REPEATED ADMINISTRATION OF EPINEPHRINE

(0.05 MG/KG EVERY FIVE MINUTES)

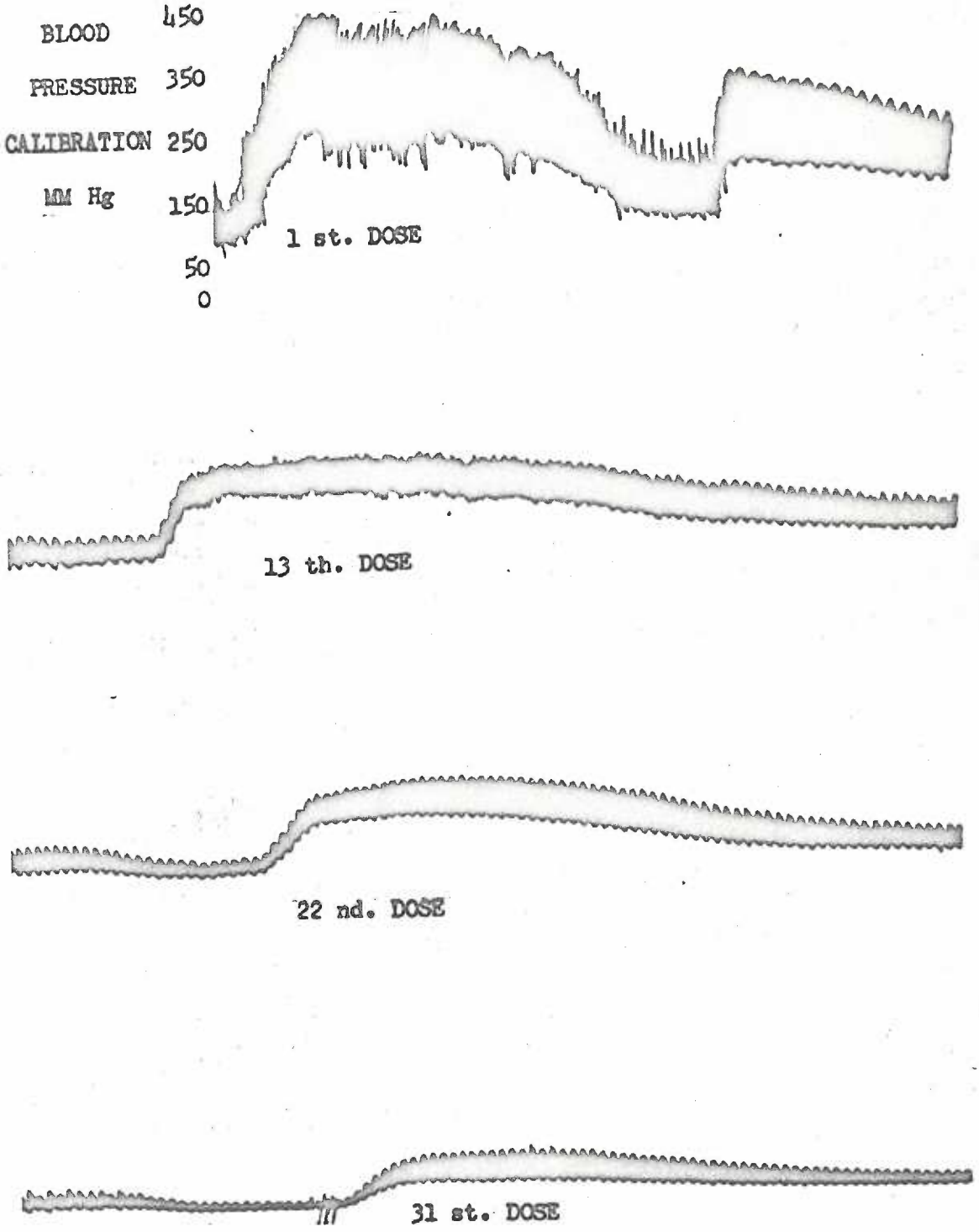

BLOOD
PRESSURE
CALIBERATION
MM Hg
450
350
250
150
50
0
1 st. DOSE
13 th. DOSE
22 nd. DOSE
31 st. DOSE

FIGURE 21

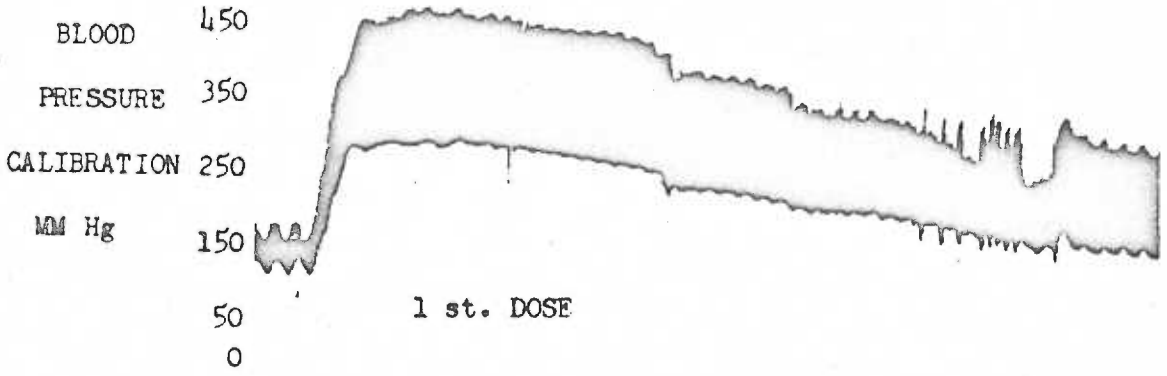
FIGURE 22

TYPICAL ARTERIAL BLOOD PRESSURE RESPONSE

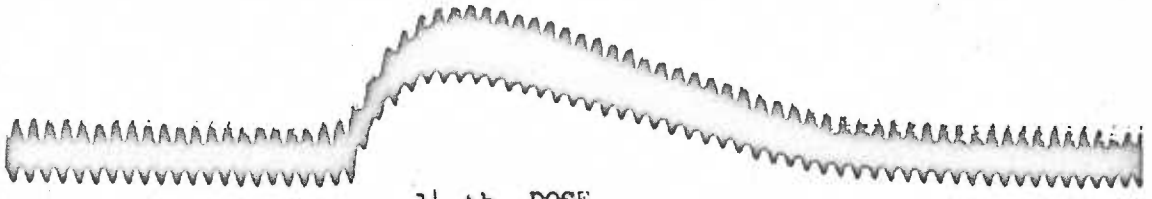
SHOWING TACHYPHYLAXIS FROM

REPEATED ADMINISTRATION OF NOREPINEPHRINE

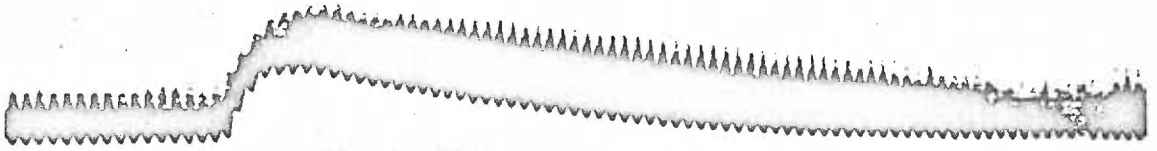
(0.05 MG/KG EVERY FIVE MINUTES)



1 st. DOSE



14 th. DOSE



21 st. DOSE

33 rd. DOSE

FIGURE 22

FIGURE 23

COMPARISON OF BLOOD PRESSURE RESPONSE

OF NOREPINEPHRINE AND EPINEPHRINE

(0.05 MG/KG EVERY FIVE MINUTES)

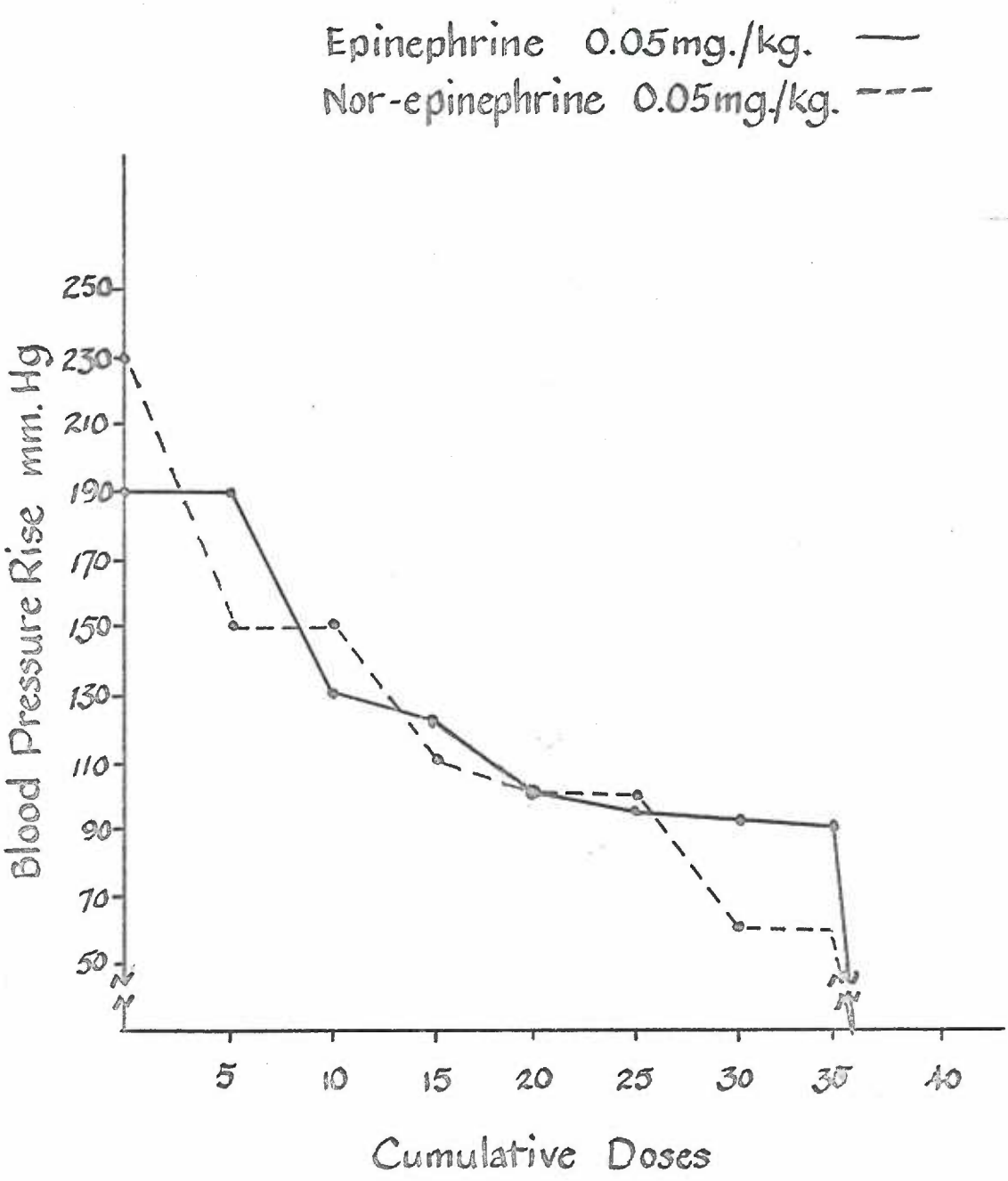

Epinephrine 0.05mg./kg.
Nor-epinephrine 0.05mg./kg.
Blood Pressure Rise mm. Hg
250
230
210
190
170
150
130
110
90
70
50
5
10
15
20
25
30
35
40
Cumulative Doses

FIGURE 23

FIGURE 24

DOG FEMORAL ARTERY SODIUM CONTENT

BEFORE AND AFTER INDUCTION OF SHOCK

BY REPEATED ADMINISTRATION OF

EPINEPHRINE AND NOREPINEPHRINE

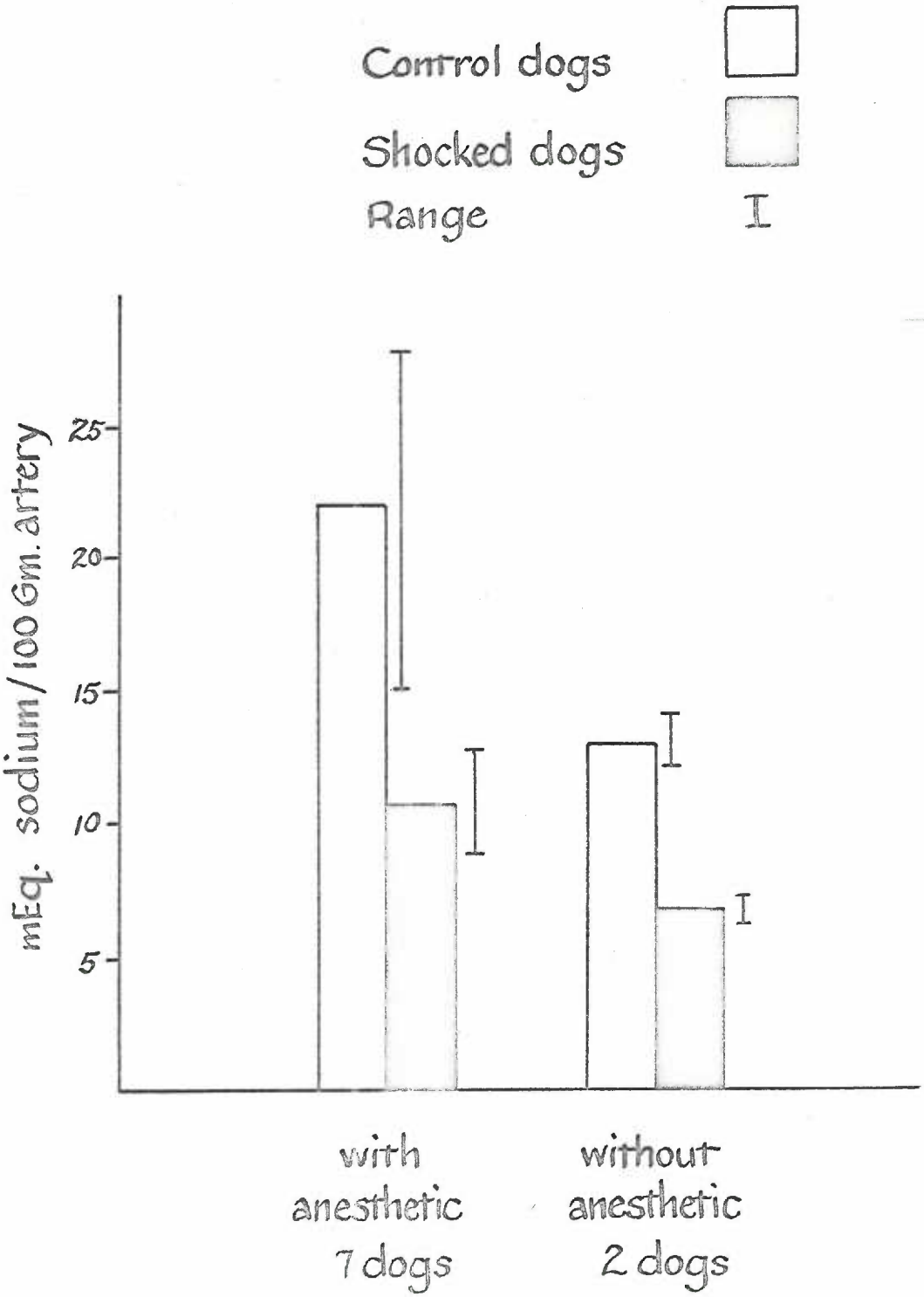

Control dogs
Shocked dogs
Range
mEq. sodium/100 Gm. artery
25
20
15
10
5
with anesthetic 7 dogs
without anesthetic 2 dogs

FIGURE 24

The potassium content of the arteries sometimes was lowered and at other times actually increased. If the average value of all the dogs is calculated, an actual increase in the potassium content is evident. This is an average of 3.38 as the control and 3.64 mEq/100 gm at the time of death. On closer inspection of the data, however, it was noted that in the animals who died early in the experiment using epinephrine, there was a decrease in the potassium content and those dying later exhibited an increase. The dogs receiving norepinephrine, however, all lived for longer than 30 doses, so here all of the dogs showed an increase in the potassium content. From this observation, along with the data from the rat experiment which showed a fall in artery potassium with a subsequent rise toward control values, it was decided to follow the course of the changes in sodium and potassium throughout the experiment. For this procedure the left carotid artery was dissected free, but allowed to remain in the sheath along with the vagus nerve and its complete blood supply. Then a polyethylene catheter about 25 cm long was inserted in both ends of the artery and the middle segment was removed for control ion determinations. Samples were removed after 10 doses had been given, after 20 doses, and after 30 doses. This procedure then gave a picture of the electrolyte changes associated with the administration of the pressor drug (Figure 25). The control sodium value was 16.73 mEq/100 gm. After 10 doses, 12.56 mEq/100 gm; after 20 doses 9.48 mEq/100 gm and at the end of the experiment, i.e., death, the sodium content had dropped to 8.93 mEq/100 gm of artery tissue. The values for potassium were 3.03 mEq/100 gm for the control period, 2.21 mEq/100 gm at the end of 10 doses,

FIGURE 25

SERIAL CHANGES OF SODIUM AND POTASSIUM
CONTENT OF DOG CAROTID ARTERY
FROM CUMULATIVE DOSES OF NOREPINEPHRINE
(0.05 MG/KG EVERY FIVE MINUTES)

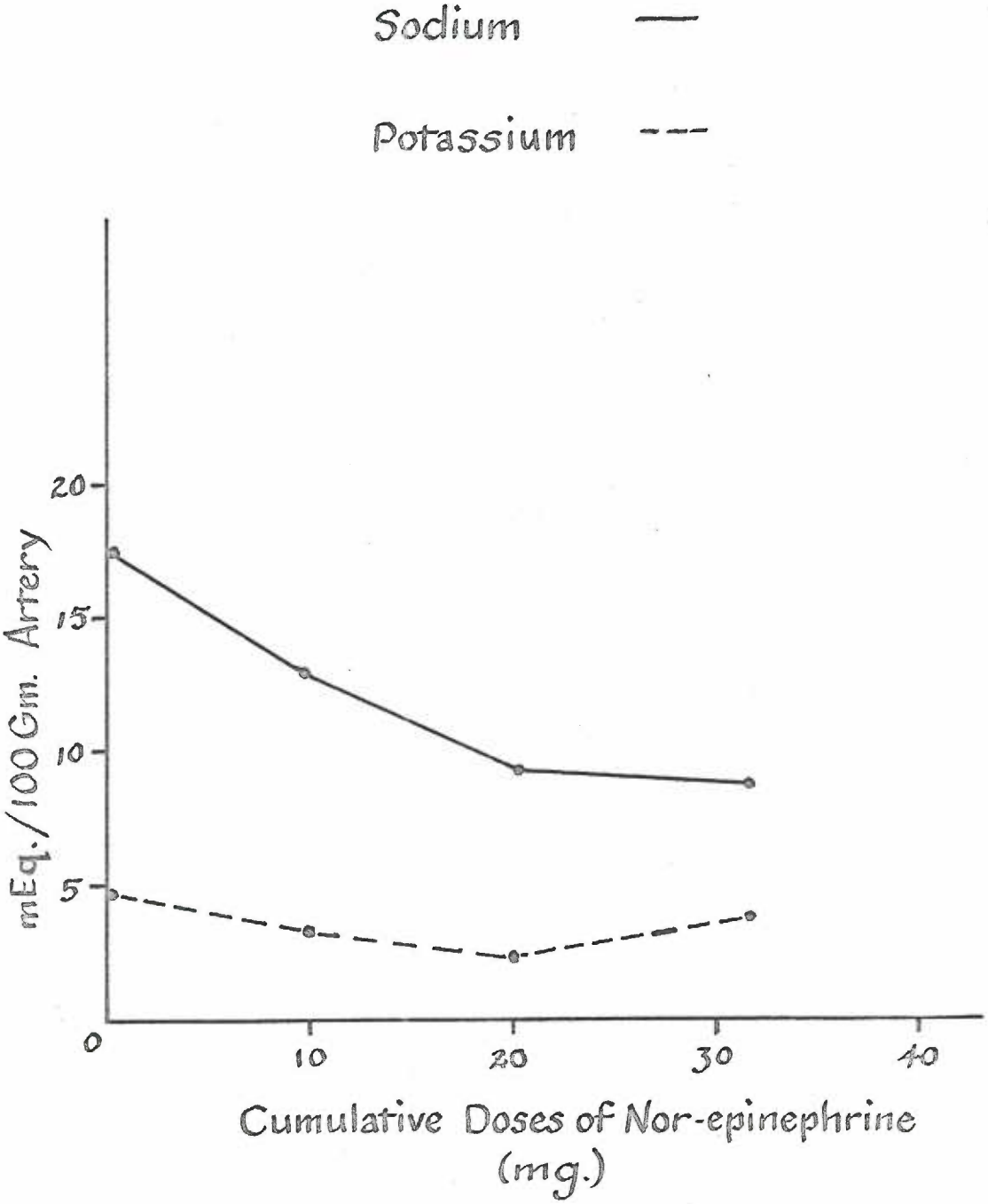

Sodium
Potassium
mEq./100 Gm. Artery
20
15
10
5
0
10
20
30
40
Cumulative Doses of Nor-epinephrine
(mg.)

FIGURE 25

2.22 mEq/100 gm at the end of 20 doses, 2.88 at the end of 30 doses, and 3.02 mEq/100 gm at the time of death.

These results appear to confirm the observation that in the dog subjected to high doses of pressor amines, there is a gradual decline of sodium, along with an initial decline in potassium, that tends to return toward normal at the time of death.

All of the dog studies were performed on dogs anesthetized with pentobarbital. To ascertain if the anesthesia over this time period had any effect on the fluctuations of arterial electrolytes, one dog was subjected to the same procedure as described above except that a volume of saline corresponding to the volume of either epinephrine or norepinephrine was given for a total of 35 injections. Any change in femoral artery electrolytes was then measured. The control sodium content of the femoral artery was 13.22 mEq/100 gm and after the last dose of saline, was 14.51 mEq/100 gm of tissue. The content of potassium also increased from 2.33 to 3.06 mEq/100 gm of fresh weight artery tissue. These results agree with those of Tobian and Fox who also studied dogs with saline infusion alone as a control (85).

Two dogs were given the corresponding doses of epinephrine in an unanesthetized state to check on the effect of the anesthetics per se. In this case the dogs were lightly anesthetized with thiopental and the area around the left femoral artery was injected with lidocaine as a local anesthetic. The dogs were then tied to a V-shaped dog board and a catheter to record femoral blood pressure was inserted. At the same time a segment of that artery was removed for control electrolyte determinations and an indwelling femoral vein catheter was

inserted for drug injections. The dog was allowed to become fully conscious (about 20 minutes) and then it was treated as previously described with the pressor drugs. The average control content of sodium in the arterial wall for the two dogs was 13.06 mEq/100 gm which was lowered to 8.03 mEq/100 gm of fresh artery sample at the end of the experiment. At the same time, the control potassium values were 3.22 mEq/100 gm and had changed to 3.19 mEq/100 gm at the time of death. The unanesthetized dogs were seemingly unconscious during the acute pressor phase of the injections, even though the rise was only 50 to 60 mm of Hg. On the other hand when the resting blood pressure between injections fell below 50 mm of Hg in the later stages of the experiment, the dogs became very apprehensive and struggled. This could have been due to myocardial ischema or decreased coronary blood flow, either of which could result in a very painful condition. The results obtained with these dogs corresponded quite well with the dogs given the barbiturate anesthetic and, as this procedure did produce a marked discomfort to the animal, the dogs in the remaining experiments were anesthetized with pentobarbital throughout the experiment.

Heparin Experiment in Rats

Heparin has been reported as being able to bind histamine, and thus remove it from the circulation in instances of histamine release (45). Histamine has been implicated in the production of the irreversible stages of shock (96), and also as being involved in the tachyphylaxis developed by norepinephrine (16).

Rats that had been subjected to 500 turns in the Noble-Collip drum were given 1000 units of heparin by tail vein injection. This was administered as 1 cc of Lipo-Hepin®. Control animals were given 1 cc of saline, also by tail vein injection. Two hours after the stress procedure, 90 per cent of the control animals were dead with only a 45 per cent mortality in the heparin-treated group. At the end of 12 hours, however, 100 per cent of the control animals were dead and 90 per cent of the heparinized animals had died (Figure 26). The heparinized rats seemed to have a somewhat longer survival time, but the overall survival from the stress procedure was not altered.

The animals which had been given the high dosages of heparin did not seem to bleed to any great extent. This seemed somewhat surprising, because at autopsy some rats were observed to have small areas of abdominal bleeding.

Hydrocortisone Experiment in Rats

Hydrocortisone, 5 mg per rat, was administered by tail vein injection immediately after tumbling rats 500 turns in the Noble-Collip drum. At the end of two hours, 90 per cent of the control animals were dead, while 70 per cent of the treated animals had died. However no more of the treated animals died after the end of the two hour period. At the end of 12 hours, 100 per cent of the control animals were dead. The animals treated with hydrocortisone thus demonstrated a 30 per cent better survival rate than did the control animals (Figure 27). They also exhibited a slower death rate in the first

FIGURE 26

SURVIVAL OF RATS GIVEN HEPARIN

(1000 UNITS PER ANIMAL)

AFTER 500 TURNS

IN THE NOBLE-COLLIP DRUM

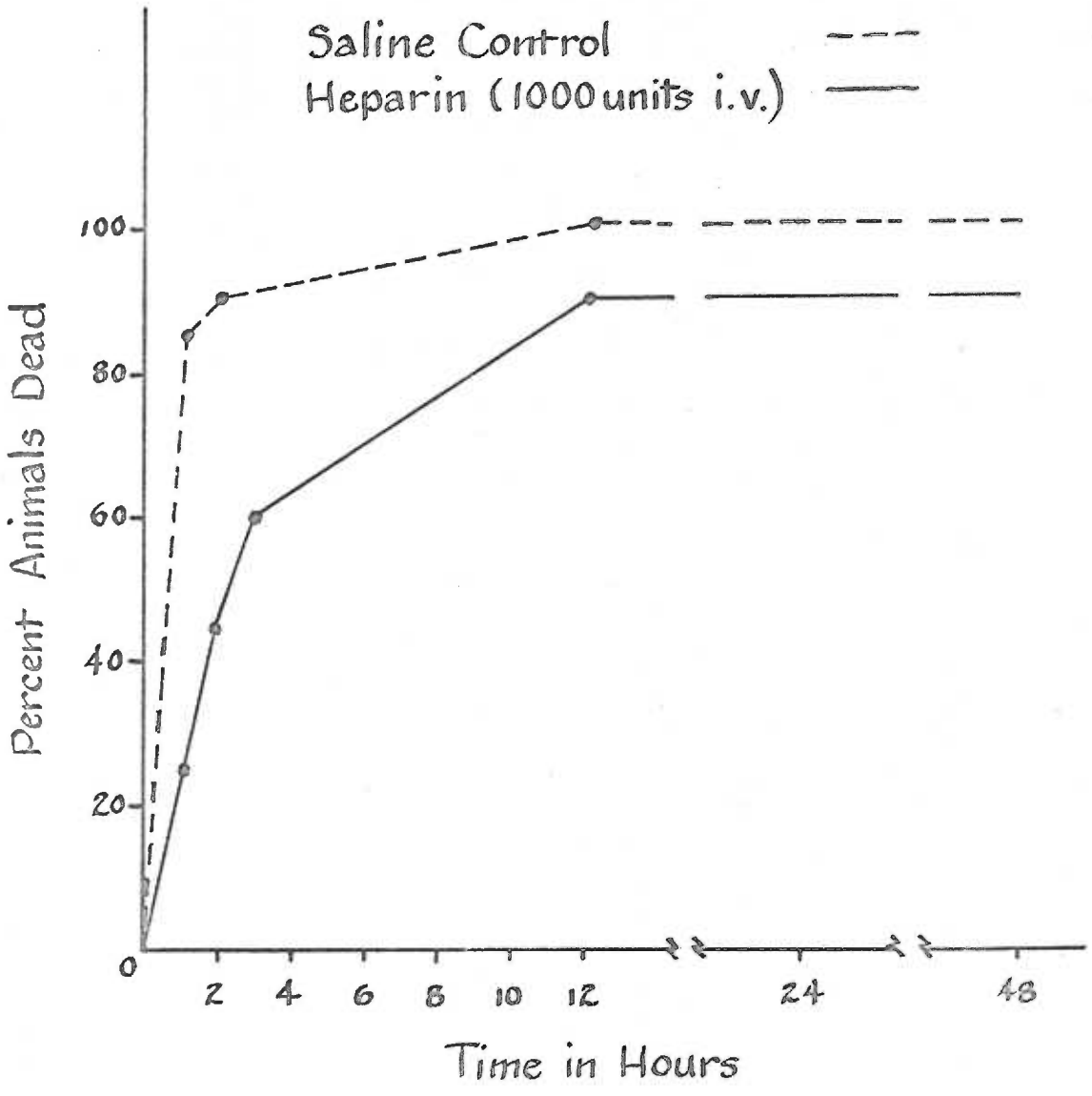

Saline Control
Heparin (1000 units i.v.)
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
48
Time in Hours

FIGURE 26

FIGURE 27

SURVIVAL OF RATS GIVEN HYDROCORTISONE

(5 MG PER ANIMAL)

AFTER 500 TURNS

IN THE NOBLE-COLLIP DRUM

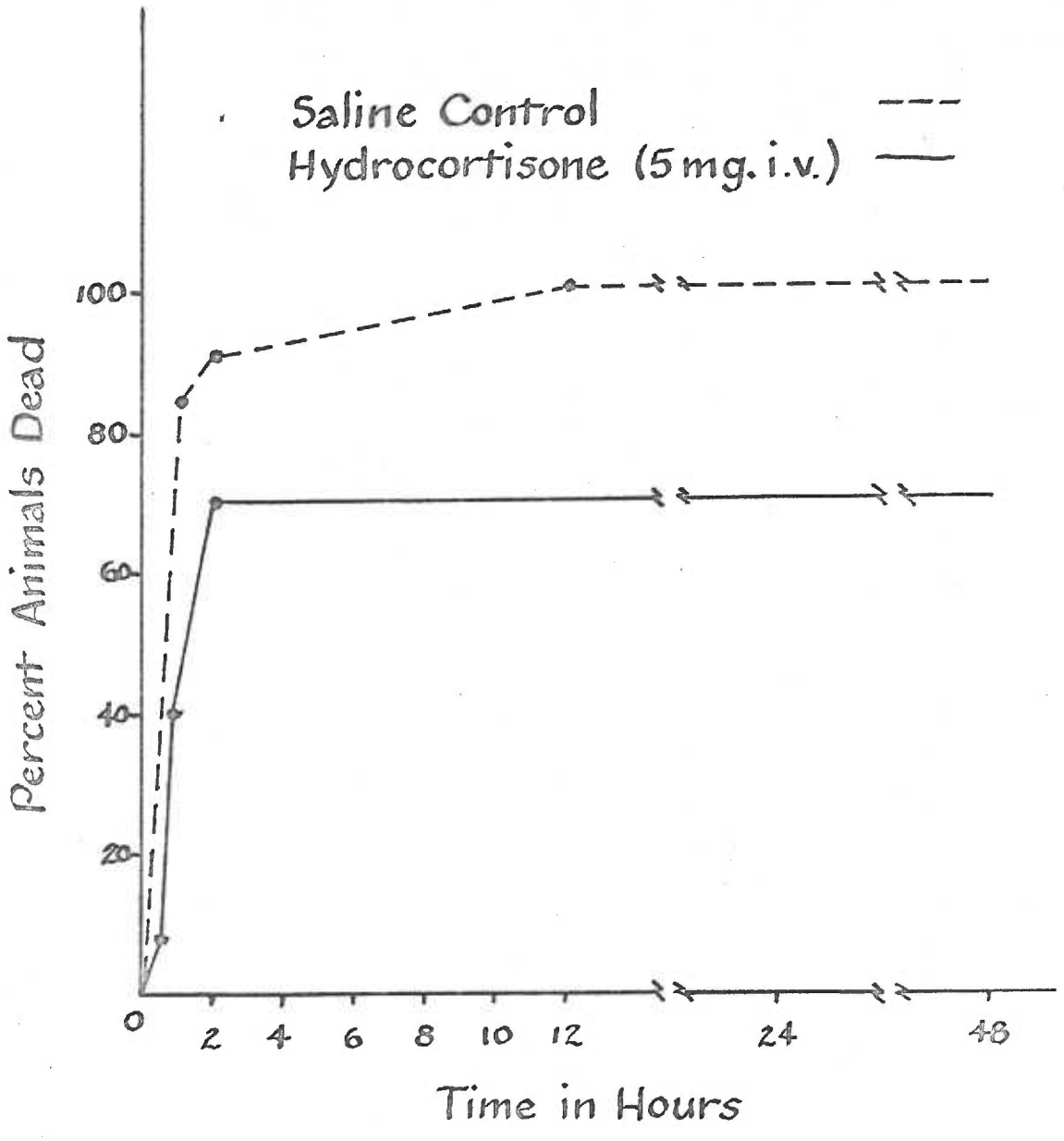

Saline Control
Hydrocortisone (5 mg. i.v.)
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
48
Time in Hours

FIGURE 27

hour after the trauma. At the end of the first hour, 85 per cent of the control animals were dead and only 40 per cent of the hydrocortisone animals had expired.

The 5 mg per animal dosage of hydrocortisone employed in this experiment would correspond to 24 to 26 mg/kg in the rat. If this figure is projected for a 70 kg man, the dose would approximate 1750 mg. This is about double the 1000 mg dosage recommended by Lillehei for humans (55).

Time permitting, this experiment would have been expanded by giving multiple doses of hydrocortisone after the stress procedure.

Aldosterone Experiment in Rats

Because of the increased ultimate survival of the animals which had received hydrocortisone, it appeared desirable to try other steroids. As it has already been shown that alterations in the content of sodium and potassium of the rat aorta occur in shock, it was decided to employ aldosterone, as the principle effect of this steroid is the conservation of body electrolytes. The aldosterone was dissolved in saline containing 10 per cent ethanol to obtain a solution. The injection of aldosterone was given via the rat tail vein immediately after tumbling 500 turns in the Noble-Collip drum. A dose of 0.1 mg per rat was administered in a total volume of 1 cc. The control animals were simultaneously given 1 cc of saline containing 10 per cent ethanol.

At the end of the first three hours, 100 per cent of the control animals were dead, whereas only 40 per cent of the aldosterone animals had died. However, at the end of 12 hours all of the treated animals

were dead (Figure 28). It seems then that aldosterone gave some early protection that was greater than that afforded by the hydrocortisone. However, it did not alter the overall mortality of the treated animals.

Intestinal Trauma in Experimental Animals

Upon autopsy of the rats which had been subjected to the experimental stress in the Noble-Collip drum, a very characteristic intestinal hemorrhagic appearance was noted. The area involved seemed to be about 25 to 35 cm in length and was located in the lower jejunal and upper ileal sections of the small intestine. At first glance these lesions seemed to be caused by trauma suffered by the animal in the tumble cage.

To test the hypothesis that these lesions were caused by direct trauma to the area and not by the stress procedure itself, rats were subjected to stress by placing them in sealed quart size specimen jars and placed in a cold environment. The temperature was from 3° to 4° C. The animals were kept in this condition for 90 minutes when they were removed from the jars and sacrificed. The abdomen was opened and the small intestinal area examined. As shown in Figure 29, these animals had a similar lesion of the small intestine. This would then indicate that the pathology of the small intestine is not totally caused by the trauma of the animals in the tumble cage, but seems to be at least in part caused by stressful situations. Similar results were observed in the dogs that were induced into shock by repeated injections of high dosages of pressor amines as described earlier.

FIGURE 28

SURVIVAL OF RATS GIVEN ALDOSTEROME

(0.1 MG PER ANIMAL)

AFTER 500 TURNS

IN THE NOBLE-COLLIP DRUM

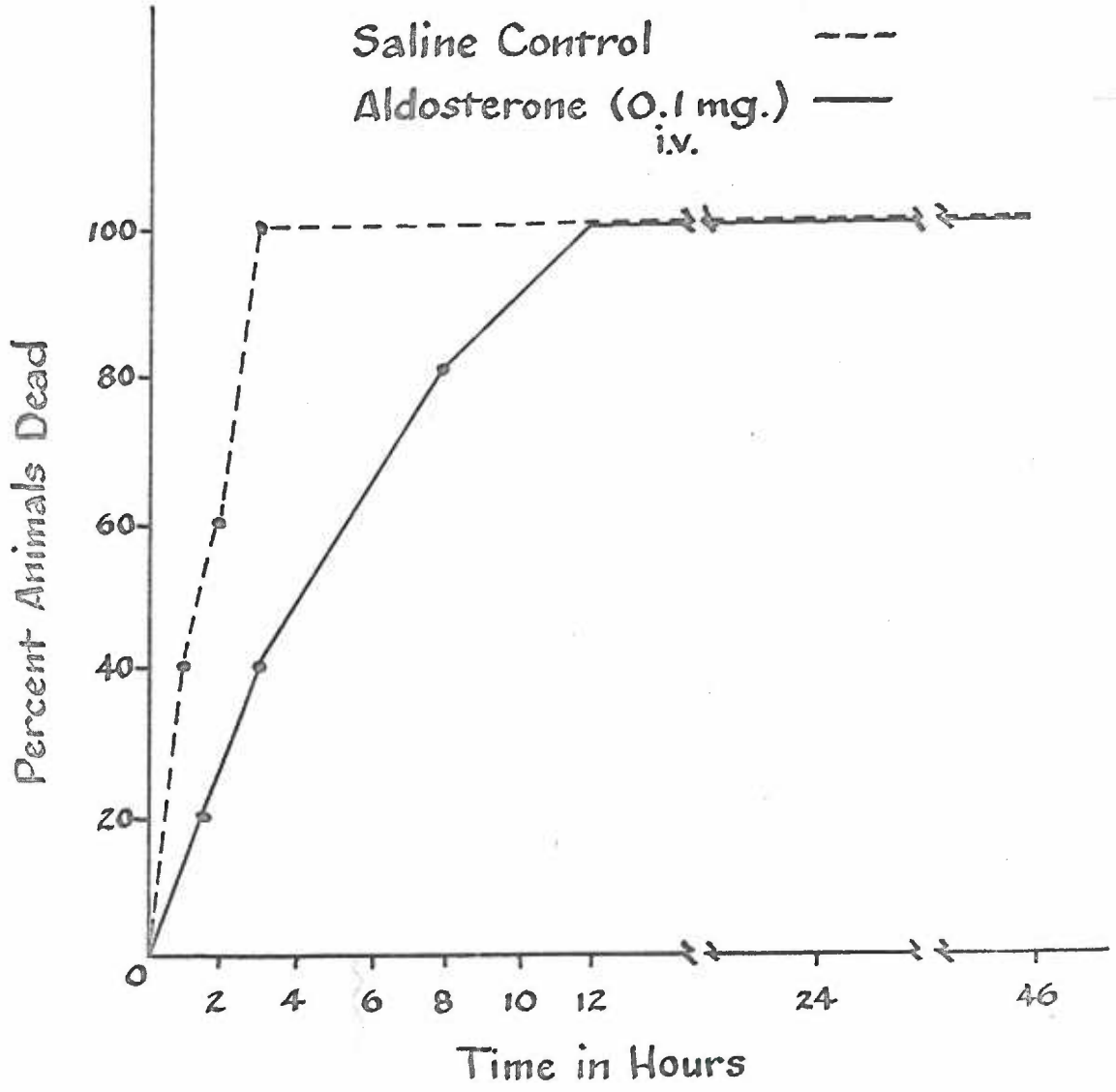

Saline Control
Aldosterone (0.1 mg.) i.v.
Percent Animals Dead
100
80
60
40
20
0
2
4
6
8
10
12
24
46
Time in Hours

FIGURE 28

FIGURE 29

GROSS RAT INTESTINAL PATHOLOGY AFTER

500 TURNS IN THE NOBLE-COLLIP DRUM

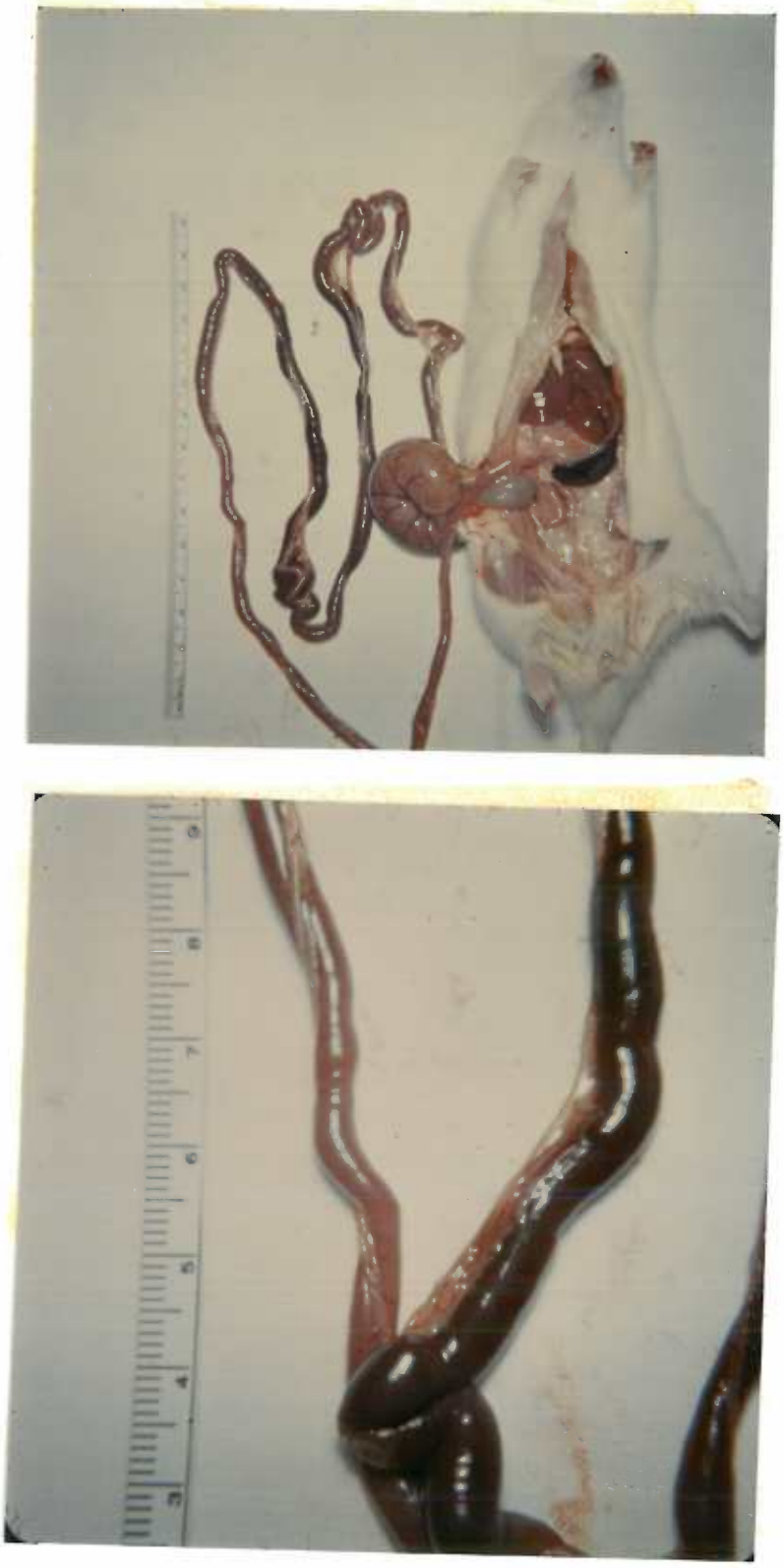


FIGURE 29

A preliminary histologic examination was made of this traumatized tissue. Rats were sacrificed about 10 minutes after tumbling 600 turns in the Noble-Collip drum and then killed by an intraperitoneal injection of 0.5 cc of 50 per cent urethane. Control animals that had not been tumbled were also sacrificed with intraperitoneal injections of 0.5 cc of 50 per cent urethane solution. Then segments of approximately 4 cm in length were removed from the control ileum and from the corresponding area of the traumatized intestine from the stressed rat. Also a segment of the lower ileum was removed from the tumbled animal which still retained a normal macroscopic appearance. The sections of tissue were then promptly placed in 10 per cent formalin solution and saved for microscopic examination.

The slides were kindly prepared by the department of Pathology and stained with hematoxolin and eosin. Microscopic observation verified the normal macroscopic appearance of the intestine from the traumatized rat. However, the area showing marked trauma grossly appeared also to have a destruction of the villi of the small intestine. This can be seen from the following photographs of the normal and traumatized rat intestine (Figure 30).

Myocardial Shock in Dogs

To study the effect of pressor amines in dogs with myocardial infarct shock, dogs were prepared as described previously. Of the eight dogs who appeared to be in shock 48 hours after the coronary ligation, five were infused with norepinephrine (4 mg in 500 cc normal saline solution) at a rate that would restore their blood pressure to the

FIGURE 30

MICROSCOPIC CHANGES IN THE
SMALL INTESTINE OF CONTROL RATS
(TOP PHOTOGRAPH)
AND OF A RAT STRESSED 500 TURNS
IN THE NOBLE-COLLIP DRUM
(BOTTOM PHOTOGRAPH)
x 200

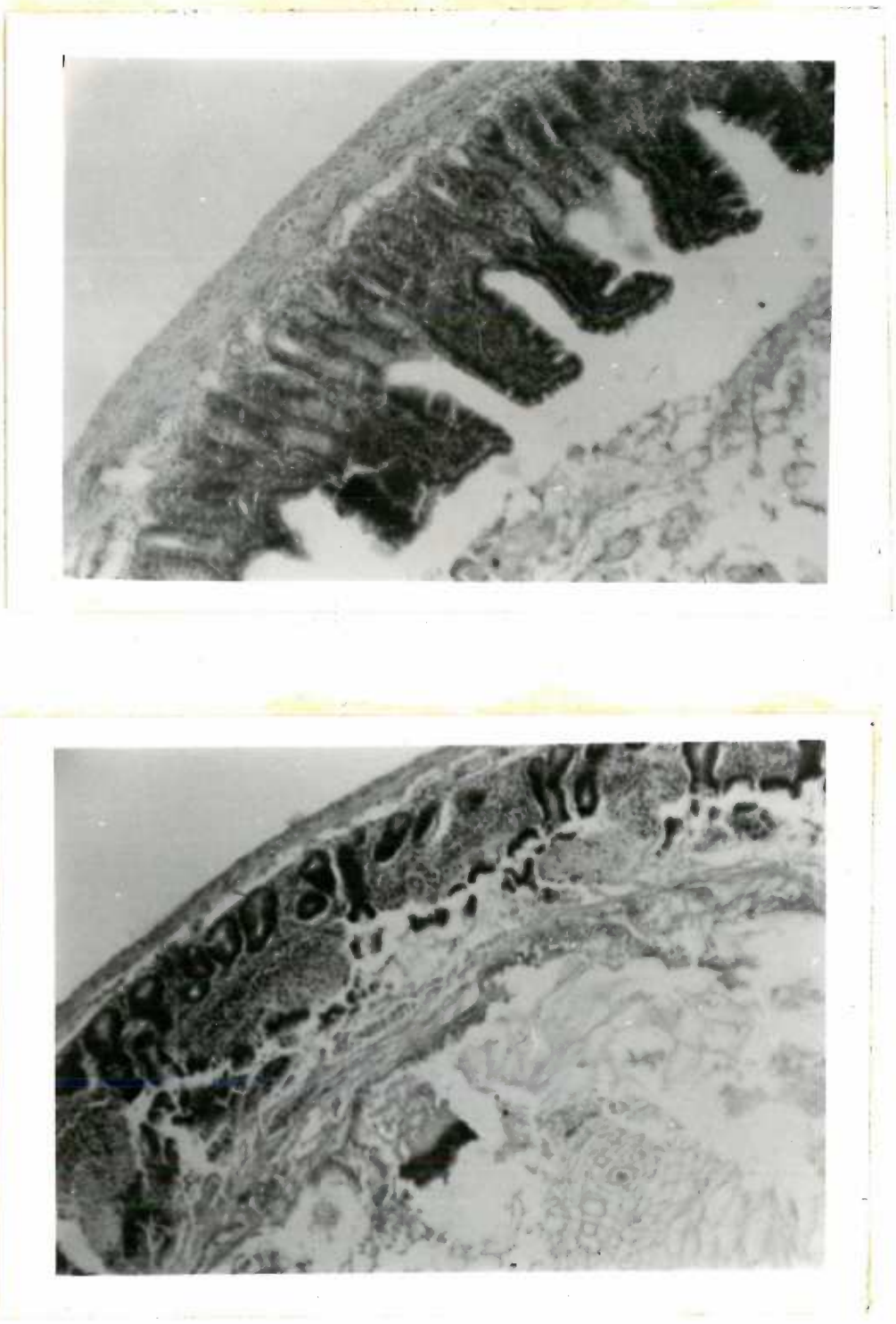


FIGURE 30

control blood pressure taken before the surgery. Three of the dogs were infused in the same manner, but instead were given a solution containing 2.5 mg of angiotensin amide in 500 cc normal saline solution.

All of these animals became tachyphylactic to both the norepinephrine and angiotensin amide and died. The infusions were given approximately for four hours before death occurred. No apparent difference was noted between the two pressor agents.

The sodium and potassium concentrations of the femoral arteries of the dogs was determined on the dogs with myocardial infarct as previously described. The sodium and potassium content of the infarct dogs still alive 48 hours after the ligation showed an increase compared with the control values (Figure 31). However, in the dogs which died within 30 minutes after the ligation of the left descending coronary artery, a marked decrease in the sodium content with a slight increase in potassium could be demonstrated. The average value of the two dogs showed a drop in sodium from 15.43 mEq/100 gm artery tissue to 7.76 mEq. The average potassium content rose from 2.21 mEq to 2.72.

Two of the dogs which were alive 48 hours after the ligation were withheld until they became refractive to the norepinephrine before removal of their femoral arteries for the determination of the sodium and potassium content. These dogs again had the marked drop in arterial sodium content that was observed after the injection of pressor amines in normal dogs. In one case, the potassium content dropped from 1.93 mEq/100 gm to 1.38 mEq/100 gm, and in the other dog it had only

FIGURE 31

ARTERIAL ELECTROLYTE STUDIES

ON DOGS SUBJECTED TO

LEFT DESCENDING CORONARY LIGATION

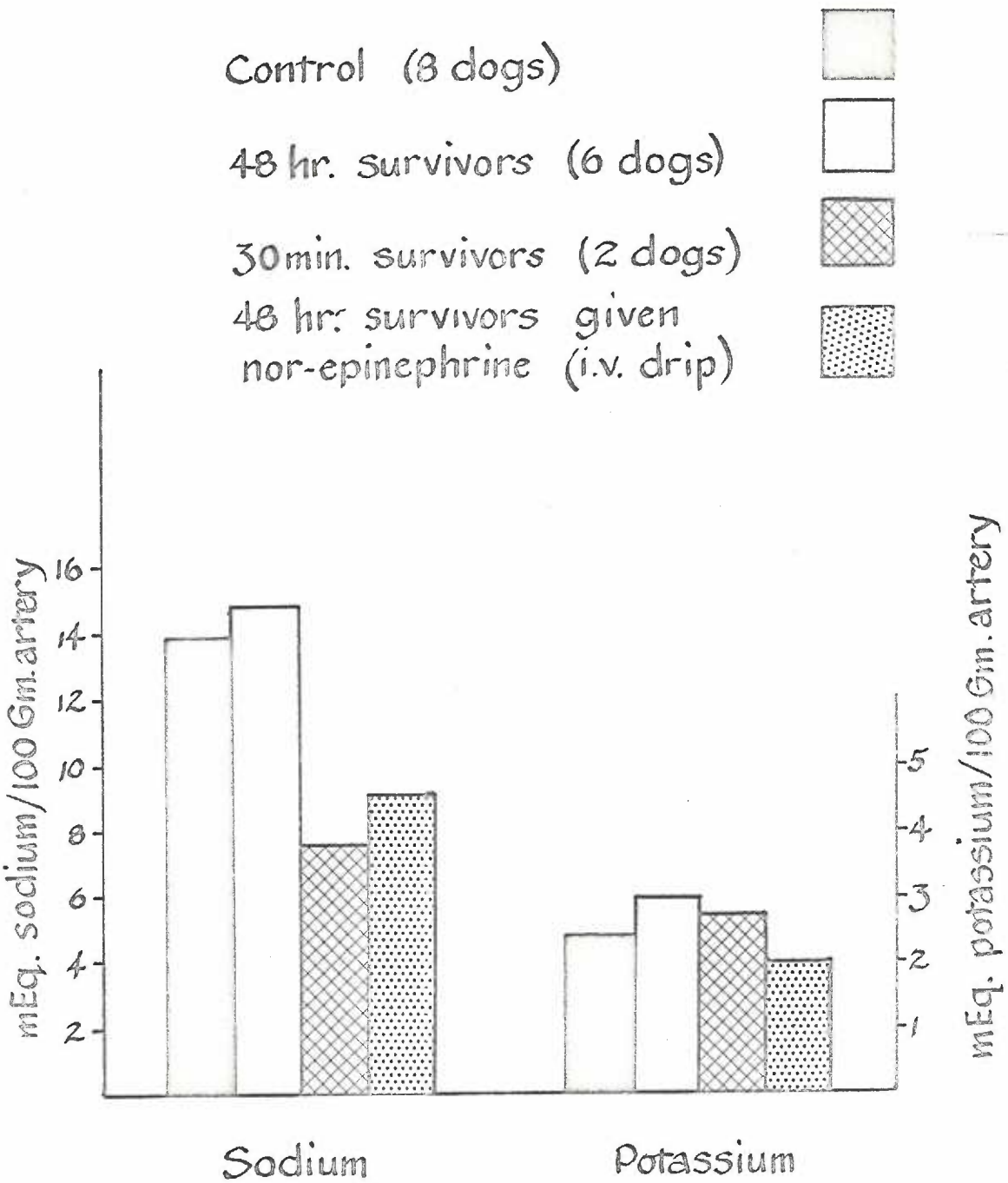

Control (8 dogs)
48 hr. survivors (6 dogs)
30 min. survivors (2 dogs)
48 hr. survivors given nor-epinephrine (i.v. drip)
mEq. sodium/100 Gm. artery
16
14
12
10
8
6
4
2
mEq. potassium/100 Gm. artery
5
4
3
2
1
Sodium
Potassium

FIGURE 31

changed from 2.73 mEq/100 gm to 2.78. This last observation is still within the experimental error of the electrolyte determinations.

DISCUSSION

The treatment of shock is currently undergoing a critical evaluation as to both etiology and therapeutic management with drugs. This is quite obvious in that a large number of papers have appeared in the last few years pertaining to these two phases of shock. It is also obvious that no general agreement has been reached as to the usefulness of all of the therapy currently employed. The main point of reference seems to be whether the commonly employed pressor drugs should or should not be the drugs of choice after the blood volume has been restored to normal. One group seems to advocate the employment of pressor agents (26)(29)(35)(65)(87) and the other side tends to favor other forms of therapy (5)(28)(30(55)(58)(68)(76)(81). This study concerns itself with the experimental evaluation of drugs employed in the treatment of shock.

As in all forms of experimental treatment of disease states in animals, it is desirable to produce in the animal a condition resembling that seen in the human counterpart. To date no animal preparation can be said to correspond to all forms of shock (95), and it is doubtful that any one is wholly satisfactory even when limited to a single form of shock.

The Noble-Collip drum technique which produces in rats a state of traumatic shock was employed in this study. It is probably not the

ideal test procedure. However, at this time, it seems to be very useful for rapidly screening drugs that may be useful in either the prevention of shock or its treatment. The advantages of this method are several:

A. It allows the use of small laboratory animals, with corresponding minimum expense.

B. The per cent lethality of the stress can be controlled with good reproducibility.

C. It allows for simultaneous controls.

There are also several disadvantages:

A. It is difficult to make detailed physiological measurement.

B. There is the question as to whether the method is applicable to human shock which has not been determined.

The method of producing myocardial shock in dogs was employed not necessarily because the shock produced mimics that of the human counterpart, but to see if the same alterations in arterial electrolytes occurring in the dogs occurred with the rats in the Noble-Collip drum and with the dogs in epinephrine shock.

In the preliminary experiment in which morphine sulfate was administered prior to the tumbling of the rats, it was observed that the animals receiving a single injection of 2 mg/100 gm body weight one hour prior to the stress were more susceptible to the damaging effect than those that had been receiving the same dose daily for five days. This observation seemed to be opposed to customary philosophy

concerning stress reactions. It has been observed repeatedly that morphine sulfate when given clinically will inhibit the release of ACTH in response to stress (7)(11)(27)(38)(61), while single injections of morphine seem to stimulate the production of this hormone (7)(67) as well as that of epinephrine (71). These results would lead to the conclusion that a single injection of morphine along with its analgesic properties should have protected the animal.

This work seems to demonstrate that in rats which have been subjected to sub-lethal amounts of stress in the Noble-Collip drum, stimulating procedures seem to be detrimental to the eventual recovery of the animal. In fact the injection of pressor amines into the animals studied seems to produce the effect that would be expected from another 100 turns in the drum. This response is to be expected however if the detrimental effects of the stress procedure are mediated by endogenous epinephrine and and norepinephrine released in response to the noxious stimuli. As has been shown by Young and Gray (94) the blood levels of these two amines increase in rats with the greater number of revolutions in the Noble-Collip drum.

It has been observed repeatedly that during shock-like states there is normally an increased blood level of epinephrine and norepinephrine. This has been reported both for human patients (49)(55)(96) as well as for experimental animals (60)(74)(75)(76)(94). It would appear that these high blood levels of pressor amines are involved in the production of the irreversible state of shock. This would be substantiated by the fact that by giving pressor amines to animals

already in shock, with high blood levels of pressor amines, the lethality of the stress is increased.

This hypothesis is also confirmed, at least in part, by the production of experimental shock-like states in dogs with the repeated injections of both epinephrine and norepinephrine. In these experiments it would have been desirous to determine the actual blood levels of these amines attained in the dogs, but the equipment for such studies was not available at this time. I do think however that this is an important point to consider.

The observation that the three different methods of producing shock in the experimental animals (the tumbling stress in the rats, the injection of high doses of pressor amines, and the production of acute myocardial shock in dogs) all produced a drop in the sodium content of the arterial wall seems to indicate that this may be a manifestation of a common defect in shock-like states. The alterations in potassium in the dog are not quite as consistent as that of the rat, but as has been pointed out, this may be due to a gradual rise prior to death of the animal. The accuracy of the method employed for the determination of the sodium and potassium is open to some criticism as an internal lithium standard was not employed. For more critical changes induced in electrolyte composition of arterial wall, it would be mandatory that a more sensitive instrument be used. However, even with the procedure employed, significant decrease in the sodium content of the aorta of the rat and of the femoral artery of the dog occurred when the animals were in shock. A significant drop in potassium content

occurred in the rat, but was not significant in the dog. Because these alterations in arterial wall electrolytes occurred both in the tumbled rat and in the epinephrine-shocked dog, it was speculated that a causal relationship occurred. This is indicative also in that when the animals were given ephedrine and sacrificed 30 minutes after the stress, the control rat seemed to be returning toward normal while the sodium and potassium content of the treated rat remained low. This observation was made in only two rats, however, as the others in the group died before the 30 minutes were up. It would be desirous to repeat this experiment until a larger number of surviving rats could be obtained.

It was also shown that in this same connection rats which were pretreated with phentolamine one hour prior to tumbling did not exhibit the drop in aortic sodium or potassium. The action of phentolamine is such that it will inhibit or depress the biological effect of catecholamines. This, coupled with the observations of Manger et al, would further implicate epinephrine, norepinephrine or some other unknown vasoactive compound that would be involved in the production of the lethal effect of shock (60).

A further observation that would tend to confirm the importance of the shift or alterations in aortic electrolytes in the shock-like states was obtained using chlorothiazide-treated rats. The proposed mechanism of action of the thiazide diuretics as anti-hypertensive agents is to remove sodium and/or potassium from the vascular wall (91)(33). This is then thought to cause a depressed response of

pressor amines on the arterial tree. When this drug was given to the rats (10 mg per day for 10 days) prior to tumbling in the Noble-Collip drum, it was found that these animals were more sensitive to the lethal effect of the stress.

This would then indicate that the actual pressor mechanism of the endogenously released amines may not be the only detrimental effect of the result of the stress reaction, since in this experiment the pressor effect of the amines was somewhat obtunded by the prior administration of the chlorothiazide. Whether this was due solely to the tendency to depress the body stores of sodium and potassium or to some other unknown toxic manifestation remains to be determined. It was demonstrated however that phentolamine (a ganglionic blocking agent) was able to protect rats from this depression in aortic sodium and potassium content.

This evidence would seem to indicate that high levels of pressor amines in the body are detrimental in cases of shock, whether they come fdom endogenously released sources as a result of stressful stimuli or from repeated injections of large amounts of these amines. At the same time, alterations in the electrolyte composition of the arterial wall occur. It seems doubtful that alterations in sodium and potassium are the only changes involved. Because these two ions are easily measured this was the only parameter studied.

It is conceivable that these changes in ionic composition are mere reflections of some more fundamental change in the chemical or physical makeup of the reactive protein in the arterial musculature.

An intriguing possibility exists that an alteration in the receptor site for epinephrine or norepinephrine may account for the loss of these ions. As proposed by Bellau, the receptor site consists of a phosphate complex that may be adenosine triphosphate (ATP). If this is true, when the ATP becomes attached to the catecholamine, one molecule of pyrophosphate would be released and as this leaked out of the cell an equivalent amount of either sodium or potassium could also leave. This is mere speculation, however, and no evidence exists to support this theory. Further work along this approach, however, may prove very rewarding.

In attempting to reverse the lethal effect of the shock-like state produced in the rats, several drugs were employed. It has been demonstrated that heparin will bind histamine and reverse it from the general circulation, especially in allergic-like conditions (45). Heparin was injected intravenously into the rats after tumbling 500 turns in the drum with limited success. The animals which had received the heparin did have a somewhat slower rate of death initially but the overall mortality was not different from the control animals. The same results were also obtained using aldosterone. However, in rats treated immediately after the stress with 5 mg of hydrocortisone only 70 per cent of the animals died as compared to 100 per cent for the control saline-treated group. This was the only drug employed that gave any lasting protection to the animals. High dosages of hydrocortisone and other anti-inflammatory steroids are being advocated for the treatment of shock (28)(55). Whether the steroids protect the animal

from high blood levels of pressor amines or whether they alter some metabolic or physical effect in the shock-like condition is at present undetermined. It is known, however, that steroids will protect animals from otherwise lethal doses of epinephrine, and it also seems to sensitize the blood vessels to small doses of epinephrine.

The observed protection of these animals with large intravenous doses of hydrocortisone combined with the observation that pressor substances appear to be detrimental to animals which are in a shocked condition prompted Dr. Melvin M. Reeves to employ this treatment in human patients.*

Endotoxin release has been implicated by many authors (5)(28)(55)(68)(81) in the production of irreversible stages in shock. This is thought to be by way of tissue destruction in the gastrointestinal system which leads to the absorption of endotoxin from the normal flora of the intestinal tract (56). The destruction of the intestinal tract is thought to be due at least in part to localized tissue destruction brought about by vasopressor or vasospastic compounds released during the development of the shock-like conditions (9). Evidence for the intestinal change was observed both in the rat and dog. The rat showed a marked hemorrhagic-appearing small intestine that upon microscopic examination revealed villi destruction. In the dog experiment whenever high dose levels of epinephrine or norepinephrine were infused,

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*To date two patients have been treated by the administration of from 200-250 mg of Solu-Cortef® intravenously. This is then followed by 100-200 mg intravenously at intervals of one to two hours until the patient shows no signs of reverting into shock.

the animal had gross intestinal hemorrhages. This is in confirmation of the work of Lillehei who also observed gastrointestinal hemorrhage in dogs subjected to endotoxin and hypovolemic shock (55).

Whether or not this intestinal lesion observed in shock-like states actually leads to systemic absorption of endotoxin has not been settled. Animals that have been raised from birth in sterile surroundings still exhibit irreversible shock (5). The question has been proposed as to whether absorption of endotoxin from dead bacteria eaten in the food may provide adequate endotoxin for the production of shock in these animals. The total elimination of bacterial products from the diet of experimental animals cannot be obtained with present-day techniques. It is also known that animals which have had intestinal sterilization prior to stress also exhibit shock conditions, so that the absorption of endotoxin from dead organisms cannot be ruled out.

The myocardial shock in dogs which was produced by ligation of the left descending coronary artery was somewhat disappointing. It was found that a prior splenectomy was necessary before the lethal effects of the ligation were apparent. The first two dogs which were operated upon did not have the splenectomy and did not seem to go into shock. Because of the unpredictability of the effects of tying the left descending coronary artery, this procedure is not, in my opinion, an ideal test method for the screening of drugs in shock-like states. This same observation has also been reported by other authors (14)(17). However it was noted that in the animals which died within the first 30 minutes after ligation, a fall in arterial sodium had occurred which would correspond to the results found in the other shock situations.

CONCLUSIONS

It has been shown that when pressor amines (epinephrine, ephedrine, norepinephrine and mephenteramine) are administered to rats which are in shock, there is a greater mortality rate than in the control animals given saline. In fact, a shock-like state can be produced in dogs by the repeated administration of 0.05 mg/kg of either epinephrine or norepinephrine.

It was also observed that in the production of traumatic shock in rats, or the pressor amine and the myocardial infarct shock in dogs, a marked reduction of both the sodium and potassium content of the vascular wall occurs. These changes in the traumatized rats and pressor amine treated dogs are progressive alterations proportional to the degree of shock. In the rats, this fall in arterial electrolytes was abolished by the prior treatment with phentolamine (an adrenergic blocking agent). To test the hypothesis that the reduction of sodium and potassium was important in the production of shock, rats were depleted of their sodium and potassium content by the intraperitoneal administration of the diuretic drug, chlorothiazide. These animals exhibited a lesser tolerance to stress when compared to saline treated control rats.

As an incidental observation, it was noted that necrotic changes of the small intestine were present in rats subjected to tumbling

stress or pressor amine shock in dogs. In the rat this appears to be mediated by villi destruction. This is not directly related to the trauma of tumbling, since rats subjected to non-traumatizing stress also exhibited the same type of lesion.

Hydrocortisone was the only drug which could be given after the stress procedure and resultant shock which was able to increase the incidence of survival.

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APPENDIX

Trade name equivalents of generic names used in this thesis.

	Generic Name	Trademark Name
1.	Angiotensin amide	Hypertensin®
2.	Carbachol	Doryl®
3.	Chlorothiazide	Diuril®
4.	Chlorpromazine	Thorazine®
5.	Epinephrine	Adrenalin®
6.	Heparin	Lipo-Hepin®
7.	Hydrocortisone	Solu-Cortef®
8.	Isoproterenol	Isuprel®, Norisodrine®
9.	Lidocaine	Xylocaine®
10.	Mephentermine	Wyamine®
11.	Norepinephrine	Levophed®
12.	Pentobarbital	Nembutal®
13.	Phenylephrine	Neosynephrine®
14.	Prednisolone	Solu-Medrol®
15.	Procaine	Novocaine®
16.	Thiopental	Pentothal®
17.	Trometh amine THAM)	Talatrol®
18.	Vasopressin	Pitressin®

DEFINITION OF TACHYPHYLAXIS AND TOLERANCE

The terms tachyphylaxis and tolerance are undergoing a process whereby they are changing somewhat in their connotation. However, in the body of this thesis usage implies the classical definition, which is a diminution of the response of a drug in relation to time. Tachyphylaxis therefore denotes a rapidly decreasing response from repeated injections of the drug independent of the mechanism, while tolerance signifies a decrease in response that may take several days to become manifest.

Currently mechanisms of action are entering the definition in that tachyphylaxis may be thought of as an inhibition in some way of receptor sites, while tolerance may be manifest by either an increased metabolism or excretion of the drug.

PER CENT WATER CONTENT OF RAT AORTA

CONTROL RATS	RATS TUMBLED 800 TURNS
53.47	64.41
55.00	61.90
60.00	61.90
63.51	61.66
60.27	61.06
58.42	66.67
Mean 58.49	62.93

SALINE TREATED RATS 1 HOUR AFTER TUMBLED 400 TURNS	EPHEDRINE TREATED RATS 1 HOUR AFTER TUMBLED 400 TURNS
60.8	56.7
63.0	57.9
63.3	60.7
60.0	62.4
62.1	57.8
Mean 61.8	Mean 59.1