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# THE AMELIORATION OF EXPERIMENTAL CHRONIC HYPERTENSION BY VITAMIN E.

BY

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## ABSTRACT

Chronic hypertension was induced in female albino rats. Amelioration by means of daily feeding of histidine and ascorbic acid, histidine and vitamin E, histidine and urease, vitamin E, urease and ammonium chloride was attempted. Blood pressures were determined under sodium pentathol anesthetic by the indirect method using the foot. Chronic hypertension was produced by two methods, (1) Injections of DCA. This method was found to give inconsistant results. Several modifications of this procedure were tried unsuccessfully. (2) The use of a choline deficient diet. This method proved to give consistant results in producing chronic hypertension. Vitamin E was the only substance found useful in ameliorating the experimental chronic hypertension. This amelioration resulted only in the presence of excess vitamin E. This fact was thought to give further evidence for the vasodilator properties of vitamin E.

An hypothesis was advanced that DCA may inhibit the sympathetic nervous system, to sensitize the vascular responsiveness to pressor and depressor substances.

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## I INTRODUCTION

One of the most controversial issues of present day medical research is hypertension. The enigma of this problem has kept some of our keenest medical minds in constant research since the year 1934 when Goldblatt showed it was experimentally possible to induce a hypertensive state. This in itself was a major step forward because it made feasible the first controllable experiments on hypertension under carefully supervised laboratory conditions.

Previous to this time the amount of work done on this problem, qualitatively and quantitatively, was from a co-ordinated viewpoint, negligible. There were various men like Bright, who precipitated the problem of hypertension which, even now, is only partially resolved. But on the whole investigation into the problem was the exception rather than the rule.

Hypertension was at one time considered a hemodynamic response to aging of the blood vessels - a final effort on the part of the body to maintain perfusion of blood through narrowed vessels. But this view has long since been superceded and to-day we firmly believe that elevation of blood pressure is only a

symptom of some preceding disorder and, as such, has numerous causes.

This change in thought coupled with the ability to artificially create hypertension has been undoubtedly responsible for the recent acceleration of research.

The various ramifications of the problem of hypertension are now approached from a number of bases. The fields of investigation are usually classified as (1) the renal origin of hypertension (2) the humoral pressor mechanisms (3) the neurogenic origins and (4) endocrine origins of hypertension. These classifications are for convenience only and must not be mistaken for separate and non-overlapping research. But even with this category simplification and corresponding research human hypertensive disease remains a baffling and mystifying disorder. The more time, thought, work and effort that is devoted to the problem the more we discover the complexity of the problem and of its ultimate etiology.

## A. THE PATHOGENESIS OF HYPERTENSION

### 1. ROLE OF THE KIDNEY IN HYPERTENSION

There is no reasonable doubt that some cases of human hypertension are of renal origin. The unsolved problem is whether many or most of those still referred to as " essential" are also of renal origin (20).

Essential hypertension has usually been defined as the persistent elevation of systolic and diastolic pressures without accompanying renal disease, and has therefore excluded the renal origin of hypertension by definition. However, numerous mechanisms have arisen which centre about the kidney as the possible initiator of hypertension.

(a) RENIN - ANGIOTONIN PRESSOR SYSTEM.

The publications of Richard Bright (6) (1827-1836) were the first to link the role of the kidney and hypertension, but it was Tigerstedt and Bergmann in 1898(83), who first demonstrated a blood pressure raising principle from a saline extract of renal tissue which they called "renin". However, it was not until such investigators as Houssay, Plentl, Taguini, Fasciolo, Braun - Merendez, Page, Munoz, Leloir, Kohlstaedt and Helmer (1937 - 1941) (9, 37, 38, 39, 47, 48, 61, 62) that the pressor response was found due to a substance they called "angiotonin" or "hypertensin". Angiotonin proved to be the result of a chemical reaction between a pseudoglobulin of the plasma and the substance "renin" found by Tigerstedt and Bergmann.

Investigations by the above mentioned authors have revealed a fundamental scheme for the renal pressor mechanism. Renin is produced by the kidney cortex, of which the exact site is unknown, but it has been associated with the juxtaglomerular apparatus and the macula

densa of the kidney. Renin has been found to have the property of a proteolylic enzyme (9, 58) and a protein. Renin is physiologically inert, but possesses the ability to break down a substrate compound known preferably as "renin - substrate" (12) or as hypertensinogen, into an active vasopressor material "angiotonin" (or hypertensin). Renin - substrate is a protein produced by the liver and is found in the alpha - 2 - globulins of the blood. Renin-substrate has a relatively large molecule, which under the influence of renin is decomposed to a smaller polypeptide, "angiotonin"

Angiotonin is decomposed by the action of another enzyme "angiotonase" (or hypertensinase). Angiotonase is found in various tissues namely, kidney, plasma, erythrocytes, and intestinal wall. It is probable that angiotonin is destroyed by a number of other enzymes, such as, proteinases of renal and intestinal origin, by amino polypeptidases found in kidney extracts and in blood plasma, by tyrosinase, by carboxylpolypeptidase as well as by oxidizing systems (15).

## WEAKNESSES OF THE RENIN THEORY.

Using the most sensitive methods of detection, some renin has been found during the acute phase of hypertension in experimental animals (30) (Haynes and Dexter 1947),

and also in some patients with acute hypertension due to glomerulonephritis and toxemia of pregnancy. In dogs with chronic hypertension of three months to four years duration, no renin has been detected, nor in the systemic blood of patients with chronic hypertension (12) (Corcoran 1948). Renin is no more commonly present in the renal venous blood of patients with essential hypertension than it is in others with no hypertension (Haynes et al 1947) (31). Failure to demonstrate the presence of renin in hypertension might be due to the lack of sensitivity of the methods (12). If this were the case, Leloir (41) raised the question as to how such a small concentration of renin in the blood could lead to such marked hypertension, as it has been shown that the methods are adequate to show its presence when in concentrations sufficient to increase arterial pressure in normal animals (12). But Page (51) has found a greater sensitivity to renin injections in hypertensive animals. Verny and Vogt (84), and Brown and Maegraith (8) have also found a greater sensitivity of hypertensive animals to pressor substances such as adrenalin and tyramine. (55, 59).

The phenomena of renin tachyphylaxis is another weakness in the renin - angiotonin theory. It is well recognized that it is difficult or impossible to maintain increased pressure by infusion of renin for periods of more than a few hours (33, 52, 82). This tachyphylaxis

to renin was thought to be due to a disappearance of blood hypertensinogen or renin - substrate. Page (51) has found however, that injection of renin - substrate does not restore the response to renin and has shown that exhaustion of the substrate is only part of the phenomena of tachyphylaxis (57, 53, 54). (See "Neurogenic Significance").

## (b) THE EFFECTS OF NEPHRECTOMY

The view that chronic hypertension as observed in the experimental animal, results from the elaboration of a circulating pressor substance has been rather generally accepted. However the experimental data of Grollman and co-workers cited in the following sections, not only are contrary to this view, but present definite evidence against it.

### (i) UNILATERAL NEPHRECTOMY

It has been generally assumed that unilateral nephrectomy has no effect on normal animals (23). Although this is usually true, a certain percentage of animals develope a moderate but definite increase in blood pressure which is evident some weeks following removal of one kidney (28, 25). Similar results have been shown to occur with unilateral operations on the kidney by application of a cloth capsule, figure-of-eight

etc. This rise may, however, not be evident for weeks or even months following the operation (Grollman et al (28,35) ) However, Halpert and Grollman (29), have recently shown that the induction of hypertension as a result of unilateral nephrectomy and unilateral operations is apparently dependent upon the existance of some lesion in the contralateral kidney. A study of kidneys of rats with chronic hypertension revealed the presence of local lesions not only in the kidneys to which a figure-of-eight had been applied but also similar lesions in the contralateral unoperated kidney. This finding is cited as evidence that unilateral injury induces hypertension only in the presence of some anatomical lesion in the contralateral kidney. Otherwise, application of the figure-of-eight and removal of the remaining kidney is necessary to induce chronic hypertension. Grollman (26) has cited these experiments as incompatable with the view that hypertension is due to the elaboration of a pressor substance by the injured kidney. They are better explained by assuming that constriction of the kidney interferes with a normal function of this organ essential for the maintenance of normal blood pressure.

Grollman (25,28), Patton and associates (60) have found that if the blood pressure of experimental animals is elevated to hypertensive levels by the application of a figure-of-eight, cellophane wrapping, etc., to one

kidney, the subsequent removal of this kidney fails to restore the blood pressure to normal levels. Also, following the removal of the single remaining kidney in a hypertensive, unilaterally nephrectomized animal, the blood pressure remains elevated for several days, until a few hours before death of the animal. They state that were the injured kidney responsible for the liberation of a pressor substance, its removal should obviously result in a return of blood pressure to normal levels.

(ii) BILATERAL NEPHRECTOMY.

The effects of bilateral nephrectomy on the blood pressure is difficult to determine, since animals deprived of all renal tissue succumb from uremia before any effect of the deficiency of renal tissue on the blood pressure can manifest itself (26). Following the removal of the remaining kidney from a hypertensive animal Grollman (28,25) has found that the blood pressure remains at its elevated level, declining only as uremia sets in during the last days before death. However, the insufficient time offered by complete nephrectomy has somewhat been overcome by Grollman and Rule (27) by utilization of the parabiotic preparation. When one of the parabions was bilaterally nephrectomized, this animal became hypertensive, while the blood pressure of the parabiont with intact kidneys remained at a normal level. More recently (Grollman (23)

has shown that bilaterally nephrectomized dogs, maintained by an artificial kidney, show an elevation of blood pressure to hypertensive levels. These experiments have been stated as evidence for the absence of renal tissue rather than the presence of an abnormal kidney that is responsible for the developement of hypertension.

According to Grollman (24) all the experiments cited above are consistent in showing the possibility of inducing hypertension by methods which reduce the total amount of renal tissue and the possibility of the existence of hypertension in the absence of any renal tissue in the organism. Grollman's alternative hypothesis to explain the role of the kidney in the pathogenesis of hypertension is to assume that this organ elaborates a humoral agent essential for the well-being of the organism, in the absence of which hypertension results.

### (c) HEPATO-RENAL VASOTROPIC FACTORS

A pressor-antipressor system has been described by Shorr & Zweifach (79) and other workers (11,86,81,80). Their experiments have shown the regular participation in the hypertensive syndrome in man and animals of a pair of vasotropic principles of hepatic and renal origin, whose opposite action on the vascular bed are of such a nature that they are said to constitute a circulatory homeostatic system. This system consists of a vasoexcitor substance (VEM) released from the kidney under stress, and which is

apparently neither renin or angiotonin, and a vasodepressor substance (VDM) formed in the liver. The vasoexcitor apparently acts by increasing the vasomotion of the metaarterioles, and enhancing these vessels to epinephrine. The vasodepressor exerts an opposite effect. VEM has been shown present in the blood of animals during acute experimental renal hypertension, and both VEM and VDM are present in high, neutralizing concentrations in the chronic stage of experimental renal hypertension. Derangements in the balance of these factors have been postulated as a possible mechanism in the pathogenesis of hypertension.

## 2. ROLE OF THE ADRENALS IN HYPERTENSION

The only endocrine organ which may possibly play a significant part in hypertension, even if only a secondary one is the adrenal system. According to Goldblatt (20), Best and Taylor (4), there is no evidence that the adrenal medulla plays a part in hypertension, but recently Mylon and Heller (46) 1948, have found that "angiotonin" apparently needs minute quantities of epinephrine, tyrosine and other tissue products to make it effective. However, there are more definite indications that the adrenal cortex plays a role in high blood pressure, as its secretions have been found essential to the experimental establishment and maintenance of hypertension (56,34).

Goldblatt (20) has found that the excision of both adrenals in dogs interferes with the developement of hypertension due to the constriction of the main renal artery; and unless supportative therapy is given high blood pressure is not established. Ogden and associates (49) and Anderson et al (3) 1944, have shown that excision of the anterior pituitary in hypertensive rats results in a fall of blood pressure, but becomes normal only if hypertension is of less than one month duration. Other workers have found no definite effect (20). Also Houssay and Dexter (34) and Munozetal (47) have shown that bilateral adrenalectomy, although it has no immediate effect, is followed by a progressive decrease in the response to intravenous injections of renin. These inhibiting effects of adrenal insufficiency are possibly due to a decreased concentration of renin-substrate, since Lewis and Goldblatt (42) have shown that the level of renin-substrate is reduced in adrenalectomy. The adrenal secretions are postulated as necessary for the production of the substrate.

Recently Zweifach and associates (85) 1947, have found that the kidneys of adrenalectomized rats, cats and dogs progressively lost the capacity to produce the vaso-excitor principal (VEM) which appeared in the blood of hypertensive animals (See "Role of Kidney"). The administration of desoxycorticosterone acetate (DCA) restored the capacity of the kidney to produce VEM. They state that the

question remains as to whether the withdrawal of the adrenal hormones fromnthe circulation produces a specific lesion in the kidney VEM mechanism, or, whether the generalized cellular dysfunction, characteristic of adrenal insufficiency, is the factor responsible for the decline in the blood pressure of both normal and hypertensive individuals.

Selye (69,70) has postulated on the basis of his work on the "adaptation syndrome", the hypersecretion of mineralo-corticoid hormones of the adrenals as a possible cause of hypertension. Experimentally it has been demonstrated that chronic exposure to non-specific damaging agents produces adrenal cortical enlargement with an overproduction of cortical hormones and simultaneous nephrosclerosis with hypertension in the rat. At present though, there is no direct evidence indicating that hypersecretion of the animal's own cortex leads to the developement of high blood pressure (70). However, hypertension has been produced experimentally by Selye and co-workers (68,71,72, 73,74,75,76) and others (40,7) by the administration of large amounts of desoxycorticosterone acetate (DCA) particularly when combined with a high salt diet and unilateral nephrectomy, with pathological changes simulating renal hypertension. Recently Selye (70) has found that doses as small as 1 mgm. of DCA per day are sufficient to produce the same results. But little evidence exists that

injections of cortical extracts or the administration of adrenocorticotropic hormone (ACTH), which mediates its effects through the adrenal cortex, will produce hypertension (16). However, both adrenal cortical extracts and ACTH have been found by Dougherty (16) to exert similar effects on the alteration of the juxtaglomerular apparatus with the similar effects ascribed to DCA, although DCA was found more effective.

## 3. NEUROGENIC SIGNIFICANCE IN HYPERTENSION.

There have always been suspicions that nervous factors were important in the genesis of hypertension, but crucial experimental evidence was lacking, and for a time it seemed decisive that hypertension was due to a humoral mechanism. However, as will be seen below, a number of investigators have thrown doubt upon the purely chemical nature of experimental hypertension.

Dock (17) has shown that pithing a hypertensive rabbit results in a drop in blood pressure. The blood pressure of the pithed rabbit, however, still increased by the administration of renin. It was concluded that the mechanism responsible for the maintenance of high blood pressure is not dependant upon circulating renin and angiotonin, and suggested the participation of the nervous system in the mechanism of renal hypertension.

Similar experiments have been carried out by Page (57) in which he found sectioning of the brain at various levels, widespread direct injury to the central nervous system, and severe shock, abolished the pressor response of angiotonin.

Even more recently Page and Taylor (57) have shown a further connection between the central nervous and the renal pressor systems. They found that previous administration of tetraethyl ammonium chloride (TEA), an agent which blocks the transmission of impulses through autonomic ganglia, overcame the phenomena of renin tachyphylaxis and augmented the response to angiotonin. TEA augmented the effects of both pressor and depressor substances. Corcoran (12) has taken this action of TEA to mean some central influence which limits vascular responsiveness. He has postulated two states of tachyphylaxis, the first resulting from the exhaustion of renin-substrate as seen in hepatectomized animals, and the other caused by some autonomic influence which inhibits the action of renin.

Evidence has been obtained by Ogden and associates (50), showing that a neurogenic mechanism may be involved in chronic hypertension. From studies on the sensitivity of experimentally hypertensive animals to sympathoparalytic agents such as phenobarbital, yohimbine, and F 933. They considered it likely that renal hypertension proceeds in two phases. (1) The first phase is of humoral origin dependant on excess liberation of renin. This phase is resistant to depressors. (2) The second phase is assumed to be one

in which neurogenic influences predominate. In this phase the blood pressure is depressed more by agents which affect the central nervous or sympathetic activity. These effects have been confirmed by other workers (63, 65).

More recently Corcoran and associates (13,14)1948, have demonstrated the dependance of clinical and experimental renal hypertension on central nervous activity. They found functional denervation (high spinal and caudal anaesthesia) of the renal vascular system will lower the blood pressure of most hypertensives to normal levels. This response was thought to arise from renal vasodilation.

## B. STATEMENT OF THE PROBLEM OF THIS THESIS.

The problem of this thesis consists basically of three parts (1) to learn the efficient use of the apparatus utilized by the previous workers of this laboratory for the determination of rat blood pressures, namely, the indirect method using the foot. (2) To find a suitable method for the establishment of chronic hypertension in female rats and (3) to find an agent for amelioration of this high blood pressure.

### 1. METHODS USED IN THE PRODUCTION OF EXPERIMENTAL CHRONIC HYPERTENSION

Success by Semple (77) and Fitch (19) of this

laboratory in producing an acute, transitory hypertension by a 1 mgm injection of desoxycorticosterone acetate (DCA) led to the method of producing chronic high blood pressure in rats by repeated administration of DCA. However, other methods, including modifications in the use of DCA, were decided upon. These were (1) a starvation diet and subsequent injectionswith DCA. (2) Injections of epinephrine and DCA given simultaneously, followed by daily injections of epinephrine. (3) The use of a choline deficient diet, with the possible acceleration of its effects by a DCA injection.

The first modification, namely, the use of a starvation diet was initiated by Samuels (66) work on estrogens. He observed that obese women frequently showed irregular menstrual cycles and found evidence that estrogens when in high concentrations in the circulating fluids, were absorbed by the fatty tissue, and diffused out when in low concentration in the circulating fluids. A similar effect was postulated for desoxycorticosterone, because of its similarities in chemical properties. This action would minimize its effects to produce hypertension.

The second modification was the administration of epinephrine with DCA. This method of raising the blood pressure was thought possible because of the recently discovered importance of epinephrine in the vasotropic factors of Zweifach and Shorr, and in the renal pressor system

(See "Role of the Kidney" and "Adrenals" p.9,10 )

The last procedure decided upon for the production of chronic hypertension was the use of a choline deficient diet. Best and Hartroft (5) discovered that hypertension developed in weanling rats approximately 4 to 7 months after the initiation of a 5 to 6 day choline deficient diet. Histological sectioning of livers and kidneys of the above rats showed irreversible damage. The kidneys had widespread tubular lesions, which were thought responsible for the elevation of blood pressure. Because of the limited time at our disposal, DCA was administered along with the diet with hope that it would accelerate the inchoate hypertension.

## 2. METHODS OF AMELIORATING EXPERIMENTAL CHRONIC HYPERTENSION.

Success by the previous workers of this laboratory (1) in ameliorating acute hypertension with the administration of histidine and ascorbic acid, led the way to further investigation in the use of this amino acid. Since the effects of histidine were studied only on an acute, temporary stage of hypertension, it was decided to investigate its depressor effects on chronic high blood pressure. The possible ameliorating effects of ammonium chloride were also investigated.

Histidine was found to have little depressor effect without the simultaneous use of excess ascorbic acid, a

strong reducing agent. Histidine, therefore, was thought to be converted to histamine in the body, probably by the action of several strains of B. Coli in the intestines; and the depressor action of histamine was thought to be protected from the oxidative enzyme, "histaminase", by the reducing powers of ascorbic acid. With this principle in mind, the replacement of ascorbic acid by some other agent was deemed possible. Two other substances were decided upon, namely: Vitamin E and urease.

Vitamin E (tocopherol) has been found essential for numerous phases of metabolism (32) (45). The action of vitamin E in many of these biochemical systems seems to be greatly dependant upon its powerful antioxidant action. Tocopherol, therefore, was thought to be a suitable replacement for ascorbic acid. The apparent clinical success of Shute and associates (78) in vitamin E therapy of many cardiovascular diseases was sufficient to assume that vitamin E may play an independant role in the amelioration of hypertension.

It has been reported that urease was helpful in the hypertensive stage during pregnancy (2). Because of the possible reducing effect of urease by the production of ammonia from urea, urease was postulated as a possible agent in replacing ascorbic acid.

It is well established that an overdosage of desoxycorticosterone acetate produces nephrosclerosis and hypertension. Biochemical studies of changes precipitated

by DCA overdosage in animals revealed striking disturbances in the electrolytic metabolism and particularly a marked rise in the serum Na/ Cl ratio (70,73). The Na/Cl ratio could be partially restored to it's normal by the administration of ammonium chloride which at the same time prevents the production of nephrosclerosis and hypertension. It was decided to confirm further this action of ammonium chloride.

## 3. THE GENERAL DELINEATION OF THIS THESIS.

In light of the foregoing considerations, a formal summary of our problem can be stated as follows:

(1) To become proficient in determining blood pressures of albino rats by the indirect method of using the foot.

(2) To try to induce chronic hypertension by repeated injections of DCA, and to try numerous modifications of this procedure, namely; the administration of DCA with a starvation diet, daily injections of epinephrine, and a choline deficient diet.

(3) To discover the ameliorating action of histidine and ascorbic acid, histidine and vitamin E, histidine and urease, Vitamin E, urease, and ammonium chloride on chronic high blood pressure.

## II APPARATUS

The apparatus used for determining the blood pressure of rats in this investigation utilized the indirect foot method. The basis for this method is described by Griffith and Farris and the numerous modifications used have been reported by Allardyce, Semple and Fitch (1), previously of this laboratory.

### A. DESCRIPTION OF THE APPARATUS.

The apparatus has been fully reported by Fitch (19), therefore the following sections on the apparatus will be dealt with briefly, and will include a few modifications decided upon to improve the accuracy of the pressure determinations. A photograph of the apparatus is shown in figure I.

#### 1. LIGHTING

The source of light used was from a 100 watt diaphram spotlight. The light was passed through the microscope condenser to the web of the foot under consideration. The field was found to be well and regularly illuminated, particularly if the condenser was regulated to give the most diffuse light.

#### 2. MICROSCOPE

The microscope was fitted with a mechanical stage

onto which a wooden platform was attached for supporting the rat. There is an aperature in the platform coinciding with the passage of light. This aperature is surrounded by plasticine onto which the web of the foot is spread and fixed to visualize the capillary movement. Because of the supporting arrangement for the web, it is only possible to use a low power objective. However, for more accurate observation and less strain on the eyes, the use of a 15 X eye-piece (magnification 150X) was found helpful.

3. CUFF.

The making of the cuff is adequately described by Fitch (19). In this investigation the cuff was made longer than previously reported to ensure complete encircling of the rat thigh. This procedure was found to give more definition in determining the systolic pressure, and thus more accuracy.

4. CUFF PRESSURE SOURCE.

Briefly, the pressure in the cuff is obtained through the use of a mercury column. The mercury tube is attached to the cuff, a pressure guage (aneroid manometer) and a syringe, for aiding the initial pressure to the cuff. The mercury column is so assembled as to be used for fine adjustment.

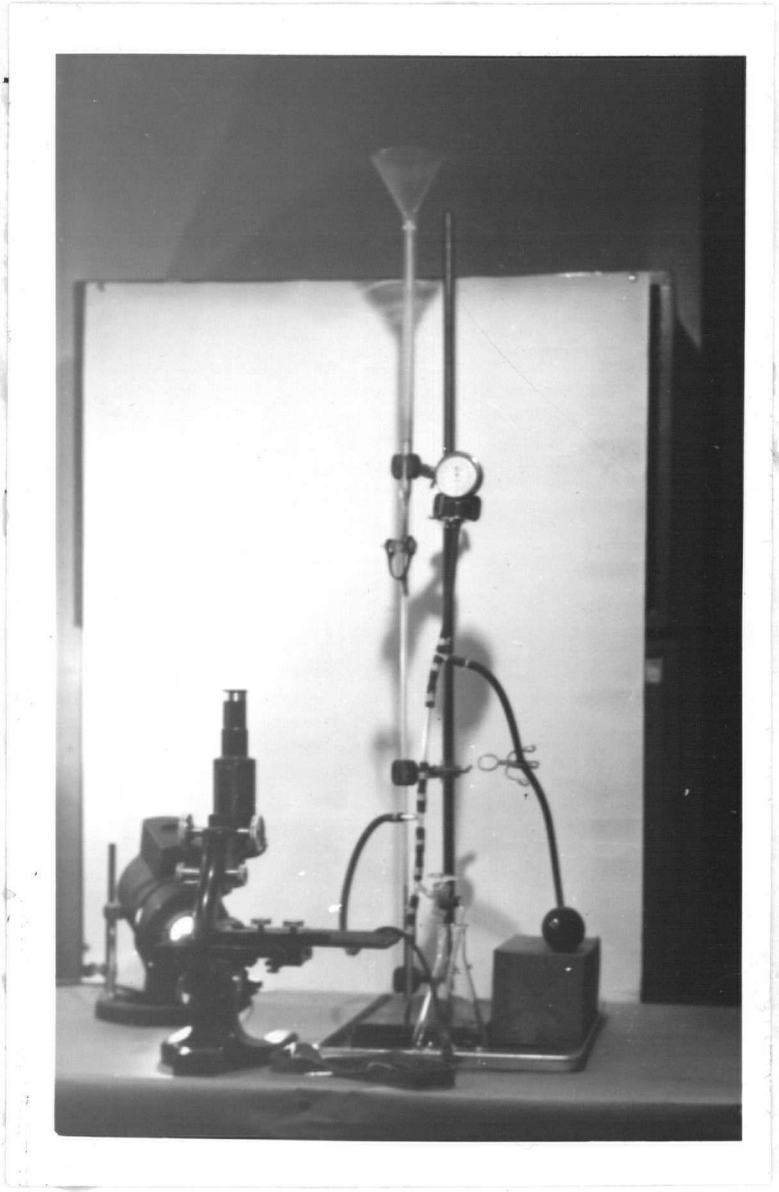

Figure 1. Photograph of the apparatus used in determining rat blood pressures.

## III METHODS

The previous work done in this laboratory investigated and established two very important problems, namely, finding a safe and effective means of anesthesia and the mastery of the microscopic technique involved in determining accurate blood pressures. The establishment of these results aided greatly in the development of our own technique. The solution to these two problems is adequately described by both Semple (77) and Fitch (19).

### A. THE PROCEDURE FOR MEASURING BLOOD PRESSURE.

#### 1. ANESTHESIA.

The anesthesia found most safe and effective by the previous investigators was sodium pentathol (monosodium salt of 5 - ethyl - 5 - (1 - methylbutyl) thiobarbituric acid), and was the anesthetic used throughout our entire experimental research.

Sodium Pentathol is obtained in ampoules containing .5 gms. Since it is necessary to have a fresh solution of pentathol daily for blood pressure determinations, with a 2.5% solution as the most reliable, it was found convenient to subdivide the .5gms into five groups of 100 mg., and store it in a refrigerator in dry, sterile bottles especially fitted with hypodermic rubber stoppers. When required, 4 cc. of water were added to make up the 2.5%

solution, an amount sufficient to do 8 - 10 rats.

From the previous work of Semple (77) and Fitch (19) and our own observations, the therapeutic dose for most Wistar rats was established. Contrary to Fitch and Semple, who did not find the administered dose influenced by such factors as body weight, we did find the dose of sodium pentathol was most dependant upon the factor of body weight. Other factors such as recent meals also effects the dose. The value of the therapeutic dose was well defined by Semple; " that volume of 2.5% sodium pentathol that is required to completely anesthetize the rat for a period lasting over 30 minutes but not longer than one hour." However, with the development of a more rapid technique, it was possible to lower the period of anesthesia from 20 - 40 minutes, thus lowering the possible effect of an overdose. Table I shows the therapeutic dose found for most animals. If the rat fails to succumb to the regular dose, it is permissable to give only .1 cc more of the anesthetic at that time. If more than .1 cc of sodium pentathol is given, the rat usually succumbs to respiratory failure.

Sodium pentathol is injected interperitoneally. Care must be taken that the needle of the hypodermic is in the peritoneal cavity and not in the abdominal wall or some visceral tissue. The administration of the anesthetic was previously given with the help of an assistant to hold the rat,

~~and an operator for the injections~~, but with practice an adequate method can be developed in which no help is required individual.

2. BLOOD PRESSURE DETERMINATIONS.

A rat is anesthetized and left for about 5 - 10 minutes. After the anaesthetic has taken effect, the animal is laid face down, and the thigh of the left leg (found most convenient) is wrapped somewhat firmly with the cuff. Holding the cuff in position, the rat is lifted onto the adjustable platform and placed in position, namely, with the web to be examined (preferably between digits 4 & 5) spread over the light aperature on the stage and fixed in place with plasticine. The web is searched under low power for a suitable field of capillaries with a rapid flow of corpuscles. When the most satisfactory field has been located, the pressure in the cuff is raised by means of the syringe to approximately 110 - 115 mm. of Hg. The pressure is then raised by the use of the syringe and by allowing mercury to run into the U-tube, until the blood flow and movements have been arrested. The pressure is released, and a second pressure reading is recorded. The average of these two values is taken as the systolic pressure. It is necessary that several groups of vessels be observed simultaneously to make sure that the blood flow has ceased throughout the web.

## B. METHODS IN EXPERIMENTAL CHRONIC HYPERTENSION.

RUN I: For the first run, 11 female rats were chosen, and their normal blood pressures established for 3 consecutive days. Of the 11 rats, two were set aside as normal controls, the other 9 were then injected intramuscularly with 1 mgm. of desoxycorticosterone (DCA). The day of DCA injections were termed day "o". Subsequent readings, starting from day "o" were taken up to day 23. Since the desired chronic hypertension was obtained by one injection of DCA, the rats were divided as follows:

GROUP (a) Two rats set aside at the start of the run for normal controls. They received no treatment.

GROUP (b) Two rats which received 1 mgm. of DCA, and received no further treatment.

GROUP (c) One rat, which received 20 mgm. of histidine and 70 mgm. of ascorbic acid daily in powdered fox chow (Purina Mills)

This was prepared as follows:

A quantity of special diet in the form of a mixture consisting of 140 gm of powdered fox chow, 140 mgm of histidine, and 490 mgm of ascorbic acid was prepared for a seven day period and was divided into 7 equal portions.

GROUP (d) One rat, which received 20 mgm of histidine in powdered fox chow, and 10 mgm of vitamin E in oil (alpha-tocopherol, British Drug Houses)

were added daily. The histidine and powdered fox chow were mixed as described above.

GROUP (e) One rat, which was given 10 mgm. of vitamin E daily on powdered fox chow.

GROUP (f) One rat, which received 20 mgm of histidine and 25 mgm of urease daily in powdered fox chow. A mixture of powdered chow, 140 mgm of histidine and 175 mgm of urease was prepared as for group (c)

GROUP (g) One rat, given 25 mgm of urease daily in powdered fox chow as described in group (c).

GROUP (h) One rat, which received 2% ammonium chloride ($NH_4Cl$) daily in powdered fox chow. Ammonium chloride was prepared with powdered fox chow as described for group (c).

All special diets were started on day 27. Pressure readings of these groups were taken up to day 48, when the special diets of groups (c) to (h) were stopped. A reading of all rats was again taken on day 62, and the diets of only groups (d) and (e) were resumed. Subsequent readings on these two groups were taken on days 71 and 78. The results of this run are shown in table 2, and figures 3 - 6.

RUN II: For this run 12 female rats were selected and their normal blood pressures established as in the previous experiment. Two rats were kept as normal controls and received no treatment. The remaining 10 rats were injected

with 1 mgm of DCA on day "o". Failure to produce high blood pressure resulted in further injections of DCA (1mgm.) on days 11 and 16. The pressure readings were continued until day 38. The results are shown in table 3 and figure 7.

RUN III: 10 female rats were chosen and their normal blood pressure determined as before. On day "o" all rats were injected with 1 mgm of DCA. Failure to obtain hypertension resulted in a second injection of 1 mgm of DCA on day 12. Pressure readings were taken up to day 32. The results are shown in table 4 and figure 7.

RUN IV: The procedure for this run was the same as for runs II and III. DCA (1mgm) injections were given to 12 female rats on days "o", "9", "29" and "52". Blood pressure readings were established up to day 66. The results are shown in table 5 and figure 7.

RUN V: Failure of runs II, III and IV in establishing hypertension by repeated injections of DCA, resulted in a modification of its use (See introduction p.15). In this run 8 female rats were chosen and their normal blood pressures established for 2 days. Two of these rats were kept for normal controls and received no treatment. The 6 remaining rats were placed on a "starvation diet" consisting of 12 gms of the regular diet (fox chow), an amount found adequate in reducing the weight. This diet was continued for 17 days with intermittent blood pressure

readings. The rats were found to lose an average of 35 gms in this 17 day period. On day 17, the six rats were injected with 1 mgm of DCA. Subsequent readings were taken up to day 33. The results are shown in table 6 and figure 8.

RUN VI: This run consists of a further modification in the use of DCA in producing high blood pressure. For this run 5 female rats were obtained and their normal pressures recorded. Two rats were set aside as normal controls and were given no treatment. The remaining 3 rats were injected with 1 mgm. of DCA and .002 mgm. of epinephrine on day "o". The adrenalin injections were given daily up to day 24. Nine pressure readings were established up to day 24. The results are shown in table 7 and figure 9.

RUN VII: Unsuccessful attempts to obtain hypertension since run I, culminated in using a choline deficient diet. Five female rats were used. After their normal pressures were established, they were placed on a prepared choline deficient diet (see figure 2) for 9 days starting on day "o". On day 9, 4 of the 5 rats were injected with 1 mgm of DCA. Readings were taken of all rats up to day 29. Results of this run are shown in table 8 and figure 10.

RUN VIII: This run was composed of 12 female rats. The normal blood pressure of each rat was established for two days. On day "o" all rats were placed on a 7 day choline deficient diet (figure 2). Chronic hypertension was established in all rats by day 20, and were then divided

| Weight in Grams | Dose in cc. |
|---|---|
| under 150 | .30 |
| 150 - 180 | .35 - .38 |
| 180 - 200 | .38 - .40 |
| 200 - 250 | .40 - .45 |
| 250 - 300 | .50 |

Table 1. Showing the dosage of a 2.5% solution of sodium pentothal necessary for anesthetizing rats as determined by their weight.

| | | | | | |
|---|---|---|---|---|---|
| Sucrose | 67.5% | *Salts | 5.0% | Beef fat | 12.0% |
| Gelatin | 7.0% | Celluflour | 2.0% | Cod liver oil | 1.0% |
| Caesin | 3.0% | *Vitamin mix | 1.0% | | |
| Fibrin | 1.0% | Cystine | 0.5% | | |

* Salts
(N.B.C. salt mix No.2)

| | |
|---|---|
| NaCl | 4.35% |
| $MgSO_4$ | 13.70% |
| $NaH_2PO_4$ | 8.72% |
| $K_3PO_4$ | 23.98% |
| $Ca(H_2PO_4)$ | 13.58% |
| Ferric Citrate | 2.97% |
| Calcium Lactate | 32.70% |

* Vitamin powder/100 gm.

| | |
|---|---|
| Thiamin | 500 mg |
| Riboflavin | 250 mg |
| Pyridoxine | 200 mg |
| Ca Pantothenate | 1 gm. |
| Nicotinic acid | 1 gm. |

-added to 997.05 gm of powdered sugar.

Figure 2 Showing the constituents of the choline deficient diet used for the production of chronic high blood pressure.

into the following groups.

GROUP (a) Two rats used as hypertensive controls and received no further treatment.

GROUP (b) Four rats, which were given 20 mgm of histidine and 70 mgm. of ascorbic acid daily /rat. in powdered fox chow. The mixture was given as described in Run I, group (c).

GROUP (c) Six rats, which received 40 mgm of vitamin E in oil added daily /rat. to powdered fox chow.

Both supplemented diets were continued for 13 days. On day 33 the special diets were stopped. The test continued for 39 days. The results are shown in table 9 and figure 11.

## IV RESULTS

The results of this investigation are listed in tabular and graphic form.

Figures 3 - 6 (table 3, Run I ) show a rise of blood pressure of ten female rats injected with 1 mgm of DCA on day "o". The blood pressure rose in four days, 170 mm of Hg above normal, to systolic pressures of over 300 mm of Hg. Pressures over 300 mm of Hg could not be recorded by the aneroid manometer. Figure (3) shows the ameliorative effect of histidine and vitamin E and vitamin E

supplement. The pressures dropped to an average of 232 mm of Hg with both diets. These diets were stopped in 21 days, after which the hypertensive levels returned to their previous state. The diets were reinstituted on day 62 for 18 days. The pressures again lowered to an average of 215 mm of Hg. Figures 4, 5 and 6 show the negative results of histidine and ascorbic, histidine and urease, urease, and ammonium chloride respectively.

Figure 7 (tables 3 - 5) shows the negative response of Runs II, III and IV to 1 mgm injections of DCA. The rats showed no response to DCA injections with the exception of Run IV, in which a temporary rise of 25 mm of Hg was observed.

Figure 8 shows the negative response of 6 rats to a 17 day starvation diet followed by 1 mgm injections of DCA to raise the blood pressure.

Figure 9 shows the negative effects on the blood pressure of 3 female rats after 24 daily injections of adrenalin with an initial 1 mgm injection of DCA on day "o".

Figure 10 shows the rise in systolic pressure of 5 rats placed on a choline deficient diet for nine days starting on day "o". 4 rats were injected on day 9 and their systolic blood pressure rose to an average of 210 mm of Hg on the 14th. day. These 4 rats remained at this level until day 20. On day 20 the uninjected DCA rat had a pressure of 200mm of Hg. From day 20 to 26 the systolic

blood pressure of all rats rose to 300 mm of Hg.

Figure 11 shows the effects of histidine and ascorbic acid and vitamin E on chronic hypertension produced by a 7 day choline deficient diet. Most of the rats had systolic pressures of over 300 mm of Hg. 20 days after the start of the choline deficient diets. Vitamin E is shown effective in lowering the blood pressure to an average of 205 mm of Hg.

| Group | Time in Days | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 2 | 1 | 0 | 1 | 2 | 3 | 4 | 6 | 7 | 9 | 13 | 16 | 20 | 23 | 27 | 30 | 35 | 38 | 41 | 44 | 48 | 48 | 62 | 62 | 71 | 78 |
| a | 126 | 127 | * | - | 131 | 134 | 132 | * | 134 | 134 | 132 | 133 | 134 | 132 | 132 | - | 136 | 134 | 134 | 133 | 131 | 13 | - | 134 | | | |
| a | 129 | 126 | 124 | - | * | 130 | 133 | 134 | 134 | 136 | 132 | 133 | 135 | 138 | 140 | - | 136 | 135 | 134 | 132 | 135 | 132 | - | 135 | | | |
| b | 120 | 126 | 122 | DCA injection | 140 | 230 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | - | 300 | 300 | 300 | 300 | 300 | 300 | - | 300 | | | |
| b | 130 | 131 | 130 | | 145 | * | * | * | 300 | 300 | 300 | 300 | 300 | 300 | 300 | - | 300 | 300 | 300 | 300 | 300 | 300 | - | 300 | | | |
| c | 108 | 109 | 108 | | 119 | 173 | 212 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | started diets | 300 | 300 | 300 | 300 | 300 | 300 | stopped diets | 300 | | | |
| d | 110 | 116 | 114 | | 144 | 210 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | | 260 | 242 | 244 | 240 | 246 | 230 | | 300 | started diets | 280 | 215 |
| e | 116 | 116 | 117 | | 136 | 275 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | | 280 | 265 | 254 | 240 | 235 | 235 | | 300 | | 284 | 220 |
| e | 125 | 128 | 128 | | 148 | 227 | 255 | 300 | # | | | | | | | | | | | | | | | | | | |
| f | 116 | 118 | 120 | | 156 | 215 | 288 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | | 300 | 300 | 300 | 300 | 300 | 300 | | 300 | | | |
| g | 120 | 122 | 124 | | 154 | 297 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | | 300 | 300 | 300 | 300 | 300 | 300 | | 300 | | | |
| h | 118 | 116 | 118 | | 148 | 190 | 260 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | | 300 | 300 | 300 | 300 | 300 | 300 | | 300 | | | |

Table 2. Showing the results of the daily feeding of histidine and ascorbic acid(groupe), histidine and vitamin E (group d), vitamin E (group e), histidine and urease (group f), urease (group g), and ammonium chloride (group h) on the chronic hypertension of female rats (Run I) produced by a 1 mgm. injection of desoxycorticosterone acetate (DCA) on day '0'. (see figures 3-6)

* not properly anesthetized.
\- no reading.
\# died under anesthetic.

| Rat No. | Time in Days | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 2 | 1 | 0 | 1 | 4 | 7 | 11 | 11 | 14 | 16 | 16 | 20 | 22 | 26 | 30 | 34 | 38 |
| 1 | 145 | 144 | 143 | Desoxycorticosterone acetate injection | 146 | 150 | 140 | 146 | Desoxycorticosterone acetate injection | 140 | 138 | Desoxycorticosterone acetate injection | 138 | 135 | 134 | 136 | 138 | 134 |
| 2 | 145 | 140 | 138 | | 140 | 140 | 155 | 135 | | 130 | 125 | | 130 | 134 | 132 | 134 | 136 | 136 |
| 3 | 144 | 145 | 143 | | 130 | 140 | 160 | 130 | | 125 | 126 | | 135 | 140 | 138 | 142 | 140 | 143 |
| 4 | 145 | 145 | 145 | | 130 | 150 | 130 | 132 | | 130 | 132 | | 136 | 146 | 145 | 142 | 144 | 146 |
| 5 | 140 | 144 | 145 | | 130 | 125 | 144 | 135 | | 130 | 132 | | 138 | 128 | 130 | 136 | 140 | 140 |
| 6 | 138 | 142 | 140 | | 135 | 135 | 150 | 135 | | 125 | 135 | | 125 | # | | | | |
| 7 | 145 | 145 | 144 | | 135 | 132 | 130 | 134 | | 120 | 125 | | 122 | 132 | 136 | 138 | 142 | 138 |
| 8 | 130 | 132 | 133 | | 125 | 140 | 135 | 125 | | 120 | 115 | | 132 | 130 | 134 | 128 | 130 | 132 |
| 9 | 145 | * | 140 | | * | 140 | 135 | 142 | | 140 | 146 | | 144 | 140 | 142 | 146 | 140 | 140 |
| 10 | 135 | 136 | 138 | | 130 | 126 | 136 | 135 | | * | 134 | | 130 | 125 | 134 | 130 | 131 | 134 |
| 11 | 142 | 140 | 144 | | 140 | 130 | 130 | 130 | | 115 | 130 | | 130 | 132 | 134 | 132 | 136 | 138 |
| 12 | 134 | 132 | 130 | | 138 | 126 | 128 | 126 | | 130 | 122 | | 128 | 134 | 134 | 132 | 136 | 135 |

Table 3. Showing the results of 1 mgm. injections of desoxycorticosterone acetate (DCA) on the blood pressure of female rats (Run II ). (see figure 7).

* not properly anesthetized.
\# died under anestetic.

| Rat No. | Time in days | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 2 | 1 | 0 | 1 | 4 | 8 | 12 | 12 | 14 | 16 | 19 | 24 | 27 | 32 |
| 1 | 1 26 | 130 | 130 | Desoxycorticosterone acetate injection | 130 | 125 | 132 | 134 | Desoxycorticosterone acetate injection | 136 | 134 | 130 | 132 | 130 | 126 |
| 2 | 126 | 128 | 130 | | 132 | 126 | 130 | 134 | | 128 | 130 | 132 | 126 | 127 | 128 |
| 3 | 134 | * | 132 | | 128 | 125 | 135 | 132 | | 129 | 131 | 134 | 132 | 130 | 126 |
| 4 | 128 | 134 | 126 | | 130 | 126 | 132 | 130 | | 132 | 138 | 132 | 128 | 128 | 132 |
| 5 | 130 | 132 | 134 | | 134 | 132 | 136 | 130 | | 128 | 129 | 132 | 134 | * | 132 |
| 6 | 132 | 134 | 138 | | 136 | 134 | 132 | 136 | | 128 | 130 | 136 | 130 | 134 | 130 |
| 7 | 134 | 132 | 128 | | 130 | 126 | 134 | 128 | | 130 | 133 | 136 | 132 | 134 | 132 |
| 8 | 135 | 136 | 134 | | 134 | * | 136 | 130 | | 138 | 134 | 135 | 130 | 134 | 138 |
| 9 | 138 | 140 | 136 | | 140 | 138 | 142 | 136 | | 136 | 134 | 138 | 138 | 140 | 135 |
| 10 | 125 | 132 | 128 | | 128 | 126 | 130 | 132 | | 129 | 125 | 128 | 122 | 130 | 128 |

Table 4. Showing the results of 1 mgm. injections of desoxycorticosterone ( DCA ) on the blood pressure of female rats (Run III) (see figure 7).

* not properly anesthetized.

| Rat No. | Time in days | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 2 | 1 | 0 | 9 | 9 | 20 | 25 | 29 | 36 | 40 | 45 | 52 | 56 | 60 | 66 |
| 1 | 125 | 128 | 126 | Desoxycorticosterone acetate injection | 130 | 124 Desoxycorticosterone acetate injection | 128 | 122 | Desoxycorticosterone acetate injection | 132 | 128 | 128 | Desoxycorticosterone acetate injection | 126 | 124 | 125 |
| 2 | 136 | 134 | 138 | | 134 | | 145 | 160 | | 165 | 150 | 145 | | 138 | 140 | 134 |
| 3 | 134 | 132 | 135 | | 134 | | 120 | 140 | | 140 | 135 | 138 | | 145 | 142 | 138 |
| 4 | 108 | 112 | 110 | | # | | | | | | | | | | | |
| 5 | 115 | 120 | 118 | | 132 | | 125 | 170 | | 150 | 145 | 135 | | 136 | 130 | 128 |
| 6 | 130 | 132 | 134 | | 138 | | 120 | 140 | | 130 | 135 | 140 | | 136 | 138 | 140 |
| 7 | 112 | 115 | 114 | | 116 | | 125 | 160 | | 130 | 125 | 128 | | 120 | 122 | 125 |
| 8 | 108 | 110 | 109 | | 140 | | 130 | 145 | | 140 | 135 | 132 | | 140 | 130 | 128 |
| 9 | 120 | 124 | 120 | | 126 | | 140 | 130 | | 160 | 152 | 135 | | 135 | 130 | 132 |
| 10 | 122 | 120 | 120 | | 125 | | 126 | 160 | | 135 | 132 | 134 | | 130 | 128 | 128 |
| 11 | 110 | 112 | 114 | | 110 | | 120 | 130 | | 135 | 128 | 130 | | 122 | 125 | 124 |
| 12 | 112 | 115 | 110 | | 116 | | 132 | 170 | | 130 | 124 | 128 | | 132 | 125 | 120 |

Table 5. Showing the results of 1 mgm. injections of desoxycorticosterone acetate (DCA) on the blood pressure of female rats (Run IV) (see fig. 7).

| Group | Time in Days | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 1 | 0 | 5 | 11 | 17 | 17 | 23 | 24 | 28 | 33 |
| a | 124 | 128 | - | 125 | 130 | 128 | - | 127 | - | 126 | 128 |
| a | 128 | 129 | - | 129 | 128 | 129 | - | 132 | 128 | 128 | 133 |
| b | 128 | 124 | Starvation diet | 130 | 130 | 133 | DCA injection | 138 | DCA injection | 138 | 132 |
| b | 128 | 130 | | 126 | 129 | 132 | | 133 | | 134 | 130 |
| b | 136 | 132 | | 134 | 128 | 130 | | 134 | | 135 | 133 |
| b | 120 | 125 | | 124 | 128 | 130 | | 125 | | 132 | 126 |
| b | 140 | 138 | | 135 | 135 | 136 | | 142 | | 140 | 138 |
| b | 133 | 134 | | 132 | 131 | 126 | | 136 | | 139 | 140 |

Table 6. Showing the results of a starvation diet (group b ) with subsequent 1 mgm. injections of DCA on the blood pressure of female rats (Run V). (seefigure 8)

\- no reading.

| Group | Time in days | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 1 | 0 | 2 | 3 | 6 | 10 | 13 | 15 | 20 | 24 |
| a | 145 | 143 | - | 142 | 140 | 143 | 143 | 142 | 144 | 140 | 142 |
| a | 132 | 130 | - | 133 | 138 | 139 | 136 | 135 | 136 | 133 | 134 |
| b | 145 | 142 | DCA and Adrenalin/day | 146 | 142 | 140 | 138 | 145 | 142 | 145 | 136 |
| b | 142 | 142 | | 145 | 144 | 141 | 144 | 138 | 140 | 139 | 136 |
| b | 136 | 138 | | 139 | 142 | 140 | 137 | 138 | 138 | 142 | 135 |

Table 7. Showing the results of a 1 mgm. injection of desoxycorticosterone acetate (DCA) on day '0', with with daily injections of .002 mgm of epinephrine(adrenalin) (group b) on female rats(Run VI). (see figure 9)

| Group | Time in Days | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 1 | 0 | 4 | 9 | 9 | 14 | 18 | 20 | 24 | 27 | 29 |
| a | 134 | 132 | Choline deficiency | 130 | 135 | - | 140 | 180 | 205 | 210 | 300 | 300 |
| b | 140 | 145 | | 142 | 150 | DOA injection | 185 | 185 | 180 | 190 | 300 | 300 |
| b | 144 | 142 | | 142 | 144 | | 180 | 200 | 195 | 225 | 300 | 300 |
| b | 136 | 138 | | 135 | 136 | | 205 | 210 | 205 | 215 | 296 | 298 |
| b | 140 | 140 | | 138 | 142 | | 230 | 235 | 245 | 260 | 300 | 300 |

Table 8. Showing the results of a 9-day choline deficient diet (group a) with a 1mgm. injection of DOA on the blood pressure of female rats (Run VII) . (see figure 10).

- no reading.

| Group | Time in Days | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 1 | 0 | 4 | 8 | 16 | 20 | 20 | 27 | 29 | 33 | 33 | 40 |
| a | 125 | 124 | - | 126 | 125 | 210 | 285 | - | 300 | 300 | 300 | - | 300 |
| a | 136 | 140 | - | 138 | 150 | 185 | 290 | - | 295 | 295 | 298 | - | 296 |
| b | 142 | 140 | Choline Deficiency | 144 | 155 | 200 | 300 | Started diets | 300 | 300 | 300 | stopped diets | 300 |
| b | 145 | 142 | | 146 | 148 | 195 | 300 | | 300 | 300 | 300 | | 300 |
| b | 132 | 130 | | 128 | 146 | 190 | 290 | | 295 | 290 | 296 | | 294 |
| b | 136 | 138 | | 134 | 152 | 215 | 285 | | 300 | 300 | 300 | | 300 |
| c | 135 | 136 | | 138 | 142 | 220 | 300 | | 240 | 220 | 210 | | 280 |
| c | 128 | 126 | | 125 | 140 | 130 | 280 | | 220 | 215 | 200 | | 285 |
| c | 144 | 142 | | 142 | 156 | 205 | 300 | | 240 | 225 | 215 | | 295 |
| c | 140 | 144 | | 145 | 154 | 215 | 300 | | 245 | 235 | 220 | | 270 |
| c | 125 | 125 | | 138 | 138 | 190 | 295 | | 215 | 200 | 190 | | 286 |
| c | 138 | 140 | | 141 | 144 | 210 | 300 | | 220 | 210 | 200 | | 290 |

Table 9. Showing the effects of histamine and ascorbic acid (group b), and vitamin E (group c) on the chronic blood pressure of female rats produced by a choline deficient diet(Run VIII). (see figure 11)

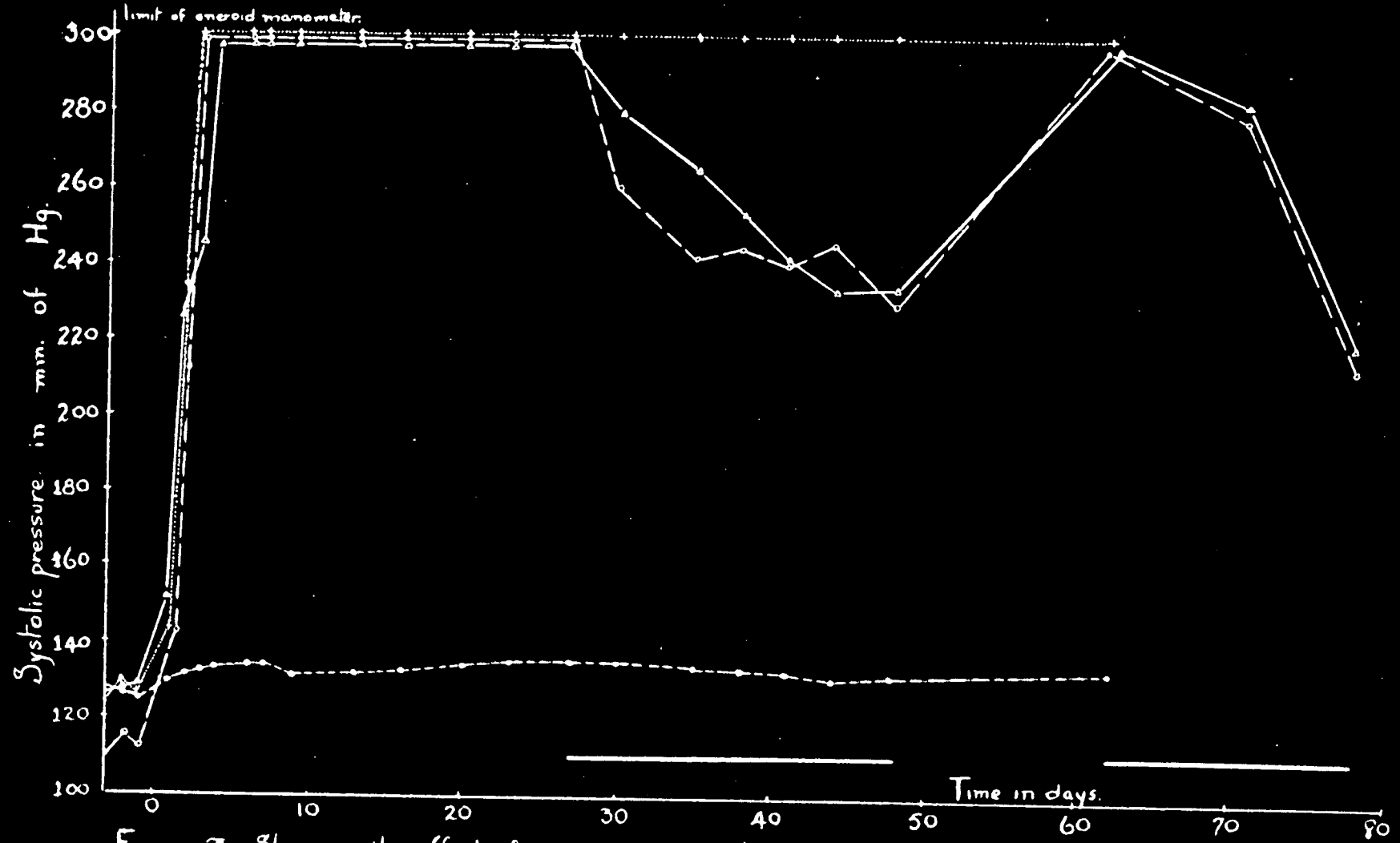


Figure 3. Showing the effect of daily feeding of histidine and vitamin E, and vitamin E on chronically hypertensive rats injected with Desoxycorticosterone acetate (1 mgm) on day '0'

legend: ---•--- group (a) average of 2 rats, normal control
.....+..... group (b) average of 2 rats, hypertensive control
——— showing periods of daily feeding.
——o—— group (d) 1 rat, histidine (20 mgm) and vitamin E (10 mgm) daily
——▲—— group (e) 1 rat, vitamin E (10 mgm) daily

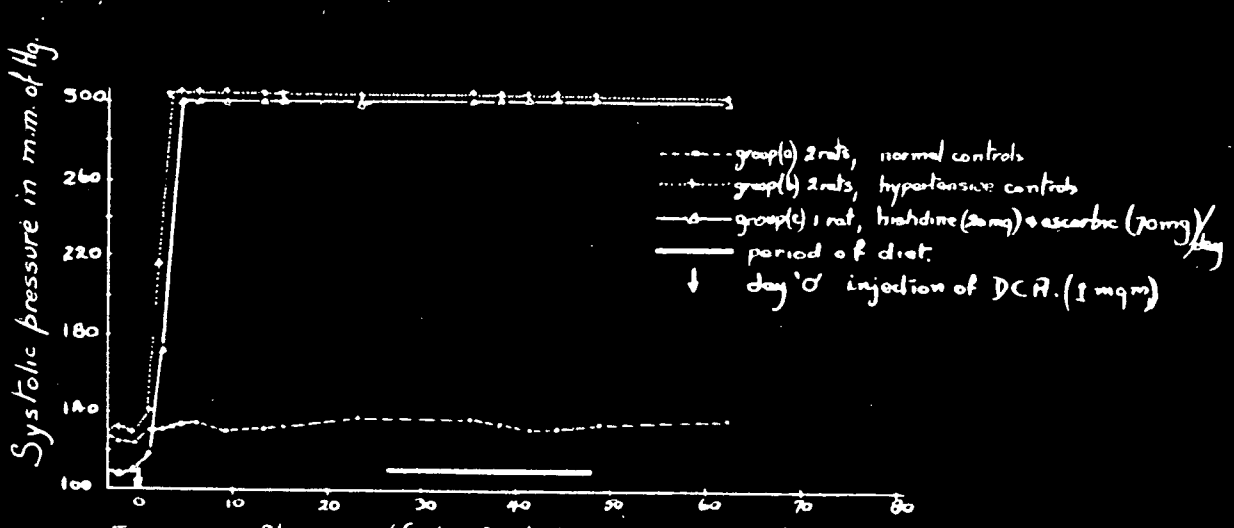


Figure 4. Showing effect of Histidine and Ascorbic acid on chronically hypertensive rats produced by a DCA injection (1 mgm.)

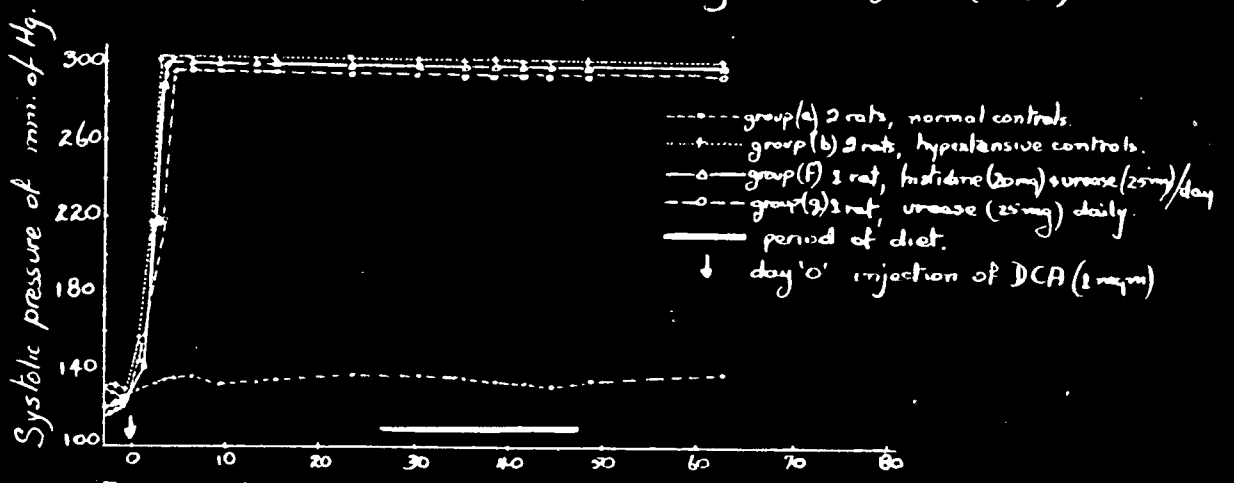


Figure 5. Showing effect of histidine and Urease, and urease on chronically hypertensive rats produced by a DCA. injection (1 mgm)

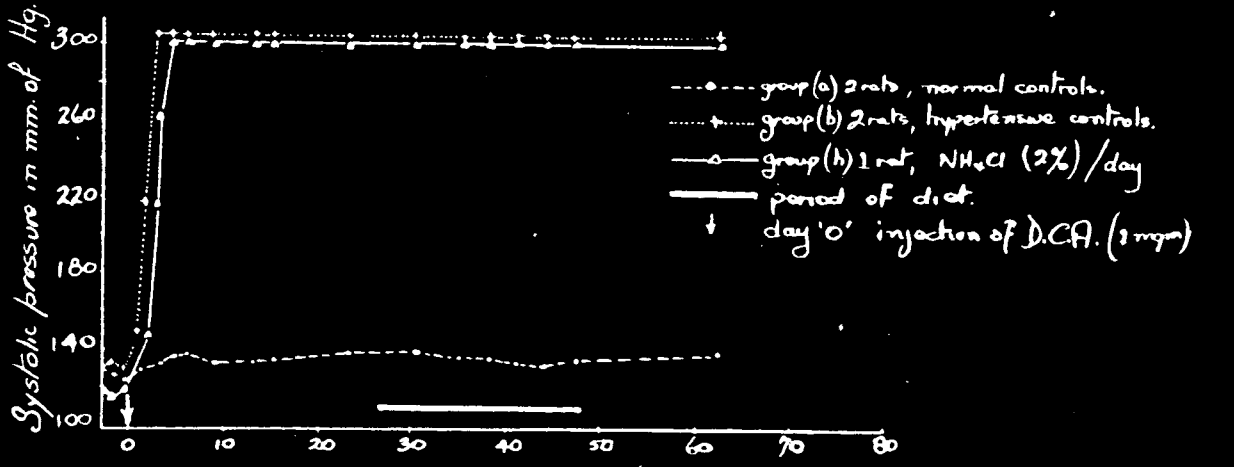


Figure 6. Showing effect of $NH_4Cl$ (ammonium chloride) on chronically hypertensive rats produced by a DCA injection (1 mgm)

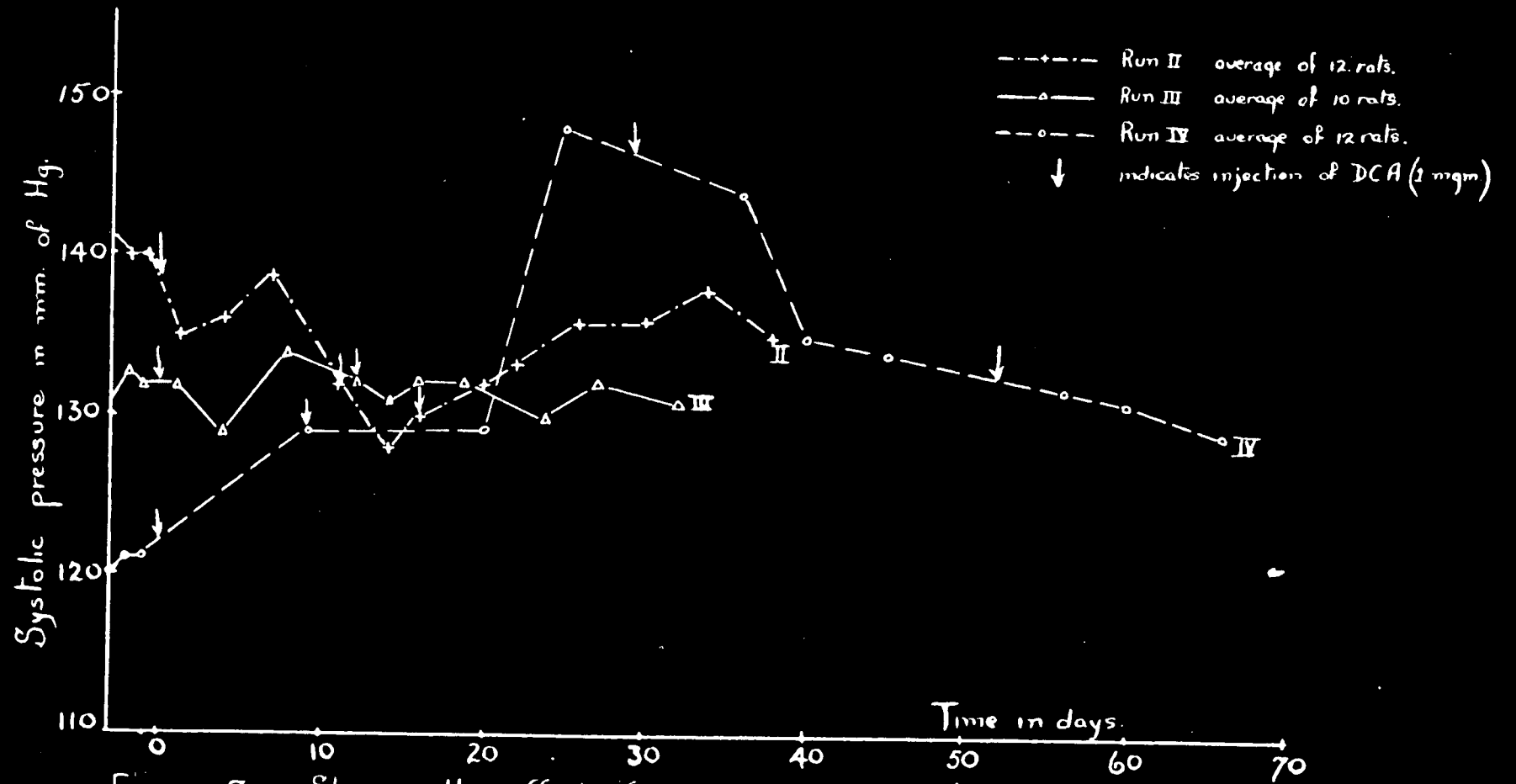


Figure 7. Showing the effect of repeated injections of D.C.A. (1 mgm.).

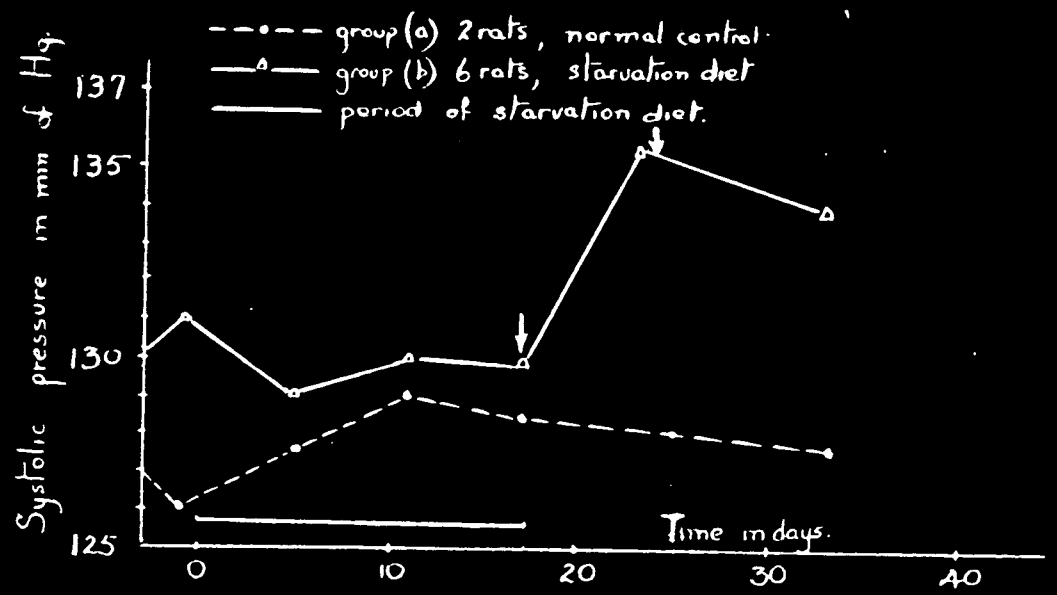


Figure 8. Showing effects of a starvation diet followed by a 1 mgm. injection of Desoxycorticosterone acetate (↓).

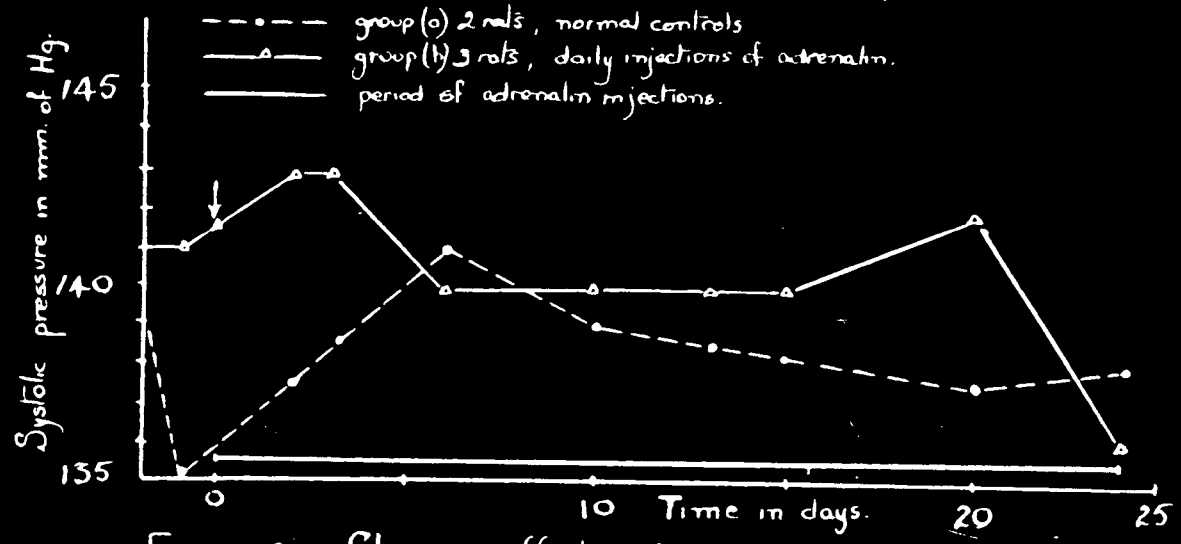


Figure 9. Showing effects of daily injections of adrenalin with a 1 mgm injection of DCA on day '0' (↓)

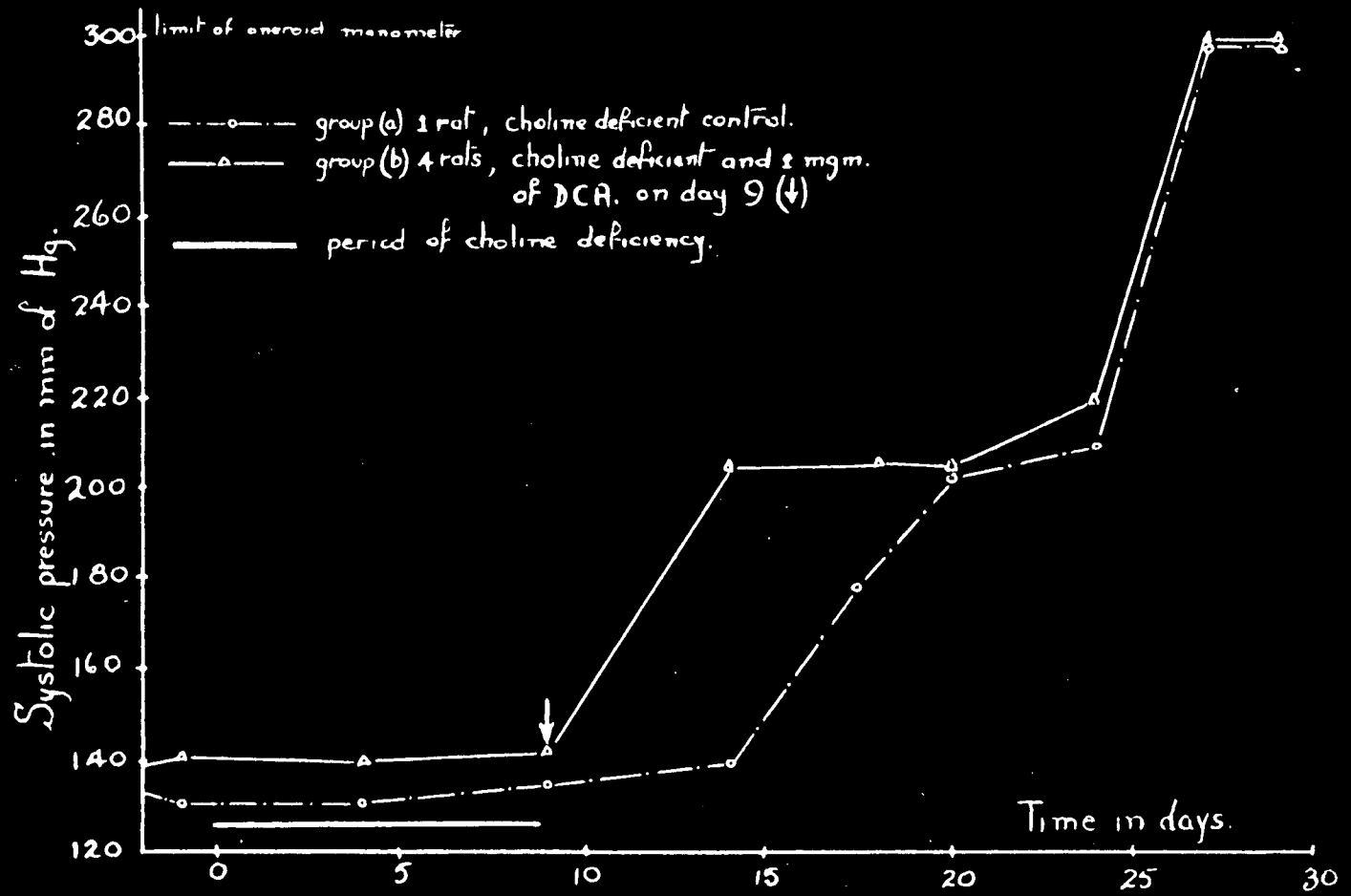


Figure 10. Showing effect of a 9 day choline deficient diet followed by a 1 mgm. injection of Desoxycorticosterone acetate.

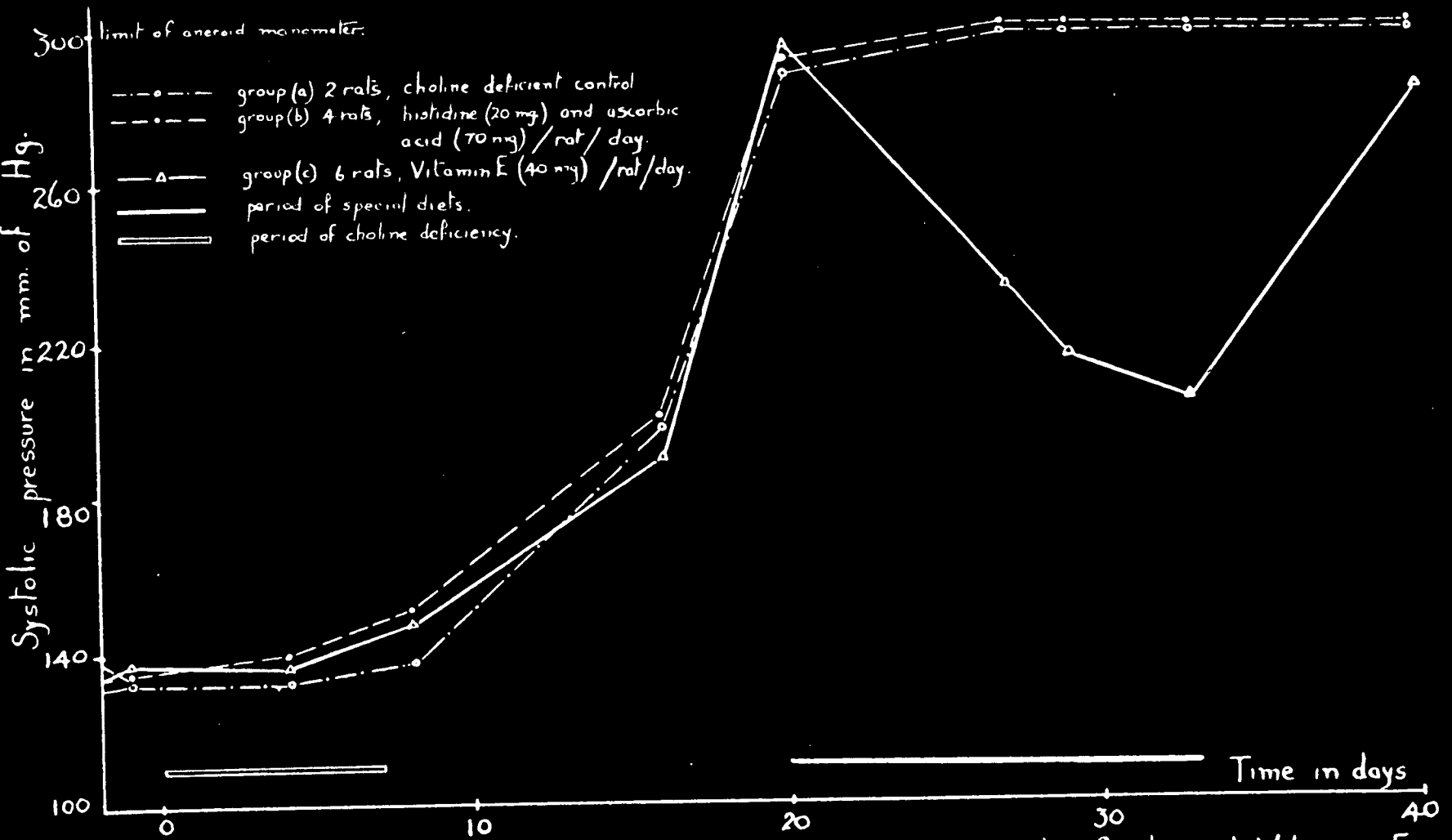


Figure 11. Showing the effects of histidine and Ascorbic Acid and Vitamin E on chronically hypertensive rats produced by a 7-day choline deficient diet.

# V. DISCUSSION OF RESULTS.

## A. METHODS IN PRODUCING EXPERIMENTAL HYPERTENSION.

### 1. DESOXYCORTICOSTERONE.

The use of desoxycorticosterone acetate has shown extremely varied results in the production of chronic hypertension. In the introduction, it was postulated on the basis of previous work, that repeated injections of desoxycorticosterone acetate (DCA) would be necessary for the production of chronic high blood pressure. But, as shown by the results of Run I (figures 3 - 6), a very marked hypertension occurred in all rats with a single injection (1mgm) of DCA. Values of 300 mm of Hg and over were recorded. Aside from the ameliorating of blood pressures in two rats with vitamin E therapy, all rats remained chronically hypertensive for the remainder of the test which lasted 68 days. Two rats from this run that were histologically sectioned by Logan (43) were still chronically hypertensive eight months after administration of DCA. These results differ from Fitch (19) and Semple (77), who obtained only acute, transitory hypertension by the same injection of DCA.

Runs II, III, and IV (figure 7) showed even more

divergence than the preceding run. No significant rise in blood pressure occurred with single or repeated injections (1 mgm) of DCA. These latter results were the ones obtained by Salter (64) of this laboratory, who worked primarily with male rats. The negative results of these experiments, however, were more in keeping with the literature, which indicate that very large repeated injections, or implants of DCA are required, with a diet supplement of sodium chloride (see introduction p.12 ). Although some workers are finding that lower amounts of DCA will produce the same effects, these amounts are still much higher than the dosage used in this experiment.

Histological inspection of renal tissue taken from animals subjected to overdosage of DCA has revealed that kidney damage in the form of nephrosclerosis is necessary for the production of hypertension. This explanation may account for the negative results in Runs II, III, IV (figure 7) in which 34 rats failed to reach hypertensive levels. However, it is inadequate to explain the results of Run I, since sectioning of kidney tissue from two rats in this Run by Logan (43), failed to show any kidney damage, even after hypertension of 8 months duration. It is clearly shown from this evidence that the hypertension produced in Run I was possibly due to some extrarenal mechanism. If it were possible to repeat the production of hypertension after the method of Run I by DCA injections, certain experiments could be carried out to determine any

neurogenic influence, namely, the use of sympatholytic drugs. These have been successfully employed by Ogden and associates ( see introducion p.14 ) in showing the influence of the nervous system in chronic hypertension.

The inability to produce even a marked rise in blood pressure by the intramuscular injection of DCA made it necessary to modify the technique. The modifications were (1), a starvation diet with subsequent injections (1 mgm) of DCA, and (2) the injection of epinephrine and DCA simultaneously, with subsequent daily injections of epinephrine (see introduction p. 16). As can be seen from figures 8 and 9, such modifications of these experiments proved unsuccessful.

However certain factors were established. Mr. Salter (64), a co-worker in these investigations, observed that both the starved rats which received DCA injections and also those which obtained no treatment with DCA, produced hypertension 3 months after the initial starvation, with average pressures of 195 mm of Hg. This evidence points to a possible dietary deficiency as an initiator of high blood pressure. Such a possibility has been suggested by various workers. Calder (10) and Durlacher and Darrow (18) have shown the production of hypertension by a diet deficient in the heat-stable fraction of the vitamin B complex, which can be reversed by restoring this fraction. However, the involvement of a choline deficiency could be evident here, as will be seen in

the following sections.

The experiments with epinephrine and DCA may show that desoxycorticosterone plays a role in counteracting the nervous system. Work done in conjunction with Mr. Salter (64) (reported in Mr. Salter's thesis) has shown that DCA injections sensitize rats to the pressor response of epinephrine. The results show that rats increase their sensitivity to the pressor response of epinephrine from 9 to 16 mm of Hg, over a 3 day observational period. But rats that were given daily injections of 1 mgm of DCA previous to a small amount of epinephrine showed a higher pressor response increasing from 16 to 32 mm of Hg. over the same observational period. These results might possibly indicate the participation of the nervous system in this pressor response. Page and Taylor (57) have recently found that injections of tetraethyl ammonium chloride (TEA), an agent which blocks sympathetic ganglia, increases the response of both pressor and depressor substances. Among these substances they found a manifold increase in the pressor response to epinephrine and angiotonin and in the depressor response of histamine. Corcoran (12) has taken this action of TEA to mean some central nervous influence which limits vascular responsiveness to pressor substances. DCA may have a similar effect in the inhibition of this neurogenic mechanism, thus increasing the response to epinephrine. However, more experimental evidence is needed to justify this hypothesis.

This will be referred to later.

2. CHOLINE DEFICIENCY.

As can be seen from figures 10 and 11 this method, as found by Best and Hartroft (see introduction p. 17 ), was the only definite means of inducing experimental hypertension. Best and Hartroft found that in a group of weanling rats which had been subjected to a 5 and 6 day choline deficient diet, hypertension occurred 4 to 7 months later, along with irreversible liver and kidney damage. However figures 10 and 11 show that in the case of this investigation only a few weeks ~~was~~ were necessary to induce hypertension by this method. Specifically in the case of Run VII, 17 days, and Run VIII, 13 days. The mean pressure of the most severe group obtained by Best and Hartroft was 195 mm of Hg, from which we can estimate the severest systolic pressure as being 260 mm of Hg, whereas systolic pressures in these experiments were consistantly above 290 with the majority over 300mm of Hg. However, several factors could possibly account for these differences in results: (1) The period of deficiency was longer in both trials; Run VII having a 9 day choline deficient diet and Run VIII a 7 day deficiency. (2) The use of weanling rats by Best and Hartroft rather than adult rats ( 6 months) as used in this investigation. Young rats may be more resistant to such changes. (3) The last factor is that the choline

deficient diet mixed for the purpose was not identical to that used by Best and Hartroft. A comparison will show a lack of "arachin" and "corn oil". However it does not seem likely that the unsaturated and saturated fatty acid metabolism would enter into the problem for such a short deficiency period.

Figure 10 represents the first attempt to obtain hypertension by a choline deficiency supplemented by DCA. As the graph shows the injection of DCA at the end of the choline deficient diet, raised the blood pressure infive days to a hypertensive level. However, this hypertensive level, an average of 205 mm of Hg, remained constant for 6 days until the blood pressure of the control, which received no DCA, rose to meet this level, at which time the blood pressures of all rats rose concomitantly beyond readable levels (300 mm of Hg). The manner in which these hypertensive levels were reached raises a very interesting point.

Is the mechanism which raises the blood pressure in choline deficient rats the same as in choline diet deficient DCA injected rats ?

We have two possible interpretations. If we assume that renal lesions produced by the choline deficienty initiates the renal pressor system as suggested by Best and Hartroft, then two possible mechanisms are involved in using DCA.

(1) DCA may act on the renal tissue causing an

earlier secretion of renin thereby allowing the renin-angiotonin mechanism to come into play more quickly.

(2) DCA may function in inhibiting a neurogenic mechanism as discussed in a previous section, in which it was noted that the inhibition of this mechanism sensitized the pressor response to angiotonin

## B. AMELIORATION OF HYPERTENSION WITH VITAMIN E.

Of the various ameliorating agents used in this investigation, only vitamin E or alpha-tocopherol seemed to be of value in chronic hypertension. In Run I (figure 3) rats which had been made hypertensive by a 1 mgm DCA injection, were given vitamin E and vitamin E in combination with histidine (see introduction p.17-18). In both cases the effects were similar in partially reducing the blood pressure. The ameliorating effects of the histidine and vitamin E combination were in the initial stages faster, although somewhat more irregular than vitamin E alone, possibly indicating the participation of histamine. However the final ameliorating results of the above procedure was similar to that obtained by vitamin E, and was considered as being primarily caused by vitamin E therapy. In Run VIII (figure 11), results almost identical with the first Run were obtained with alpha-tocopherol treatment on rats made hypertensive by a choline deficiency.

Although these latter finds seem more effective, it is explanable on the basis of a higher dosage of alpha-tocopherol.

The cessation of the vitamin E supplement re-established the hypertension to its former level. This fact shows very clearly that the presence of Vitamin E in excess amounts is needed for amelioration. To further substantiate this fact the special diets of the rats in Run I (figure 3) were reinstituted with previously noted results. This singular effect of vitamin E indicates its independant action, particularly if the hypertension induced by a choline deficiency and DCA are assumed to be different.

Recently Shute and associates (45) 1949, have shown a similar ameliorating effect by vitamin E therapy on hypertensive patients.. They state that all their evidence indicates vitamin E is a capillary dilator. However, they have found that alpha-tocopherol does not always show ameliorative action, and have come to the conclusion that some hypertensives have constriction higher in the vascular tree, perhaps at arterialar levels. They have found some evidence that estrogens may act as arteriolar dilators. In this respect, combinations of alpha-tocopherol and estrogens have proved useful.

From the results of our experiments this dilatory action of vitamin E seems quite probable, Also, the

presence of arteriolar constriction could very possibly account for the partial amelioration effects of vitamin E on these chronically hypertensive rats. It would therefore be of interest to find the effects of combined vitamin E and estrogens. Mr. Salter (64) of this laboratory, however has found estrogen injections capable of inducing high blood pressure. Nevertheless McGrath and Hermann (44) and Kaiser (36) have found prolonged and considerable dilation of capillaries resulting from intramuscular injections of estrogen.

## C. CORRELATION INADEQUACIES BETWEEN THE DATA OF ACUTE AND CHRONIC HYPERTENSION.

Attempts to ameliorate chronic hypertensions on the whole, have been unsuccessful. Fitch (19) and Semple (77), who worked exclusively on acute hypertension had definite ameliorative success. These workers found that histidine and ascorbic acid had a definite effect on lowering the systolic pressure of acute hypertension. In comparison, these substances were found to be ineffective in chronically hypertensive rats (Run I and VIII; figures 3 and 11) of a DCA and choline deficiency origin. The explanation probably lies in the basic dissimilarities between these two forms of hypertension.

## D. NEGATIVE RESULTS OBTAINED WITH UREASE AND AMMONIUM CHLORIDE.

The individual effects of urease and its effective replacement of ascorbic acid in combination with histidine (see introduction p. 18) has been found unsuccessful in ameliorating DCA - chronically hypertensive rats (Run I, figure 5). The use of histidine and ascorbic acid was found ineffective in the chronic stage but successful in ameliorating the acute stage of hypertension; therefore the inability of urease in replacing the reducing action of ascorbic acid and its singular effect in ameliorating acute hypertension is yet to be determined.

Ammonium chloride was also unsuccessful in ameliorating chronic hypertension produced by a DCA injection (Run I, figure 6). The literature finds that acidifying salts, such as ammonium chloride did lower the blood pressure of hypertension produced by larger doses of DCA. This hypertension, however, was always accompanied by nephrosclerosis. The effects of ammonium chloride are attributed to its balancing effects on the sodium metabolism (see introduction p.18). Since the DCA - chronic hypertension of Run I (figure 6) no such kidney damage was observed (Logan 43), therefore it would seem that sodium metabolism was not involved in this type of experimental hypertension.

## E. SUGGESTIONS FOR FURTHER RESEARCH.

Examination of the results of this investigation and their subsequent discussion, ~~raises~~ the following suggestions for further research:

1. The use of vitamin E in ameliorating hypertension needs to be investigated further.
   (a) Experiments could be conducted to discover the effects of vitamin E in preventing the production of experimental hypertension, and its ameliorative effects in acute high blood pressure.
   (b) To discover the maximum ameliorating action of vitamin E by prolonged therapy on hypertension.
   (c) To discover the effectivness of combined estrogens and vitamin E therapy on chronic hypertension.
   (d) To discover further the vasodilator action of vitamin E, by testing its ability to lower the blood pressure of normal rats. Such information would add further confirmation to this action.

2. The effect of histidine and ascorbic acid has been found ineffective in chronic hypertension and a comparable

situation arises in the replacement of ascorbic acid by urease. However, histidine and ascorbic acid were successful in ameliorating acute hypertensive. An experiment could then be carried out to establish the effectiveness of histidine and urease in acute high blood pressure.

3. To investigate the possibility of a dual mechanism between acute and chronic hypertension produced by (1) a choline deficienty and (2) DCA injections. Such experiments could be carried out by the use of sympatholytic drugs such as phenobarbitol and yohimbine. This procedure would possibly determine the influence of the nervous system on the problem, particularly in chronic hypertension.

4. Evidence in the effect of DCA in inhibiting the nervous system, as previously discussed, could be obtained in paralleling the response of pressor and depressor substances in rats singularly injected with either DCA and TEA (tetraethyl ammonium). The pressure response of angiotonin and adrenalin, and the depressor action of histamine might be determined.

## VI CONCLUSIONS.

1. The production of experimental hypertension by 1 mgm injections of desoxycorticosterone acetate has been found to be unreliable in female albino rats.

2. A choline deficient diet was found dependable in producing severe hypertension in adult female albino rats.

3. The daily feeding of vitamin E to female rats was shown to be partially effective in ameliorating experimental hypertension caused by both desoxycorticosterone acetate injections and a choline deficient diet.

4. The partial amelioration of hypertension resulted only in the presence of excess vitamin E. This fact was thought to give further evidence to the vasodilator properties of vitamin E.

5. The feeding of histidine in combination with ascorbic acid and with urease were ineffective in ameliorating chronic hypertension produced by a choline deficiency and DCA injections.

6. The feeding of urease and ammonium chloride individually have been shown in effective in DCA - chronic hypertensive rats.

The hypothesis was advanced that DCA may inhibit the sympathetic nervous system to explain the increased pressor response of epinephrine in rats injected with desoxycorticosterone acetate.

## SUMMARY

This investigation deals with the possibility of ameliorating chronically hypertensive female rats by means of the daily feeding of histidine and ascorbic acid, histidine and vitamin E, histidine and urease, vitamin E, urease, and ammonium chloride.

The apparatus used in determing the blood pressures was the indirect method using the foot, of Griffith and Farris, as modified by Allardyce, Fitch and Semple. Sodium pentathol was used as anesthetic.

Two methods were used to induce chronic hypertension (1) desoxycorticosterone acetate (1 mgm) injections. This method was found to give very diverse results, and was disregarded as unsatisfactory. Modifications of this method were used involving a starvation diet and epinephrine injections. Both of these modifications failed to produce hypertension. (2) The feeding of a choline deficient diet. This method proved consistant in producing a severe hypertensive state. A DCA injection was tried with the purpose of initiating an earlier hypertensive state.

Vitamin E was the only substance found useful in ameliorating the experimental chronic hypertension. This amelioration resulted in the presence of excess vitamin E. This fact was thought to give further evidence for the vasodilator properties of vitamin E.

An hypothesis was advanced that DCA may inhibit the sympathetic nervous system, to sensitize the vascular responsiveness to pressor and depressor substances.

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