The Amelioration of Experimental Hypertension by Histidine and Ascorbic Acid.

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ABSTRACT

Hypertension, induced in rats by desoxycorticosterone acetate, has been rapidly ameliorated by the daily feeding of histidine and ascorbic acid. Blood pressures were determined under pentothal sodium by the indirect method using the foot. Desoxycorticosterone acetate (1 mgm/rat) was administered intraperitoneally. Blood pressure elevations obtained in 2 separate experimental runs were nearly identical: an average increase on the 3rd day of 67 mm. of mercury above the pre-injection level, followed on the 4th day by a depression back to pre-injection levels, a rise on the 8th day to a maximum increase of 148 mm. and remaining above the hypertensive level from the 6th to the 14th days when daily readings were discontinued. Some of these rats were still hypertensive 27 days after the injection of the desoxycorticosterone acetate. The effect of daily feeding of histidine (20 mgm/rat) and ascorbic acid (75 mgm/rat) on the hypertension produced by desoxycorticosterone acetate indicated that this feeding could maintain the blood pressure below pre-injection levels whether the diet was started before, at the same time as, or after the injection and that the effect became apparent within 24 hours. Another experimental run showed that neither histidine nor ascorbic acid, when used separately, is capable of preventing the hypertension in rats caused by desoxycorticosterone acetate. These results suggest that: (1) the added histidine in the diet increased the histidine content of the intestinal tract;

(2) the decarboxylation of the histidine in the intestinal tract yielded histamine of which the subsequent detoxification by histaminose was inadequate in the presence of the added ascorbic acid; (3) the vasodilatory action of the histamine escaping detoxification effected the reduction of the hypertensive blood pressures.

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I. Introduction

Dr. Irvine H. Page, of the Cleveland Clinic, has said, "Medical research usually passes through four distinct stages: the first, awareness that a problem exists, but widespread indifference to it; the second, attempted formulation of the problem along with much speculation; the third, widespread intellectual and emotional disagreement among those concerned with putting the problem to experimental test; the fourth and last, agreement on the fundamental nature of the mechanism, application of the new knowledge to the care of patients, and commercialization." It is into the third stage that the problem of hypertensive or high blood pressure investigation has entered. While this stage is one rich with promise, it is essential that the confusion, arising out of its many conflicting points of view, should be counteracted by a thorough knowledge of the principles involved and a critical analysis of the present work.

With this consideration in mind, the following sections have been devoted to a discussion of the classification and pathogenesis of hypertensive diseases and a survey of the recent work in experimental hypertension.

A. Classification and Pathogenesis of Hypertensive Diseases.

Ever since Richard Bright observed, more than one hundred years ago, that hypertension implied by hypertrophy of the heart often resulted from certain renal diseases, blood pressure and the functioning of the kidney have been inescapably linked together. Since that day, so many conflicting theories and classifications of hypertension have been put forth, that a worker, new to the field, must approach the problem through the more conservative teachings of a basic text in physiology. The importance placed, by current workers, on the kidney in the genesis of high blood pressure is shown by the classification of hypertensive diseases given by Best and Taylor, (2), 1945.

These authors separate hypertension, on a clinical basis, into three main divisions: hypertension secondary to renal disease, essential or primary hypertension and malignant hypertension.

1. Hypertension Secondary to Renal Disease.

It is an accepted fact that sooner or later, chronic glomerulonephritis is followed by a general rise in the arterial blood pressure, consequent hypertrophy of the left ventricle, and atherosclerosis of the vascular tree. It is well established that the mechanism for this change in blood pressure lies in an increased peripheral resistance and is not due to an increase in the cardiac output or viscosity nor to a change in the quantity of the blood. Abramson (1), 1940, has found this increased resistance to involve the splanchnic area mainly.

Two prominent theories have been put forward on the mechanism by which the increased resistance is brought about.

(a) Reduction of the total vascular bed of the body by a reduction in the vascular bed of the kidney due to destruction of a large number of the glomerular capillaries. Best and Taylor (3), 1945, claim that this is unlikely since, in most animals (rat excluded), removal of one kidney and part of the other does not cause any rise in the blood pressure, the great adaptability of the circulatory system allowing for compensation upon removal of large parts of the vascular area.

(b) The second theory put forth by Tigerstedt and Bergman (40), 1898, is remarkably similar, as will be shown, to the modern conception proposed by Goldblatt (14), 1934. Tigerstedt and Bergman isolated a pressor substance from the normal kidney tissue of rabbits and postulated the theory that the faulty excretion of this substance was responsible for a generalized vasoconstriction.

2. Essential or Primary Hypertension.

As in the first type, the mechanism for this group of hypertension depends on an increased peripheral resistance and not on the other factors which can affect the hemodynamics of the body. By definition, essential or primary hypertension implies an increased blood pressure with no demonstrable kidney disease or change in kidney function. In time, degenerative changes will occur in the whole vascular tree and renal insufficiency may develop, but, as Albutt first proposed and, as Best and Taylor (6), 1945, maintain, this is entirely secondary to the hypertension. However, the primacy of the high blood pressure is not accepted by all workers.

The cause of the increased peripheral resistance in essential hypertension is unknown, but several theories have been advanced.

(a) Arteriosclerosis.

It is generally accepted that the usual senile sclerosis involving the larger arteries does not cause hypertension but many workers have attributed the cause of essential hypertension to a diffuse sclerosis of the systemic arterioles. Whether this sclerosis is primary or secondary to the hypertension is, at present, a matter of conjecture. However, Josue (24), 1903, by raising the blood pressure in a vessel by adrenalin injections or postural changes produced sclerotic changes in the vessel walls and showed that, in the rabbit, strain due to increased pressure can cause arteriosclerosis. This gives some indication that hypertension is primary to the arteriosclerosis.

(b) Nervous Influences.

For a long time, some workers have held that essential hypertension is due to an inherent hypersensitivity of the vasoconstrictor nerves and an exaggeration of the vasomotor responses. This theory is partially sustained by the fair degree of success achieved by sympathectomies where *hypertension is suspected of being of a neurogenic origin.

Although sectioning of the aortic branch (cardiac depressor) of the vagus nerve and the sinular branch of the glossopharyngeal nerve does produce hypertension, Thomas (39), 1934, has shown it to differ from essential hypertension in that increased cardiac output is the important factor in its production and that elevated blood pressures are of a transient nature. The bulk of essential hypertension has no apparent nervous connections and experimental neural hypertension appears unrelated. Investigators consequently have had to turn elsewhere in the search for increased knowledge of the etiology of essential hypertension.

*Local anaesthesia of the ulnar nerve at the carpal level visably increases the blood supply to the hand of a patient suffering from hypertension of a nervous origin.

While most workers regard this as a closed field, there are indications that prolonged experimental renal hypertension may have a neurological basis. This will, no doubt, provide the stimulus for further experimental work.

(c) Toxic Substances.

Probably the most frequently blamed substance is cholesterol which produces experimental hypertension and arteriosclerosis in rabbits. Best and Taylor (6), 1945, however, claim that no causal relationship between cholesterol and hypertension has yet been shown in man. Despite the fact that cholesterol has been discarded by some authorities, investigation is still proceeding in this field.

Pressor amines (tyramine and isoamylamine) formed in the colon by bacterial decomposition have been suspected and will be treated as a problem in a later section of this work.

(d) Endocrines.

The lack of success in demonstrating a pressor effect from the blood or serum of subjects of essential hypertension has been used by most workers to prove that the mechanism for essential hypertension is not hormonal. That this is not conclusive evidence against an endocrine mechanism will be discussed in the section dealing with the revent experimental work concerning the relationship between the adrenal cortex and hypertension.

While the pressor effect of adrenalin is well known, the speed with which adrenalin is used up in the body precludes the adrenal medulla from being a factor in any chronic high blood pressure except in rare cases of adrenal adenoma.

According to Best and Taylor (6), 1945, there is no evidence that

either the anterior lobe or the posterior lobe of the pituitary play any part in essential hypertension.

3. Malignant Hypertension.

Malignant hypertension is a condition in which acute high blood pressure comes suddenly while degeneration and occlusion of the peripheral vessels is wide spread. Glomerular destruction with renal failure and death in anuria may occur but more often death from cerebral hemorrhage takes place. Best and Taylor (8), 1945, find this condition to be the same as essential hypertension except more severe.

B. Survey of Current Experimental Work.

In the last twenty years, there has been a profound increase in the knowledge of secondary, essential and malignant arterial hypertension, largely through investigation in experimental hypertension. At present, high blood pressure is believed to represent a distinct pathological state rather than an effort on the part of an aging body to maintain circulation through narrowing vessels. It is no longer considered a disease of the heart, blood vessels, kidney or brain but a systemic process, damaging each organ to the degree that their blood vessels suffer morbid change.

The increased knowledge of arterial hypertension unfolded by the acceleration of research in experimental hypertension has opened up many new fields for investigation. Probably the role of the kidney, the participation of the endocrine system and the toxic effect of metabolic products are the most important and have received the most attention. Therefore in this section, discussion will be centered about these approaches.

1. Experimental Renal Hypertension.

Because of the work of Richard Bright it was natural that the earliest investigators would turn to the kidney in endeavouring to obtain the experimental hypertension so necessary for comprehensive work in this field. Thus we have the stimulus which started renal-vascular research some fifteen years ago.

(a) Experimental Work.

Goldblatt et al (14), 1934, were the first to demonstrate that experiments, supposedly producing renal ischemia, caused substantial and prolonged hypertension in laboratory animals (dog, rat and rabbit). His experiments consisted principally in partial occlusion of one renal artery with a silver clamp. This caused ischemia of the kidney on the same side and produced a moderate hypertension after three or four days with the blood pressure dropping back to normal in about two or three months. If the ischemia was bilateral or, after unilateral constriction, the normal kidney was excised, permanent hypertension resulted. The degree of hypertension corresponded to the degree of constriction of the renal artery. While a mild constriction caused hypertension with no observable change in renal function, severe constriction resulted in a severe hypertension but was followed by renal insufficiency and death in uremia. In the severe cases, changes in the vascular tree together with hypertension and renal insufficiency indicate a close relationship between the experimental condition and clinical malignant hypertension. Encapsulation of the kidney with cellophane by Page (31), 1939, and with gauze and collodion by Greenwood et al (16), 1939, produced the same results with fewer animal fatalities.

(b) Proposed Mechanism.

Goldblatt and most of the many workers in this field put forward a working hypothesis which is very well accepted today.

The ischemic kidney produces a substance known as renin which results in pressor or vasoconstrictor action. The hypertensive effect of renin is not dependent on the nervous system since denervation of the kidney, cutting of the splanchnic nerve, destruction of the anterior spinal nerve roots and total sympathectomy do not affect the pressure levels. This indicates that it is a humoral mechanism working directly on the peripheral vessels rather than on the vasoconstrictor center in the brain.

Renin, itself, is free from any pressor action. It is thermolabile and non-dialyzable.

Renin is found in both the normal kidney and in the normal urine but to a lesser degree than in the kidney and urine of the animal with experimental renal hypertension.

Helmer and Page (21), 1939, found that purified renin had no pressor effect until the addition of blood or plasma. This and further experimental work, established the theory that the blood contains a renin activator, believed to be a pseudoglobulin and called angiotonin-precursor (hypertensinogen). The reaction between angiotonin-precursor and renin, produces angiotonin (hypertensin) which is thermostable and dialyzable. This substance is active and exerts its vasoconstrictor action directly on the minute systemic vessels.

The experimental facts indicate that normal kidney tissue elaborates a substance with an inhibiting effect on the pressor principle, angiotonin. Such an inhibitory enzyme was isolated from normal kidney tissue and

received the names: hypertensinase, by its discoveror, Braun-Menendez et al (13), 1940; angiotonase by Page (32), 1940; and antirenin, by Wakerlin et al, (41), 1941.

It is suggested by Best and Taylor (4), 1945, that the reaction between angiotonin and angiotonase represents a normal body mechanism for balancing the blood pressure, as is shown by the liberation of renin into the blood of patients suffering from shock or any other condition giving an acute hypotension.

(c) Weaknesses in the Renin Theory.

(i) Despite the fact that serum from the vein of an ischemic kidney shows marked vasoconstriction in frog perfusion and despite the hypertensive effect on the host of a transplanted ischemic kidney as shown by Houssay et al, (22), 1938, workers since Katz (25), 1939, have been unable to show a pressor substance by means of transfusion and cross-circulation with hypertensive animals.

(ii) The actual stimulus for the production of renin is somewhat obscure. Since, according to Best and Taylor (5), 1945, constriction of the renal artery is very quickly compensated for by dilatation of the intrarenal vessels, there is nothing more than a momentary decrease in the flow of blood. This is against the conception of the ischemic kidney of Goldblatt (14), 1934, and sustains the belief of Page (26), 1940, that it is a change in pulse pressure that causes the liberation of renin. Goldblatt counteracts this by claiming that there is no pulse pressure in the capillaries where renin is liberated.

The theory that renin production depends on anaerobic conditions and that constriction causes this by depriving the kidney of some of its oxygen has been popular but Levy et al (29), 1937, have refuted this by

observing that there was no change from the normal arterio-venous oxygen difference during constriction. Because of the uncertainty of this point many workers now refer to the renal "ischemia" by the more general terms "changed intrarenal hemodynamics". While such vague terminology relieves much disagreement, it does little to further the knowledge on this point.

(iii) A serious shortcoming of the of the renin theory, as pointed out by Leloir (27), 1946, and supported by Wakerlin (42), 1946, is that, while there are definite amounts of renin in the blood of hypertensive animals for a short time after renal constriction, no renin has been demonstrated in the blood of animals subjected to chronic experimental renal hypertension of more than three or four weeks standing. Also, no renin has ever been isolated in the systemic vessels of humans suffering from chronic essential hypertension. These facts indicate that, while renin may play a rold in the instigation of hypertension, it is doubtful that it plays any apparent role in the maintenance of high blood pressure.

(iv) One final criticism of the renin theory is that, while Page (33), 1946, was able to observe renin in the peripheral blood of patients with acute nephritis or shock and severe eclampsia, no angiotonin or any other vasopressor has ever been demonstrated in the peripheral blood. It is Page's opinion that until vasopressor substances are found in the blood bathing the vessels which are chiefly responsible for the increased periferal resistance in hypertension, belief in a humoral mechanism must remain a matter of conjecture.

2. Role of the Adrenal Cortex.

The great emphasis placed on the role that the kidney may play in the pathogenesis of high blood pressure has to some extent distracted thought from other possible approaches. However, the adrenal cortex has

received a good deal of attention and is apparently involved in both the initiation of hypertension and the mechanism of experimental renal hypertension.

(a) The adrenal cortex as an initiator of hypertension.

In the last few years, the drug, desoxycorticosterone, an adrenal-hormone-like substance, has come into prominence as a replacement therapy for Addison's disease and experimental adrenalectomies. It has been subsequently noted by several workers (34, 37, 38), that desoxycorticosterone causes a marked rise in blood pressure along with destructive lesions in the vascular tree.

Selye (36), 1946, in his work on the adaption syndrome, observed that when experimental animals are subjected to the continuous stress of any non-specific damaging agent (e.g. cold) certain adaptive changes occur among which is a definite, persistent rise in the blood pressure. This hypertension was accompanied by a hyperplasia of the adrenal cortex. The mechanism for this, while unknown, is very naturally suspected to parallel that of the hypertension caused by injection of desoxycorticosterone.

Best and Taylor (7), 1945, maintain that, if the hypertension is caused by the over-elaboration of adrenal cortical hormones, transfusion of the blood serum into a second subject should show a vasopressor reaction. Because this apparently does not occur, the above authors have discarded the adrenal cortex as having any possible place in the etiology of hypertension. In the face of Selye's work, Best and Taylor may have been too hasty. This is especially true, since transfusion and cross-perfusion experiments should not be taken as absolutely conclusive evidence. The reason for this lies in that when the products of an unbalanced system in

one organism are transferred to a normal organism with its counteracting systems intact, lack of an expected reaction may be attributed to the counteraction of the normal organism.

(b) The adrenal cortex in experimental renal Hypertension.

Page (30), 1937, and Goldblatt (15), 1942, discovered that hypophysectomy lowered to normal the blood pressure of animals made hypertensive by renal ischemia and that replacement of the adrenal corticotrophic hormones brought the pressure up to the original hypertensive level. It was then found that a unilateral adrenalectomy would bring the experimental hypertension down to normal pressures and bilateral adrenalectomy would lower the pressure to lethal levels. Replacement therapy by desoxycorticosterone was found to bring the pressure up to its former hypertensive level. Following this work which indicated that the adrenal cortex was essential to the renin mechanism, Houssay et al (22), 1938, found that in short term experiments, transplantation of the ischemic kidney would make the host hypertensive even after total adrenalectomy. Lewis and Goldblatt (28), 1942, Houssay and Dexter (23), 1942, reconciled these conflicting reports by observing that after an adrenalectomy the concentration of angiotonin precursor was reduced. It is thus apparent that the cortical hormone is necessary for the production of the substrate upon which renin reacts.

In summarizing the role of the adrenal cortex, it would appear that this gland is able to initiate experimental hypertension by oversecretion and inhibit experimental renal hypertension by undersecretion. Since this must be done by the artificial injection of the hormone or extirpation of the gland, the clinical significance is not apparent at the present.

3. Role of the Toxic Metabolic Products in Hypertension.

A great deal of work has been carried out in an attempt to link toxic products of metabolism with hypertension. The most frequently suspected substances are pressor amines.

Bing and Zuchar (12), 1941, have shown that the pressor amine, hydroxytyramine, is formed in the acutely ischemic kidney by the decarboxylation of l-dihydroxyphenylalanine. The effect of tyrosinase (a phenolic oxidase) lends support to the theory that the pressor amine, tyramine may be the responsible agent in renal hypertension. This enzyme, as shown by Schroeder et al (35), 1941, lowers the blood pressure of animals with experimental renal hypertension and inactivates renin, adrenalin and tyramine, in vitro.

It has also been shown that extracts prepared from the ischemic kidney and tested for their enzymatic activity (amine oxidase and polyphenol oxidase) show less activity than those prepared from the normal kidney. An amino acid, carboxylase, which acts under anaerobic conditions has also been demonstrated in the kidney.

The above obeervations suggest that excessive amounts of pressor amines may be produced by the ischemic kidney as a result of an increase of anaerobic carboxylase activity and depression of oxidative enzymatic processes and that this, in part, may be responsible for the hypertension following constriction.

Among the arguments, which may be cited against this theory, are the facts that the ischemic kidney does not suffer anoxia, Levy et al (29), 1937, and that carboxylase is absent from the kidney of the rat, an animal in which renal hypertension is readily produced.

Since histamine has a known vasodilatory action, Best and Taylor (11), 1945, it would appear that amines may also play a part in reducing hypertension or in maintaining normal blood pressure. This is supported by the report that Wirtschafter and Widman (44), 1947, have ameliorated severe peripheral vascular disease and gangrene by injections of histidine and sodium ascorbate. The relief is believed to be achieved through the vasodilatory action of the histamine that is produced.

C. Statement of the Problem of This Thesis.

The complexity and the breadth of the field of hypertension investigation made difficult the task of narrowing the problem into a unit which would be in harmony with the limited time and experience of this worker. In considering the choice of a single phase of this many-sided problem and at the same time keeping in mind the above limitations, several conclusions were arrived at.

Firstly, in the study of experimental renal hypertension, all the work, results and conclusions are based on an artificially induced anatomical condition which apparently does not exist in the clinical study of hypertension. In view of this and the high degree of technical training required for the chemical isolation and assay of the substances involved in experimental renal hypertension, it was decided to investigate either the hormonal aspect of hypertension or the role played by the toxic products of metabolism.

Secondly, it was felt that, with the exception of Selye's work on the adaptation syndrome, recent investigation of the endocrines as a possible causative agent of hypertension was rather unproductive and suggested few

new points of attack.

Thirdly, the work done on the possible pressor effect of amines absorbed as toxic products of metabolism seemed both stimulating and full of promise.

Because of these considerations, it was then decided to investigate the possibility of affecting the blood pressure through action of pressor or depressor amines, by oral feeding of large doses of their respective amino acids.

Having come to this decision, a formal statement of our problem was formed, as follows:-

(i) To select and become practiced in the most suitable method of measuring the arterial blood pressure of white rats.

(ii) To select and become efficient in the most suitable means of raising the arterial blood pressure of white rats, to a hypertensive level.

(iii) To study the effect on the arterial blood pressure of white rats, with experimental hypertension, of oral feeding, both separately and together, of histidine and ascorbic acid overdoses.

It was felt that by overloading the system with histidine, there would be a larger amount of histidine converted to histamine in the colon by putrefaction processes. Histaminase, the enzyme responsible for the destruction of the histamine is an oxidizing agent and it was hoped that the ascorbic acid, a reducing agent, would prevent the destruction of the histamine, and hence, the vasodilatory action of the extra histamine might ameliorate the experimental hypertension.

II. Apparatus

Before any construction or development of apparatus could be carried out, the three possible methods of blood pressure measurement had to be considered and the most suitable one selected. The advantages and disadvantages of each method were very well discussed by Griffith and Farris (17), 1942.

A. Selection of the Most Suitable Method.

Because of the small size of the rat, the problem of reading its arterial blood pressure involves many technical difficulties not found in the simple auscultatory method for man. None of the three available methods are any more than adequate and selection of one merely means accepting the method with the fewest disadvantages.

1. Direct Method.

This method, which is standard laboratory procedure for large animals, involves the insertion into a large artery, of a cannula or needle which is connected, through physiological saline and anticoagulant, to a mercury manometer.

(a) Advantages.

This method is more accurate than either of the indirect methods since it gives a direct reading of the pressure within the artery. As well as being more accurate, it permits the reading of both the systolic and diastolic pressures.

(b) Disadvantages.

Because of the size of the rat, limb vessels are not available for cannulization and the worker is therefore limited to the abdominal aorta and the common carotids. The possibility of mechanical trauma influencing the blood pressure through the sinular apparatus of the carotid sinus, necessitates entering the abdominal aorta which usually results in the animal bleeding to death after the cannula is withdrawn.

2. Indirect Method Using the Tail.

In this method a pressure cuff is applied to the base of the tail of a rat, the pressure is suddenly increased well above systolic pressure and then gradually lowered to the systolic level when blood may enter but not leave the tail. The resulting increase in volume is measured on a simple water plethysmograph.

(a) Advantages.

(i) The same animal may be used over and over again.

(ii) Little technical skill is necessary in the use of the apparatus.

(iii) Anaesthetization not essential. Williams, Harrison and Grollman (43), 1939, were able to use this technique on unanaesthetized rats after warming and preliminary training.

(b) Disadvantages.

This method depends on lowering the pressure to a point where the flow of blood will start once more. Because of the inertia of the blood, pressures obtained will be lower than the true systolic pressure. This inaccuracy is greatly multiplied in reading hypertensive rats in which the systolic peak is thin and sharp.

3. Indirect Method Using the Foot.

This method involves the microscopic visualization of the blood flow in the cutaneous minute vessels of the foot and raising the pressure in a cuff around the thigh to the point where the flow is seen to stop.

(a) Advantages.

(i) Since the pressure is read as it increases, the error due to the inertia of blood is omitted and, thus the method is more accurate than that of the plethysmograph.

(ii) Measurements may be repeated indefinitely.

(iii) Relatively inexpensive equipment is required.

(iv) Due to vascular spasms there is occasionally a tendency to make the reading too low; a consolation in hypertensive work.

(b) Disadvantages.

(i) The rat must be anaesthetized, a condition which involves both an error due to anaesthesia and a risk to the animal.

(ii) The method requires considerable training for the microscopist.

(iii) The method is poorly adapted for conditions involving peripheral vascular spasm, i.e. neurogenic hypertension or hypertension from adrenalin injections.

4. Reasons for Selection of Indirect Method Using the Foot.

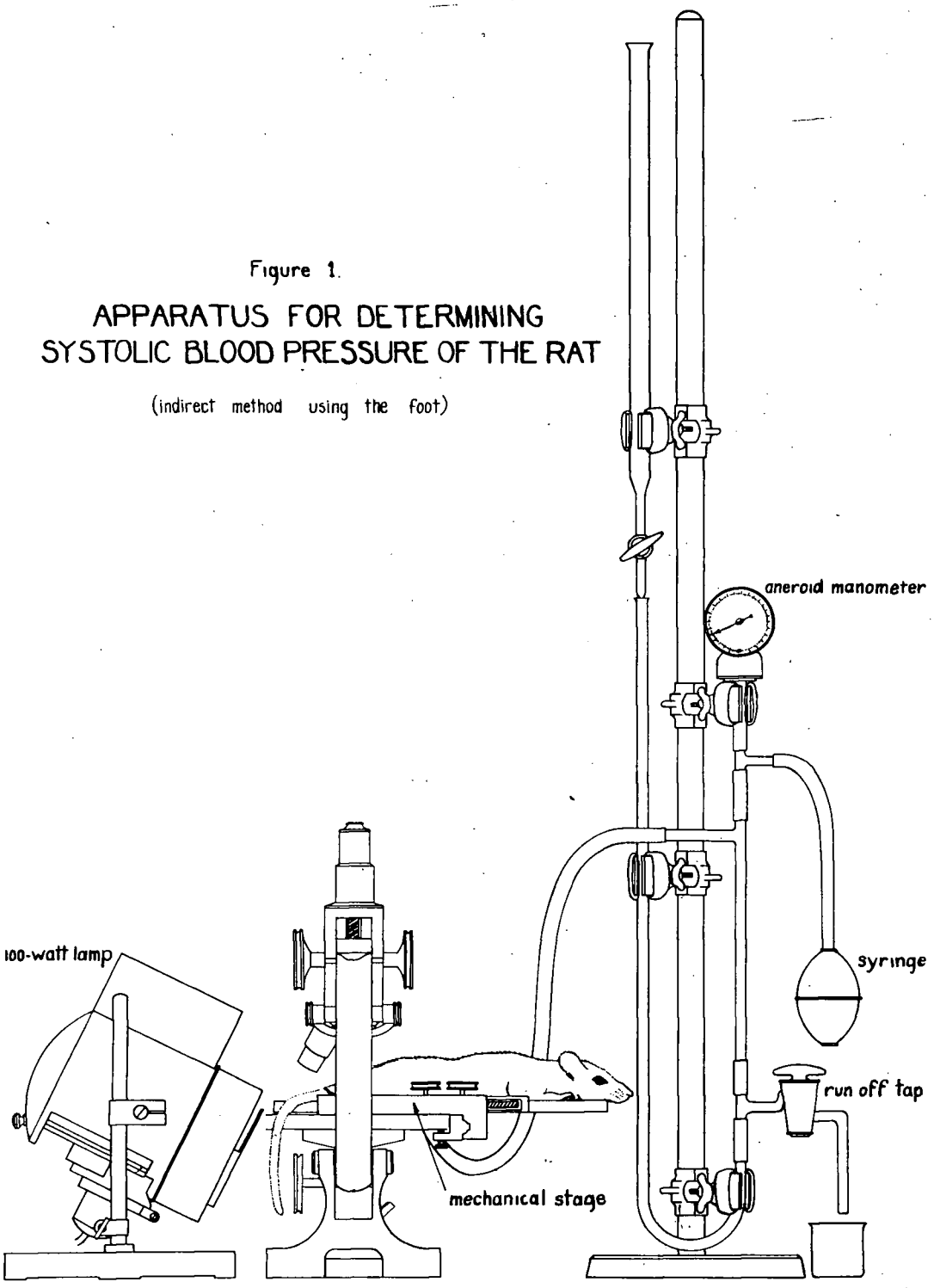
After considering the three methods, it was decided to use the indirect method of the foot. This method allows repeated readings on one animal, which the direct method does not. It has greater accuracy than the plethysmograph method. Further, any difficulty in giving the anaesthetic or in observing the flow of blood in the cutaneous minute vessels can be overcome with training and experience.

PLATE I.

Figure 1.

APPARATUS FOR DETERMINING SYSTOLIC BLOOD PRESSURE OF THE RAT

(indirect method using the foot)



B. Description of the Apparatus.

Griffith and Farris (17), 1942, gave an excellent description of the apparatus they proposed for this method of measuring blood pressure. Because of the amount of learning and practice that was ahead, it was decided to start with a simplified form of their apparatus until the technique was developed.

In the following sections, each unit of the apparatus will be discussed, dealing briefly with that used by Griffith and Farris, then indicating briefly the changes made to this and finally giving a description of the apparatus incorporating all the improvements decided upon.

A schematic diagram of the final apparatus is found on Plate I.

1. Lighting.

Griffith and Farris illuminated the field, on the rat's foot, directly with a 500 watt lamp passing through a 500 cc. flask filled with water to reduce the heat. This method of direct lighting was found to be greatly inferior to that of passing light up through a thin portion of the interdigital web of the foot. By the latter means, a 100 watt lamp proved quite satisfactory after a little experience.

One suggestion, to add a green filter to make the vessels stand out better, was tried and found no noticeable improvement.

2. Supporting the Foot.

The foot must lie supported, plantar surface upwards, in such a manner as to permit the unobstructed passage of light upwards through one of the webs. At the same time, there must be enough support to fix the foot in one position and to stretch the toes apart in order to show enough of the web.

At the start, the foot was held in place by three small artery clamps fastened to three of the toes. This method did not permit adjustment of the foot, required a deeper degree of anaesthesia and damaged the toes. Because of these disadvantages, it was decided to support the foot on plasticine as is shown in figure 3. The hind leg rests in the V-shaped notch of a rubber bar, .75 cm. above the level of the stage, while the toes are stretched over a plasticine crescent at the same level as the bar. The plasticine crescent circles around the light aperture in the stage. The toes are then lightly pressed down into the plasticine and held in place by small strips of plasticine. This method proved easy to perform, did not damage the foot, and did not bring the rat out of a light anaesthesia.

3. Making the Cuff.

In the indirect method using the foot, 4 mm., 7 mm., and 16 mm. pressure cuffs have been used. Since the 16 mm. cuff is reputed to give higher and more accurate results, it was adopted for this work. The preparation of this cuff is shown in figure 2 (a-f) and will be discussed below.

A sheet of thin latex rubber (glove rubber will do) 42 x 45 mm. was folded with a 1 cm. overlap to give a tube 45 mm. long and 16 mm. wide when flattened. The overlap was sealed with liquid rubber glue. One end of the tube was then squeezed together, the inside being moistened with glue, and 5 mm. of that were folded over and glued into place, this sealing off one end. Into the open end was inserted 1 cm. of 3 mm. rubber tubing reinforced from within by a short piece of glass tubing. The end of the cuff was moistened with glue, crimped around the rubber tubing and the joint wrapped with cotton thread. This produced an air-tight cuff of

PLATE II

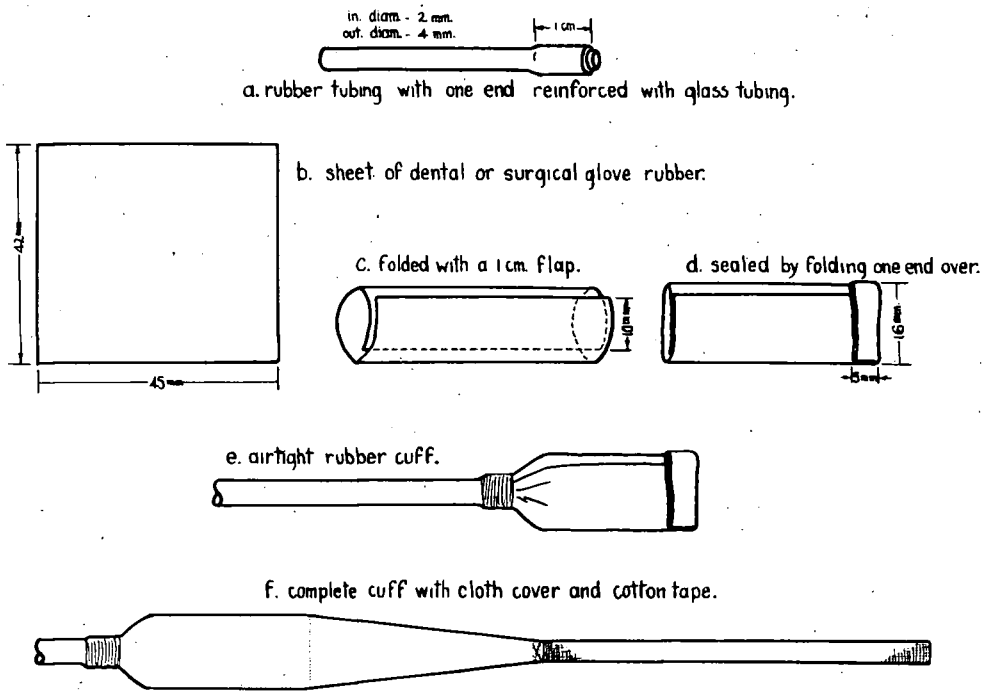


Figure 2. Preparation of the cuff.

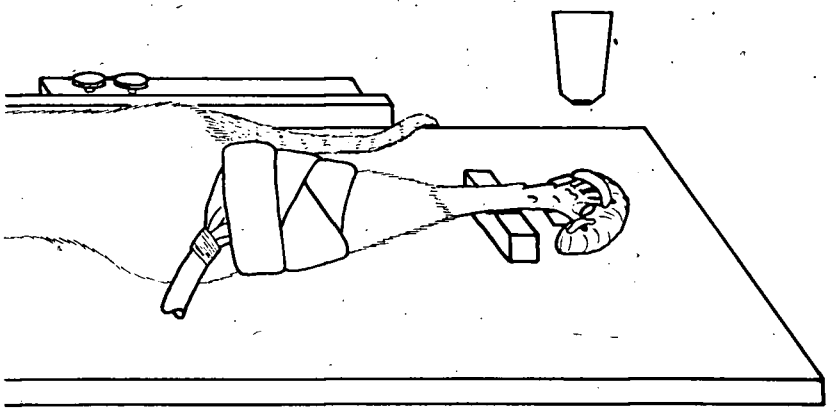


Figure 3. Arrangement with cuff attached and foot in place.

rubber. A snug cloth cuff was made to fit over the rubber cuff and taper out to a point in the same manner as the cuff of a sphygmomanometer. The entire cuff was 12 cm. long but difficulty in wrapping and fastening it around the rat's leg necessitated the addition of 10 cm. of .75 cm. cotton tape.

4. Supplying Pressure to the Cuff.

Due to the small amount of enclosed air in the cuff and tube, the syringe of a sphygmomanometer is not a gradual enough source of pressure. Therefore, following the apparatus of Griffith and Farris, a wooden box was made, 18x18x18 cm., inside dimensions, with a top which could be depressed by means of a centrally located screw. Inside the box, a standard football bladder was placed with a T-tube connecting the bladder to the cuff and to a pressure gauge. By depressing the lid of the box, 9 cm., the maximum thrust of the screw, it was possible to obtain pressures of the order of 80 mm. of Hg. Higher pressures could be reached by starting with an initial pressure in the bladder but it was difficult to turn the stiff screw with one hand against high pressures. Because of this, an adjustable mercury column was adopted as a source of pressure.

A glass U-tube was set up with one end open and the other leading to the cuff, a pressure gauge and a pressure syringe. A run-off tap for the mercury was placed at the bottom of the U-tube. A burette full of mercury was placed above the open end of the U-tube to permit a gradual and controlled increase in pressure. The pressure syringe was used to give the initial pressure in the cuff in cases of hypertension.

An aneroid manometer, 0 - 300 mm. of Hg. obtained from a sphygmomanometer made a very satisfactory pressure gauge.

5. Supporting the Rat.

Griffith and Farris describe a rather complicated mechanical stage. It is a bulky piece of apparatus which replaces the microscope stage. Because of the time required to make such an instrument, a simple immobile wooden stage was made to serve the purpose of supporting the rat until the requirements of this task became certain.

This stage consisted of a piece of three ply, 30 x 18 cm., one end of which lay upon the microscope stage, the other end was supported on the right by a block of wood. A hole was bored through the three ply to coincide with the hole in the microscope stage and to allow the light to pass up and through the foot of the rat.

Since the stage was immobile, readjustment of the rat's foot in the microscopic field became a continual delay. Because of this, it was decided to build a mechanical stage on which the whole animal could be moved under the microscope without disturbing the arrangement of the foot.

Accordingly, a piece of three ply, 22.5 x 9 cm. was attached to a Bausch and Lomb mechanical stage No. 31-59-53 in such a manner as to slide freely over the microscope stage. The plywood was oriented in the same position as the original stage, one end directly over the microscope stage, the other end projecting to the right. A square hole, 1 x 1 cm. was cut through the plywood to coincide with the hole in the microscope stage. The rubber bar and plasticine crescent for supporting the foot were attached around this hole.

A further improvement that might be suggested for this stage would be a support for the rat's head while it lies in an anaesthetized condition, belly downward on the stage. To insure drainage of saliva, the rat's head should hang down at a slight angle and a cradle might be made at the end of

the stage to support the rat's head in this position. Care should be taken in making such a cradle that there will be no pressure put on the trachea of the rat.

III. Methods

This section will contain a discussion of the problems encountered after the completion of the apparatus. The methods used to overcome these problems and to carry out the main problem of this thesis will be described in the order in which they occurred.

A. Measurement of the Normal Blood Pressure.

Anaesthesia presented some difficulties which had to be worked out before blood pressures could be determined. The use of the apparatus required considerable training before accurate results could be obtained.

1. Anaesthesia.

Over a period of 4 months, several anaesthetics were tried with very little success. The various anaesthetics, their methods of use, their effects on the rat and the final method that was adopted will be discussed below.

(a) Unsuccessful Methods of Anaesthesia.

(i) Pentothal sodium (first attempts). It was decided to try pentothal sodium (Abbott) (sodium ethyl-(1-methyl butyl) thiobarbiturate) because of its ease in handling, the speed with which it acts and is used up in the body, and its low toxicity. The pentothal sodium was made up in a 2.5% solution and injected intraperitoneally.

In the first attempt to anaesthetize the rats, 1 ampoule (.5 gm.) was dissolved in 20 cc. of hot water to give 20 cc. of 2.5% solution. Following the therapeutic dosages laid down by Griffith and Farris (19), 1942,

.4 cc. (10 mgm.) were injected intraperitoneally into two male rats. The only effect of this was a very slight paralysis of the hind limbs lasting for 30 to 45 minutes.

One ampoule of pentothal sodium was next dissolved in 20 cc. of cold sterile water to prevent any decomposition due to heat. When 3 rats were injected with .4 cc. of this solution, 1 was very nearly anaesthetized and the other 2 were little affected.

It was then suspected that the dosages of Griffith and Harris were too light and consequently two rats were injected with .6 cc. of 2.5% solution. Since neither of these rats was deeply enough anaesthetized 15 minutes after the injection, both received another .4 cc. of solution. Both rats were under enough to work within 5 minutes after the second injection but within 10 minutes, one had died of a respiratory failure.

Due to the possibility that the pentothal might have been absorbed too quickly, the next ampoule of pentothal was made up with a cold isotonic saline solution. This solution was then injected intraperitoneally into two rats, starting with .2 cc. of 2.5% pentothal and giving them repeated injections at 10 to 15 minute intervals until they went under. One rat was completely workable in 15 minutes with a dose of .4 cc. while the other rat received 1 cc. before he went under and then died almost immediately of respiratory failure.

Over the next few days, five rats were injected in this manner, small initial doses with extra injections every 10 minutes until the rats became sufficiently anaesthetized. The five rats received the following total amounts of pentothal before becoming workable; .4, .7, 1.0, 1.0 and 1.7 cc. The last four rats died of respiratory failure and tracheal blockage 5 to 30 minutes after becoming anaesthetized.

On the basis of the above results in which 6 attempted anaesthesias were not effective, 4 were effective and 6 resulted in death of the rats from respiratory collapse, it was decided to drop pentothal sodium as a means of anaesthesia and to adopt an anaesthetic involving less risk for the rats. It was felt, at the time, that the failure with pentothal sodium was due to one or more of the following causes: the solutions became progressively more toxic and less potent with time; there was not enough spread between the therapeutic dose (40 mgm./Kg. wt.) and the lethal dose (120 mg./Kg. wt.); the individual resistance to the drug's effect was extremely variable.

(ii) Ether. Ether was used as an anaesthetic for the next two months while apparatus for measuring the blood pressure was being developed and tested. At the same time a number of other anaesthetics were tried.

Each rat was placed in a cylindrical cardboard box, 18 cm. in diameter and 12.5 cm. high. Then a cotton sponge, wet with ether, was added. The rat was completely workable in 3 to 4 minutes and could be kept in this condition by intermittently putting a paper funnel with an ether-soaked sponge in the apex over its nose.

While this method was fairly effective (45 to 50 anaesthetizations with 3 fatalities), it was not desirable to make it a standard part of the technique since the volume of ether administered could not be measured and hence its effect on the blood pressure could not be treated as a constant. Further, the need for intermittant administration of ether while the rat was under the microscope required too much of the operator's attention. Also, constant surveillance was necessary to guard against a lethal overdose. However, the use of ether did make possible the

opportunity to work on the apparatus and, at the same time, try several other forms of anaesthesia.

(iii) Seconal. One capsule of seconal (sodium propyl methyl carbinyl allyl barbiturate) was added to 20 cc. of water. It only partially dissolved. Two rats were injected intraperitoneally, one receiving .5 cc. of this mixture, the other receiving 1 cc. The seconal reacted very quickly, causing loss of the voluntary control of the hind limbs, but the anaesthesia never became any deeper and its effects were gone within 20 minutes.

(iv) Carbritol. One capsule (3.6 gm.) of carbritol (Parke, Davis) was added to 20 cc. of water. Since it was even less soluble than seconal, it formed a mixture of fine particles suspended in the water. Two rats were injected intraperitoneally, each with .5 cc. of the mixture. This amount of carbritol produced little or no effect on the rats.

(v) Luminal sodium. One ampoule (.13 gm.) of luminal sodium, (Winthrop) sodium phenyl ethyl barbiturate) was dissolved in 2 cc. of water and 1 cc. of the solution was injected intraperitoneally. There was no effect in 10 minutes so an additional 1 cc. was injected. It took 30 minutes for the luminal to become effective. The rat was under the anaesthesia for 90 minutes, then died.

(vi) Paraldehyde. The knowledge that paraldehyde is widely used to induce anaesthesia in children resulted in its trial on rats. For children, it is given rectally in linseed oil. One cc. of a solution (2 parts paraldehyde, 1 part raw linseed oil) was injected intraperitoneally into a male rat. Complete anaesthesia developed in 11 minutes and the animal died in 130 minutes. Blood in the urine indicated an acute

inflammatory nethritis, death probably occurring through a profound visceral shock. One cc. of a solution (2 parts paraldehyde, 1 part Ringer's solution) was injected into a female rat with no better results. A second male rat was injected with .75 cc. of the paraldehyde and linseed oil solution and was dead within three or four minutes.

After these rather discouraging results, 5 rats were injected with smaller amounts (.3 and .4 cc.) of the paraldehyde solution. Only 2 of the rats were sufficiently anaesthetized to be usable. However, there were no deaths. Four days later 3 of the 5 rats were noticed to have open fistulas in the abdominal wall at the point of injection and, subsequently, had to be destroyed.

(b) Successful Method Using Pentothal Sodium.

After the unseccessful results from the trial of these anaesthetics, it was decided that probably the fault did not lie with the anaesthetic but rather in the manner in which it was prepared and injected and in the fact that the rats were placed belly up on the stage, thus increasing the risk of tracheal blockage. Accordingly, pentothal sodium was given another trial using a more careful technique and adding improvement gained from the series of unsuccessful attempts. In its second trial, pentothal sodium proved to be entirely satisfactory.

(i) Procedure for Successful Anaesthesia.

The first step in its successful use is to ensure that the pentothal is freshly prepared each day. The single ampoule contains enough powdered pentothal (.5 gm.) to make up into 20 cc. of solution, enough for several days of work. Because of this, the pentothal should be weighed out in smaller amounts and put in sterile glass containers sealed

with the type of rubber top through which a needle may be passed. A satisfactory amount of pentothal was found to be .175 gm. which added to 7 cc. of water makes enough solution for 12 to 14 injections. With this type of container, sterile water may be injected into the vial, the contents mixed and withdrawn in small amounts without being contaminated.

In injecting the pentothal, extreme care must be taken that the needle enters the peritoneal cavity and does not empty its contents into the abdominal wall. The slower rate of absorption of the pentothal in the body wall reduces its effect, yet, if multiple injections are attempted, the summation of the rates of absorption at each point soon exceeds the lethal limit.

An assistant holds the rat in a horizontal position, with the abdomen up, the head and front legs of the rat in one hand with the thumb under the rat's chin to prevent biting and the hind legs and tail in the other hand. He should be careful not to stretch the rat and should keep the abdominal muscles slack, permitting the easy insertion of the needle. The needle should be inserted, bevel-face downward, in a cephalo-dorsal direction, making a 45^0 angle with the body wall. The needle is then thrust inward until it is felt to penetrate through the body wall. It is then tilted toward the head of the rat and thrust forward 1.5 to 2 cm. being careful not to enter the liver. If any resistance is felt against expelling the contents of the syringe, the needle still lies in the body wall and must be withdrawn partially and thrust downward. If any injection appears only partially effective, the worker must conclude that the needle has not entered the intraperitoneal cavity.

After the injection the animal should be returned to a cage,

preferably separated from the other rats, and not disturbed until 2 or 3 minutes after the anaesthetic has taken affect, a total time of about 10 minutes.

As soon as the rat is ready to handle, it should be placed upon its belly and maintained in that position during the experiment and until the anaesthetic starts to wear off, 30 to 40 minutes after it becomes effective. This is extremely important in preventing blockage of the trachea with saliva and mucus.

As for arriving at a suitable dosage for each rat, if the pentothal is fresh and is correctly injected, .35 to .45 cc. of 2.5% solution will effectively anaesthetize 80% of the rats. It is advisable to start each rat with .4 cc. If this dose is insufficient for 2 consecutive days, it should be increased by .05 cc. each day until the rat goes under just enough to be usuable.

If at any time, after an effective dose for a particular rat has been determined, that rat should fail to respond to the dose, no more than .1 cc. should be injected on top of the regular dose. If the rat should fail to be anaesthetized with the regular dose and an extra injection of .1 cc., he should be left without being read until the next day and then the dose should be increased.

(ii) Rules for Safe Anaesthesia of Rats With Pentothal.

On the basis of the information gained from experimentation with the methods of anaesthesia and practice in the above, four important rules were developed for safe employment of pentothal sodium:

- The pentothal must be freshly prepared each day.
- The operator must be sure that the needle has entered the

peritoneal cavity.

- Once anaesthetized, the rat must be kept off its side or back to prevent tracheal blockage.

- The operator should never keep injecting pentothal until the rat becomes anaesthetized. If .1 cc. on top of the regular dose does not work, do not give any more that day.

It was the ignorance of all four of these necessary precautions that made the first attempts with pentothal so unsuccessful and it was the careful compliance with all four rules that gave the final record of 374 anaesthesias with only two fatalities.

2. Determination of Blood Pressure.

The rat is anaesthetized and laid, belly downward, on the stage, in the position indicated in Plate I. The position should be such that when one of the rat's legs is extended (the left hind leg for the purpose of description) the foot will fall directly over the light aperture in the stage.

The deflated cuff is then firmly wrapped around the thigh of the left leg. If there is a tendency for the cuff to slip downward, the end of the cotton tape should be brought forward and stuck to the stage with a piece of plasticine.

The light is then turned on and the foot properly oriented and attached to the stage as previously indicated. The web between digits iv. and v. has been found to afford the best view of the capillaries.

It is more convenient to arrange the cuff and the foot while the tube assembly of the microscope is removed.

The entire procedure with the microscope is carried out under low power, (x100).

The entire web should then be searched quickly, with the microscope, for the most satisfactory view of the vessels. Whenever possible, the operator should select the field with the largest number of capillary and precapillary vessels with a rapid visible flow. Since the flow in an individual vessel may appear to stop at low pressures because of arterial spasms, it is essential that more than one vessel be viewed and that the pressure be raised until the flow in all of them has stopped. Since the flow in vessels with a rapid initial movement of corpuscles continues at pressures which appear to stop the flow in vessels with a slow initial movement, only vessels with a rapid movement should be used in making the determination.

When the most satisfactory field has been located, an initial pressure of 100 mm. of Hg. should be built up in the cuff by means of the syringe shown in Plate I. Mercury is then run into the open end of the U-tube from the burette while the vessels are under observation. When the systolic pressure is reached and all flow has stopped the manometer is read and the pressure reduced by running off the mercury. After waiting 1 minute, another reading is obtained in the same manner, observing different vessels if possible. If the spread between the two readings is greater than 10 mm. of Hg., a third reading should be taken and the average of the higher two accepted.

3. Determination of the Normal Blood Pressure.

Before the rats could be made hypertensive and the amelioration of this hypertension attempted, it was first necessary to establish the normal pressure levels as a basis for subsequent experimentation. It was not

until the technique for the determination of the blood pressure had been developed that the experimental work could be planned.

So much time had been required in the development of these techniques that certain phases of the work had to be curtailed. The problem of determining how much certain environmental factors (time of day, time of feeding, temperature, effect of anaesthesia, etc.) influenced the blood pressure, had to be faced, not by carrying out a long experimental run on each factor, but by making each factor as constant as possible.

At the start of each experimental run the blood pressure of each individual rat was determined over a period of 2 to 6 days, the readings for each rat were averaged and the result accepted as the average or "normal" blood pressure for that rat.

B. Achievement of Hypertensive Levels Through the Use of Desoxycorticosterone Acetate.

In the selection of a suitable method of making the rats hypertensive, three considerations must be made. First, the hypertensive levels reached should fall well outside the limits of the experimental error and and daily variation of the normal. Second, the hypertension must be of a long enough duration to allow other experimentation to proceed during its maintenance. Third, the results of making the animal hypertensive must be standard and regular enough to be treated in a quantitative manner.

1. Selection of Desoxcorticosterone Acetate.

There are three main methods of raising the blood pressure of rats; the experimental renal hypertension of Goldblatt and Page, experimental neurogenic hypertension and the hypertension of Selye from desoxycorticosterone.

It was decided not to use experimental renal hypertension because it was based on an extremely unnatural anatomical condition and because the constriction of the renal artery or the encapsulation of the kidney would involve considerable operational risk for the rats.

Thomas (39), 1934, showed that experimental neurological hypertension differed from essential hypertension in that it is chiefly due to an increased cardiac output. Because of this and the operative technique necessary to produce the hypertension, it was discarded as a method of inducing hypertension.

Desoxycorticosterone acetate was accepted as a method of obtaining hypertensive levels. There is little technical skill necessary for its administration. Because it is injected, the degree of high blood pressure may be quantitatively controlled. Also, the use of desoxycorticosterone acetate, apparently follows a more normal mechanism than does experimental renal hypertension.

2. Method of Using Desoxycorticosterone Acetate.

Desoxycorticosterone acetate (Ciba) was obtained, dissolved in sesame oil, and suitable for intramuscular injection.

(a) First Experimental Run.

Since the available literature on the use of desoxycorticosterone acetate dealt with either its replacement after adrenalectomy or with gross overdoses to produce cardiac and renal lesions, the dosage necessary to produce a prolonged hypertension was not known.

It was decided to observe the effect of a single dose of 1 mgm. of the drug. Six rats, 3 female and 3 male, 11 to 12 months of age were selected. Their blood pressures were determined on 2 consecutive days and then 5 hours before their pressures were read on the third day, each was

given 1 mgm. of desoxycorticosterone acetate intramuscularily in the right thigh.

Because the method of anaesthesia had not been perfected, at this time, 3 rats died on the third day from the improper use of pentothal sodium.

The experimental run proceeded with the remaining rats, 2 males and 1 female. Their pressures were read daily for 17 days and again on the 28th and 30th days.

In figure 4, the graph represents the average of all rats for that day. Because of the rather unusual shape of the curve and the small number of rats used, it was decided to repeat this run.

(b) Second Experimental Run.

At this time, the determination of the blood pressure of 3 or 4 rats still took about 3 hours to complete, which imposed a severe limitation on the number of rats that could be used.

Consequently, for the second run, 4 rats were selected, 2 males and 2 females. As before, normal blood pressures were determined on two consecutive days. Immediately, after their blood pressures were determined on the second day, each was given 1 mgm. of desoxycorticosterone acetate. The rats' pressures were read daily for 17 days and again on the 27th day. As before, the readings were averaged and plotted (figure 5).

Since the two curves were remarkably similar and the hypertension was pronounced and maintained for a sufficient period, it was felt that the problem of the amelioration of this hypertension could now be attempted.

C. Methods Used in Studying the Amelioration of Experimental Hypertension.

Both the ascorbic acid and the histidine which were given to the rats are water soluble and could have been injected. However, the more natural method of oral feeding was adopted in an effort to keep as close as possible to natural conditions and thus throw more light on the mechanisms involved.

1. Feeding of Histidine and Ascorbic Acid.

In order to make the rats accept the histidine and ascorbic acid, it was necessary that it be mixed with a small amount of their regular food. Accordingly, amounts of histidine and ascorbic acid were weighed out and mixed with a given volume of the regular Fox-Chow feed (Purina Mills). The amounts and volume were such that when fed the mixture at the rate of 1 cc. per day, per rat, each rat would receive 20 mgm. of histidine and 75 mgm. of ascorbic acid. Sufficient of the Fox Chow was well ground and to this was added the histidine and ascorbic acid and the whole thoroughly mixed.

Since this small amount of prepared food was eaten more quickly when the rats were hungry, it was necessary to maintain a strict schedule with them. They were anaesthetized and their blood pressures determined in the late morning, then they were given their regular diet immediately afterwards. What was left of this was removed in the evening. After fasting over night they were hungry and ready to eat the small portion or prepared diet before being anaesthetized the following morning.

2. Using Histidine and Ascorbic Acid Together.

Since it was decided to determine the effect on experimental

hypertension of daily feeding of histidine and ascorbic acid, a group of 8 rats were selected and the following procedure carried out.

The rats were divided into 3 groups: group A, 1 male and 1 female; group B, 2 males and 2 females; group C, 1 male and 1 female. While receiving their regular diet of Fox-Chow, the blood pressure of each rat was read daily for 6 days. On day 6 each rat of groups A. and B. was given a daily feeding of histidine (20 mgm.) and ascorbic acid (75 mgm.) while group C. was continued on the regular diet. After being read on day 15, each rat of groups B. and C. received 1 mgm. of desoxycorticosterone acetate intramuscularly. On day 23, group C. was put on the histidine and ascorbic acid until day 31.

In this manner, group A., showed the effect of histidine and ascorbic acid on the normal blood pressure, group B. showed the effect of histidine and ascorbic acid on the blood pressure before injection of 1 mgm. of desoxycorticosterone acetate and group C. the effect after the injection of 1 mgm. of desoxycorticosterone acetate.

3. Using Histidine and Ascorbic Acid Separately.

Since it was necessary to determine the effect of feeding the histidine and ascorbic acid separately, another experimental run was planned.

Eight rats were again selected and divided into 3 groups: group D, 2 males and 1 female; group E, 1 male and 2 females; group F, 1 male and 1 female.

The blood pressure of the rats was determined daily for 6 days. Each rat was then injected intramuscularly with 1 mgm. of desoxycorticosterone acetate and the groups were fed as follows: group D, 20 mgm. of

histidine per day; group E, 75 mgm. of ascorbic acid per day and group F, 20 mgm. of histidine and 75 mgm. of ascorbic acid per day. The experimental run was continued until day 19.

D. Histological Methods.

It was felt that an investigation should be made of the amount of glomerulosclerosis caused by the desoxycorticosterone acetate and the possibility that the histidine and ascorbic acid therapy might partially prevent this sclerosis. With this in mind the kidneys of the rats in the 3rd and 4th experimental runs were prepared for sectioning. At a later date it was decided that the number of kidneys was too small to allow for the extensive statistical treatment that the evaluation of sclerosis required.

The kidneys have been put aside until such time as more material is available.

IV. Results

The results obtained fall into two main groups. First, the scattered blood pressure readings obtained while the apparatus and technique were being developed. Second, the readings obtained during the 4 experimental runs in which the hypertensive effect of desoxycorticosterone acetate and the ameliorative effect of histidine and ascorbic acid were investigated.

A. Results of the Initial Determinations of the Blood Pressure.

During the search for a satisfactory anaesthesia, it was possible to obtain only a few readings from the rats. Because the visualization of the minute vessels was seldom satisfactory and often vague, the readings obtained were extremely inconstant and low. They are discussed here, merely to give an indication of the amount of improvement that has taken place in the accuracy and consistency of the results.

During this period of development, 34 readings were obtained with values from 54 to 140 mm. of Hg. with 77% of these falling below 80 mm. The largest spread between 2 readings obtained from the same rat at the same time was 22 mm. While this indicates considerable inaccuracy, yet on two occasions, 3 readings falling within 5 mm. were obtained from one rat. This standard of accuracy was usually reached after the apparatus and technique had been perfected.

A probable explanation for the lowness of these readings (normal systolic blood pressure of the rat should fall around 120 mm. of Hg.) is that any error due to poor visualization tends to cause the operator to assume that the flow in the vessel has stopped before it actually has. Also, it has been noticed that the flow in large vessels appears to stop before that of the minute vessels. This phenomenon is probably due in the first place to the fact that the flow in large vessels is less apparent than the flow in minute vessels. Because of their prominence, large vessels were observed almost exclusively at first.

B. Results from Use of Desoxycorticosterone Acetate.

The results from the 2 experimental runs in which desoxycorticosterone acetate was used are presented in tabular form (tables 1 and 2) and in graphical form (figures 4 and 5).

The tables show the 2 daily readings per rat, giving the systolic blood pressure in mm. of Hg., and indicate the time at which the 1 mgm. of desoxycorticosterone acetate was injected.

The graphs show the deviation of the daily reading from the normal systolic blood pressure measured in mm. of Hg. and indicate the time at which the 1 mgm. of desoxycorticosterone acetate was injected.

Table I

Showing the systolic blood pressure, in mm. of Hg., two readings per day, of a group of rats injected with 1 mgm. of desoxycorticosterone acetate, 5 hours before being read on the day 3.

Rats	Time in days 1	2	3	4	6	7	8	9	10	11	12	13	14	15	16	17	28	30
1(f)	120	132	130	172	202	118	138	225	280	292	198	178	210	140	167	142	140	138
	126	128	138	178	206	114	134	230	286	296	192	174	205	146	170	152	142	142
2(f)	130	128	142	*														
	134	130	140															
3(f)	134	128	150	144	*													
	132	130	142	144														
4(m)	120	122	124	168	198	144	134	280	210	240	144	288	216	196	170	*		
	128	122	132	165	200	148	136	285	202	246	145	292	222	198	170			
5(m)	130	128	148	170	*													
	136	134	138	172														
6(m)	128	112	132	172	152	124	138	260	217	250	176	172	118	110	140	126	160	156
	124	118	124	168	155	124	140	268	208	258	169	166	122	114	142	134	152	150

* died from improper use of anaesthesia

Table II

Showing the systolic blood pressure in mm. of Hg., two readings per day, of a group of rats injected with 1 mgm. of desoxycorticosterone acetate after being read on the second day.

Rats	Time in days I	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	27
1(m)	126	128	110	145	160	148	203	192	174	240	226	175	166		280	160	262	208
	136	130	118	142	162	150	210	188	170	245	232	180	170		276	162	258	200
2(m)	138	138	172	*	205	100	144	178	230	286	270	245	200		150	248	268	190
	140	132	178		200	102	148	181	236	280	274	250	192		145	252	260	180
3(f)	117	126	180	185	218	118	*	230	250	286	290	220	180		286	205	236	134
	119	132	172	190	220	118		230	260	292	286	224	182		292	210	228	142
4(f)	114	122	138	150	192	124	188	218	170	292	270	202	176		200	240	272	176
	117	112	145	154	195	120	192	220	174	290	272	200	180		205	246	278	165

* went into collapse from anaesthesia (profound hypotension)

C. Results from the Use of Histidine and Ascorbic Acid.

The results from the 2 experimental runs using histidine and ascorbic acid, first together, then separately, are presented in tabular form (tables 3 and 4) and in graphic form (figures 6 and 7).

The tables give the 2 daily readings per rat of the systolic blood pressure in mm. of Hg., while the graphs show how the average of the 2 daily readings per rat deviate from the normal systolic blood pressure of each rat. The values for each separate group have been averaged and then plotted.

Table III

Showing the effect of histidine(20 mgm. per day) and ascorbic acid (75 mgm. per day) on the normal blood pressure (group A) and on the hypertensive blood pressure (groups B & C).

		Time in days																						
		1	3	4	5	6	7	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	27	29	31
A group	(m)	122	176	146	186	138	140	176	112	147	155	130	146	128	124	185	155	115	150	140	150	110	148	142	112	150
		127	168	154	184	144	156	180	118	151	148	132	146	124	126	178	160	120	149	138	158	118	145	145	120	140
	(f)	156	172	158	144	132	124	118	120	150	134	170	120	150	138	162	#									
		164	180	150	140	130	126	120	120	160	130	165	118	145	148	162										
B group	(m)	145	155	148	140	165	136	128	142	120	172	146	*	186	165	145	192	95	138	130	124	150	120	148	112	94
		142	162	150	132	156	134	126	146	120	176	140		184	172	150	196	105	140	132	130	158	120	146	116	96
	(m)	118	126	192	188	186	186	138	138	138	142	130	132	155	118	202	200	156	120	160	160	110	132	150	118	110
		122	134	184	182	188	188	144	138	140	142	136	134	150	122	210	200	156	124	156	154	120	132	148	114	105
	(f)	132	165	136	150	172	138	110	116	106	160	130	162	138	122	152	120	160	*	148	180	120	118	145	98	140
		128	163	132	150	165	134	112	110	110	158	125	158	140	124	152	125	155		156	186	118	120	155	102	136
	(f)	158	160	160	142	132	124	118	96	130	165	145	185	134	192	140	132	176	145	160	120	105	110	120	86	100
		158	166	164	136	134	126	122	104	128	170	150	182	128	196	136	140	180	145	156	118	98	102	114	78	104
C group	(m)	158	173	136	176	158	162	120	145	150	148	175	180	160	162	225	245	255	160	168	170	220	180	162	100	156
		152	181	148	176	162	175	124	146	152	152	178	182	158	166	232	250	260	168	170	174	216	176	166	98	162
	(f)	152	142	188	172	150	*	180	155	140	178	156	171	187	155	160	250	195	*	158	188	215	134	140	116	132
		150	146	194	166	156		182	160	145	186	160	168	192	160	160	248	200		168	180	210	140	140	118	124

A.regular diet	A.reg.diet & hist.,asc.ac.	A.reg.diet & hist.,asc.ac.	A.reg.diet & hist.,asc.ac.
B.regular diet	B.reg.diet & hist.,asc.ac.	B.reg.diet & hist.,asc.ac. (1mgm.desoxycort. on day 15)	B.reg.diet & hist.,asc.ac.
C.regular diet	C.regular diet	C. regular diet (1mgm.desoxycort. on day 15)	C.reg diet & hist.,asc.ac.

*insufficiently anaesthetized. #died from anaesthesia.

Table IV

Showing the effect of histidine (20 mgm. per day), group D, ascorbic acid (75 mgm. per day), group E, and histidine and ascorbic acid (20 and 75 mgm. per day), group F, on hypertensive blood pressure.

		Time in days													
		1	2	4	5		7	8	10	11	12	15	17	19
D group	(m)	101 96	*	156 151	100 102	histidine added daily from day 6.	120 128	134 136	188 192	155 165	182 180	190 190	175 178	150 146
	(f)	132 130	144 148	162 158	102 138		*	*	210 202	*	192 190	156 154	184 176	160 155
	(f)	*	166 166	134 132	120 120		152 152	172 180	140 146	*	180 180	208 208	186 186	210 205
E group	(m)	118 122	165 162	158 160	122 124	ascorbic acid added daily from day 6.	158 154	178 188	144 144	204 200	192 196	*	225 230	180 188
	(m)	130 134	145 142	168 166	128 132		142 144	210 205	250 250	*	262 272	244 246	*	162 168
	(f)	172 168	160 164	110 108	152 156		180 186	135 138	192 192	*	244 140	162 160	152 148	#
F group	(m)	178 172	118 112	122 122	120 118	histidine and ascorbic acid added daily from day 6.	140 142	131 130	160 162	104 110	154 148	134 132	112 112	102 92
	(f)	96 98	152 148	126 132	122 127		142 134	179 175	110 100	*	110 122	100 98	148 144	120 122

NB. all rats received 1 mgm. desoxycorticosterone acetate on day 6.
*insufficiently anaesthetized
#died from anaesthesia

PLATE III.

Figure 4.-Showing the effect of 1 mgm. of desoxycorticosterone acetate on the systolic blood pressure of a group of 3 rats.

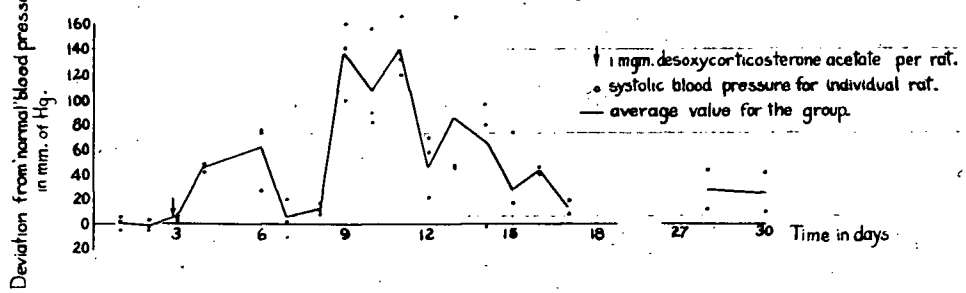


Figure 5.-Showing the effect of 1 mgm. of desoxycorticosterone acetate on the systolic blood pressure of a group of 4 rats.

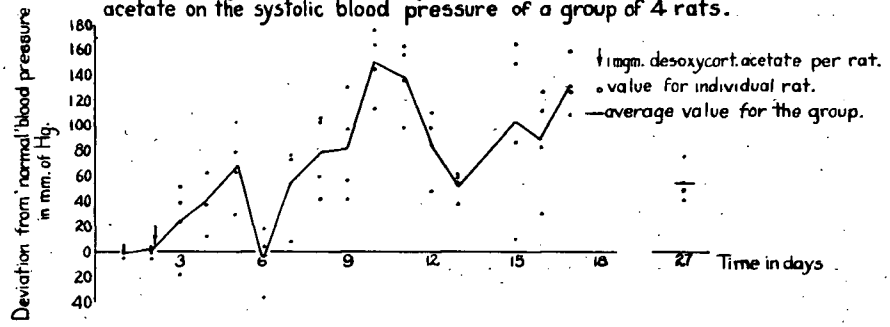


Figure 6.-Showing the effect of daily feeding of histidine + ascorbic acid on the blood pressure of rats injected with 1 mgm. desoxycort. acetate

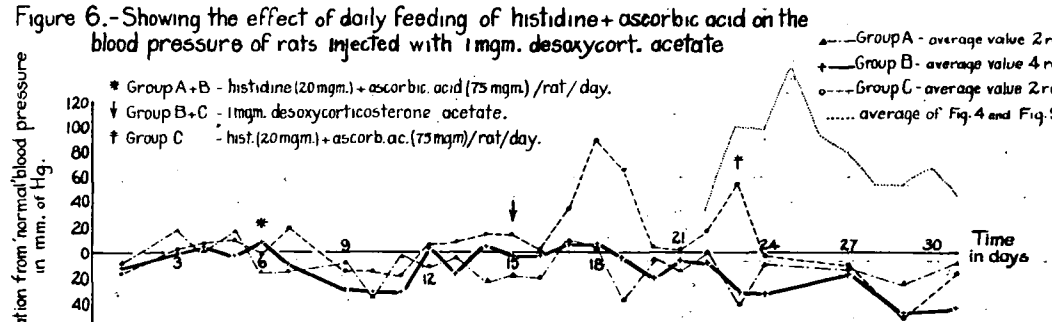
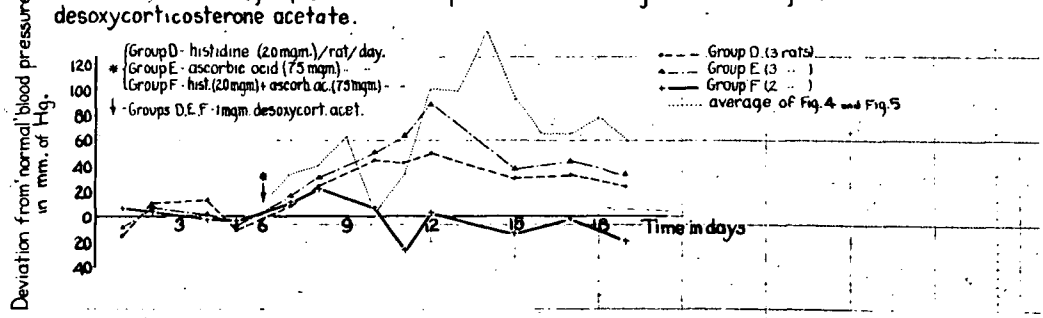


Figure 7.-Showing the effect of daily feeding of; histidine (group D), ascorbic acid (group E.) and hist. + ascorb. ac. (group F) on the blood pressure of rats injected with 1 mgm. of desoxycorticosterone acetate.



V. Discussion of the Results.

In this section, the value and significance of the results will be discussed. A few suggestions are made for further work and some clinical possibilities considered.

A. The Value of the Results.

The value of any experimental biological work lies, primarily, in the accuracy of the results obtained and in the number of animals upon which these results are based.

1. Accuracy of the Results.

There are 2 errors involved when the accuracy of these results are considered: (i) an error, chiefly human, which causes inconsistency in the 2 readings obtained from the same rat at the same time;

(ii) an error, lying in the apparatus and procedure, which causes a constant difference between the values obtained and the true arterial blood pressure. The evaluation of the second error is extremely difficult.

(a) Evaluation of the Human Error.

This error which is caused by the difficulty in observing the minute vessels is responsible for most of the spread in the 2 readings obtained from each rat each day. Under good conditions, 4 consecutive determinations have been made with a spread of only 2 mm. of Hg. However, under poor conditions, 2 readings may have a spread of over 10 mm. which necessitates a third reading.

In order to eliminate too much detail, the analysis of this spread has been limited to 1 experimental run. By random selection the second run has been chosen (table 2 and figure 5).

In this run, 66 determinations were carried out, 2 readings of the blood pressure per determination. The spread between these 2 readings was from 0 to 10 mm. of Hg. with 94% of these determinations falling within 8 mm. The mean spread was 4.9 mm.

While this spread indicates a definite error, it appears negligible when the increases in blood pressures investigated are of the order of 40 to 160 mm. of Hg.

(b) Evaluation of the Constant Error of the Apparatus.

Since all the conclusions drawn from the results will be based on changes in blood pressure, constant absolute error in this pressure is unimportant. However, it is of interest to evaluate the apparatus. This can be done directly by determining the blood pressure by cannulization. This method, however, offers no opportunity of estimating the surgical shock involved. It can also be done by comparing the normal blood pressures found with those obtained by other workers in the field. Unfortunately, the only data available was for dogs by cannulization or for rats by plethysmograph.

Griffith and Farris (18), 1942, working on young rats under ether obtained normal pressures ranging from 80 to 140 mm. of Hg., with an average of 110 mm. by means of the indirect method using the foot. By comparison, the 7 rats used in experimental runs 1 and 2, had normal pressures ranging from 116 to 137 mm. of Hg. with an average of 125 mm. Experimental runs 3 and 4, carried out on rats 4 months older, had normal

pressures with a range from 118 to 162 mm. of Hg. with an average of 146 mm.

2. Value of the Results Based on the Number of Animals Used.

In arranging the 23 rats used in the 4 experimental runs, it was possible to investigate the following conditions with the number of rats as indicated: the effect of desoxycorticosterone acetate on the normal blood pressure (9 rats); the effect of histidine and ascorbic acid on the normal blood pressure (6 rats); the effect of histidine and ascorbic acid on the hypertensive pressures when started before the injection of desoxycorticosterone acetate (4 rats), when started at the same time (2 rats), when started after (2 rats); the effect of histidine on the hypertensive pressures when started at the same time as the injection of desoxycorticosterone acetate (3 rats); and the effect of ascorbic acid when started at the same time as the injection of desoxycorticosterone acetate (3 rats).

While the small number of rats used for each condition does not allow a statistical treatment, the numbers are large enough and the results constant enough to give a clear, qualitative picture and an indication of what the quantitative picture will be.

B. Important Points in the Results and their Significance.

There are several salient features of the results of the 4 experimental runs. These will be discussed below along with their probable significance.

1. Features in the use of Desoxycorticosterone Acetate.

The interesting features in the results of the use of desoxycorticosterone acetate are: the degree of hypertension achieved, the duration of this hypertension and the shape of the curve showing the relationship between the degree of hypertension and time (figures 4 and 5).

(a) Degree of Hypertension Achieved.

The peak of the hypertension, which occurred in both experimental runs on day 8, showed an average increase in the systolic blood pressure of 143 mm. of Hg. in the first run and 151 mm. in the second run. At this time, 4 of the 7 rats showed a systolic blood pressure of over 280 mm. while the lowest blood pressure was 243 mm.

This would indicate, as far as the degree of hypertension is concerned that the use of desoxycorticosterone acetate is entirely satisfactory as a means of producing experimental hypertension in the rat.

The fact that the average blood pressure of the 7 rats on day 8 was 271 mm. may have a significance, since Grimson et al (20), 1944, showed that pressures from experimental renal hypertension averaged 200 mm. while pressures from experimental neurological hypertension (section of the moderator nerve) averaged 275 mm.

(b) Duration of Hypertension.

While a rise of 15 mm. of Hg. above that of the normal for a given age, is termed hypertensive in man by Best and Taylor (2), 1945, a 40 mm. rise is usually required in animal experimentation, Wakerlin et al (42), 1946. Accepting the 40 mm. rise as a standard, the hypertensive level was exceeded: in the first experimental run, from day 1 to day 3 and from day 6 to the end of the run; in the second

experimental run, from day 2 to day 3 and from day 6 to the end of the run, (all times measured from the day of the injection of desoxycorticosterone acetate).

The uninterrupted hypertension, 8 days in length, might well have been found to be longer if daily readings had been kept up, since 20 days after the start of this period, 3 of the 7 rats were found to be still hypertensive.

These results show the use of desoxycorticosterone acetate to be satisfactory in the production of acute hypertension and indicate that, by spacing the injections, it could be used in the production of chronic hypertension.

(c) The Similarity Between the Graphs in Figures 4 and 5.

The similarities between figures 4 and 5 indicate that desoxycorticosterone acetate has a constant and predictable effect on the blood pressure of the rat.

Both experimental runs reach a small peak on the third day after injection. The averages of each group are only 6 mm. of Hg. apart at this peak. Both averages have a large depression on the 4th day with only a difference of 9 mm. Both averages reach their maximum peak on the 8th day with a difference of 7 mm. Considering the variability of the blood pressure of the individual rat and the small number of rats used, the 2 graphs are remarkably similar.

The only marked dissimilarity between the 2 graphs is that the blood pressures of the rats of the second experimental run did not return as nearly or as quickly to the pre-hypertensive levels as did those of the first experimental run.

(d) The shape of the graphs in figures 4 and 5.

The result that would be expected from the injection of any substance responsible for raising the blood pressure is a direct rise in the blood pressure to a point of maximum effectiveness of the substance followed by: (i) a maintenance of this pressure if permanent damage is done to the body,

(ii) a smooth return to pre-hypertensive levels as the substance is used up, or,

(iii) a fluctuating return to the pre-hypertensive levels, indicating that a blanaced mechanism has been interfered with.

The latter condition appears to occur in the use of desoxycorticosterone acetate since the decrease in blood pressure of the individual rat from the 8th day is not staady but shows considerable fluctuation.

However, the increase of the blood pressure to a maximum peak also shows the effect of a very sensitive balancing mechanism. There is a constant increase to a peak (average, 67 mm. of Hg. above normal) on the third day after injection followed by a sharp drop back to the pre-injection level on the 4th day. This would indicate that the depressor factors of the balancing mechanism had been made hypersensitive and were able to overcome the first effects of the desoxycorticosterone acetate. The depression on the 4th day was followed by a more rapid increase to a higher peak (average, 100 mm. of Hg. above normal) on the 6th day. By the 7th day, the blood pressure of 4 of the 7 rats had dropped to 65 mm. above the normal. The maximum peak for all of the 7 rats was reached on the 8th day after injection of desoxycerticosterone acetate. It is the picture of a sensitively balanced mechanism which,

when disturbed by an outside influence, becomes more sensitive. The maximum effect of that outside influence is arrived at by a series of fluctuations of increasing frequency and magnitude.

2. Features in the Use of Histidine and Ascorbic Acid.

The important points of experimental runs 3 and 4, and their significance will be discussed under the 2 headings, "Histidine and ascorbic acid given together" and "Histidine and ascorbic acid given separately".

(a) Histidine and Ascorbic Acid Given Together.

The important features in the use of histidine and ascorbic acid given together are: their effect on the normal blood pressure, the degree to which they ameliorate hypertension caused by desoxycorticosterone acetate, the speed of the amelioration and the duration of this amelioration.

(i) Effect on the Normal Blood Pressure.

The effect of histidine and ascorbic acid on the normal blood pressure of rats is shown by group A (2 rats) and group B (4 rats) in table 3 and figure 6 between day 7 and day 15. The average blood pressure for each group was determined from day 1 to day 6. The average blood pressure for groups A and B while receiving histidine and ascorbic acid was 13 mm. of Hg. below the level at the start of the treatment.

The fact that histidine and ascorbic acid appear to cause a definite but not great depression of the normal blood pressure would indicate that the mechanism involved does not depend on the presence of an abnormal condition.

(ii) The Degree of Amelioration.

The main problem of the thesis was to investigate the ability of histidine and ascorbic acid to reduce hypertensive blood pressures. The success of this treatment is shown by the 9 rats of groups B, C and F, in tables 3 and 4 and figures 6 and 7.

The average blood pressure of group B (4 rats) went above the normal levels only on the second and third day after the injection of desoxycorticosterone acetate, and then only by 9 mm. of Hg. During the next 13 days, it went down below normal from 7 to 48 mm., with an average drop of 23 mm.

This would indicate that a daily feeding of 20 mgm. of histidine plus 75 mgm. of ascorbic acid per rat was more than is necessary for the total amelioration of the profound hypertension caused by 1 mgm. of desoxycorticosterone acetate.

Group C (2 rats) which received no histidine and ascorbic acid but was injected with 1 mgm. of desoxycorticosterone acetate showed the first peak (average of 85 mm. of Hg. above normal) followed by a depression (average of 12 mm. below normal) and began the second as was expected. On the 8th day after the injection of desoxycorticosterone acetate, they were put on the daily treatment of histidine and ascorbic acid. Within 24 hours, their average blood pressure was reduced from 55 mm. of Hg. above to 4 mm. below their normal level. For the remaining 8 days, their average blood pressure stayed well below their normal levels by an average of 20 mm.

This would indicate that the histidine and ascorbic acid diet is just as effective in reducing this hypertension when it is commenced after the blood pressures have been allowed to reach the hypertensive

level. It also shows that its effect appears within 24 hours of the start of the treatment.

Group F (2 rats) received 1 mgm. of desoxycorticosterone acetate and at the same time was started on a daily diet of histidine plus ascorbic acid. The blood pressure was above the normal level for the first 4 days after the injection, showing an average increase of 13 mm. and reaching a maximum of 24 mm. above the normal level. On the 5th day it dropped and showed during the remaining days, an average of 12 mm. below normal.

This indicates that a histidine and ascorbic acid diet, when started at the same time as the injection of desoxycorticosterone acetate is effective in preventing the blood pressure from reaching hypertensive levels. However, it is slightly more effective when started before the injections.

On the basis of these 3 groups, it is noticed that the histidine and ascorbic acid were least effective during the second and third day after the injection of desoxycorticosterone acetate. While this is not the period during which desoxycorticosterone acetate has its maximum effect, it is probably the period during which the most of it is absorbed.

(iii) The Speed of Amelioration.

On the basis of the results of group C (table 3, figure 6), it would appear that the depressor effect of histidine and ascorbic acid is shown within 24 hours. While this is not the time for the maximum effect of these two substances, it is enough, in the case of group C to reduce the average pressure 59 mm. and to a point below the normal levels.

(iv) The Duration of the Amelioration.

On the basis of the results of groups B, C and F (tables 3 and 4, figures 6 and 7), the effect of the histidine and ascorbic acid continued over a period of 25 days (group B), 8 days (group C) and 13 days (group F).

Moreover, the effect continued to become greater in all 3 cases rather than less. Whether this is due to an accumulation of histamine in the blood, an increase in sensitivity to the histamine, or to some other mechanism, is, of course, a matter of conjecture at this time.

(b) Histidine and Ascorbic Acid Given Separately.

As is shown in figure 7, while neither the feeding of histidine nor of ascorbic acid separately was effective in preventing the hypertension due to injection of desoxycorticosterone acetate, both showed some effect in preventing the maximum peak on the 8th day after injection and both appeared partially effective after this peak, with histidine slightly more effective than ascorbic acid.

The results of groups D, E and F would appear to support the theory that the amelioration which has been effected is due to the vasodilatory effect of histamine in the blood and that histidine is essential in its production and that ascorbic acid is essential in the prevention of its destruction. That the both are necessary has been clearly indicated.

C. Suggestions for Further Research.

Re-examination of the results obtained and of the theory upon which the present problem was based will indicate the direction of further work.

1. Proposed Theory Behind the Effectiveness of Histidine and Ascorbic Acid.

The parts of the theory that have been advanced to explain the mechanism involved in the ameliorative power of histidine and ascorbic acid include the following:-

(a) Diet can affect the blood pressure.

(b) Daily feeding of excessive amounts of histidine can overload the system with histidine and the content of the latter in the colon will increase.

(c) Histidine can be converted into histamine in the colon by bacterial decarboxylation without deamination and then be absorbed into the blood.

(d) Destruction of the histamine by histaminase, a biological oxidizing agent, can be offset or prevented by the presence of sufficient ascorbic acid, a biological reducing agent.

(e) The known vasodilatory action of histamine can reduce the hypertension produced by the desoxycorticosterone acetate.

2. Further Work Suggested by This Theory.

(a) Does the histidine content of the colon increase?

The assumption that the histidine content of the colon increases, while being logical, is nevertheless a matter of conjecture. It must remain so until a specific test for histidine has been applied to the contents of the colon.

(b) Does the histamine content of the blood increase?

Since the whole theory is based on the premise that the histamine content of the blood increases, conclusive proof of this would seem essential. This should be done, if possible, by a specific test for blood histamine. Since such a test is not available at the present, it could be demonstrated imdirectly by the use of an antihistaminic such as antistine (Ciba), 2 - phenylbenzylaminomethyl-imidazoline.

(c) Is it the reducing power of ascorbic acid that is essential?

This could be tested by trying other biological reducing agents in place of ascorbic acid.

With the answering of the above 3 questions, the theory would become tenable and the worker could turn to the consideration of the possible clinical applications of this theory.

D. Possible Clinical Applications of This Theory

There would appear to be a possible clinical application of this theory both in the reduction of acute and chronic hypertension, and as a prophylaxis against the start of essential hypertension. However, the possible harmful reactions of histidine and ascorbic acid must be considered.

1. Possible Harmful Reactions from Histidine and Ascorbic Acid.

Certain of the effects of histamine in the body are not desirable.

(a) Since histamine liberation appears to be the harmful factor in anaphylactic disturbances (e.g. asthma, hayfever), further

increase in its production would not be desirable for sufferers of an anaphylactic condition.

(b) The vasodilation by histamine is most pronounced in the cerebral vessels, Best and Taylor (9), 1945. Because of this, large doses are likely to cause cortical congestion, an increase in the cerebrospinal fluid pressure and severe headaches.

(c) Histamine is a gastric secrotogog and large doses have been used to induce experimental gastric ulcers in animals, Best and Taylor (10), 1945.

While the above 3 are illustrations of the harmful effects of histamine, they are the effects produced by large injections of histamine into the blood. It is doubtful if the gradual production of histamine by means of histidine and ascorbic acid would be great enough to cause these harmful effects.

2. Possible Use in the Amelioration of Acute Hypertension.

The degree and speed with which the daily feeding of histidine and ascorbic acid was able to reduce the profound hypertension in rats caused by desoxycorticosterone acetate would suggest that the same treatment might be beneficial in temporatily reducing the blood pressure in acute hypertension.

3. Possible Use in Controlling Chronic Essential Hypertension.

While it is not known, on the basis of this work, whether the effectiveness of histidine and ascorbic acid would decrease with time, there is nothing to indicate that this would happen. It is quite possible that histidine and ascorbic acid might be useful in controlling chronic high blood pressure. The duration of its effect on rats could easily be

tested by carrying the treatment over a period of months on rats subjected to repeated injections of desoxycorticosterone acetate.

4. Possible Use as a Prophylaxis Against Hypertension.

While this section is based largely on conjecture, it may prove stimulating to thought and it can be readily put to test.

The traditional treatment for high blood pressure for many years has been the removal from the diet of as much of the protein content as possible. This treatment is empirical and the mechanism uncertain.

The results submitted in this thesis indicate that the amino acid, histidine, in the presence of ascorbic acid can cause an extreme reduction in the hypertensive blood pressure of the rat. Since tyramine has a known pressor effect, Best and Taylor (11), 1945, it is possible that tyrosine, in the presence of ascorbic acid, could cause corresponding rises in the normal blood pressure. On the basis of this, it would appear that all amines arising from metabolism of protein should be tested for their vasodilator or vasoconstrictor properties.

In considering the classic treatment for hypertension, it would seem possible that the regular protein diet was made up of more tyrosine-like amino acids than histidine-like amino acids.

If this were the case, most of the common protein sources could be analysed for their amino acid content and a sufferer of hypertension might be able to eat a selected diet containing food high in histidine-like amino acids. This would have the dual advantage of helping to ameliorate the hypertension and not subjecting the body to an almost protein-free diet.

Such a diet might be used as a prophylaxis against that small rise in blood pressure which so often initiates the vicious circle of hypertension and arteriosclerosis.

VI. Conclusions.

In considering this problem, as laid down in the introduction, and the results as obtained from the investigation, the following conclusions can be made:

(i) The indirect method (using the foot) of determining the blood pressure of the rat has been developed, as described, and found to be satisfactory.

(ii) Desoxycorticosterone acetate has been found to be an effective method of raising the blood pressure of rats. It caused a profound degree of hypertension, gave easily reproducable results and required much less skill and care than does the experimental renal method of Goldblatt.

(iii) The daily feeding of histidine and ascorbic acid was shown to be an extremely effective method of ameliorating the hypertension in rats caused by the injection of desoxycorticosterone acetate.

(iv) The histidine and ascorbic acid was found to be effective under the 3 following conditions: when started 10 days before the injection of desoxycorticosterone acetate; when started at the same time as the injection; when started 8 days after the injection.

(v) Histidine and ascorbic acid, when fed separately, showed little ability to reduce hypertension caused in rats by the injection of desoxycorticosterone acetate.

The theory has been advanced that the amelioration of this hypertension is due to vasodilation by histamine in the blood. The histamine is believed to be produced in the colon by the bacterial decarboxylation

without deamination of the amino acid, histidine. The ascorbic acid, a biological reducing agent, is believed to retard or prevent the destruction of histamine by histaminase, an oxidizing agent.

While the conclusions drawn above support this theory, conclusive proof would rest upon the demonstration of an increased amount of histidine in the colon and of histamine in the blood and that it is the reducing action of the ascorbic acid that is effective.

VII. Summary

It was decided to investigate the possibility of ameliorating hypertension in rats by means of the daily feeding of histidine and ascorbic acid.

The indirect method, using the foot, of Griffith and Farris (17), 1942, was adopted as the method of determining the blood pressure of the rats. The apparatus was modified, as shown in Plate I. After trying several anaesthetics, pentothal sodium was finally accepted and proved to be entirely satisfactory.

Desoxycorticosterone acetate was injected intraperitoneally into the rats (1 mgm./rat) in order to produce the hypertension. By this method, 7 rats were treated in 2 separate experimental runs (tables I and II, figures 4 and 5). The results of the 2 runs were nearly identical: an average increase, on the third day, to 67 mm. of Hg. above the pre-injection level, followed by a depression back to pre-injection levels on the 4th day, a rise to a maximum increase of 148 mm. of Hg. on the 8th day, remaining above the hypertensive level from day 6 to day 14 when the runs were stopped. Of the 7 rats, 3 were found to be hypertensive 27 days after the injection of desoxycorticosterone acetate.

An experimental run of 8 rats was then started to investigate the effect of the daily feeding of histidine (20 mgm./rat) and ascorbic acid (75 mgm./rat) on the hypertension produced by desoxycorticosterone acetate. It was shown that this feeding could maintain the pressure below pre-injection levels when: (i) the diet was started 9 days before the injection (4 rats), (ii) at the same time as the injection (2 rats),

(iii) 8 days after the injection (2 rats). It was also shown (2 rats) that the effect of this diet became apparent within 24 hours.

Another experimental run of 8 rats was started to investigate the effect of daily feeding of either histidine (20 mgm./rat) or ascorbic acid (75 mbm./rat) on hypertension caused by the injection of 1 mgm. of desoxycorticosterone acetate. This run showed that neither the daily feeding of histidine nor ascorbic acid separately is capable of preventing the hypertension caused by the injection of desoxycorticosterone acetate.

These results support the theory that has been proposed that:

(i) by overloading the system with histidine, the histidine content in the colon is increased.

(ii) the histidine in the colon is converted to histamine by the bacterial decarboxylation without deamination of the amino acid.

(iii) the ascorbic acid, being a biological reducing agent, prevents the destruction of the histamine by histaminase, an oxidizing agent.

(iv) the vasodilatory action of histamine effects the reduction of the hypertensive blood pressure.

While the experimental results support this theory, conclusive proof would rest on the demonstration of an increased blood histamine content.

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