The Amelioration of Experimental Hypertension by Histidine and Ascorbic Acid

by

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I Introduction

Although hypertension has received a great deal of attention from many investigators in recent years, and although several different experimental approaches have been made to the problem, the etiology of most forms of the disease in man remain uncertain. The unusual problems facing the experimental worker in this field have had a lot to do with the slow progress that has been made. One of these problems consists of the inability, up to the present, of the worker to induce in an experimental animal, a state that is truly representative of hypertension in man. However, in spite of this and other obstacles, some progress has been made.

A. Classifications of Hypertension and Review of Literature

There have been a number of suggested classifications for hypertensive diseases, the most recent of which has been presented by the Russian investigator Kogan-Yasny (24). This classification is based on: a) local tissue humeral changes; b) circulating pressor substances in the blood; and, c) changes in the vascular walls. Best and Taylor (2) recognise only two forms: the first of these classes results from, or is secondary to, renal disease, while the second is essential, or primary, hypertension. The etiology of the latter form, which is also the more common form, is not yet clear. Malignant hypertension is also included under essential

hypertension, being considered a severe form of this type.

Experimentally, the problem has been approached from a number of angles. Fitch (11) reporting in 1947 the early work done on hypertension in this laboratory, gave a very detailed review of these approaches and the theories resulting therefrom. Consequently the account given here will not repeat his review but will place emphasis on reports published during the past year.

Among the more important experimental approaches to hypertension, the author would enumerate the following:

1. Renal Origin.

This method was first used in 1934 by Goldblatt and his co-workers (15). Hypertension was induced by means of unilateral nephrectomy in combination with partial occlusion of the renal artery above the remaining kidney. Page (27) achieved a similar result by encasing the kidney in a cellophane capsule to produce nephritis. An improvement on this latter method has been recently suggested by Abramms (1). This consists of using latex for the capsule, and it is reported to be a relatively safe and simple procedure to attain renal constriction. Bilateral application of these capsules resulted in rises in the systolic blood pressure of white rats of up to 100 mm. Hg. over normal.

As a result of investigations along these lines, the reninangiotonin theory to explain renal hypertension was advanced. Essentially this theory postulates that the kidney elaborates renin, a proteolytic enzyme inert in itself but

possessed of the ability to catalyse the breakdown of the substrate, hypertensinogen. This substrate, also hypertensively inert, is elaborated in the liver. The breakdown of hypertensinogen, however, results in the liberation of angiotonin (hypertensin), the active pressor substance. Angiotonin, in turn, is destroyed by the enzyme angiotonase (hypertensinase). The latter is found in kidney, erythrocyte, and other tissues.

Goldblatt (14) has recently summarized the evidence supporting this theory, and the concept of a renal origin for the essential hypertension in man. He concludes that it would be very surprising, in view of this evidence, if eventually hypertension did not prove to have a renal origin. At the same time he concedes a possible secondary role to the hormones of the adrenal cortex.

The theory of a renal origin has been supported by some clinical evidence. For example, Yuile (40) reports several cases of hypertension accompanied by obstructive lesions in one or more renal arteries.

Renal hypertension itself is claimed to be established by means of a humeral mechanism. Ogden et al (26) feel that this has been established. Recently these workers have reported that the hypertension that follows the application of a clamp to the renal artery disappears if the clamp be removed within 10 days. The hypertension becomes chronic if the clamp is applied for longer periods. One of their conclusions from this work is that the nervous system plays no

part in the establishment of renal hypertension, but may have a role at a later stage of the condition.

The renal theory has not gone unchallenged, however, and one of the strongest arguments against it has been the phenomenon of tachyphylaxis. (The progressively diminishing pressor responses to intravenous injections of renin.) Page and Taylor (29) have found that the administration of tetraethylammonium ion overcame this phenomenon. Thus the action of angiotonin, itself musculotropic in function, was affected by a substance that acts upon the nervous system by blocking the sympathetic ganglia.

These latest investigations, then, have raised some interesting questions, principal amongst which is the problem of why the action of a musculotropic substance should be affected by the use of a neural drug. This work with tetraethylammonium also raises doubts as to the validity of the conclusions drawn by Ogden and his co-workers (26), in regard to the role of the nervous system.

2. Nervous Origin.

The concept of nervous influences playing a major role in the etiology of hypertension has existed for some time. It has been pointed out that the strain of living under modern civilized conditions could bring on such a condition. Best and Taylor (3), however, show that Life Insurance statistics, on comparing urban and rural people, fail to substantiate such an argument.

A good deal of work has nevertheless been done along

this line. Schafer (32) and Thomas (36) have reported achieving hypertension by the complete section of the aortic depressor and carotid sinus nerves. In opposition it is pointed out by Wiggers (38) that the overall pathological picture in such a neurogenic hypertension is very different from that of essential hypertension. In the former case peripheral resistance remains practically unaltered and the hypertension induced is the result of cardiac acceleration, with a resultant increase in cardiac output. The reverse is true of essential hypertension.

Holt et al (21) found that stimulation of the carotid sinus nerve results in the lowering of the systolic blood pressure by 47%. This is accompanied by a decreased cardiac output as well as decreased peripheral resistance.

The action of the sympatholytic drug, pentaquine, has been investigated clinically by Fries (13). It is said to exhibit a pronounced depressor effect; a dose of 120 mg./day lowering, in 10 days, the systolic blood pressure of a typical hypertensive patient from 220 to 150 mm. Hg. The drug induced hypotension in patients with normal blood pressures. Pentaquine was not recommended, however, for clinical use because of its toxic effects.

What would seem the most convincing evidence favouring a neural genesis of hypertension comes from Medhoff (25) and Farris (9). These workers have exposed rats, from weaning onwards, to noise blasts of 10 minutes duration daily. Hypertension developed with age. This work would support Selye(33)

who has referred to hypertension as one of the possible "diseases of adaptation", in connection with the general-adaptation-syndrome.

3. Vascular Origin.

The suggestion has been made that the arteriosclerosis of hypertension may be the cause, rather than the effect, of the disease. Actually there is little evidence for this belief to date, although King (22) has shown that there is a definite thinning out of the elastomeric constituents of the aortic wall and an accumulation of collagenous fibres and other deposits with age. Freedman (12) suggests that a hyaluronidase deficiency, if long sustained, might actually change the vessel walls to such an extent that a compensatory increase in blood pressure takes place in order to maintain the normal tissue-blood fluid exchange.

4. Hormonal Origin.

In spite of the fact that authorities like Best and Taylor (3) discard the possibility of an endocrine origin for hypertension, and others allot such a mechanism only a possible secondary role, Goldblatt (14), there remains a certain amount of evidence to be explained before such a decision can be made with certainty.

Knowlton et al (23) at Columbia University have confirmed previous results that overdosage of rats with desoxycorticosterone acetate (DCA) resulted in renal and cardiac hypertrophy, atrophic changes in the subcapsular zone of the

adrenal cortex, and hypertension. Such rats received normal amounts of NaCl. If, however, the intake of NaCl was drastically reduced, the effects of the steroid were abolished or reduced to a minimum. Grollman (20) has had considerable clinical success in ameliorating hypertension in man by means of drastically reducing the NaCl intake. Although the practical value of such treatment was questioned because of the difficulty in establishing the restrictions necessary, nevertheless the possible connection between these two investigations is of the greatest interest.

The work of Soto (34), in Mexico, is of interest in this connection. He reports that overdosing mice with such substances as DCA failed to produce the typical pathological symptoms (renal lesions, etc.) that have been repeatedly confirmed with rats.

Further evidence involving the adrenal cortex has come from Victor (37) who obtained immediate and sustained hypertension in dogs by a unilateral partial ligation of the periadrenal blood vessels and tissues. Such treatment produced elevations in the systolic blood pressure within three days. Six months later, the systolic blood pressures of the animals operated on were reported to average 125 mm. Hg over normal. Goldblatt et al (16) reported that they were unable to induce hypertension by repeating this procedure.

However, that adrenal cortex function and blood pressure are interrelated is indicated by Cawadias (5). He reports the case of a man suffering from hypotension and other "classic

symptoms of adrenal insufficiency". A post-mortem on this case revealed advanced carcinoma of both adrenals, with metastases in one kidney.

Selye's "general-adaptation-syndrome" (33) suggests that hypertension may be endocrine as well as neural in origin. (See neural origin.) He states that the disease may "...represent the by-products of the endocrine reactions that are at play in the general-adaptation-syndrome." On the basis of this evidence alone, it would seem too early to write off the possibility of an endocrine origin for essential hypertension.

B. The Approach to the Problem in This Investigation

The success achieved by Wirtschafter and Widman (39) in ameliorating peripheral vascular disease by injections of histamine and sodium ascorbate, together with the well-known vasodilatory action of histamine, Best and Taylor (4), pointed the way to the investigation of the action of histamine in experimental hypertension.

1. Previous Report From This Laboratory.

Fitch (11) has shown that hypertension can be induced, in the albino rat, by a single small intramuscular injection of DCA. Such hypertension was subsequently ameliorated by the feeding of excess histidine and ascorbic acid; moreover, if such feeding was started at the same time as, or before, the DCA injection, no hypertension resulted. If either the histidine, or the ascorbic acid were omitted, hypertension

developed.

The amelioration described above resulted, it was thought, from the action of excess histamine. Fieser (10) and other authors have confirmed the fact that the decarboxylation of histidine can be accomplished in the intestine by several strains of B. coli. Normally such histamine would be destroyed by the oxidative enzyme histaminase. However, this could be prevented by the presence of ascorbic acid, a potent reducing agent, in excess.

Reports recently published by Ellinger (7) (8) were of interest in the light of the results obtained by Fitch (11). Mice, irradiated with lethal doses of X-rays, developed characteristic pathological conditions including fatty degeneration of the liver. These changes, it was determined, were the result of the elaboration of total body tissue breakdown products, the most important of which was histamine, or a histamine-like substance. The daily administration of DCA showed a protective action against the liver degeneration, and brought about a reduction in the mortality rate. These two effects were correlated, and, within limits, their magnitude was proportional to the dose of DCA. It seemed possible that the phenomenon reported above was the reverse of that under observation in this laboratory. In work reported by Ellinger (7) (8) the effects of an excess of histamine were ameliorated by daily dosing with DCA, whereas in the work previously reported from this laboratory the effects of excess DCA were ameliorated by making excess histamine

available.

C. Statement of the Problem of This Thesis

Inasmuch as this work was initiated in association with Fitch (11), the factors that author took into consideration in the formulation of an objective for his work apply equally to this investigation. Time and experience, as is usual with a thesis of this nature, were limited. Accordingly it was necessary to focus attention on a very limited phase of hypertensive work. The phase chosen was the possibility of affecting blood pressure by the oral feeding of amino acids, through the pressor or depressor action of their respective amines.

The author also shared the problem of developing, and learning to use efficiently, a suitable apparatus for measuring blood pressure in the albino rat.

Taking the above facts into consideration, a statement of the problem was formulated as follows:

1. To develop a suitable method of measuring the arterial blood pressure of the albino rat, and

2. To confirm the report that the oral feeding of excess histidine and ascorbic acid ameliorated the hypertension induced by a small injection of DCA, and

3. To investigate the mechanism of such amelioration if confirmed.

II Apparatus and Methods

A. Apparatus

It was decided to measure the blood pressure of the rats by the indirect method using the foot. The basis for this method is described by Griffith and Farris (17), and the modifications found useful in this laboratory have been reported by Fitch (11).

Essentially the apparatus was designed to allow the visual observation of the blood flow in the capillaries of one of the interdigital webs. This flow was blocked by means of an inflatable cuff which encircled the thigh of the leg under observation. The pressure in the cuff could be adjusted coarsely with an ordinary syringe, or finely by means of a mercury column. The stoppage of blood flow so induced was observed under the low power magnification (x 100) of the microscope. The systolic blood pressure thus obtained was read directly from an anaeroid manometer.

The assembly of the apparatus used by the author is pictured in Fig. 2a.

B. Methods

1. Preliminary Work.

The work done during the early stages of this investigation was concerned almost in its entirety with two problems. The first of these was the problem of finding a safe and

efficient means of anaesthesia, the second concerned the mastery of the technique involved in obtaining accurate blood pressure readings, in reasonable time, with the apparatus described above.

Anaesthesia:

The lack of success with the several anaesthetics first tried, and the success finally achieved with Pentathol Sodium (Abbott), as well as the reasons for these results, have been reported in detail by Fitch (11). It will suffice here to list those tried, over a period of three months, with little or no success (from a point of view of consistent day to day work):

a) An initial trial of sodium ethyl (1-methylbutyl) thiobarbiturate. (Pentathol Sodium) (Abbott).

b) Ethyl ether.

c) Sodium propylmethylcarbinylallylbarbiturate. (Seconal).

d) Carbritol (Parke Davis).

e) Sodium phenylethylbarbiturate (Luminal Sodium) (Winthrop).

f) Paraldehyde.

As mentioned previously, success was finally achieved with Pentathol Sodium. The measure of this success can be judged by the fact that during the early attempts with the anaesthetics enumerated above, one in every three attempted anaesthesias resulted in the death of the rat, and the majority of the remainder were ineffective. However, during the four hypertensive runs to be described, there were 280

satisfactory (as defined below) anaesthesias, for a total of seven fatalities.

Pressure Readings:

Early blood pressure readings were also unsatisfactory. Systolic pressures obtained varied from 50 to 140 mm. Hg., but were generally low (50 to 85 mm. Hg.). Then too, these early readings were inconsistent. A range of 20 to 30 mm. Hg. for two separate determinations on an individual rat was not unusual. Both the low values for normal pressure, and the inconsistency of the readings, were eliminated when the errors induced by the three faults outlined below were corrected.

a) Faulty Capillary Observation:

Because they were conspicuous, and because blood movement in them was rather more obvious, the larger capillaries were observed almost exclusively at first. When attention was turned to the finer vessels, more satisfactory results were obtained. The ideal objective was found to be several small vessels (approximately 8μ in diameter) in which the corpuscles could be seen streaming rapidly in typical erythrocyte chains. Although at first difficult to locate, they were found to give very consistent results. The problem of locating such vessels was solved by practise. Actually it was found later that with experience on the part of the operator, the larger vessels could give equally constant results, and that such results do not differ from those obtained from the observation of smaller vessels. However,

constant and careful focus adjustments were necessary. The flow of the corpuscles near the vessel walls was arrested before that of those in mid-stream. This was the source of the early low values.

b) Visual Fatigue:

In the author's opinion, the inconsistency of the early readings were mainly a result of visual fatigue. During this period a great deal of time was spent attempting to obtain a single pressure reading. The long searching of the webs by the untrained eye inevitably resulted in eye-strain. This problem, too, was solved by experience. Practise made the detection of movement in the very fine vessels readily discernible, with the result that several readings could be taken before any strain was felt.

c) Improper Lighting:

Lighting was found to be of great importance inasmuch as it affected directly the two factors first mentioned.

The web under observation had to be well, <u>but not intensely</u>, lit from beneath. The best results were obtained if such light was reduced by means of the sub-stage diaphragm of the microscope until it affected only the specific area of the web under examination. All other lights in the room were cut to a minimum, and whenever possible the shades were drawn over the windows during daylight.

Having observed these precautions, the author found the following rules a help in obtaining satisfactory results:

i) Determinations should be made when the observer is

fresh and avoided when he is fatigued, and avoided particularly when suffering from any eye-strain from other work.

ii) Lights should be adjusted carefully before undertaking any observation.

iii) When observing the web, the operator should search quickly for suitable vessels and if such are not noted immediately, the eye should be rested and another portion of the web, or another web, examined.

2. Routine Procedure.

After preliminary exploration of possible techniques, a set procedure was adopted. The routine followed is set forth below:

a) Records:

Each rat, on selection for experimental work, had a serial number allotted to it which was tattooed on the ear, males on the left ear, females on the right. At the same time a filing card was prepared, on which the statistical data concerning the animal was noted (age, sex, weight, etc.). Subsequently this card was used as a record of the time and nature of any treatment, the results of such treatment, as well as any unusual condition noted in the animal. The card record for one of the rats of Run IV is shown in Fig. 1.

b) Anaesthesia:

Pentathol Sodium was received in powder form, put up in vials containing 0.5 gm. This powder was made up to 2 1/2% aqueous solution. Such a solution had to be prepared within three hours of use, any longer period in solution rendering

the anaesthetic ineffective. For convenience and economy, therefore, these 0.5 gm. lots were subdivided into 0.125 gm. samples, and these smaller amounts were placed in vials fitted with rubber stoppers especially prepared for hypodermic use. The vials thus prepared were stored at 5°C. When they were required 5 cc. of water were added to make a 2 1/2% solution. This amount was found convenient when working with six to twelve rats.

It was found very early in the work that the therapeutic dose of Pentathol Sodium varied considerably from one animal to another. Furthermore, there was no definite correlation between this dose and the variable factors that would seem to be indicated, factors such as age, sex or body weight. (Table 1). As a result, the therapeutic dose for each animal had to be established before experimental work could begin. This "therapeutic dose" for the purposes of this investigation was defined as: that volume of 2 1/2% Pentathol Sodium that was required to completely anaesthetise the rat for a period lasting over 30 minutes, but not longer than one hour. This dose was established by starting with a minimum dose (usually 0.35 ml.) and if this was found to be ineffective, it was raised by 0.05 ml. daily until the required effect was obtained. Whenever an animal failed to succumb to a specific dose, it was replaced in its cage and given no further treatment on that day.

The actual administration of the anaesthetic was straightforward. An assistant was required to hold the rat,

TABLE 1.

This table shows the therapeutic dose in ml. of 2 1/2% Pentathol Sodium required to induce 30 to 60 minute anaesthesia in six typical rats.

Rat Serial Number	Weight (gms)	Sex	Dose (ml)
III L 3	200	M	0.60
III L 5	200	M	0.40
II L 5	270	M	0.55
III R 5	176	F	0.50
III R 1	180	F	0.40
IV R 8	170	F	0.35

ventral surface up, and the operator passed the hypodermic needle through the body wall into the peritoneal cavity. It was important to be sure of actual penetration, otherwise a subcutaneous injection resulted. Such an injection caused the animal considerable distress and furthermore was never effective as an anaesthetic. The correct dose, properly administered, caused the animal to succumb in three to five minutes, and remain under the influence of the anaesthetic for 30 minutes to one hour. There were, however, day to day variations in the response of a given animal to a specific dose of Pentathol Sodium. These variations resulted in the loss of a number of readings, for where an animal failed to start to recover from anaesthesia within one hour, any readings that had been taken on it were discarded. Although this loss of occasional readings was a definite disadvantage, the method used offered the requisite amount of control over the depth of anaesthesia induced.

c) Pressure Determinations:

The animal was left undisturbed for about five minutes after the anaesthetic had taken effect. It was then removed from the cage to the apparatus and laid, belly down, in front of the microscope. The inflatable cuff was wrapped about the thigh of the hind leg, care being taken not to bind it tightly enough to impair circulation, or loosely enough to allow subsequent cuff inflation to be ineffective. The animal was then placed, again belly down, on the platform of the microscope and the foot placed in position. The web to be examined was spread, then stretched slightly, the toes on either side being held in place by strips of plasticene. This mounting

is shown in Fig. 2b. (The tube of the microscope was removed for this picture to permit a clearer view of the animal's position.)

After the rat was in position under the microscope, the instrument was focused and the web searched quickly for small vessels showing rapid blood movement (see above). When these were detected, the cuff was inflated by means of the syringe until a pressure of about 90 to 100 mm. Hg was reached. The pressure was then adjusted upwards by raising the head of mercury until blood flow in the vessels ceased. The manometer was read at this point and the pressure in the cuff immediately released. A minute or two was allowed to elapse before the cuff was inflated again and a second reading taken. If these two readings differed by more than 10 mm. Hg a third reading was taken. A maximum of four on a rat at one time was set. Where more than two readings were taken, the average of the two highest was accepted as the determination for that day. This procedure was in accordance with the procedure adopted by Griffith and Farris (18) and other workers (11) (35).

3. Methods in Experimental Hypertension.

a) Normal Pressures:

The establishment of the normal blood pressures of the animals was given careful consideration. It was realised that a number of factors, normally variable, might conceivably influence such blood pressure and, as a result, be a source of error in the subsequent hypertensive work. Such factors included age, sex (and the sex cycle in the female), time of

feeding, time of day, and anaesthesia. The last named, while not a factor affecting "normal" pressure as such, nevertheless had obviously to be considered as affecting normal pressures for the purposes of this investigation.

Two possible methods were considered for investigating the effect of these factors. The better method would have been to make experimental runs against each individual factor. Such a procedure alone would have taken more time than was at the disposal of the author for the whole problem, and so had to be ruled out of the question. It was decided, therefore, to establish as far as possible, the effect of these variables in conjunction with the main experimental runs. Before an animal was subjected to any procedure that was likely to induce abnormal pressure, at least four readings were taken on it to establish its normal systolic blood pressure. Such readings were recorded along with all relevant data, (Fig. 1) and at the conclusion of the four experimental runs all the normal values of the 29 rats used were checked in an effort to establish a correlation between any of the variables under discussion and systolic blood pressure.

b) The Injection of DCA:

It was decided first of all to confirm the characteristic pattern of the pressor response to a single injection of DCA as reported by Fitch (11).

For Run I, therefore, three males and three females were selected, and their normal blood pressures established in the manner indicated above. All rats were then injected

intramuscularly with 1 mgm. DCA in sesame oil (Ciba). The day on which this injection took place was termed Day '0' in relation to the period during which observations were taken. This terminology was retained throughout subsequent runs.

With the exception of Days 2 and 5, two blood pressure readings per day were taken on each rat up to and including Day '9'.

c) The Use of Histidine and Ascorbic Acid and the Injections of Antistine:

Run II: Eight rats were selected, four of each sex, and their normal systolic blood pressures determined. All then received injections of DCA (1 mgm.) and the resultant pressures determined daily as described for Run I. On Day 5, six of the eight animals were found to be hypertensive (systolic pressures 20 mm. Hg or more over normal) but readings were not obtained on the other two. The run was then divided into three groups as follows:

Group 'a', consisting of one male and one female, received no further treatment.

Group 'b', one male and one female, were fed 20 mgm. histidine and 70 mgm. ascorbic acid daily. These amounts were offered each morning mixed with a little Fox Chow (Purina Mills). When this mixture had been consumed the animals were given additional Fox Chow ad libitum, but any food remaining by late afternoon or evening was removed and the animals required to fast overnight to ensure consumption of the histidine mixture on the following morning. Such feeding was carried on from

Day 5 to Day 10 inclusive.

Group 'c', two rats of each sex, were fed as were those in Group 'b', and in addition received a total of four intramuscular injections each of the anti-histamine, antistine (2-phenylbenzylaminomethylimidazoline) (Ciba), in aqueous solution. The first two injections were 0.05 mgm. doses administered following the pressure determinations on Days 5 and 7 respectively. The two final doses were smaller (0.0125 mgm. each), and were injected 4 to 6 hours before the pressure determinations of Days 10 and 11 respectively.

Run III: This run involved ten rats, five of each sex, and was carried out over a period of five weeks. The normal pressures and DCA injections were carried out as for the previous runs, and on Day 5 the animals were again subdivided into three groups:

Group 'a', one male and two females, received no further treatment.

Group 'b', two males and one female, were fed histidine and ascorbic acid in the manner described for Run II and this feeding was continued over a period of 16 days (Day 5 to Day 20 inclusive).

Group 'c', two animals of each sex, were fed as were Group 'b' and in addition received a total of nine 0.0125 mgm. intramuscular injections of antistine each. The days on which these injections were administered, and their timing in relation to the daily determinations of blood pressure, are shown by Fig. 6. During Run III, determinations were taken daily

up to and including Day 8. Subsequent readings were taken as shown by Table 4 and Fig. 6. The run was discontinued on Day 26.

Run IV: Three rats of each sex were used for this run, which was carried out as a control run on the effect of antistine. After normal pressures had been determined, each rat received a total of five 0.0125 mgm. intramuscular injections of antistine each. These injections were timed to correlate with the first five antistine injections (also 0.0125 mgm.) given Group 'c' of Run III. For example, the first injections in both runs were given immediately following pressure determinations of the day, the second lot were given 26 hours later, (2 hours after the pressure determinations of the following day).

Thus these procedures allowed for a study of the effect of DCA in raising normal systolic blood pressures, the effect of histidine and ascorbic acid in lowering this experimental hypertension, and finally the effect of antistine on rats which had received histidine and ascorbic acid treatment.

III Results

A. Normal Systolic Blood Pressures

The systolic blood pressures of the 29 rats under observation ranged from 106 to 145 mm. Hg, with 24 of the 29 coming within the 120 to 140 mm. Hg range. Two animals showed systolic blood pressures of less than 120 mm. Hg, while three were over 140 mm. Hg.

B. Results From the Use of DCA, Histidine and Ascorbic Acid, and Antistine.

Results are given in detail by Tables 2 to 5 inclusive, and are shown graphically by Figs. 4 to 7 inclusive. These graphs show the deviation from normal systolic blood pressure in mm. Hg. Each point represents the average of the two daily readings taken on an individual rat. Day '0' on all graphs and tables represents the day on which the first injection was given. In Runs I, II, and III this first injection was DCA, in Run IV it was antistine.

In reference to Figs. 6 and 7, the period of feeding (see methods) is denoted by the heavy black line at the base. Pressure determination times are denoted by vertical lines below, and injections of antistine are shown by arrows above the heavy line.

TABLE 2. The Results of Run I.

The variation in the systolic blood pressures, in mm. of Hg, of each of five rats. Two readings per day are given covering a period of two weeks. Each rat received, on Day '0', an intramuscular injection of 1 mgm. DCA. See also Fig. 4.

Rat	Sex	Time in Days											
		-4	-3	-2	-1	0	1	3	4	6	7	8	9
L.1	M	126 ·	131 ·	128 126	128 130	1 mgm. injection DCA	132 134	-	116 120	134 140	-	170 170	160 155
L.4	M	-	-	127 125	125 130		125 125	97 100	-	164 158	150 160	240 233	170 172
R.2	F	127 133	128 133	130 133	-		126 128	124 122	184 176	118 123	#		
R.3	F	126 134	140 134	135 125	148 139		-	100 100	-	-	156 165	170 165	140 142
R.5	F	-	-	128 130	134 134		134 136	118 123	140 135	144 144	150 152	160 160	129 128

Note: (-) anaesthesia either ineffective or too long in duration.
(#) died in anaesthesia.

TABLE 3. The Results of Run II.

The variations in systolic blood pressures of eight rats over a period of sixteen days. All rats were injected on Day '0' with 1 mgm. DCA. Group 'a' received no further treatment, Group 'b' were fed 70 mgm. ascorbic acid and 20 mgm. histidine daily from Day '5' to Day '10' inclusive. Group 'c' received the same treatment as did Group 'b' and in addition received four intramuscular 0.0125 mgm.@ injections of antistine. For the times and spacing of these injections see Fig. 5.

Rat	Sex	Gp.	Time in Days														
			-5	-4	-3	-2	0	1	2	3	4	5	7	8	9	10	11
L.7	M	a	-	137 143	125 125	130 140	Desoxycorticosterone	125 120	105 105	116 120	nr	186 184	-	190 190	-	158 162	148 148
R.7	F	a	130 135	140 130	135 140	145 148		-	115 115	144 130	nr	175 175	170 170	#			
L.9	M	b	-	130 130	-	114 120		100 106	-	-	124 115	176 174	160 170	136 140	120 120	110 116	140 140
R.9	F	b	-	110 110	130 115	120 115		100 112	104 100	125 133	-	-	140 144	140 140	100 100	125 135	148 152
L.6	M	c	-	120 130	105 106	118 120		-	-	118 118	-	155 146	-	108 112	120 126	120 120	140 140
L.8	M	c	-	107 108	114 110	100 100		105 101	-	-	110 130	130 126	115 110	120 120	130 130	140 146	#
R.6	F	c	-	140 150	130 126	-		-	120 130	110 104	120 120	-	-	#			
R.8	F	c	130 130	120 124	-	-		-	97 100	121 115	-	141 155	144 137	130 130	100 106	180 176	130 138

Note: (-) anaesthesia either ineffective or too long in duration.
(#) died in anaesthesia.
(nr.) no reading.
(@) the first two of the four injections were 0.05 mgm.

TABLE 4. Results of Run III.

The variations in systolic blood pressures of ten rats over a period of 5 weeks. All were injected on Day '0' with 1 mgm. DCA. Group 'a' received no further treatment, Group 'b' were fed 70 mgm. ascorbic acid and 20 mgm. histidine from Day '5' to Day '20' inclusive. Group 'c' received the same treatment as did Group 'b', and in addition received nine 0.0125 mgm. injections of antistine intramuscularly, the first being given on Day '5'. For the times and spacing of subsequent antistine injections see Fig. 6.

Gp.	Rat	Sex	Time in Days																					
			-8	-7	-6	-5	-4	-1	0	1	2	3	5	6	7	8	9	10	12	13	14	16	20	21	26
a	L5	M	118 126	-	-	135 140	nr	nr		136 140	140 140	104 104	-	154 158	164 180	180 180	158 161	-	120 116	nr	nr	128 128	-	134 128	96 102
	R2	F	126 136	-	-	-	-	130 130		-	126 124	100 108	146 150	116 120	-	150 158	-	128 118	140 146	nr	nr	-	140 140	-	102 104
	R5	F	-	120 124	-	-	116 120	nr	DCA	112 118	104 96	110 108	108 110	150 150	156 168	158 162	-	108 118	-	nr	nr	100 100	132 136	-	120 128
b	L1	M	-	-	138 135	140 146	nr	nr	injection	-	-	121 125	160 160	-	136 130	150 150	nr	110 110	nr	150 160	nr	118 123	nr	106 110	nr
	L2	M	-	-	-	140 150	142 140	nr	-		112 110	160 154	168 173	190 180	198 210	-	nr	102 102	nr	150 140	nr	100 110	nr	144 144	#
	R1	F	-	120 120	-	130 140	150 156	131 135		-	120 120	120 120	-	144 143	#										
c	L3	M	-	-	140 150	155 140	148 138	nr	1 mgm	140 146	116 118	-	140 144	146 154	164 174	120 125	nr	120 124	-	nr	166 176	nr	nr	130 130	115 104
	L4	M	-	-	140 148	-	145 155	140 146		130 140	-	116 120	160 168	166 174	220 225	170 174	nr	128 134	148 150	nr	-	nr	nr	110 110	154 158
	R3	F	-	-	-	-	130 142	146 136		-	130 132	156 156	116 120	144 148	168 162	-	nr	144 150	144 142	nr	140 140	nr	nr	166 166	#
	R4	F	-	-	140 146	-	130 136	nr		-	130 130	126 130	165 158	148 150	-	140 140	nr	110 106	144 150	nr	-	nr	nr	150 154	174 180

Note: (-) anaesthesia either ineffective or too long in duration.
(#) died in anaesthesia.
(nr.) no reading.

TABLE 5. Results of Run IV.

The systolic blood pressures of six rats taken twice daily over a two-week period. These rats received a total of five 0.0125 mgm. intramuscular injections of antistine, the first injection being on Day '0' (immediately after readings). Subsequent injections were timed to correspond with identical injections given Group 'c' of Run III. See also Figs. 6 and 7.

Rat	Sex	Time in Days											
		-9	-7	-6	-4	-3	-2	0	1	2	3	5	7
L.7	M	-	-	-	136 138	120 122	136 138	120 120	-	116 116	105 114	128 134	125 129
L.8	M	132 123	120 123	-	120 112	-	-	128 138	114 108	108 118	100 102	90 92	100 100
L.9	M	-	116 118	-	120 110	106 106	-	128 122	134 128	108 118	-	-	100 100
R.6	F	140 132	138 140	154 158	132 140	120 122	nr	-	110 108	-	-	100 114	100 98
R.7	F	130 132	136 140	118 124	126 118	116 120	nr	-	84 92	122 128	118 120	120 122	106 108
R.8	F	-	130 138	124 128	128 128	nr	nr	-	134 138	90 90	107 110	96 92	96 96

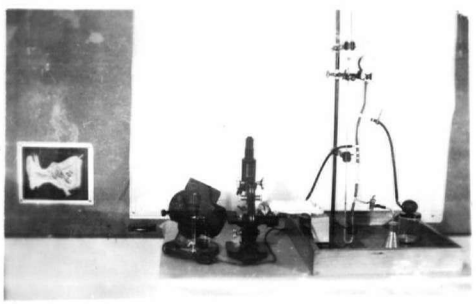
Note: (-) anaesthesia either ineffective or too long in duration.
(nr.) no reading.

PLATE I

L-7 ♂ RUN IV Age: 10 mo. Wt: 220 gms. (20/3)

20 Mar (0900): 0.45 cc - ~~—~~ ineffective 217 gms (7/4)
22 ✓ (1645) - 0.50 cc - ✓ (just)
25 ✓ (1200) - 0.55 cc - OK. 136, 138
26 ✓ (1550) - ✓ - 120, 122
27 ✓ (1630) - ✓ - 136, 138
28 ✓ (1420) - ✓ - 120, 120.
29 ✓ (1400) ✓ - 126, 130. Norm. pressure est. at 129 mm.
(1730) - intramuse. inj. antetine (0.0125 mgm)
30 Mar (1200) .0125 mgm antetine (1430) 0.55 cc - 134, 128. (over anesthetized ??)

Fig. 1. Record of Rat in Run IV



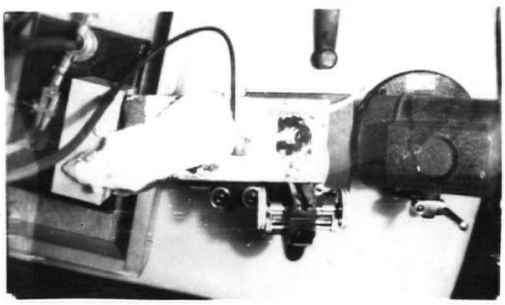
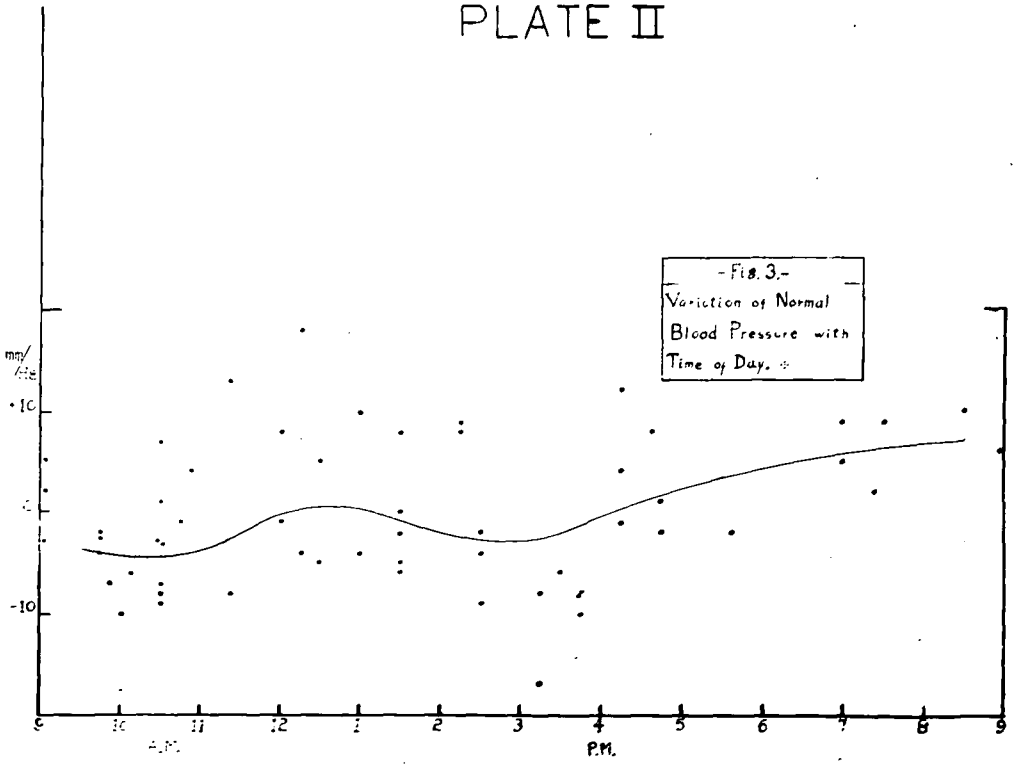


Fig. 2a: Apparatus Assembly (above).
Fig. 2b: Position of Rat for Pressure Reading.

PLATE II



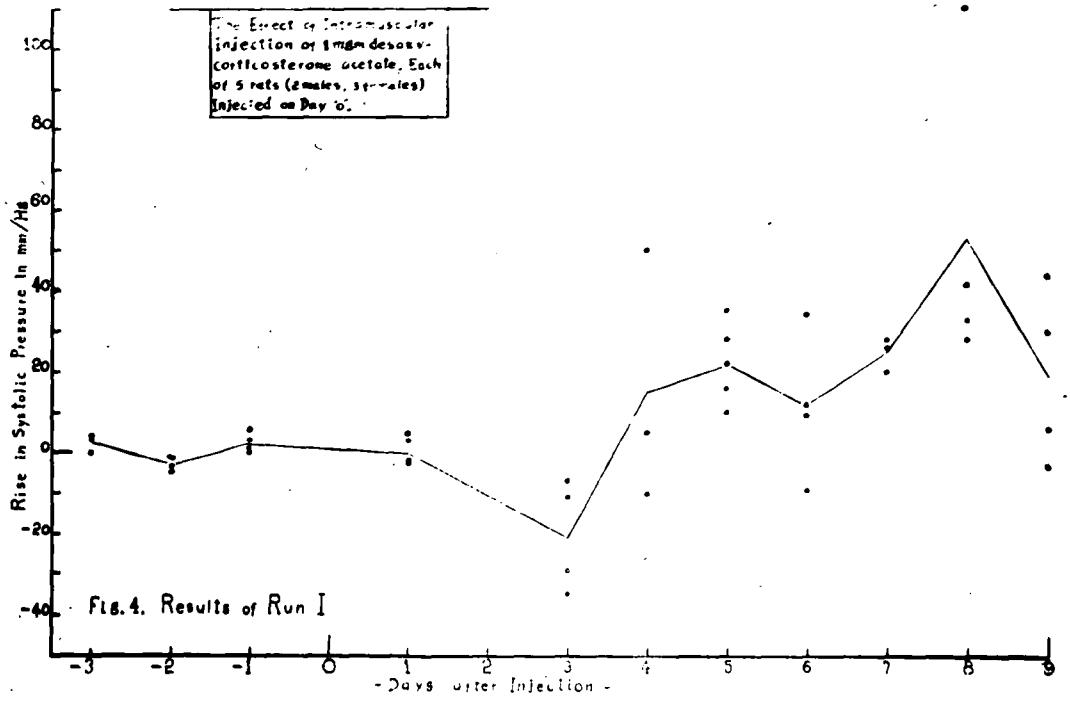


Fig. 4. Results of Run I

PLATE III

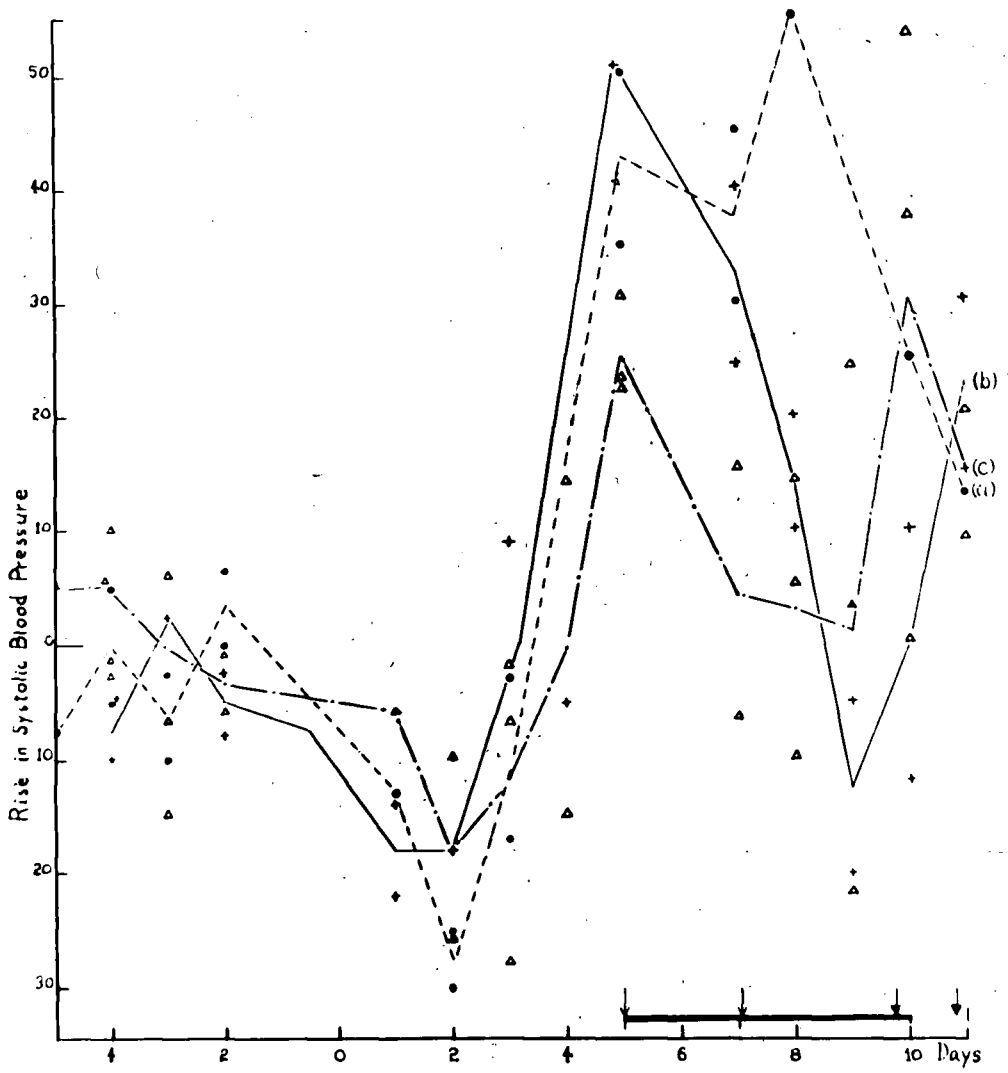


Fig. 5. Results of Run II

All rats injected intramuscularly with 1mgm desoxycorticosterone acetate on day '0'.

(a)_____ no further treatment. (1♂, 1♀).

(b)_ . _ . _ 10 mgm ascorbic acid + 80 mgm histidine daily in food during period marked ▬▬▬ (1♂, 1♀)

(c)_ . . _ . . _ as in (b) plus intramuscular injections of .05 mgm (⇓) or .025 mgm (↓) antistine. (2♂, 2♀).

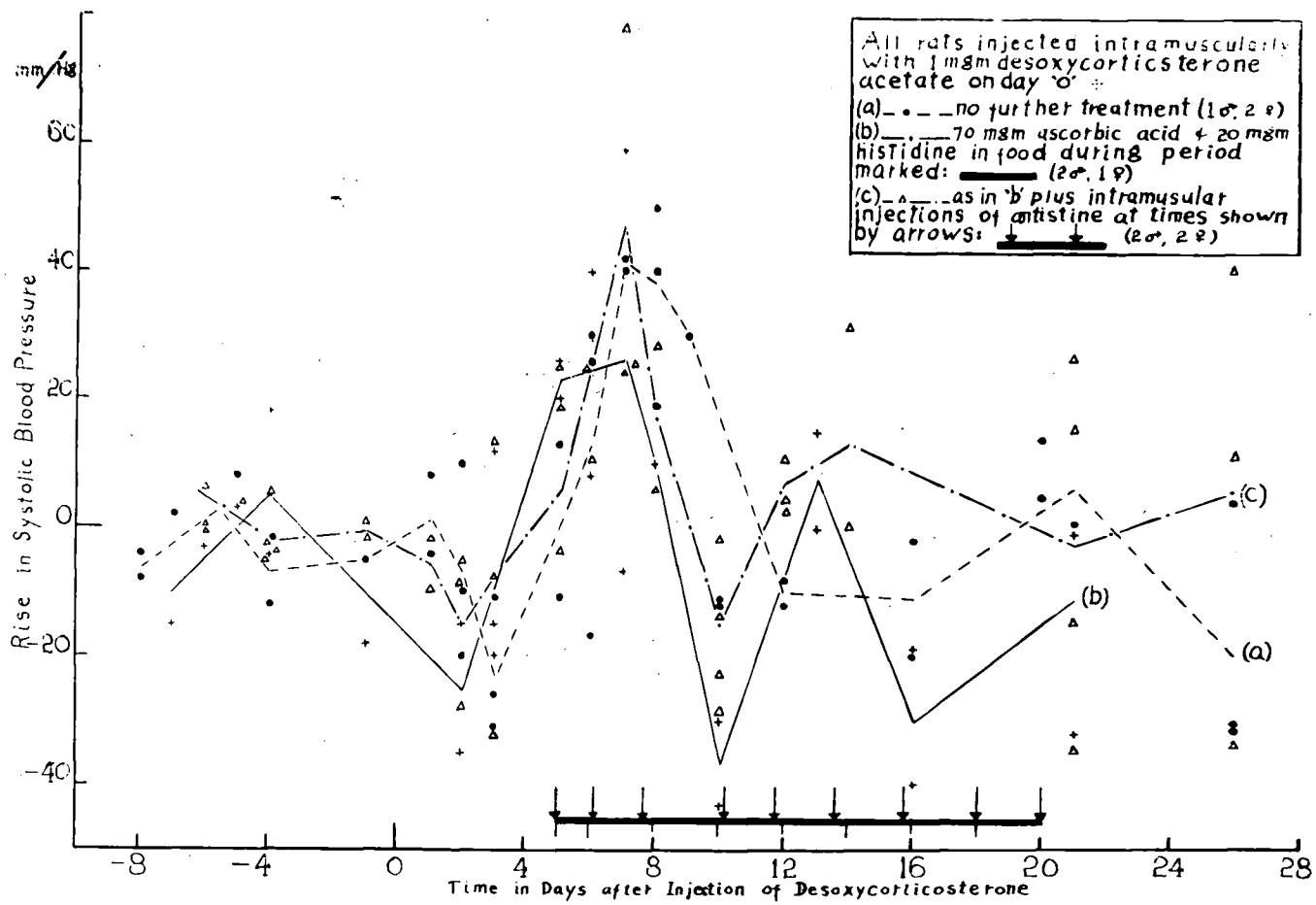


Fig. 6. Results of Run III

PLATE IV

PLATE V

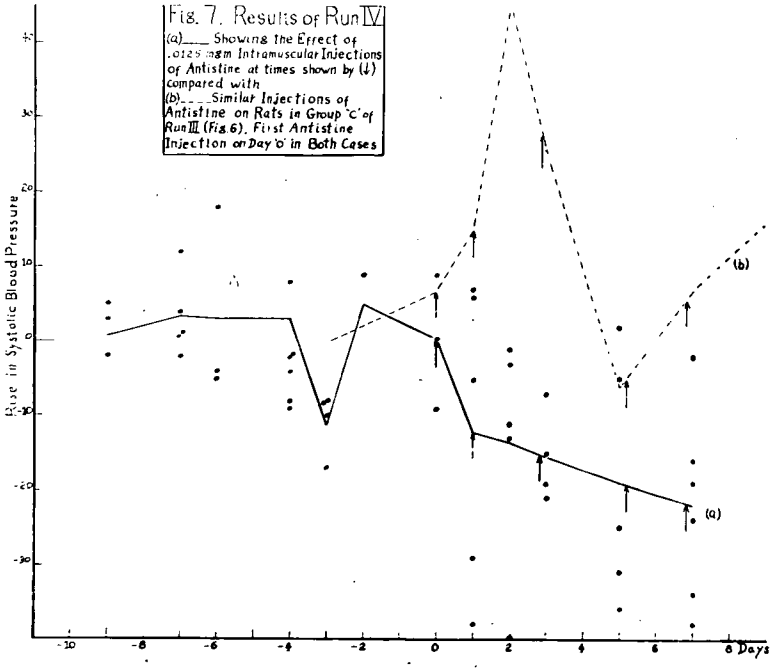


Fig. 7. Results of Run IV
(a)____ Showing the Effect of .0125 mgm Intramuscular Injections of Antistine at times shown by (↓) compared with
(b)_ _ _ _ Similar Injections of Antistine on Rats in Group 'C' of Run III (Fig. 6). First Antistine Injection on Day '0' in Both Cases
Rise in Systolic Blood Pressure
(b)
(a)
-10
-8
-6
-4
-2
0
2
4
6
8 Days

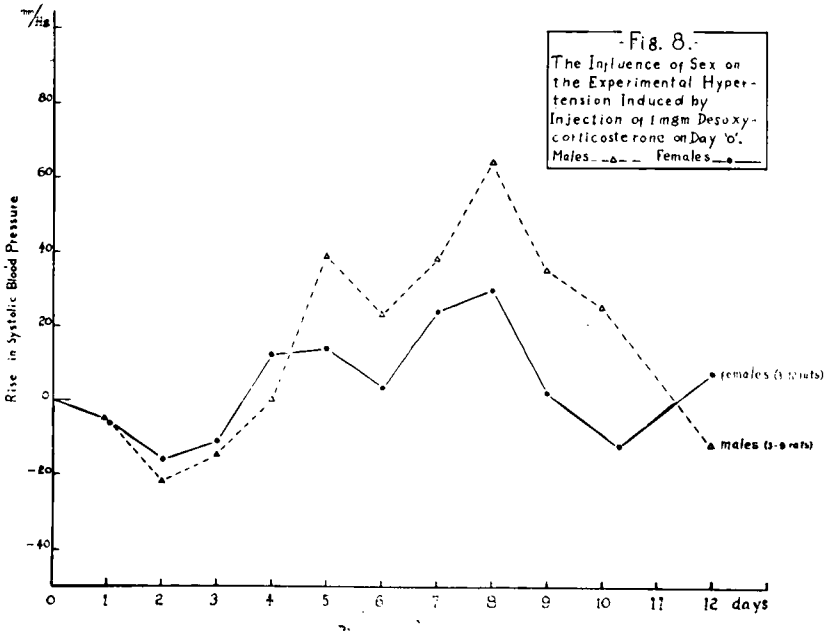


Fig. 8.
The Influence of Sex on the Experimental Hypertension Induced by Injection of 1 mgm Desoxycorticosterone on Day '0'.
Males _ _Δ_ _ Females _●_
Rise in Systolic Blood Pressure
80
60
40
20
0
-20
-40
females (5 rats)
males (5 rats)
0
1
2
3
4
5
6
7
8
9
10
11
12 days

PLATE VI

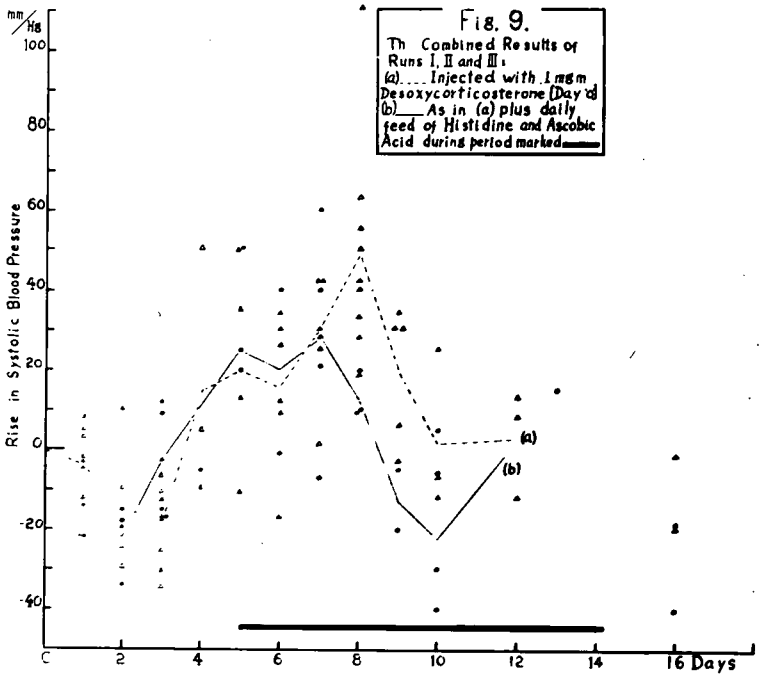
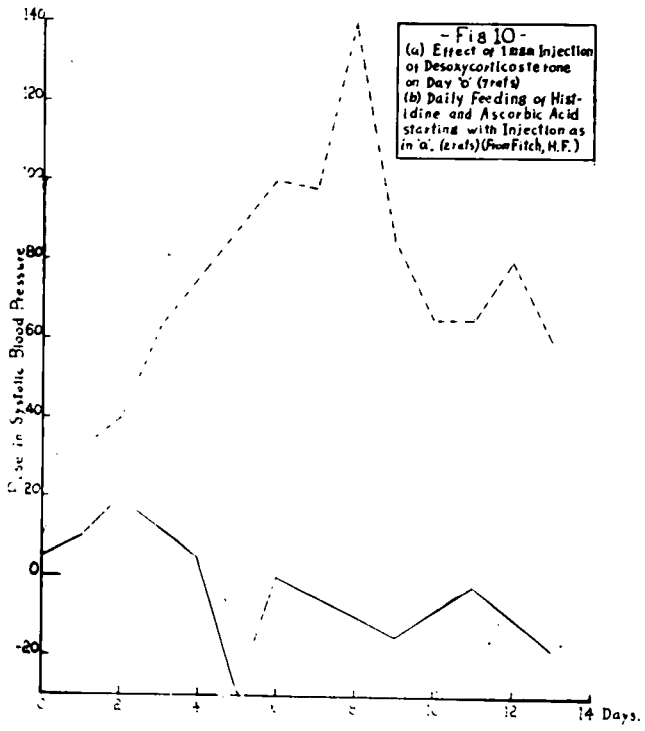


Fig. 9.
Th Combined Results of Runs I, II and III:
(a)---- Injected with 1 mgm Desoxycorticosterone (Day '0')
(b)____ As in (a) plus daily feed of Histidine and Ascobic Acid during period marked ▬▬



- Fig 10 -
(a) Effect of 1 mgm Injection of Desoxycorticosterone on Day '0' (7 rats)
(b) Daily Feeding of Histidine and Ascorbic Acid starting with Injection as in 'a'. (6 rats) (From Fitch, H.F.)

IV Discussion of the Results

A. The Validity of the Results

In evaluating the validity of the results obtained, the author has taken into consideration several factors which might effect systolic pressure. As far as possible these factors will be discussed separately in order to decide which of them can be disregarded, and which, if any, must be considered as significant sources of error.

1. Consistency of the Results.

Theoretically, the two readings that were taken daily on the same rat at the same time should agree perfectly. This was seldom the case. These deviations must be considered as errors. In this regard, Run II (Table 3) can be taken as typical and is analysed below to illustrate the extent of this error.

Of 73 pairs of readings, two pairs showed a deviation of more than 10 mm. Hg, and six others showed deviations of exactly 10 mm. Hg. The average deviation was 4 mm. Hg, or 3% of the average systolic pressure (127 mm. Hg). Such a deviation is small in relation to the elevation in blood pressure of 48 to 60 mm. Hg (38 - 47% increase) brought about by DCA.

Further analysis shows that the average deviation for normal pressures was similar to the deviation observed when the rats had been treated. Thus these deviations have no significant bearing on the hypertensive results obtained.

2. Validity of Determinations of Normal Pressures.

The average normal systolic pressure of the 29 rats (14 males and 15 females) was 127 mm. Hg, with individual normals ranging from 106 to 145 mm. Hg. In assessing the accuracy of these figures the author has turned for confirmation to the results of other workers.

Prado et al (30) have given a statistically derived figure for mean blood pressure of the albino rat, a figure reported by them to include 95% of any group of rats of statistical size. This figure was 108 ± 14 mm. Hg. Converting to systolic pressure, a range of 115 to 145 mm. Hg is obtained. Only one of 29 rats used in the experiments under discussion exhibited a normal systolic pressure outside of this range.

Sulkin and Brizzee (35) report that anaesthesia lowers the blood pressure of experimental animals, and "normal" pressures taken under such conditions do not, in fact, represent true normal pressures. These workers report an average systolic pressure of 124.8 mm. Hg for the unanaesthetised rat, and lower values for anaesthetised animals. They report depressions ranging from 7 mm. Hg with light ether, to 61.5 mm. Hg with morphine. They do not report on pentathol sodium, but other barbiturates, nembutal and amytal which are similar in action to pentathol, gave depressions of 20.7 and 21.3 mm. Hg respectively. However, it will be noted that the normal pressures as established in the investigation reported here agree not with the pressures of their rats under the influence of

barbiturates, but rather with the figures they give for un-anaesthetised animals.

Some figures for normal systolic blood pressure in the albino rat as reported by a number of workers are given below:

Abramms (1) reports a mean of 125.7 mm. Hg for three anaesthetised rats, by the indirect method using the tail (and plethysmograph), and 129.2 for three unanaesthetised rats by a direct method with a manometer reading directly from the femoral artery.

Page and Reed (28), with five rats, report a range of 105 to 125 mm. Hg.

Fitch (11) after work with 26 rats reports an average value of 120 mm. Hg.

Pratt et al (31) after clinical studies of the action of pentathol sodium, report that blood pressure usually falls the moment after a dose of pentathol sodium has been injected, and that after a small dose the return of the blood pressure to its previous level occurs quickly.

As a result of the consistency of the readings obtained in this investigation and as a result of checking the reports mentioned above, the author is satisfied that the normal systolic blood pressures as established were correct and that the anaesthesia, as administered in this experiment, played no significant part in subsequent pressure changes.

3. Effect of Variable Factors.

a) Age:

In general there was a very rough correlation between

age and systolic blood pressure, the older and heavier rats tending to have higher normal pressures than the younger, lighter rats. Inasmuch as rats were selected with a view to uniformity, the range of age and weight was small. Eight male rats used weighed less than 225 gms. and these showed an average systolic pressure of 128 mm. Hg; whereas the other six males investigated, all of which weighed more than 225 gms., had an average pressure of 135 mm. Hg.

Griffith and Farris (19) state that there is a slight but definite rise in blood pressure with age. Medhoff and Bongiovanni (25) concur. The latter give figures on this rise in terms of the percentage of rats of a given age group, that are found to be in certain arbitrary pressure groups. The rise indicated is, however, so slow that it is neglible for the periods over which the rats in this investigation were observed.

b) Sex:

There was no appreciable difference between the two sexes in regard to systolic blood pressure. Further, the females failed to show any day to day, or rythmic pressure changes, indicating that the sex cycle was not a factor to be considered. Medhoff and Bongiovanni (25) are in agreement on these points.

c) Time of Day:

The normal pressures obtained for all rats used are plotted against time of day of determinations in Fig. 3. The pressures tend to be a little low in the morning, rise at

noon, drop again in the afternoon and rise a little more definitely in the evening. All these variations, however, are very small (Fig. 3). It will be noted that only 4 of 48 determinations show a deviation from normal of over 10 mm. Hg. The general diurnal variation as shown by Fig. 3 is, therefore, negligible from the standpoint of induced hypertension. It is possible that the slight rise about noon was correlated with feeding (which was carried out between 8.30 and 10.00 A.M.) and the rise in the evening with the natural nocturnal activity of these animals.

d) Statistical and Human Factors:

Although the more obvious variables have been shown to have little or no effect on the results of the experiment, two further factors must be considered. These are the statistical value of the results, and the human error involved in the determination of blood pressures.

The number of rats used in each run was admittedly small. This was necessary because of the limited time available. When this work was first started, two to three hours a day were required to carry through a run in which four animals were involved. Practise reduced this time, but nevertheless two or three hours were still required at the end to handle a run of ten rats.

The author is of the opinion that this small sampling is the weakest point of the investigation.

The human factor is rather difficult to evaluate, as there are openings for error in the procedure. Mention has

already been made of the possibility of error from faulty capillary observation. Localized vascular spasms could be mistaken for true stoppage of blood flow, and fatigue on the part of the observer was always a potential source of error. However, if the consistency of the results obtained, and their agreement with other workers can be used as a yardstick, then these sources of error can have little significance in the final results of the investigation.

B. The Hypertension Resulting From DCA Injection, and Its Amelioration With Histidine and Ascorbic Acid

1. The Pressor Effect of DCA.

The effect of a 1 mg. intramuscular injection of DCA on systolic blood pressure is summarized in Fig. 9 for all rats so treated. The general shape of the graph agrees with that reported by Fitch (11), as shown by Fig. 10a. It will be noted that in both cases the systolic blood pressure rises rapidly to reach a pronounced peak about the 7th or 8th day after an injection of DCA, (although the author found that there was an initial lowering of pressure immediately following the injection, whereas Fitch (11) reports an immediate rise). In degree of hypertension achieved, however, the reports disagree. Fitch reports systolic blood pressures averaging 140 mm. Hg over normal on the eight day after injection while the author has found it to average 50 mm. Hg.

It is difficult to find confirmation in the literature for these values. Prado et al (30) achieved hypertensive

levels of a degree similar to that reported by Fitch (11), (150 to 160 mm. Hg above normal), but as a result of massive dosing (40.0 mg. pellets subcutaneously implanted). Other workers, all using similar large doses, show rough agreement (23). With the exception of the present work, and that done by Fitch there are no reports known on the effect of small doses of DCA.

a) The Influence of Sex on the Effect of DCA:

There was found to be a marked difference between the sexes in their response to DCA. This is shown graphically by Fig. 8. The number of animals represented by this graph varies with time, due to the nature of the experiments. Inasmuch as all rats in all runs except Run IV received the same DCA injections with no further treatment until Day 5, the graph up to this point represents 9 males and 12 females. At this point several animals of Runs II and III were started on other treatments (see methods), so as a result the number of animals represented drops to 4 males and 6 females. Day 12 represents only 3 rats of each sex.

Up to and including Day 8 the number of rats available for readings is sufficient to give a good indication of the influence of sex on this pressor response. It will be noted that the initial drop in the systolic blood pressure of the males is a little more pronounced on the average than that of the females, and that the eventual maximum rise, reached about Day 8, is twice as great on the average in the males as in the females.

This finding could be explained by the fact that in the female there are found significant amounts of progesterone, pregnandiol, and allopregnandiol. These are found only in very small amounts in the male (as measured by urinary excretion). These hormones, especially progesterone, are closely related chemically to DCA, and the progesterone-like activity of DCA, has been demonstrated (Best and Taylor, (4)). Thus it would seem logical to expect an animal that is naturally "buffered" against such substances to react less violently than one that is not so "buffered", or at least not nearly as well "buffered".

2. Amelioration With Histidine And Ascorbic Acid.

A composite picture of the amelioration of the DCA-induced hypertension that was achieved by histidine and ascorbic acid feeding is given in the graphs shown in Fig. 9. Fig. 10 shows the results obtained by Fitch (11) when the feeding was started on the same day that the DCA was administered, that is before any hypertension had developed. With such a procedure hypertension failed to develop.

From these results there seems little doubt that this form of experimental hypertension can be ameliorated by the treatment outlined. It has already been suggested by Fitch that this amelioration is a result of the elaboration of excess histamine by intestinal bacteria from the histidine administered. This theory will be referred to later.

3. The Effect of Antistine.

If the theory mentioned above is tenable, it seems logical to expect that the administration of an anti-histamine would destroy such histamine, and as a result rats so treated would become hypertensive in spite of the histidine feeding. With this in mind, the results of Runs II and III were examined. (In order to avoid repetition, the three groups of rats treated in these runs will be referred to in this discussion as groups 'a', 'b', and 'c'. The reader is referred to the outline of the methods used (page 21) or to the legends to Figs. 5 and 6 for details.)

The response of the animals to intramuscular injections of antistine was rather variable. Thus, in Run II, (Fig. 5) it will be noted that the pressures of the rats in group 'c' tended to follow those of group 'b' after the first two, relatively large, injections of the anti-histamine. At that point it would seem that the antistine was having no effect. However, inasmuch as the antistine was in aqueous solution, and would therefore, be readily absorbed, it was felt that a different result could be obtained if the injections were given a few hours before the blood pressures were read, when its effect would not have had time to wear off. Actually 48 hours elapsed before a determination was taken after the first injection of antistine, and 24 hours elapsed after the second. This delay was necessary because the size of dose used proved too large and resulted in considerable distress to the animals concerned. They exhibited a partial temporary

paralysis of the leg injected, abnormal excitability and anorexia. Pressure determinations were therefore delayed until these symptoms had subsided. The third injection of antistine was given when the animals had fully recovered, and it and the fourth injection were reduced from 0.05 mgm. to 0.0125 mgm. Readings were taken in these cases a few hours after the injections and the result was that the pressures of the antistine rats went up instead of down, and thereafter showed a correlation with the pressures of the rats in group 'a'.

In Run III the mistake in regard to dosage (of antistine) was avoided and as a result this run (Fig. 6) shows a better indication of the effect of the antistine. Dosage was again 0.0125 mgm., and these doses were given more frequently.

It was noted in general that the rats of group 'c' tended to give a pressure graph similar in shape to that given by the rats of group 'a'. The effect was still slightly variable, but it was again noted that whenever pressure readings were taken within 24 hours after an antistine injection, the systolic blood pressures of the rats thus treated tended to approach the hypertensive line represented by the blood pressures of the rats of group 'a'. Thus, although the rats of group 'c' received the treatment that otherwise brought about amelioration of the experimental hypertension, their pressures nevertheless continued to rise after the start of this treatment, apparently due to administration of antistine, reaching a maximum equal in degree

with the pressures of the rats of group 'a' at the same time. The pressures, on the other hand, of the rats of group 'b', ceased to rise shortly after amelioration treatment was started and failed to show the characteristic 7 or 8 day peak of the other two groups. After Day 8 the pressures of group 'c' fell rapidly and tended to follow those of group 'b' as closely as they did those of group 'a'. It was considered again that the injection timing was of importance here, for, when subsequently the antistine injections were given 4 to 8 hours prior to pressure determinations (on Days 12 and 14), the systolic blood pressure determinations of the animals so injected rose again.

To summarize, the antistine definitely exerted a pressor effect, but this effect was shortlived and was not apparent if pressure determinations were not taken within 24 hours of an injection.

The question now arose, "was this pressor effect a result of a byplay with another substance such as histamine, or was there a pressor action inherent in the antistine itself?" The answer to this question is found in the results of Run IV.

All animals in this run received antistine injections to correspond to the injections given group 'c' of Run III (see methods and Fig. 7). It was noted (Fig. 7a) that antistine injections alone did not result in a rise in blood pressure, but rather the pressures of the rats so treated dropped steadily. This result was compared with the results of similar injections to rats of group 'c', Run III (Fig. 7), where a definite rise in blood pressure took place after several of

the injections of antistine.

The conclusions drawn from these results are discussed below.

C. Suggestions For Further Research

As a result of this investigation, suitable subjects for further work along the same lines present themselves.

1. Further Investigation of the Histamine Theory

The results discussed above give definite indications that the amelioration of the DCA-induced hypertension was due to the action of histamine, but further work on this is required. Several lines of approach suggest themselves, including:

a) Oral Administration of Antistine:

It is possible that a clearer picture of any histamine-antistine balance could be obtained if the antistine were administered orally instead of by intramuscular injection. A preparation suitable for such administration is now available (Ciba). The injection method was chosen by the author because it promised more accurate control of dosage. However, in the light of experience, it would seem that this is more than balanced by the fact that, with injections, the rate of absorption is erratic. Absorption from the digestive tract, on the other hand, would be relatively constant and the results of such absorption should show definitely the effect of this drug. Oral administration, too, would have the

advantage of avoiding the physically irritating effects of intramuscular injections of antistine.

b) Histamine Occurance:

Proof that body histamine rises after the oral administration of histidine and ascorbic acid would be important evidence in the confirming of the role of histamine.

For example, the contents of the colon could be analysed for histamine, and an analysis of blood for histamine would be interesting. Best and Taylor (4) quote Anrep as reporting on the histamine content of the blood, and the method used by this investigator may be applicable to the work under discussion.

2. The Action of Histamine.

a) Vasodilatory Action:

If it is conclusively shown that histamine is responsible for the amelioration of the experimental hypertension as described above, it would be interesting to investigate, in more detail, the mechanism through which it acts.

In reporting the formulation of the theory, Fitch (11) suggested that the amelioration might be due to the vasodilatory action of histamine. In addition to this property, histamine possesses other physiological (or chemical) properties that would make it a blood pressure reducer under the conditions of these experiments.

b) DCA Antagonism:

Ellinger's work(7) (8) on mice, previously noted, shows that DCA seems to act as an anti-histamine. This effect,

therefore, must be considered as a possible mechanism in the amelioration of any DCA-induced hypertension.

c) Effect on Cardiac Output:

Best and Taylor (3a) state that histamine increases the cardiac output in man, but recent work, including that of Deyrap and Root (6) seems to establish an opposite effect, at least on some animals. These investigators injected, subcutaneously, 10 mgm. histamine per Kgm. body weight into unanaesthetized dogs. The result of such injections was a definite increase in peripheral resistance, with a very marked decrease in cardiac output, and blood pressure.

The possibilty thus exists that histamine caused the amelioration of hypertension by decreasing the cardiac output.

3. Effect of Progesterone.

In view of the different responses to a DCA injection shown by the two sexes, it would be instructive to note the effect of progesterone administration on the systolic blood pressure. Because of the chemical and physiological similarities between DCA and progesterone, it might be expected that the latter would cause a similar hypertensive response.

The theory that the difference in response to DCA between the sexes, in the white rat, is due to the relatively large amounts of progesterone and progesterone-like hormones in the female could be confirmed by administering DCA to ovaryectomized females, and checking the resultant systolic blood pressures against those obtained when DCA is administered to complete females and to males.

V Conclusions

The conclusions drawn from the results of this investigation in the light of the problem set out in the introduction are as follows:

An indirect method of determining the systolic blood pressure of the rat, which entails the use of the foot, has been found to be satisfactory.

Incidental to the conclusions to the assigned problem of this thesis, several minor conclusions of some value have been drawn:

1. Pentathol sodium, when carefully administered, is a satisfactory anaesthetic for hypertensive work with rats.
2. The normal systolic blood pressure of albino rats between the ages of 10 months and 18 months ranges from 115 to 145 mm. Hg, with an average of 127 mm. Hg. There are no significant differences in systolic blood pressure between the sexes.
3. The pressor effect of DCA is more pronounced in males than in females.

Fitch's report that oral feeding of excess histidine and ascorbic acid ameliorates the experimental hypertension brought on by an injection of DCA, has been confirmed. Moreover it has been shown that this amelioration is generally counteracted by the use of an anti-histamine (antistine), which when used on non-DCA treated rats acted as a depressor.

Hence this anti-amelioration (a pressor action) may ←

presumably be due to an interaction of antistine with DCA and/or histidine (or their metabolic products). Antistine is a known anti-histamine, and histamine has already been mentioned as a logical metabolic product of histidine. In view of this it is logical to conclude that the amelioration effect of histidine and ascorbic acid is due to the production of histamine in the rat.

VI Summary

This investigation was an attempt to confirm the report that oral feeding of histidine and ascorbic acid was effective in the amelioration of hypertension induced by small injections of desoxycorticosterone acetate (DCA). If such amelioration was confirmed, its mechanism was to be investigated.

An indirect method using the foot has been described for measuring systolic blood pressure in rats lightly anaesthetized with pentathol sodium.

Normal systolic blood pressures were established for all rats used.

Single small doses of DCA were injected intraperitoneally into the rats to induce hypertension. Such injections resulted, on the average, in an initial drop in systolic blood pressure, followed by a steady rise in pressure until a maximum of 40 to 60 mm. Hg over normal was reached 7 or 8 days after the injection of DCA.

When other rats so injected had become hypertensive, but

had not reached the peak of hypertension, they were fed 20 mgm. histidine and 70 mgm. ascorbic acid with food. This treatment resulted in the rise in blood pressure being arrested, and on the eighth day after DCA injection these rats showed blood pressures that were almost normal.

When rats injected with DCA, and subsequently fed histidine and ascorbic acid as above, were treated with intramuscular injections of 0.0125 mgm. of the anti-histamine substance "antistine" (the hydrochloride of the methane-sulphonate of 2-phenylbenzylaminomethyl-imidazoline), the result, in general, was to nullify the effect of the histidine feeding described above. There were some exceptions to this general effect.

Similar intramuscular injections of antistine alone instead of generally raising the blood pressure, effected a steady lowering of the systolic blood pressures of six rats so injected.

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Abstract

Albino rats have been made hypertensive by single 1 mgm. intramuscular injections of desoxycorticosterone acetate (DCA). Pressures were determined by the indirect method using the foot. Eight days after the injections male rats showed an average systolic pressure 60 mm. Hg above normal, and females 40 mm. Hg above normal. After the eighth day the pressures dropped rapidly to normal. Daily feeding of 20 mgm. histidine and 70 mgm. ascorbic acid, started when rats were already hypertensive (on the fifth day after injection of DCA), arrested the rise in blood pressure and on the eighth day after injection, rats so fed showed almost normal pressures. It was believed that the amelioration of the experimental hypertension was due to the elaboration of histamine from histidine. To confirm this rats injected with DCA and fed as above were injected intramuscularly with periodic small doses of the anti-histamine compound "antistine" (the hydrochloride of the methane-sulphonate of 2-phenylbenzylaminomethyl-imidazoline). In general rats so injected showed subsequent systolic blood pressures resembling those of rats that had received DCA but had received no ameliorating treatment. There were exceptions to this effect. The conclusion is drawn that the amelioration of the DCA-induced hypertension by histidine and ascorbic acid feeding is, in all probability, due to the elaboration of histamine from histidine.