THE AMELIORATION OF EXPERIMENTAL HYPERTENSION WITH GRANULESTIN

by

ROBERT BARKER

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF ARTS

in the Department
of

BIOLOGY AND BOTANY

We accept this thesis as conforming to the
standard required from candidates for the
degree of MASTER OF ARTS.

Members of the Department of
BIOLOGY AND BOTANY.

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1953.
ABSTRACT

Granulestin, added to the basal diet of the rat, was found to be effective in lowering the blood pressure where an elevation had been obtained by nicotine injections and choline deficiency. No significant decrease in blood pressure could be found in the case of male or female rats treated with estradiol dipropionate.

Caffeine and desoxycorticosterone acetate injections were found to result in a slight transient hypertension and the effectiveness of Granulestin feeding could not be tested.
ACKNOWLEDGMENTS

I would like to express my thanks to:

Dr. A.H. Hutchinson, Head of the Department of Biology and Botany, under whose authority this work was carried out.

Dr. J. Allardyce, under whose personal supervision this investigation was made, and whose continued interest and constructive advice were invaluable.

Miss E. Jow, for her instruction in the method used to determine blood pressures.
CONTENTS

ABSTRACT......................................................... 1
INTRODUCTION.................................................... 2
RECENT THEORIES ON HYPERTENSION.............................. 3
THE APPARATUS AND METHOD USED IN DETERMINING BLOOD PRESSURES... 11
EXPERIMENTAL.................................................. 15

I. THE EFFECT OF DIETARY SUPPLEMENTS OF GRANULESTIN ON
   THE BLOOD PRESSURE OF RATS MADE HYPERTENSIVE WITH
   SMALL INJECTIONS OF NICOTINE............................ 15

II. THE EFFECT OF DIETARY SUPPLEMENTS OF GRANULESTIN ON
   THE BLOOD PRESSURE OF FEMALE WISTAR RATS MADE
   HYPERTENSIVE BY A PERIOD OF CHOLINE DEFICIENCY...... 21

III. THE EFFECT OF DIETARY SUPPLEMENTS OF GRANULESTIN ON
     THE BLOOD PRESSURE OF MALE AND FEMALE WISTAR RATS MADE
     HYPERTENSIVE BY THE ADMINISTRATION OF ESTROGENS..... 24

IV. THE EFFECT OF SMALL INTRAPERITONEAL INJECTIONS OF
    CAFFEINE ON THE BLOOD PRESSURE OF FEMALE WISTAR RATS.. 28

V. THE EFFECT OF DESOXYCORTICOSTERONE ACETATE ON THE
    BLOOD PRESSURE OF FEMALE WISTAR RATS.................. 32

GENERAL DISCUSSION............................................. 34
SUMMARY.......................................................... 35
CONCLUSION...................................................... 37
BIBLIOGRAPHY.................................................... 38
INTRODUCTION

During the past twenty years a great deal of research has been done on the production of a hypertensive state in experimental animals by such workers as Goldblatt (19), Selye (42), Shorr (48), Grollman (22) and Kendall (29). On the basis of these experimentations many classifications of hypertension have been suggested. In general, experimentally produced hypertension is classified under the headings:

I. Renal Hypertension  
II. Hormonal Hypertension  
III. Neurogenic Hypertension.

In 1950 Braun-Menendez (?) speaking at a symposium on hypertension suggested a fourth class:--

IV. Metabolic Hypertension.

This heading covers cases in which the hypertensive state might be attributed to a disruption of normal fat, protein, water or electrolyte metabolism.

With this view in mind and having considered recent work by Dietrich (10) who demonstrated that feeding diets high in phospholipids and tocopherol to diabetics usually resulted in an improved vascular condition and a decrease in insulin requirements, it was thought probable that phospholipid treatments might to some extent correct faulty metabolism. It was decided to test this hypothesis by feeding large doses of phospholipids to rats made hypertensive by various treatments taking a decrease in blood pressure as being indicative of an improved metabolism.

* The phospholipids used in this work were supplied by the American Lecithin Co. Inc., L.I. 77, N.Y. under the trade name "Granuleatin" and will be referred to as such throughout this paper.
RECENT THEORIES ON HYPERTENSION

The following sections contain a brief review of work and theories in the field of experimental hypertension using the classification suggested by Braun-Menendez (7).

I. RENAL HYPERTENSION

Kidney damage was first associated with vascular disorders by Richard Bright (6) in 1836. He noted that patients with renal lesions usually possessed an enlarged heart which he took to indicate vascular involvement. Tigerstedt and von Bergman (51) in 1898 were able to extract a substance, renin, from the kidney which when injected into normal animals caused an elevation of the blood pressure. It was not until 1934 that interest in the kidney as a causative factor in hypertension came to the fore. At this time Goldblatt and his associates (19) were able to produce permanent hypertension by restricting the blood flow to the kidney causing ischemia of that organ. They further showed that hypertension can occur in the absence of kidney damage and in the absence of renal innervation.

On the basis of this evidence Goldblatt (18) postulated a humoral factor as being responsible for the change in blood pressure. Since an increase in the renin content of the kidney and the blood was found immediately following the partial occlusion of the renal artery it was stated that renin was the factor released.

The renin hypothesis as it stands today states that renin is produced in the cortex of the kidney and is released
into the circulation where it acts as an enzyme on hypertensinogen, a blood globulin, converting it to hypertensin the active pressor substance.

The major flaw in this hypothesis lies in the fact that in cases of chronic hypertension it has been impossible to demonstrate an increase in the renin content of either kidney or blood (9).

Other workers have ascribed the elevated blood pressure in cases of renal deficiency to factors other than renin (5, 41, 44, 47). Grollman, Muirhead and Vanata (23) produced hypertension in dogs by total nephrectomy. They postulate that the pressor effect is due to the inability of the animal to remove, via the kidney, circulating vasoactive materials.

Floyer (14) and Ledingham (30) have shown that in the rat, the hypertensive effects of total nephrectomy can be reversed by bilateral adrenalectomy or increased by feeding high salt diets. This points to changes in the electrolyte balance being the effective factor.

Bing (5), Shipley and Helmer (47), Shorr (48), Grollman (21) and Schroeder (41) have all demonstrated pressor substances, other than renin, present in the blood in cases of hypertension due to renal involvement. Most of these findings have not yet been confirmed.

To summarize, it can be stated that renal damage may be a primary factor in the production of hypertension though the proof of this is not conclusive.
II. HORMONAL HYPERTENSION

Prado and Dontigny (36) adopted the term "hormonal hypertension" to describe the hypertension obtained by the administration of hormones or non-specific noxious substances. These factors may play a role in the development of the other forms of hypertension.

A. THE ADRENAL GLANDS.

Adrenal medulla.

Adrenalin is known to increase the blood pressure but its effect is transitory and thought to have little connection with the development of hypertension. Goldenberg (20) has shown that adrenalin acts as a vasodilatory and cardiotonic agent and in a hypertensive animal may lower the blood pressure. Noradrenalin which has been identified with sympathin is known to be a powerful vasoconstrictor with little effect on the heart. Adrenalin and noradrenalin are normally antagonistic in action and Goldenberg believes that some cases of spontaneous hypertension may be due to a disturbed balance between the two substances.

Adrenal cortex.

Selye (42) working with chicks showed that desoxycorticosterone acetate (DCA) caused renal lesions and that in rats prolonged injection or implantation of DCA resulted in the development of chronic hypertension. Diets high in salt tend to aggravate the condition and Sapirstein (40) was able to produce an apparently similar hypertension in rats by excessive salt feeding over a long period.
These results together with those of Floyer (14) and Ledingham (30) seem to point to the kidney and the adrenal cortex being closely linked through electrolyte metabolism in the genesis of hypertension.

B. THE HYPOPHYSIS.

Anterior lobe.

Extracts of the anterior lobe of the hypophysis exhibit pressor activity when injected into normal animals. The work of Selye (45), Prado, Dontigny et al (36) and Masson et al (31) indicates that the pressor action of this extract is due to adrenocorticotrophin which stimulates the adrenal cortex to secrete mineralo corticoids of similar function to DCA.

Posterior lobe.

Extirpation of the posterior lobe of the hypophysis does not alter the blood pressure in normal or hypertensive animals. However, pitressin one of the principles of the gland has a slight pressor action and antidiuretic effect and is thought by Ellis and Grollman (11) to be active in the development or maintenance of hypertension. These workers have shown a significant increase in the elimination of the antidiuretic principle in hypertensive men.

C. THE GONADS.

Testes.

Testosterone has not been shown to have any direct pressor activity (33).

Ovaries.

Sapeika (39) showed that stilbestrol injections caused
an elevation in the blood pressure of rats but Page (33) showed that moderate doses of estradiol had no effect on hypotensive adrenalectomised rats. Grollman (23) was able to produce hypertension in the rat with large doses of estrogen and his results have been confirmed in this laboratory (38).

It is not known in what way estrogens produce hypertension but the similarity of these substances to the hormones of the adrenal cortex suggests that their mode of action may be similar.

Selye et al (44) have been unable to produce hypertension in the rat with progesterone overdosage even though there is a close structural similarity to both estrogens and DCA.

D. THE THYROID.

Thyroxin does not seem to have any direct effect on the blood pressure but may have an indirect sensitizing effect due to its influence on protein metabolism (43).

III. NEUROGENIC HYPERTENSION

Hypertension has been produced experimentally by stimulation or section of various parts of the pressor regulator system. Heller (26) by intracisternal injections of kaolin, Nowak (32) by ligation of the spinal, carotid and vertebral arteries and Farris et al (12) by submitting rats to intermittent explosive noises, produced hypertension.

Little work has been done to explore the exact cause of the hypertension thus produced but it has been shown that denervation of the kidney in these cases prevents any rise in
blood pressure (52).

IV. METABOLIC HYPERTENSION

The preceding discussion has pointed to the kidney as being involved in the genesis or maintenance of all experimental hypertension. The concept of hypertension as due to a disturbance of metabolism does not overlook this fact but attempts to explain it at a cellular level.

The following section contains a short review of some of the experimental work which might be used to support this concept.

Parpart and Ballentine (34), using the plasma membrane of the erythrocyte, have shown:

a. That this membrane is composed of lipoproteins.

b. The ratio of protein to cholesterol to phospholipid is constant.

c. The permeability of the membrane can be altered by altering the structure of the component molecules.

d. Enzymes present within the cell are capable of causing a change in the molecular anatomy of the membrane.

Since it is believed that the plasma membranes of all living cells are composed of lipoproteins similar properties may be accorded to all cell types.

Anitschow (2), Gofman (17), Katz (23), Barr (3), Simms (49), and Kendall (29) have recently shown:

a. That feeding diets high in cholesterol produces
atherosclerosis (2, 17, 28, 29).

b. Feeding these diets alters the ratio of cholesterol to phospholipid in the blood (3, 17, 29).

c. That lipoproteins are a lipid transport mechanism in the blood (3, 17).

d. The incidence of atherosclerosis is not dependant on an overall increase in serum cholesterol but is closely linked with the appearance of a specific lipoprotein fraction in which the cholesterol/phospholipid ratio is increased (17).

e. That in most cases of hypertension a marked increase in the abnormal lipoprotein fraction can be demonstrated (17a).

The occurrence of atherosclerosis is dependant on a disturbance of normal metabolism which causes the appearance in the serum of a lipoprotein fraction in which the protein, cholesterol, phospholipid ratio is altered.

Results obtained by other workers and previously mentioned in this survey indicate that:

a. The kidney is involved in most forms of hypertension (18, 40, 42, 52).

b. Supplements of mineralo corticoids or salt accentuate the effect of other treatments (15, 40, 42, 43).

c. Renal involvement results in the increased production or decreased elimination of pressor substances (5, 18, 24, 41).

An increase in the production of pressor substances could only be due to an alteration in the normal metabolic pattern of the kidney. Decreased elimination of pressor
substances could be caused by an alteration in the molecular anatomy of the cell membranes.

The hypothesis of metabolic hypertension may be stated: Hypertension is primarily due to an altered protein or fat metabolism or to a change in the water-electrolyte balance in the body. Both of these are intimately associated with cell permeability the latter being dependant on it, the former possibly governing it.
THE APPARATUS AND METHOD USED IN DETERMINING BLOOD PRESSURES

Blood pressures were determined by the indirect method using the foot as previously described by Allardyce, Fitch and Semple (1).

Throughout the experiments readings were taken using the tail plethysmograph as described by Friedman and Friedman (15a) and by direct carotid cannulation as described by D'Amour and Blood (8a). (Plates I, II, and III).

In all cases an aqueous solution of sodium pentothal was used as an anaesthetic. An intraperitoneal injection of 0.3 ml. of freshly prepared 2.5% pentothal solution was found to produce light anaesthesia in a 150 gm. rat enabling blood pressure determinations to be made by either indirect method. The determinations of pressures by the direct method required doses of 0.8 to 1.0 ml. of 2.5% sodium pentothal for a rat of the same weight.
PLATE 1

INDIRECT METHOD USING THE FOOT

A. Aneroid Manometer.

B. Mercury Column.

C. Syringe.

D. Run-off tap.

E. Mechanical stage.
PLATE 2

TAIL PLETHYSMOGRAPH

A. Aneroid Manometer.

B. Pressure Cuff.

C. Capillary Tube.

D. Glass Plethysmograph.

E. Syringe.
PLATE 3

DIRECT METHOD. CAROTID CANNULATION.

A. #20 Hypodermic Needle.

B. Heavy Rubber Membrane (finger from rubber glove).

C. Light Rubber Membrane (dental dam).

D. Aluminum writing point.

E. Lever Fulcrum.
EXPERIMENTAL

I. THE EFFECT OF DIETARY SUPPLEMENTS OF GRANULESTIN ON THE BLOOD PRESSURE OF RATS MADE HYPERTENSIVE WITH SMALL INJECTIONS OF NICOTINE

INTRODUCTION.

Previous work by Fung (16) in this laboratory has shown that small intraperitoneal injections of dilute aqueous solutions of nicotine produce a sustained pressor effect in the rat. This investigation was undertaken to confirm these findings and to test the effect of dietary Granulestin supplements on the course of hypertension thus induced.

METHOD.

Trial 1.

Eight Wistar rats, five male and three female, 6 to 8 months old were injected intraperitoneally with 0.00001 mg. nicotine in 0.5 ml. of sterile water on days 0, 1, 2, 3, 4, and 5. The systolic blood pressures of these animals, and three control animals injected with 0.5 ml. of sterile water were determined at intervals for a period of 134 days.

Three of the four rats remaining on day 93 were fed 0.5 gm. Granulestin* per day for a period of 12 days.

On day 106 the remaining animal was started on a similar treatment.

* Granulestin contains 80% phospholipids comprising roughly equal amount of lecithin, cephalin, and lipositol.
Trial 2.

The blood pressures of eleven Wistar rats, eight males and three females were determined on three consecutive days to establish normal values. The rats were then divided into three groups.

Group 1. Four rats received intraperitoneal injections of 0.00001 mg. nicotine in 0.5 ml. of sterile water on days 4, 5, 6, 7, 8, and 9, and 0.5 gm. Granulestin per day, days 4 - 24 inclusive.

Group 2. Four rats received intraperitoneal injections of 0.00001 mg. nicotine in 0.5 ml. of sterile water on days 4, 5, 6, 7, 8, and 9, and 0.5 gm. Granulestin per day, days 27 - 37 inclusive.

Group 3. Three rats were fed 0.5 gm. Granulestin per day on days 4 - 27 and were injected with 0.00001 mg. nicotine in 0.5 ml. sterile water days 28 - 35 inclusive.

The blood pressures of all rats were read at intervals for a period of 40 days.

Trial 3.

Eight female Wistar rats were given intraperitoneal injections of 0.00001 mg. nicotine on days 8, 9, 10, 11, 12, and 13. Starting on day 81 the stock diet was supplemented by the addition of 0.5 gm. Granulestin per day. This treatment was continued for 40 days. The systolic blood pressures of these animals were determined at intervals for a period of 150 days.
RESULTS. (Figs. 1, 2 and 3)

In all cases it was shown that rats treated with small intraperitoneal injections of nicotine developed sustained hypertension. There was no significant difference noted between the sexes.

Granulestin had a definite effect in lowering the blood pressures of these animals and in preventing an elevation in blood pressure in those animals receiving both treatments simultaneously.

Granulestin feeding does not cause a drop in normal blood pressure nor does pretreatment with Granulestin protect against the hypertensive effect of nicotine.

DISCUSSION.

The role of nicotine in the genesis of hypertension has not been widely studied. Short term experiments have shown nicotine to have a pressor effect but the literature contains no reference to sustained responses being obtained.

In vitro and in vivo studies have shown that nicotine acts as a ganglionic blocking agent affecting the body of the nerve cell and not the post ganglionic fibre (23a). It is possible that nicotine acts to block the synaptic release of acetylcholine or to alter the permeability of cell to various substances.

On the basis of what is known of the action of nicotine it is impossible to state in what way lecithin could counteract its hypertensive effect. Does lecithin supply acetylcholine or
Fig. 1 (Trial 1) The Effect of Granulestin on the Blood Pressures of 4 Rats made Hypertensive with Intraperitoneal Injections of .00001 mg. of Nicotine on days 0, 1, 2, 3, 4, and 5.

- 3 rats fed Granulestin days 93 - 104 inclusive.
- 1 rat fed Granulestin days 106 - 117 inclusive.

Period of Granulestin treatment.

Fig. 2 (Trial 2) The Effect of Granulestin on the Development of Hypertension in the Rat Due to Nicotine Injections.

- 4 rats receiving .00001 mg. nicotine intraperitoneally days 4, 5, 6, 7, 8, and 9, and 0.5 gm. Granulestin days 27 - 37.

- 4 rats receiving .00001 mg. nicotine intraperitoneally days 4, 5, 6, 7, 8, and 9, and 0.5 gm. Granulestin days 4 - 24.

- 3 rats receiving 0.5 gm. Granulestin days 4 - 27 and injected with .00001 mg. nicotine days 28 - 33 inclusive.
does it become part of the plasma membrane of the nerve cell?

CONCLUSION.

Small injections of nicotine will produce sustained hypertension in rats.

The addition of large amounts of Granulestin to the diet rapidly ameliorates this hypertension even when it has been sustained for 100 days.

Granulestin supplements protect against the hypertensive action of nicotine though pretreatment with Granulestin does not seem to have any effect.
**Fig. 3**  (Trial 3) The Effect of Granulestin Supplements on the Blood Pressure of Female Wistar Rats made Hypertensive with Six Injections of .00001 mg. Nicotine.

- ○--○ 3 rats injected with .00001 mg. nicotine on days 8 - 13 inclusive.

- •---• 5 rats injected with .00001 mg. nicotine on days 8 - 13 inclusive and fed 0.5 gm. Granulestin days 81 - 121.

--- Period of Granulestin treatment.

**Fig. 4** The Effect of Granulestin Supplements on the Blood Pressure of Female Rats made Hypertensive by a Period of Choline Deficiency.

- ○--○ 3 rats fed a choline deficient diet days 6 - 15 inclusive.

- •---• 5 rats fed a choline deficient diet days 6 - 15 inclusive and 0.5 gm. Granulestin per day from day 77 to day 116.

--- Period of choline deficiency.

--- Period of Granulestin treatment.
II. THE EFFECT OF DIETARY SUPPLEMENTS OF GRANULESTIN ON THE BLOOD PRESSURE OF FEMALE WISTAR RATS MADE HYPERTENSIVE BY A PERIOD OF CHOLINE DEFICIENCY

INTRODUCTION.

Salter (38), in this laboratory, showed that a short period of choline deficiency would result in an increase in systolic pressure of the rat after it has been returned to a normal diet.

This experiment was carried out to confirm this work and to determine whether the administration of Granulestin would ameliorate hypertension thus induced.

METHOD.

Eight female Wistar rats, 3 - 4 months old, were subjected to a period of choline deficiency from day 6 to 15 inclusive and were then returned to stock diet. (Deficient diet identical to that used by Best and Hartroft (4)(Table I).

Five of the rats were fed 0.5 gm. Granulestin per day from day 77 to day 116.

The blood pressures of all animals were determined at intervals for a period of 140 days.

RESULTS. (Fig. 4)

Six days after the animals had been returned to the stock diet the average blood pressure of the group was 210 mm. Hg. These hypertensive levels were maintained showing little tendency to fall.
Treatment with Granulestin resulted in a marked decrease in the blood pressures of all the test animals. These animals had pressures within the normal range 28 days after the addition of daily Granulestin supplements.

DISCUSSION.

Best and Hartroft (4a) have recently shown that rats maintained on a choline deficient diet for life did not become hypertensive while those returned to a stock diet after a 6 day deficiency did become hypertensive. A comparable degree of renal damage was demonstrated in both groups of animals. The hypertensive animals usually developed a degree of hepatic damage while the others did not. These findings indicate that hypertension in this case was secondary to liver damage.

Granulestin was found to effectively ameliorate hypertension due to a period of choline deficiency. The phospholipids are important in that they are the lipid transport molecules. A deficiency in these substances or their precursors usually results in the deposition of fat in the liver and a consequent upset in the metabolic pattern. The Granulestin supplements would increase the rate of lipid turnover in the liver causing regression of any fat deposited there (8).

CONCLUSION.

The hypertensive action of a 9 day period of choline deficiency can be completely reversed by the addition of Granulestin to the stock diet.
<table>
<thead>
<tr>
<th>Constituent</th>
<th>Percentage</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>69.5%</td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>7.0%</td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>3.0%</td>
<td></td>
</tr>
<tr>
<td>Fibrin</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>Salts</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Vitamin Mix</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>Cystine</td>
<td>0.5%</td>
<td></td>
</tr>
<tr>
<td>Beef fat</td>
<td>12.0%</td>
<td></td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>1.0%</td>
<td></td>
</tr>
</tbody>
</table>

1 Salts (N.B.C. Salt mix No. 2)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>4.35%</td>
<td></td>
</tr>
<tr>
<td>MgSO₄</td>
<td>13.70%</td>
<td></td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>8.72%</td>
<td></td>
</tr>
<tr>
<td>K₃PO₄</td>
<td>23.98%</td>
<td></td>
</tr>
<tr>
<td>Ca(H₂PO₄)₂</td>
<td>13.58%</td>
<td></td>
</tr>
<tr>
<td>Ferric Citrate</td>
<td>2.97%</td>
<td></td>
</tr>
<tr>
<td>Calcium Lactate</td>
<td>32.70%</td>
<td></td>
</tr>
</tbody>
</table>

2 Vitamin Mix

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Percentage</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamin</td>
<td>500 mg.</td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>250 mg.</td>
<td></td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>200 mg.</td>
<td></td>
</tr>
<tr>
<td>Ca Pantothenate</td>
<td>1 gm.</td>
<td></td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>1 gm.</td>
<td></td>
</tr>
<tr>
<td>Powdered sugar</td>
<td>997.05 gm.</td>
<td></td>
</tr>
</tbody>
</table>
III. THE EFFECT OF DIETARY SUPPLEMENTS OF GRANULESTIN ON THE
BLOOD PRESSURE OF MALE AND FEMALE WISTAR RATS MADE
HYPERTENSIVE BY THE ADMINISTRATION OF ESTROGENS

INTRODUCTION.

Grollman (23) has shown that the administration of large
doses of estrogens to rats results in hypertension, hypertrophy
of the kidneys and salt and water retention. Salter (38) and
Rixon (37) in this laboratory were able to confirm these results
using intact and castrate male rats.

This experiment was carried out to confirm and extend
these results and to determine what effect if any the administra­
tion of Granulest in would have on hypertension induced in this
way.

METHOD.

Eight male and eight female Wistar rats were given
subcutaneous injections of 0.5 mg. estradiol dipropionate in
1 ml. sesame oil on days 5, 6, 7, 8, and 9.

Daily supplements of 0.5 gm. Granulest in were added to
the diets of three of the five remaining male rats on days 51 to
101 inclusive.

Due to lack of space the females could not be placed
on Granulest in supplements until day 86. From day 86 to day
136, four of the six remaining female rats received 0.5 mg.
Granulest in per day.

In both tests the untreated animals were used as
controls.
Blood pressures of all animals were read at intervals for a period of 150 days.

RESULTS. (Fig. 5)

A marked increase in the average blood pressure of both males and females was observed within 10 days of the last injection of estradiol dipropionate.

The average pressure for the male rats at this time was 290 mm. Hg, while the average pressure for the female rats was 234 mm. Hg. The blood pressure of the male rats remained within 20 mm. Hg. of this value for 150 days showing no tendency to return to normal with or without Granulestin supplements.

The average blood pressure of the female rats had fallen to 194 mm. Hg. by day 35 and remained at this value for 150 days showing no tendency to fall during the period of Granulestin feeding.

Three male rats and two female rats died during the first 10 days of the experiment and values reported are the averages for those remaining in the group.

DISCUSSION.

These experiments showed that the administration of estradiol dipropionate results in a sustained elevation in the systolic blood pressure of both male and female rats. This elevation is more pronounced in the male than in the female.

Previous investigators have shown that estrogentic substances cause hypertrophy of the kidney and salt and water retention. Such anatomical changes indicate that in all
Fig. 5  The Effect of Granulestin Supplements on the Blood Pressure of Male and Female Rats made Hypertensive with Five Subcutaneous Injections of 0.5 mg. Estradiol Dipropionate on days 5, 6, 7, 8, and 9.

- 2 male rats receiving no Granulestin.
- 3 male rats receiving 0.5 gm. Granulestin days 51 - 101 inclusive (shown ).
- 2 female rats receiving no Granulestin.
- 4 female rats receiving 0.5 gm. Granulestin days 86 - 136 (shown ).
FIG 5
probability estrogens are converted to some other steroid in the
body (23, 35), probably a mineralo corticoid.

Treatment with Granulestin did not significantly lower
the blood pressure probably because the estrogen treatment had
resulted in irreversible liver and kidney damage.

The fact that the female rat normally secretes and
metabolizes more estrogens than does the male rat may explain
the difference in response of the two groups of animals.

CONCLUSIONS.

Subcutaneous administration of estrogens to male and
female Wistar rats results in a sustained hypertension which
did not respond to treatment with Granulestin.
IV. THE EFFECT OF SMALL INTRAPERITONEAL INJECTIONS OF 
CAFFEINE ON THE BLOOD PRESSURE OF FEMALE WISTAR RATS

INTRODUCTION.

Jow (27) in this laboratory has shown that weak 
solutions of caffeine administered intraperitoneally will cause 
an elevation in the blood pressure of both male and female rats. 
This experiment was carried out to confirm these 
results and to study the effect of further doses of caffeine.

METHOD.

Eight female Wistar rats were injected intraperitoneally 
with 0.001 mg. caffeine in 0.5 ml. sterile water on days 0, 1, 3, 
5, 6, and 7, and with 0.01 mg. caffeine in 0.5 ml. sterile water 
on days 96, 97, 98, 99, 110, 111, 112, and 113.

The blood pressures of all animals were read at 
intervals for a period of 130 days.

RESULTS. (Fig. 6)

Eight days after the first injection it was found that 
there had been an average increase in blood pressure from 125 to 
158 mm. of Hg. The average pressure 13 days after the first 
injection was 220 mm. of Hg. with a range from 167 to 242 mm. of 
Hg. These pressures fell gradually until on day 95 the average 
pressure was again 125 mm. of Hg.

Further injection of caffeine in 10 times the dosage 
failed to produce any further increase in blood pressure.
DISCUSSION.

Jow (27) was able to show that the administration of 2, 3, or 4 injections of 0.001 mg. of caffeine intraperitoneally within a period of 11 days would cause a striking rise in blood pressure within 15 days of the first injection to levels over 300 mm. of Hg. This elevation held for 20 to 35 days and pressures returned to normal within 80 days. Further work showed that injection given at less frequent intervals would produce an initial response lasting 10 to 15 days but that in some cases later injections were ineffective.

This experiment showed that six injections of a similar strength given in the first seven days of the experiment resulted in a marked elevation in the systolic pressure. This elevation was not however comparable to that obtained by Jow with fewer injections.

It was found that further injections of a stronger solution produced no further change in the blood pressure of these animals after their pressure had returned within the normal range. This does not entirely support the results previously obtained yet it does not invalidate them since there is a marked difference in the two experimental procedures. In this experiment larger and more frequent injections were given which may have led to a type of "immunity" being developed by the rats. Further experimentation may throw some light on this question.
CONCLUSION.

Intraperitoneal injections of dilute solutions of caffeine were found to produce a transient hypertension in female Wistar rats. After the blood pressure of these rats had returned to normal further injections even when increased tenfold in strength failed to re-elevate the blood pressure.
Fig. 6 The Effect of Repeated Injections of Caffeine on the Blood Pressure of Female Wistar Rats.

Average pressure of eight female Wistar rats receiving 0.001 mg. caffeine intraperitoneally on days 0, 1, 3, 5, 6, and 7, (○) and 0.01 mg. caffeine intraperitoneally on days 96, 97, 98, 99, 110, 111, 112, and 113, (□).

Fig. 7 The Effect of DCA and Salt Overdosage on the Blood Pressure of Eight Female Wistar Rats.

8 female Wistar rats receiving 1.0 mg. DCA intramuscularly on days indicated (●●●●) and 1.5 mg. DCA intramuscularly on days indicated (●●●●●).

Period in which 2% saline was used instead of tap water.

3 female Wistar rats receiving no treatment.
FIG 6

FIG 7
V. THE EFFECT OF DESOXYCORTICOSTERONE ACETATE ON THE BLOOD PRESSURE OF FEMALE WISTAR RATS

INTRODUCTION.

Rixon (37) in this laboratory was able to produce transient hypertension in Wistar rats with a single intramuscular injection of 1 mg. DCA. Semple (46) and Fitch (13) were later unable to reproduce these results using the same strain of rats. Salter (38) found that substituting 2% sodium chloride solution for drinking water caused a sustained hypertension. Other investigators have shown that much more drastic treatment than this is required to induce hypertension with DCA (15, 42).

This experiment was carried out to further clarify these findings.

METHOD.

Eight female Wistar rats were injected intramuscularly with 1 mg. DCA in oil on days 6, 9, 21, 22, and 23, and with 1.5 mg. DCA in oil days 27, 28, 29, 30, 31, 32, 33, and 34. 2% sodium chloride was substituted for drinking water days 25 to 40.

Blood pressure determinations were made on all animals at intervals for a period of 40 days.

Three female Wistar rats which were not treated were used as controls.
RESULTS. (Fig. 7)

Following the first two injections of DCA a slight rise in blood pressure was obtained. This elevation lasted for approximately 14 days. Further injections of DCA and the addition of 2% sodium chloride to the drinking water did not result in any further changes in pressure.

DISCUSSION.

Selye (42), Braun-Menendez (7), and Friedman (15) have all shown that in the rat, overdosage of DCA must be long continued before a permanent hypertension can be obtained. These investigators have subjected their test animals to unilateral nephrectomy, prolonged treatment with DCA and high salt diets.

The results obtained in this experiment indicate that the rat after a transient response is resistant to short periods of overdosage with DCA and high salt diet.

CONCLUSION.

Small intramuscular injections of DCA may result in a transient elevation of blood pressure in female Wistar rats. Once the blood pressure has returned to normal further injections of DCA and the addition of salt to the drinking water have no effect on the blood pressure.
GENERAL DISCUSSION

The fact that two groups of animals, those made hypertensive by nicotine injections and those made hypertensive with a period of choline deficiency, responded to treatment with Granulestin while those treated with estrogens did not, points to different mechanisms being involved in the genesis of hypertension.

Most investigators consider that kidney damage, either structural or functional, is necessary for the production of hypertension (4a, 15, 18, 43). In most experimental procedures this damage is inevitable. The reports of Grollman (22) indicate that estrogen treatments do result in massive kidney damage as does prolonged administration of DCA.

It is not known in what way nicotine injections produce hypertension but gross autopsy has revealed no macroscopic renal disturbances. Choline deficiency, according to Sobin and Landis (53), produces hypertension secondary to renal damage. This point has not been confirmed. It is probable that in this case the primary hypertensive effect is due to a disturbed metabolism of fat and protein in the liver (4a).

It is reasonable to postulate that in the case of hypertension induced by estrogens irreversible changes are produced in the kidney or other organs. Hypertension induced by nicotine injections and choline deficiency is probably due to reversible changes in the metabolic pattern of the animal. Such changes could affect cell permeability, detoxification of pressor substances, or blood composition.
SUMMARY

Sustained hypertension was produced in female Wistar rats by the following methods:

1. Six daily intraperitoneal injections of 0.00001 mg. nicotine in 0.5 ml. sterile water.
2. Feeding a diet deficient in choline and its precursors for a period of 9 days.
3. Five daily subcutaneous injections of 0.5 mg. estradiol dipropionate in 1 ml. of sesame oil.

Sustained hypertension was produced in male Wistar rats by 5 daily subcutaneous injections of 0.5 mg. estradiol dipropionate in sesame oil.

Treatment with Granulestin (0.5 gm/day) for a period of 40 days lowered the blood pressure of the female rats made hypertensive by nicotine injections and choline deficiency.

Similar treatment for a period of 50 days did not result in any lowering of the blood pressure in either male or female rats made hypertensive with estrogen injections.

Six daily intraperitoneal injections of 0.001 mg. caffeine in 0.5 ml. sterile water were found to produce a transient (80 days) elevation in blood pressure in female Wistar rats. Further injections even when increased tenfold in strength did not re-elevate the blood pressure.

Two intramuscular injections of 1 mg. of DCA in 0.2 ml. sesame oil resulted in a transient increase in the blood pressure of female Wistar rats. This increase lasted 15 days. Subsequent
injections of 1.5 mg. of DGA in 0.3 ml. of sesame oil and substitution of 2% sodium chloride solution for drinking water produced no re-elevation in blood pressure after the blood pressure had returned to normal.
CONCLUSIONS

1. Small injections of nicotine or estrogens or a period of choline deficiency will result in a sustained elevation in the blood pressure of female Wistar rats.

2. Small injections of nicotine or estrogens produce a sustained elevation in the blood pressure of male Wistar rats.

3. Small injections of caffeine and DGA result in a transient elevation in the blood pressure of female Wistar rats.

4. Dietary supplements of Granulestin ameliorate hypertension in the rat when induced by nicotine injections or a period of choline deficiency.

5. Dietary supplements of Granulestin do not affect the blood pressure of normotensive rats or rats made hypertensive by overdosage with estrogens.
1. ALLARDYCE, J., F. FITCH, and R. SEMPLE.  

2. ANITSCHKOW, N.  

3. BARR, D. P.  
   In Zweifach and Shorr: Factors Regulating Blood Pressure.  

4. BEST, C.H., and W.S. HARTROFT.  

4a. HARTROFT, W.S.  
   In Zweifach and Shorr: Factors Regulating Blood Pressure.  

5. BING, R.J.  

6. BRIGHT, R.  

7. BRAUN-MENDEZ, E.  

8. CHAIKOFF, I.L., C. ENTENMAN, and M.L. MONTGOMERY.  

8a. D'AMOUR, F.E., and F.R. BLOOD.  
   Manual for Laboratory Work in Mammalian Physiology.  

9. DEXTER, L., and E.W. HAYNES.  

10. DIETRICH.  
    Personal Communication with J. Eichberg. American Lecithin Co.  
    Inc. L.I. 77, N.Y.

11. ELLIS, M.E., and A. GROLLMAN.  

12. FARRIS, E.J., E.H. YAEKEL, and H.S. MEDOFF.  

13. FITCH, F.  
14. FLOYER, M.A.

15. FRIEDMAN, S.M., C.L. FRIEDMAN, and M. NAKASHIMA.

15a. FRIEDMAN, S.M. and C.L. FRIEDMAN.

16. FUNG, E.
   Unpublished data.

17. GOFMAN, J.W., F. LINDGREN, H. ELLIOTT, W. MANTZ, J. HEWITT, 
   B. STISOWER, and V. HERRING.
   Science 111: 166, 1950.

17a. GOFMAN, J.W.

18. GOLDBLATT, H.
   In Zweifach and Shorr: Factors Regulating Blood Pressure.

19. GOLDBLATT, H., J. LYNCH, R.F. HANZAL, and W.W. SOMMERVILLE.

20. GOLDENBERG, M., K.L. PINER, E. de F. BALDWIN, D.G. GREENE, 
    and C.E. ROCH.


22. GROLLMAN, A.
    Essentials of Endocrinology. J.B. Lipincott Co., Philadelphia, 

23. GROLLMAN, A., E.E. MUIRHEAD, and J. VANATTA.

23a. GROLLMAN, A.

24. GROLLMAN, A.
    In Zweifach and Shorr: Factors Regulating Blood Pressure.

25. HALL, C.E., and H. SELYE.

26. HELLER, H.
    Ref. BRAUN-MENENDEZ, E.

27. JOW, E.
    Unpublished data.
29. KATZ, L.N., and STAMLER, J.

29. KENDALL, F.E.
In Zweifach and Shorr: Factors Regulating Blood Pressure.

30. LEDINGHAM, J.M.

31. MASSON, G.M.C., A.C. CORCORAN, and I.H. PAGE.

32. NOWAK, S.J.G., and I.J. WALKER.

33. PAGE, E.W., E. OGDEN, and E. ANDERSON.

34. PARPART, A.K., and R. BALLENTINE.
In Baron: Modern Trends in Physiology and Biochemistry.

35. PASCHKIS, K.E., and A.E. RAKOFF.

36. PRADO, J.L., P. DONTIGNY, E.C. HAY, and H. SELYE.

37. RIXON, R.H.
Master's Thesis.

38. SALTER, J.M.
Master's Thesis.

39. SAPIRSTEIN, L.A., W.L. BRANDT, and D.R. DRURY.

40. SELYE, H.

41. SELYE, H., E. BELAND, and H. STONE.

46. SEMPLE, R. Master's Thesis.


49. SIMMS, H.S. J. Gerontol. 6: 160, 1951.


