CAFFEINE AS A HYPERTENSIVE REAGENT

by

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ABSTRACT

Caffeine has been shown to induce a transient hypertensive state in Wistar rats. The height to which the blood pressure rises in this caffeine-induced hypertension, and the duration of this hypertensive state was found to be dependent on the concentration of caffeine administered.

Caffeine exposed to negatively ionized air was shown to undergo a loss in its pressor activity. This loss in pressor activity was found to be greater when the caffeine was exposed in solution than when it was exposed in the crystalline state.

Once the blood pressures of Wistar rats were elevated with injections of caffeine and had again returned to normal levels there were no further rises in blood pressures with the administration of an equal number of injections of this drug.

TABLE OF CONTENTS

TABLE OF FIGURES, TABLES, and PLATES

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INTRODUCTION

The study of experimental hypertension has, within the past quarter century, progressed enormously. Although great growth has been achieved in the knowledge of the mechanism of hypertension, most investigators know that the end is not yet, and that hypertension will not yield its secrets easily.

As a result of the extensive work done in the field of experimental hypertension, there have been postulated a vast number of theories as to the mechanism of the disease. These concepts may be classified, in general, into three main categories:

I Neurogenic
II Nephrogenic
III Endocrinal

Attention must be drawn to the fact that although these concepts may be classified into the three categories mentioned above, this has been done only to facilitate an easy approach to the study of the genesis of the disease, and that each category should not be considered as a separate entity.

To describe the situation in the words of Page (99) "We believe a more useful way to think about the problem is in terms of equilibrated mechanisms."

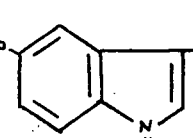
RECENT CONCEPTS OF EXPERIMENTAL HYPERTENSION

NEUROGENIC

Within the brain there are three areas which initiate or transmit sympathetic discharges to regulate vasomotor tone. These are: the cortex, the hypothalamus, and the vasomotor center. The manner in which these areas are involved in the production of hypertension is not clearly understood. However it is evident that several influences, such as ligature of the main blood vessels to the brain (35),(114), intracranial pressure (37), auditory stimulation (33),(115), or puncture of the third ventricle (142), can cause hypertension.

a. Cerebral role

Schroeder (119) has suggested four possibilities to explain the cerebral role in human hypertension. However, since three of these are mere hypotheses without any substantiating evidence, clinical or experimental, only the fourth will be considered. He believes that the peripheral metabolic abnormalities associated with hypertension may cause stimulation of cerebral metabolism. It is known that many primary amines, such as amphetamine, norepinepherine and serotonin, can cause central excitatory effects. Therefore, he states that some primary amines circulating in the blood may induce cerebral stimulation.

Serotonin,  a derivative of tryptophane, has received the greatest interest in this regard. This primary amine occurs in the brain and is believed to have a definite function in nervous tissue (17). It has been discovered to be involved in cerebral interneurone transmission (119) and apparently has a specific affinity for cortical pathways to the posterior and lateral hypothalamus.

Intravenous injections of serotonin have been reported to cause hypertension (110). However, Page and McCubbin have reported it to be hypotensive (101), and diphasic (100) (causing a fall followed by a slight rise in blood pressure). Haddy et al (58) found that serotonin has a bidirectional response. When small vessels are already neurogenically dilated, serotonin continues to constrict the large vessels with a net effect of constriction. However, when the small vessels are highly constricted, serotonin dilates small vessels more than it constricts the large vessels with a net effect of dilation. Brodie et al (17) reported that intravenous injections of serotonin are not valid since serotonin passes the blood-brain barrier with great difficulty. Bulle (18) found that when serotonin was injected into the sub-arachnoid space in dogs there was an elevation in blood pressure.

The use of serotonin antagonistic drugs in the treatment of hypertension has tended to add weight to Schroeder's hypothesis that primary amines, serotonin in particular, may induce cerebral stimulation thus causing hypertension. Reserpine, a member of the Rauwolfia family, is probably the most commonly used drug in the treatment of hypertension of a neurogenic nature. Its anti-hypertensive effects have been shown in patients with severe as well as in those with mild hypertension (24). Reserpine has been reported to cause mobilization and depletion of cerebral serotonin in experimental animals (122). Its locus of action appears to be in the posterior hypothalamus, and it is thought that reserpine affects the brain sites responsible for binding serotonin (81).

b. Carotid sinus

The regulatory function of the carotid sinus in restraining excessive rises and falls in blood pressure was first clarified by Hering (66). In 1927, his demonstration of the importance of the reflex regulation of the blood pressure from the stretch receptors of the carotid sinus led him to

speculate on the possible role of disturbance of this reflex in the genesis of hypertension in man (16). This ability of the carotid sinus receptors in affecting the arterial blood pressure, due to stretching of the sinuses, was later confirmed by Haus et al (60).

Koch and Mies (74), and Boucket and Heymans (10) obtained experimental neurogenic hypertension by section of the sino aortic buffer nerves. Heymans (67) obtained chronic sustained hypertension in dogs by ablation of the carotid sinus and aortic depressor nerves. Crandall et al (25) produced hypertension in dogs by bilateral constriction of the carotid sinus area.

Wakerlin et al (141) found that by altering cerebral hemodynamics due to reduction of volume pulse of the sinus, and of the internal and external carotid arteries, hypertension could be produced in dogs. Hawthorne and Green (62) later confirmed this finding of Wakerlin.

c. Psychogenic factors

Moses et al (93) stated that the possible origin of essential hypertension indicated that the arteriolar constriction characteristic to this disease may be due to psychogenic factors. The site of psychic precipitation is believed to be the cortex and the stimuli originating there act upon the hypothalamus, which initiated excessive sympathetic discharges.

Lang (83) has also suggested that essential hypertension is initiated by disturbance of the normal regulatory (inhibitory) effect of the cerebral cortex on the hypothalamic vasomotor center. This alteration in the cerebral cortex first produced labile and later stable elevation of blood pressure with secondary renal and cardiac involvement. The loss in initial cerebral cortical inhibition resulted from prolonged psychic stress, particularly suppressed "negative" emotions. This theory of Lang is

substantiated by a considerable mass of evidence, both experimental and clinical (123).

Goldberger (46) stated that hypertension was a sequel to continued stress. Grollman (55) disagrees with this on the basis of lack of factual support, and evidence which negates this (84).

NEPHROGENIC

The association of the kidney with hypertension was first suggested by Richard Bright (16) in 1836. However, it was not until 1934 that this association seemed to become more tangible when Goldblatt (44) succeeded in producing hypertension in the dog by applying a clamp to its renal artery. At present there are two possibilities of the causation of nephrogenic hypertension: (1) loss of a protective action by the kidney, and, or lack of a renal anti-pressor substance that keeps the blood pressure down, (2) secretion of a renal pressor substance.

a. Loss of a protective action by the kidney

In 1938, Fasciolo (34) found that a rise in blood pressure of dogs with a unilateral renal artery clamp, was transitory as long as the contralateral intact kidney was present; but on removal of the intact kidney a permanent rise in the blood pressure was obtained. From this he deduced that there was a "protective action by the normal kidney." Pickering and Prinzmetal (107) also found that in rabbits, clamping of one renal artery produced a temporary state of hypertension with a decrease in size of the clamped kidney and an enlargement of the normal one. Like Fasciolo they found that removal of the hypertrophied kidney caused permanent hypertension which they ascribed to the inability of the clamped kidney to remove some

substance from the blood stream. Kolff et al (77) using dogs, implanted the ureters into the vena cava and found that when renal tissue was present no hypertension developed, even though the excretory function of the kidney was thwarted by leading the urine into the blood stream. From this they concluded that hypertension was not due to the secretion of a pressor substance, nor by a substance excreted by the kidney, but to a substance produced elsewhere and normally destroyed by the kidneys.

Braun Menendez (12) believed that this substance is renotrophin, an intermediary metabolic substance which is influenced by protein rich diets, pituitary extracts, testosterone, and thyroid hormones (13). The existence of hypertension is believed by him to be due to an upset in equilibrium between the production of renotrophin and the ability of the kidney to cause its destruction, utilization, or transformation. Increase in the size of the normal kidney is believed to be due to the extra effort of that kidney to remove renotrophin.

Further evidence of the protective action of the normal kidney was observed by Grollman (52) who found that nephrectomy of one of a pair of rats in parabiosis resulted in hypertension in that animal but not in its normal partner. Moreover, it has been observed that parabiotic union of a chronically hypertensive rat with a normal one has resulted in a reduction in blood pressure to normal levels of the hypertensive rat. However, when a hypertensive rat was united in parabiosis with another hypertensive rat or with a nephrectomized rat there was no reduction in blood pressure (12).

Hamilton and Grollman (59) found that renal extracts administered to hypertensive animals and patients resulted in a lowering of the blood pressure. Rondell et al (113) have shown that a constrictor substance in

the blood of completely nephrectomized rats is present. However, no characterization of the substance has as yet been achieved.

b. Renoprival hypertension

Braun Menendez and von Euler (15) were the first to demonstrate hypertension in rats, by complete nephrectomy. However, better success in this field was obtained by Grollman (54) with completely nephrectomized dogs, by feeding the animals a low protein diet, electrolyte free diet, or by dialysing the blood by peritoneal lavage, or by the use of an artificial kidney. This type of hypertension was termed renoprival hypertension, and is believed to be due to an absence of a protective action of the kidney (15). Most investigators characterize renoprival hypertension as being a new type of hypertension completely different from that obtained by Goldblatt.

A reduction of blood pressure in dogs with renoprival hypertension, after vascular transplantation of a normal kidney, has been reported (92). The same results have also been reported in humans with malignant hypertension (9).

c. Secretion of a renal pressor substance

Tigerstedt and Bergman (136) in 1898, were the first to demonstrate that the kidney contained some substance that was capable of producing an elevation in blood pressure. They found that by injecting a saline extract of rabbit kidneys into anesthetized rabbits, an elevation in blood pressure was elicited. This active substance they named renin.

In 1938, it was shown that kidney extracts did not contain a direct acting pressor substance as such (75) but a proteolytic enzyme (75) which was capable of initiating a series of reactions which eventually culminated in an elevation of blood pressure. This enzyme inherited the name renin since it was believed that this enzyme was synonomyous with the

substance referred to by Tigerstedt and Bergman. It has been shown that this enzyme is associated with the juxtaglomerular apparatus (56).

Renin acts on a specific group of a specific alpha-2-globulin (104), renin substrate, to produce a 10 amino acid polypeptide Hypertensin I. This decapeptide had been reported to have had a pressor potency when tested in the rat (127), but due to advances in purification technique it is now considered to be vaso-inactive (22).

Skeggs et al (127) found another equally specific enzyme, "converting enzyme", which is believed to be a metallo-protein. This enzyme, which requires chloride ions for its activation, acts on Hypertensin I to split off histadylleucine. The octapeptide which results from this reaction has been recently named Angiotensin (69), a contraction of Angiotonin and Hypertensin II. Angiotensin is now recognized as the highly vaso-active agent which is responsible for the elevation of arterial blood pressure in renal hypertension (63). Page et al (105) and Rittel (111) have both been able to synthesize Angiotensin and they have found that the synthetic compound has much the same pressor activity as its natural analog.

d. Initiation of renal hypertension

The fact that hypertension could be elicited by a clamp constricting the renal artery led almost inevitably to the thought that lack of blood, or ischemia, was the immediate cause of renal hypertension. However, in experimental animals, renal ischemia has not been found necessary to elicit hypertension (20),(27).

Hawthorne (61) found that reducing femoral arterial pulse pressure, without concurrently reducing mean pressure, caused a significant rise in mean femoral pressure. Kohlstaedt and Page (76) showed that renin was liberated from a perfused dog's kidney when pulse pressure was reduced,

but mean arterial pressure and renal blood flow were kept constant. Corcoran and Page (23) have suggested that a dimunition in pulse pressure may be the effective stimulus. However, from the data mentioned above it seems that a reduction in pulse pressure and not ischemia acts as a stimulus for triggering off experimental renal hypertension.

e. Validity of the renal pressor system

That the renal pressor system is the operating force in chronic experimental hypertension was established by Wakerlin (138). Skeggs and Kahn (125) have found a significant increase of angiotensin in the blood of hypertensive humans and also in sufficient amounts in dogs with experimental hypertension to produce hypertension in normal dogs (126).

Taquini and Fasciolo (9) have found that although the renin content of blood and of the kidneys of hypertensive patients and dogs with chronic renal hypertension was the same as in controls of both species, clamping the renal artery caused an increase in the renin content in the kidney, which reached a peak 60 minutes after the initiation of the ischemia. Braun Menendez and his group (14) have found renin in the blood of acutely hypertensive animals and in a few patients.

Anti-renin produced by Wakerlin (139) has been shown by Goldblatt (45) to reduce blood pressure in hypertensive dogs when the concentration of two units of anit-renin per millileter of blood was maintained. Wakerlin (140) found that the concentration of 14 anti-renin units per millileter of plasma was required to cause a fall in blood pressure of hypertensive dogs to their normal levels.

ENDOCRINAL

Except for the fact that tumours of the adrenal cortex and the adrenal medulla can cause hypertension, evidence of endocrine imbalance in the genesis of hypertension is rather rare. The role of the endocrine glands appears to be secondary, conditioning, or permissive, and not primary, especially in humans. The work of Wakerlin (138) typifies the conditioning or permissive role of the endocrine glands in renal hypertension.

a. Adrenal medulla

Adrenalin is known to increase the blood pressure, but since its effects are transitory it has been thought of as having little connection with the genesis of hypertension. Labbe et al (82) were the first to demonstrate the presence of adrenalin in benign tumours of the supra-adrenal medulla. Beer et al (7) believed that a vasoconstrictor substance was present during the paroxysms of this affliction, but was absent after removal of the tumour. This substance resembled adrenalin, but since the elevation of blood pressure obtained in patients with pheochromocytoma, the name given to this irregularity, was not in keeping with that produced by adrenalin, it was thought that some other pressor substance was also secreted by these tumours.

The dilemna was resolved by Holton (68) who discovered that the chromaffin tumours contained large excesses of nor-adrenalin in addition to adrenalin. Barnett et al (5) found that circulatory changes induced by infusion of nor-adrenalin into normal subjects closely resembled the phenomenon of pheochromocytoma hypertension. It was also found that the subjective effects of adrenalin were much greater than with the same dose of nor-adrenalin. De Langly et al (29) have shown that when an equal mixture

of adrenalin and nor-adrenalin was administered to humans the effects of adrenalin predominated.

The causation mechanism of this type of hypertension is not clear, but removal of the tumour, in general, abolishes the elevation in blood pressure. Green (51) thinks that the chronic state of hypertension in pheochromocytoma is mediated by a humoral mechanism and not by the development of a secondary phase of hypertension mediated through the kidneys, nor of the addition of a self perpetuating mechanism associated with vascular sclerosis. However, Goldenberg et al (47) believe that since surgical removal of the tumour does not always cause a lowering of the blood pressure there is a development of a self perpetuating mechanism as a late result of pheochromocytoma.

b. Adrenal cortex

Clinically, the role of the adrenal cortex in initiating hypertension has been based on the work of Oppenheimer and Fishberg (97), and more recently that of Russi et al (116). These investigators found that in tumours of the adrenal cortex there was a relationship of the adrenal cortex to the genesis of hypertension. Moreover, hypertension has been recognized as one of the cardinal features in Cushing's syndrome.

Selye (120), Braun Menendez (11), and Knowlton et al (73) have shown that desoxycorticosterone (DOC), a salt retaining hormone, which is secreted by the adrenal cortex, will cause hypertension in rats when added sodium chloride is given. Selye (120) and Knowlton (73) both considered that salt was necessary for the hypertension which followed the administration of DOC, since by restricting salt the hypertensive action of this compound can be prevented. Cortisone (73) and Compound F (40), two adrenocortical hormones, have been shown to cause an elevation in blood pressure without

the concurrent administration of salt.

The relationship of the adrenal cortex and the administration of salt was shown by Goldman (48), who stated that salt restriction apparently induces adrenal cortical hyperactivity. Since hypertension, caused by the mineralocorticoids of the adrenal cortex in the rat, is salt dependent to a degree, it has been suggested that salt excess acts as a primary mechanism in these hypertensive states, as in the syndrome produced in rats by severe salt excess alone (91) and its possible equivalent in salt eating hypertensive American men (26).

Hypertension caused by the adrenal cortex has been suggested to be due to electrolyte disturbance (86) and not simply to the retention of sodium and chloride. Adrenal cortical steroids apparently act at cellular levels to regulate the amount of sodium, potassium, and possibly magnesium within the cell (28),(41). DOC has been reported to increase the sensitivity of vascular smooth muscle to the pressor substances epinephrine and norepinephrine (108).

c. Salt and its effect on hypertension

Sapirestein et al (118) were able to produce hypertension in rats by feeding them salt in excessive quantities. Stamler and Katz (133) have also shown that the excessive feeding of salt can produce hypertension in chicks. The blood pressure of hypertensive dogs, and humans has been shown to be reduced by low sodium diets (38). The sodium chloride metabolism of patients with essential hypertension has been shown to be abnormal (143). Natruretic agents such as Chlorathiazide, which causes fluid and sodium depletion by its diuretic effect on the kidneys (144), and organic mercurial drugs (90) have been shown to cause a decrease in blood pressure in patients with essential hypertension (57).

Although the exact role which the disordered salt metabolism plays in hypertension is not clear, there are two possibilities postulated for its action: (1) narrowing of the arteriolar lumen, and (2) increasing the reactivity of arterial smooth muscle (109). The arterial wall of hypertensive patients and animals have been shown to contain increased amounts of sodium and water causing swelling and thus increase peripherial resistance (137). Friedman (39) has noted that the sodium concentration gradient between the outside and the inside of the smooth muscle cell is a basic determinant of tone. An increase in gradient of Na(outside)/ Na(inside) leads to a decrease in tone, whereas a decrease in gradient leads to an increase in tone. He also noted that extracellular sodium decreased as pressure rose and increased as pressure fell. From these findings it can be argued that the transfer of sodium appears to be the regulator of blood pressure by regulating vascular smooth muscle tone.

d. Adrenal regenerated hypertension

Skelton (132) has stimulated the interest in the adrenal origin of hypertension by eliciting a salt dependent hypertension during adrenal regeneration in young rats. The mechanism is unexplained. It occurred concomittantly or as a sequel to cortical hypofunction in which no other presently characterizable hormonal factors had been demonstrated (89). This type of hypertension has been shown to develop after surgical enucleation of the adrenal gland when the cortex is most rapidly regenerating, and once it developed it persisted until the animal died (120). This type of hypertension strongly resembled that produced in the uninephrectomized salt treated rat by administration of DOC (121) and corticosterone (130).

It has been shown to be prevented by hypophysectomy, and the presence of one intact adrenal (131), also by adrenal cortical

secretory depressants such as testosterone propionate (94) and Amphenone B (21).

It has been suggested that pathogenesis of this hypertensive disease might involve some functional alteration of the adrenal cortex induced by the enucleation. However, it has also been suggested that this form of hypertension is not mediated directly through the adrenal cortex but rather through some other mechanism which is dependent upon the presence of an adequate degree of adrenal cortical function. Regardless of the mechanism employed in initiating this type of hypertension it was certain that the regenerating adrenal was an essential factor for the development of this disease (131).

e. Anterior pituitary

Pitt-Rivers (106) has reported to have induced hypertension in rats by injections of large doses of crude anterior pituitary extracts, and Johnson et al (71) have reported to have caused hypertension in rats with injections of somatotrophic hormones. Hypertension provoking properties of anterior pituitary factors, other than the syndrome evoked by ACTH (adreno-cortico-tropic hormone) are largely attributable to growth hormone supposititiously acting on a mineralocorticoid such as aldosterone which might involve the pineal (32).

INTERPLAY OF SYSTEMS

Experimental hypertension has been produced by means which involve the neural, endocrine, and renal systems. The primary mechanisms of each of these differ, secondarily however, each seems to involve the others, so that, hypertension which might have been initially renal in origin

becomes secondarily sustained by neural, or endocrinal components. The secondary mechanisms tend to explain the persistency of some remediable types of hypertension after the removal of the primary causes.

a. Neurogenic-Nephrogenic

Taquini and Fasciolo (134) found that the renin content of the blood, and of the kidneys, of patients with hypertension and dogs with chronic hypertension due to renal ischemia was similar to that found in controls of both species. From this it was suggested that renin plays some role during the early acute phase of hypertension in cases in which there is an impairment of the renal circulation, but that there were serious doubts as to its possible participation in chronic hypertension. Ogden (95) suggested that a neurogenic mechanism might have taken over in the chronic stage of the renal hypertension.

McCubbin et al (87) have demonstrated that the carotid sinus and the aortic depressor mechanisms are "set" at a higher level of pressure in renal hypertensive dogs than in normal dogs. This higher setting was shown to maintain the hypertensive state even when the initiating mechanism was removed. Kezdi (72) has also shown that in chronic renal hypertension there was a resetting of the baroceptors in the carotid sinus at a higher level, and that this resetting of the baroceptors played a role in the maintenance of chronic renal hypertension since it counteracted any decrease of the blood pressure below the hypertensive level.

Page and McCubbin (102) found that when TEAC (tetra-ethylammonium chloride) was administered to subjects with induced neurogenic hypertension there was a sharp and consistent fall in blood pressure, whereas in those with renal hypertension there was a slight fall followed by a rise. In patients with renal parenchyma lesions it was found that TEAC, when

administered, resulted in a depressor response as though the hypertension was primarily neurogenic in origin.

b. Nephrogenic-Endocrinal

Wilson (145) stated that since adrenalectomy prevented the rise of blood pressure due to nephrectomy in parabiotic rats, this fact together with the enhanced hypertension found by some investigators when salt was given, suggested that an excessive secretion of the adrenals may be concerned in the chronic phase of hypertension following renal arterial constriction. Floyer (36) believed that this influence of the adrenals was closely linked with the control of salt metabolism. Olsen (96) noted that adrenal hypertrophy accompanied experimental nephrogenic hypertension. Goldman et al (48) also observed that salt restriction apparently induced adrenal cortical hyperactivity.

In rats, both renal hypertension and an injection of renin have been observed to cause hypertrophy of the zona glomerulosa of the adrenal cortex (96). Angiotensin causes sodium loss and the response to this loss may be adrenal glomerulosa hypertrophy, with a greater production of corticoids which tend to counteract the natruretic effect. The increased sodium retention and the production of corticoids can result in further vascular disease and eventually greater secretion of renin, thus initiating a vicious circle.

Goldblatt (43) found that in totally adrenalectomized dogs, constriction of the renal arteries failed to produce a rise in blood pressure, and that adrenalectomy abolished a pre-existing hypertension in animals maintained on a sodium diet but not given cortical extracts. Page (98) found that some rise in blood pressure could be obtained in such animals when cortical extracts were given. Braun Menendez (14) found

that adrenalectomy reduced the sensitivity of dogs to renin. This was attributed to a reduction of the renin substrate content of the blood. Lewis et al (84) found that renin substrate formation was deficient in adrenal cortical failure. Helmer and Griffith (63) found that DOC stimulates the formation of renin substrate in rats.

APPARATUS AND METHOD IN DETERMINING BLOOD PRESSURE

a. Apparatus

The blood pressure of the rats was determined by the indirect method using the capillary network in the interdigital web of the left hind leg. This leg was used as it was the most convenient. (See plate I.)

The apparatus used was a modification of that described by Allardyce et al (2). (See plate II.)

The mercury column described by the above mentioned workers was detached from the apparatus since accurate reproducible values of blood pressure could be obtained by using the rubber bulb alone.

b. Anesthesia

The anesthetic used throughout this investigation was sodium pentothal. It was prepared as a 2.5% aqueous solution according to Rixon (112). The weight-dose correlation described by this worker was found to be lethal in animals between 100 and 120 grams body weight, especially when these animals were being anesthetized for the first time.

Table 1 indicates the weight-dose correlation that was found most satisfactory throughout this investigation.

When anesthesia was being induced for the first time it was found that any proneness to succumb to respiratory failure could be reduced by administering a dose of the anesthetic suggested for a body weight of 10 grams below the actual weight of the animals being anesthetized.

It was also found that when the suggested dose failed to produce anesthesia, provided anesthesia was not being induced for the first time, a further supplement of 0.05 to 0.10 ml. could be given without any deleterious

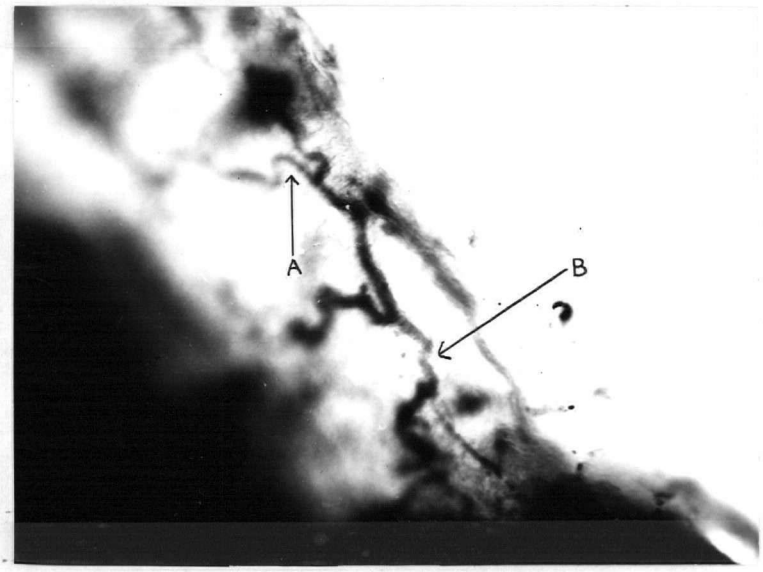


PLATE I. Photograph of a portion of the capillary network in the interdigital web of Wistar rats as seen under low power. (A and B represent capillaries of the size used in determining blood pressure).

Body weight in grams	Dose in cc.
Under 90	.10
90 - 120	.15
120 - 130	.20
130 - 145	.25
145 - 170	.30
170 - 185	.35
185 - 195	.40
195 - 250	.45 - .55
250 - 300	.55 - .70

Table 1. Showing the dose of a 2.5% aqueous solution of sodium pentothal necessary to induce anesthesia in Wistar rats as determined by their body weight.

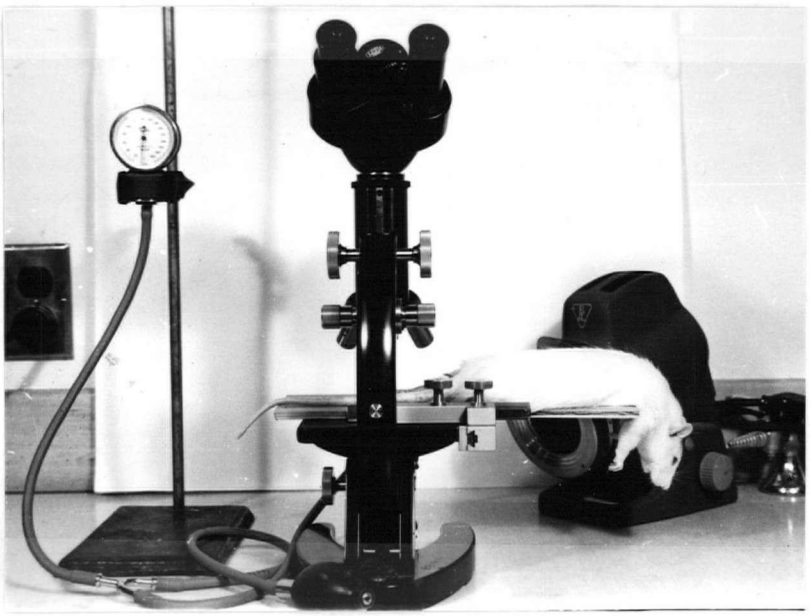


PLATE II. Photograph of the apparatus used in determining the blood pressure of Wistar rats in this investigation.

effects.

c. Blood pressure determination

Blood pressure determinations were carried out in the manner described by Allardyce et al (2), except that whereas these workers utilized a mercury column in addition to a rubber bulb to inflate the pressure cuff, a rubber bulb alone was employed to inflate the pressure cuff.

ESTABLISHMENT OF CONTROL VALUES AND A CRITERION OF HYPERTENSION

a. Control values of blood pressure

Prior to the injections of caffeine, the blood pressures of all animals were taken at intervals over a period of two weeks. The average value obtained for each animal over this period was taken as the normal blood pressure value of that animal. Table 2 shows a sample record of the blood pressures of six Wistar rats over a period of two weeks.

b. Criterion of hypertension

The criterion of hypertension was taken as any elevation in blood pressure over 20 mm. Hg above the normal blood pressure value. This arbitrary value was deduced from the variations between the average blood pressure value over a period of two weeks and the individual value at any time during this period. (See table 2.)

RAT NO.	TIME IN DAYS							AVERAGE
	1	3	5	8	10	12	14	
1	110	110	104	100	110	106	110	107
2	130	128	124	120	120	130	120	125
3	120	122	120	120	126	130	124	123
4	122	122	120	126	128	122	122	123
5	110	108	112	100	110	112	110	109
6	132	130	130	132	128	130	130	130

Table 2. Showing the blood pressure of each of six Wistar rats over a period of 14 days, and the average blood pressure of each animal for this period.

EXPERIMENTAL

THE EFFECT OF NEGATIVELY IONIZED AIR ON THE PRESSOR ACTIVITY OF CAFFEINE

a. Introduction

Studies on the biological effect of ionized air have received much attention within the last decade. In 1955, Kornblueh et al (78) found that by exposing twenty seven patients suffering from hay fever, asthma, and related conditions, to negatively ionized air seventeen of these reacted favourably to this treatment. This worker later reported (79) that exposure to negatively ionized air elicited favourable results in persons suffering from hay fever, whereas exposure to positively ionized air resulted either in no relief or in increased distress.

Kruger et al (80) found that protective or lethal effects could be obtained by varying the concentration of negative or positive air ions on staphylococci.

Gorriti and Medina (50) reported that there was an average reduction of 39 mm. Hg in twenty four hypertensive patients who were exposed to negatively ionized air.

Allardyce (1), in this laboratory, found that the hypertensive effect of nicotine could be ameliorated in Wistar rats by exposing the rats to negatively ionized air. Butler (19), also in this laboratory, found that when Wistar rats were exposed to negatively ionized air, caffeine-induced hypertension was much reduced.

Intraperitoneal injections of caffeine were found by Barker (4) to induce a hypertensive state in Wistar rats. On the basis of this worker's findings this investigation was undertaken to determine the effect of negatively ionized air on the pressor activity of caffeine.

b. Source of negatively ionized air

The negatively ionized air utilized in this investigation was produced by a tritium ion generator by Beckett and Hicks (6) manufactured by the Wesix Electric Heater Company of California. (See plate III.)

This apparatus employs beta radiation from tritium to ionize the air. Equal positive and negative ions are produced but selection of the ions of the desired charge is accomplished by collecting the undesired ions on an electrode of opposite polarity. By virtue of the charge on this electrode positive ions are absorbed and negative ions are driven in the opposite direction by electrostatic force. Martin (88) reports that oxygen readily forms negative molecular ions as the free electrons become attached to oxygen molecules. However, none of the electrons become attached to nitrogen molecules.

EXPERIMENT I.
PRESSOR ACTIVITY OF NORMAL CAFFEINE

a. Method

100 mls. of a caffeine solution of concentration 0.1 mg. caffeine per ml. was prepared from caffeine crystals obtained from the Eastman Kodak Company, Toronto, Ontario.

Six female Wistar rats were each injected intraperitoneally with 1.0 ml. of this solution on days 1 to 4 and again on days 35 to 38 inclusively.

The blood pressures of these animals were taken at intervals over a period of 60 days.

b. Results (See fig. 1 and table 3.)

On the sixth day following the administration of the first injection of caffeine the average increase in blood pressure was 68 mm. Hg above the normal average value. This increase did not show any further rise but rather continued to decrease until day 25 at which time the average blood pressure was again back

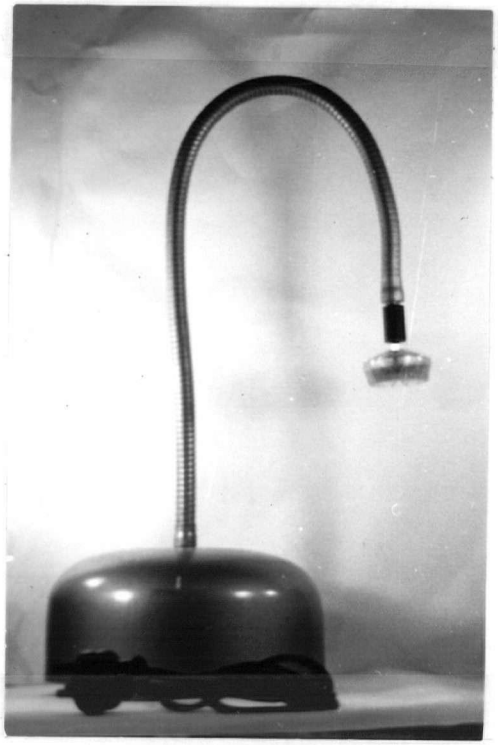


PLATE III. Photograph of a tritium ion generator. (Used in the production of negatively ionized air).

to normal levels. During this hypertensive state individual increases in blood pressure varied from 28 mm. Hg to 106 mm. Hg above their normal values.

There was no increase in blood pressure following the administration of the second set of injections of caffeine.

EXPERIMENT II.
PRESSOR ACTIVITY OF CAFFEINE EXPOSED IN SOLUTION TO NEGATIVELY IONIZED AIR

a. Method

100 mls. of a caffeine solution of concentration 0.1 mg. caffeine per ml. was prepared as described in experiment I. This volume of solution was exposed to negatively ionized air for a period of 168 hours.

During this period of exposure any loss in volume due to evaporation was restored by the addition of distilled water to the solution.

Six female Wistar rats were each injected intraperitoneally with 1.0 ml. of this "exposed" solution from day 1 to day 4 and from day 35 to day 38 with an unexposed solution of caffeine of the same concentration as that injected on days 1 to 4.

The blood pressures of these animals were taken at intervals over a period of 60 days.

b. Results (See fig. 2 and table 4.)

Following the administration of the four injections of the "exposed" caffeine solution there was no appreciable rise in the blood pressure of the animals indicative of a hypertensive state.

Four subsequent injections of an unexposed caffeine solution having the same concentration as the "exposed" solution were also ineffective in eliciting any elevation in blood pressure.

EXPERIMENT III.
PRESSOR ACTIVITY OF CAFFEINE EXPOSED IN THE CRYSTALLINE STATE TO NEGATIVELY IONIZED AIR

a. Method

Four grams of crystalline caffeine was evenly distributed on the bottom of a dry 50 ml. beaker. The beaker and contents were placed directly under the plastic head of the tritium ion generator for a period of 168 hours. The caffeine was separated from the plastic head of the generator by a distance of about 4.0 cms.

During the period of exposure the beaker was frequently tapped to ensure, as much as possible, complete exposure of all the caffeine crystals.

A weight of this "exposed" caffeine necessary to make 100 mls. of a caffeine solution of concentration 0.1 mg. per ml. was dissolved in 100 mls. of distilled water.

1.0 ml. of this solution was injected into each of six female Wistar rats from day 1 to day 4. From day 35 to day 38 each of these animals received daily intraperitoneal injections of 1.0 ml. of an unexposed caffeine solution of the same concentration as that administered on days 1 to 4 inclusively.

The blood pressures of these animals were taken over a period of 60 days.

b. Results (See fig. 3 and table 5.)

Seven days after administration of the first injection the average blood pressure of the rats rose from 129 mm. Hg to 147 mm. Hg. Although at this time there was an increase in the average blood pressure of the animals

there were still a few animals whose blood pressures were within normal levels.

On the fifteenth day, however, all animals were hypertensive. At this time the average increase in the blood pressure was 42 mm. Hg above the average normal value. There was a continued decrease in the average blood pressure from day 15 to day 27. On day 27 the blood pressures of all animals were again within normal levels. However, this return to normal levels was observed in a few animals as early as day 20.

Administration of the four injections of an unexposed caffeine solution of the same concentration as that prepared from the exposed crystals failed to elicit any rise in the blood pressure of the animals.

DISCUSSION

Figure 4 depicts in graphic form the changes in the average blood pressures of the rats after receiving intraperitoneal injections of caffeine solutions prepared from unexposed caffeine, and caffeine exposed in the crystalline state, and in solution, to negatively ionized air.

A marked increase in the average blood pressure was observed in those animals injected with a solution of unexposed caffeine, and also in those animals injected with a caffeine solution prepared from exposed crystals. There was no increase in the average blood pressure of those animals which received injections of an exposed caffeine solution.

There was a pronounced difference in the average maximum height to which the blood pressure rose in each group of hypertensive rats. This average maximum height was greater in the animals which received injections of a caffeine solution prepared from unexposed caffeine.

The periods at which the peak of average maximum increase in blood pressure was reached also showed much variance in the two hypertensive groups.

In those animals which received injections of a caffeine solution prepared from unexposed caffeine, this peak of average maximum increase was attained within six days after the first injection, whereas in those animals which received injections of a caffeine solution prepared from exposed crystals, this peak occurred within fifteen days of the first injection of the solution.

Irrespective of the time at which the peak of average maximum increase in blood pressure occurred the average normal level was re-established at approximately the same time in both sets of animals.

Once the rats were injected with a caffeine solution, whether the caffeine was exposed to negatively ionized air or not, and had regained their normal levels of blood pressure there was no further rise in blood pressure with subsequent injections of unexposed caffeine. This ability of the rats to show no rise in blood pressure to subsequent injections of unexposed caffeine was observed to occur whether there was an increase in blood pressure with the first set of injections or not.

The possibility that beta radiation emanating from tritium could act directly on the caffeine when it was exposed has to be ruled out. Glasser (42) reported that the maximum range of beta radiation emitted from tritium is 1.7 cms. The distance between the tritium foil and the caffeine was always greater than 4.0 cms. thus allowing a safety factor to insure that no direct effects of beta radiation on the caffeine were obtained.

The response obtained with the animals which received injections of caffeine solutions prepared from caffeine exposed to negatively ionized air agrees, in general, with the results of Gorriti and Medina (50), and with Allardyce (1) and Butler (19). Although these workers obtained an anti-hypertensive effect by exposing their patients and animals to negatively

ionized air, it would appear that the anti-hypertensive effect of negatively ionized air can be obtained by exposing the pressor substance as well as the rats to negatively ionized air.

The manner in which negatively ionized air exerts an anti-hypertensive effect is not fully understood. Tchijevsky (135) and Edstrom (30) reported that inhalation of the charged air ions triggers within the body physiological action on the cardio-vascular system. Kornblueh et al (78) stated that negative ionization of offending airborne substances apparently diminished their allergic toxicity by changing their electric potential.

Failla (31) stated that chemical actions are usually facilitated by the existence of an "excited" state. This "excited" state is often induced in molecules that have just missed being ionized and as a result of this they have considerable amounts of energy imparted to them. Therefore in the study of the biologic effects produced by radiation the possibility of excitation of molecules as well as ionization must be considered.

The apparent complete loss of the pressor activity of caffeine exposed in solution to negatively ionized air as compared with the partial loss of the pressor activity of caffeine exposed in the crystalline state, is probably due to the degree of interaction between the negatively ionized air and the molecules of caffeine in these two phases.

Molecules in solution tend to be more widely dispersed than molecules in a crystalline lattice. Therefore molecules of caffeine in solution will, of necessity, afford a greater degree of interaction with negatively ionized air than molecules of caffeine which are compactly integrated in a crystalline lattice.

Fig. 1 The changes in the average blood pressure of six female Wistar rats after receiving four injections of .10 mg. caffeine on days 1, 2, 3 and 4, and again on days 35, 36, 37 and 38.

Fig. 2 The changes in the average blood pressure of six female Wistar rats after receiving injections, on days 1, 2, 3 and 4, of .10 mg. caffeine previously exposed in solution to negatively ionized air for 168 hours, and injections of .10 mg. unexposed caffeine on days 35, 36, 37 and 38.

Fig. 3 The changes in the average blood pressure of six female Wistar rats after receiving injections, on days 1, 2, 3 and 4, of .10 mg. caffeine previously exposed in the crystalline state to negatively ionized air for 168 hours, and injections of .10 mg. unexposed caffeine on days 35, 36, 37 and 38.

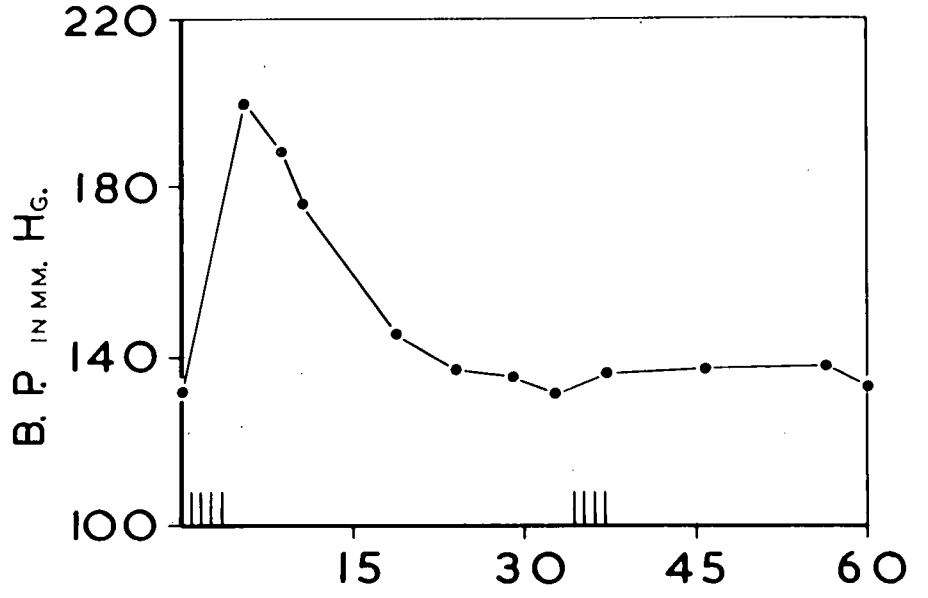


FIG. 1

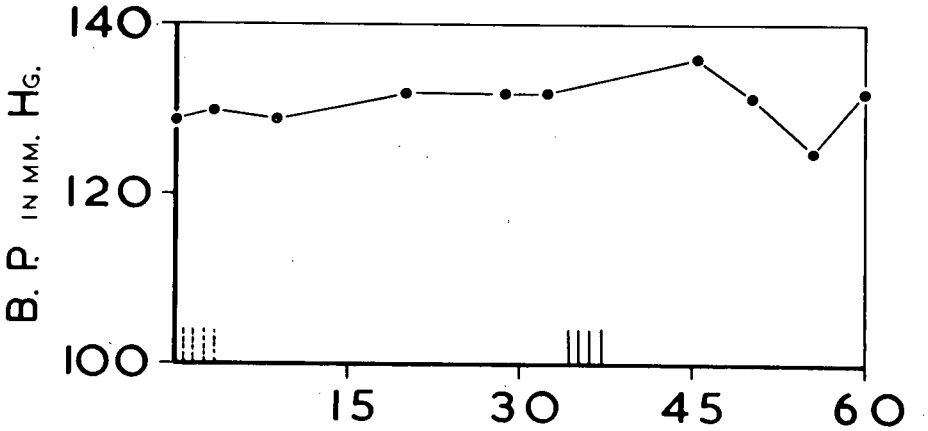


FIG. 2

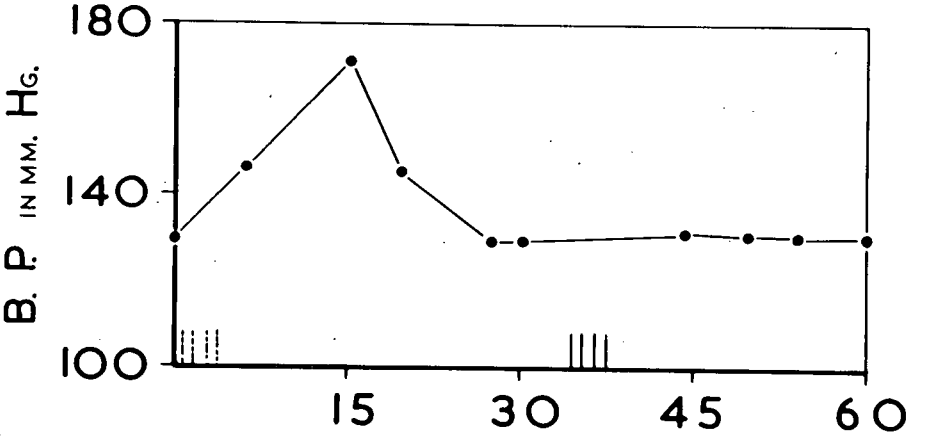


FIG. 3

RAT NO.	TIME IN DAYS													
	0	1--4	6	10	12	19	25	29	33	35-38	38	46	56	60
1	130	4 injections of unexposed caffeine of conc. 0.1 mg./ml.	168	184	210	160	132	140	130	4 injections of unexposed caffeine of conc. 0.1 mg./ml.	132	140	130	132
2	116		182	164	210	170	130	124	120		134	124	134	120
3	122		N.R.	160	150	170	140	120	124		128	124	128	120
4	138		190	244	150	130	130	140	138		142	136	140	138
5	131		240	190	164	124	140	134	130		131	140	134	130
6	146		210	184	168	110	142	148	150		144	150	154	147

Table 3. Showing the blood pressures of six female Wistar rats after receiving four injections each of a caffeine solution of concentration 0.1 mg./ml., prepared from caffeine unexposed to negatively ionized air, and four subsequent injections of the same concentration 35 days after administration of the first injection.

RAT NO.	TIME IN DAYS											
	0	1--4	4	10	20	27	32	35-38	44	50	56	60
1	135	4 injections of exposed caffeine (soln. exposed) of conc. 0.1 mg./ml.	135	135	145	140	150	4 injections of unexposed caffeine of conc. 0.1 mg./ml.	150	135	124	130
2	135		131	131	130	128	125		133	N.R.	138	133
3	134		134	136	130	136	130		142	142	120	138
4	130		140	130	132	130	130		130	130	129	134
5	130		130	130	150	146	142		130	140	119	137
6	112		112	112	110	110	120		132	120	120	118

Table 4. Showing the blood pressures of six female Wistar rats after receiving four injections each of a caffeine solution of concentration 0.1 mg./ml., prepared from a caffeine solution exposed to negatively ionized air for 168 hours, and four subsequent injections of an unexposed caffeine solution of the same concentration 35 days after the first injection of the exposed solution.

RAT NO.	TIME IN DAYS											
	0	1--4	7	15	20	27	30	35-38	44	50	55	60
1	129	4 injections of exposed caffeine crystals of conc. 0.1 mg./ml.	138	155	130	125	128	4 injections of unexposed caffeine of conc. 0.1 mg./ml.	130	132	128	128
2	129		160	160	150	123	130		130	128	130	130
3	127		138	174	131	130	128		128	130	130	130
4	130		161	172	134	130	130		132	128	127	130
5	134		151	180	187	139	126		136	130	134	134
6	128		129	187	139	127	128		128	132	129	128

Table 5. Showing the blood pressures of six female Wistar rats after receiving four injections each of a caffeine solution of concentration 0.1 mg./ml. prepared from caffeine crystals exposed to negatively ionized air for 168 hours, and four subsequent injections of an unexposed caffeine solution of the same concentration 35 days after the first injection of the exposed solution.

Fig. 4 Changes in the average blood pressure of female Wistar rats with unexposed caffeine, and caffeine exposed in solution, and in the crystalline state, to negatively ionized air.

●—● Changes in the average blood pressure of six female Wistar rats after receiving injections of .10 mg. unexposed caffeine on days 1, 2, 3 and 4, and again on days 35, 36, 37 and 38.

▲— —▲ Changes in the average blood pressure of six female Wistar rats after receiving injections on days 1, 2, 3 and 4, of .10 mg. caffeine previously exposed in solution to negatively ionized air for 168 hours, and injections of .10 mg. unexposed caffeine on days 35, 36, 37 and 38.

O---O Changes in the average blood pressure of six female Wistar rats after receiving injections, on days 1, 2, 3 and 4, of .10 mg. caffeine previously exposed in the crystalline state to negatively ionized air for 168 hours, and injections of .10 mg. unexposed caffeine on days 35, 36, 37 and 38.

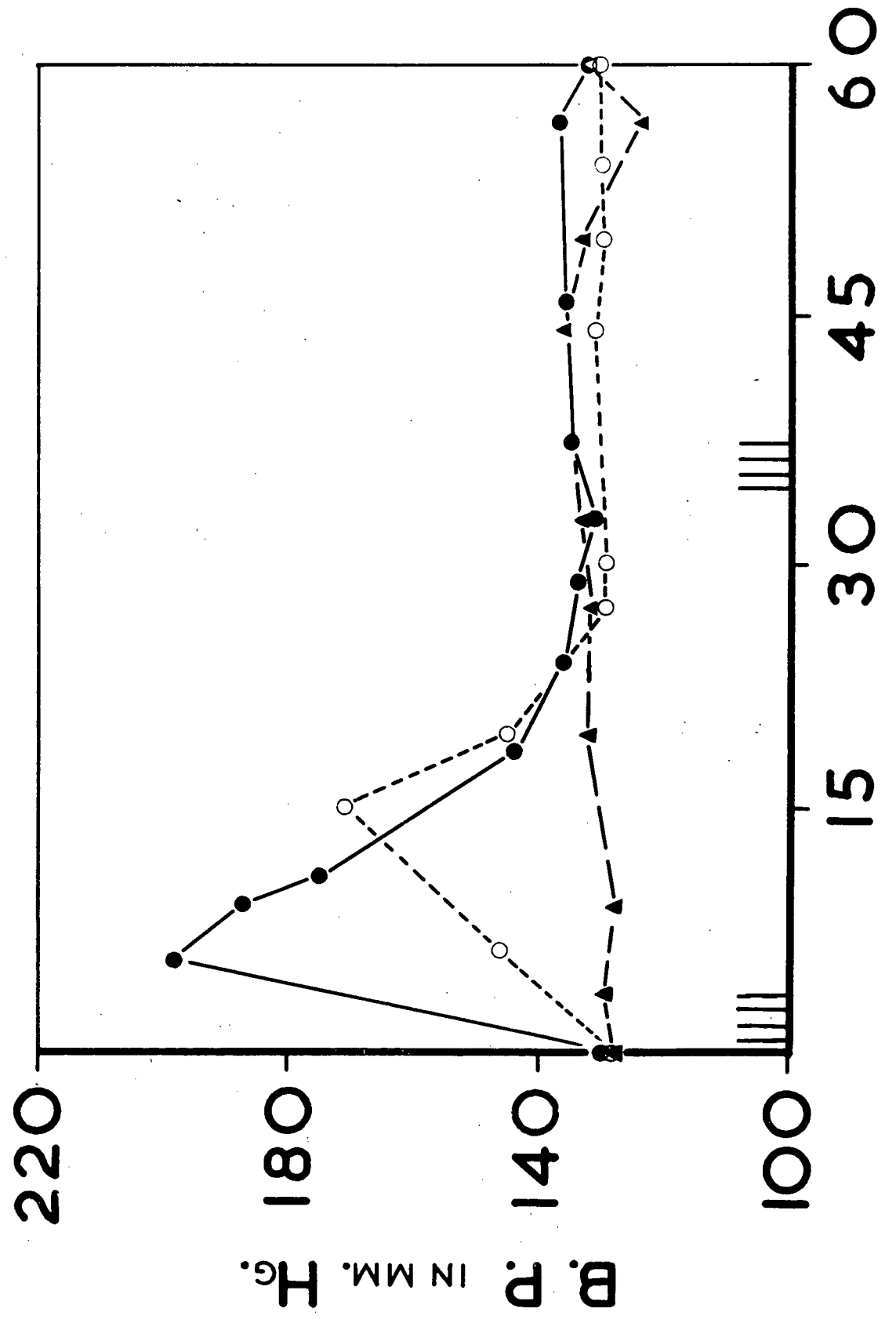


FIG. 4

THE EFFECT OF THE CONCENTRATION OF CAFFEINE ON THE DEGREE AND DURATION OF CAFFEINE-INDUCED HYPERTENSION

Introduction

The ability of caffeine to cause an increase in blood pressure was reported by Grollman (53) in 1930, and later by Horst et al (70). These workers found that when caffeine was administered to humans there resulted an increase in blood pressure concomitant with an increase in pulse rate.

Barker (4), in this laboratory, found that when Wistar rats were injected intraperitoneally with a caffeine solution there was initiated a transient hypertensive state in these animals.

This investigation was undertaken to determine whether there existed a correlation between the degree, and duration of this caffeine-induced hypertension and the concentration of caffeine administered.

EXPERIMENT IV.
THE HYPERTENSIVE EFFECT OF A CAFFEINE SOLUTION OF CONCENTRATION 0.1 MILLIGRAM PER MILLILITER.

a. Method

100 mls. of a caffeine solution of concentration 0.1 mg. per ml. was prepared from caffeine crystals obtained from Eastman Kodak Company, Toronto, Ontario.

Four female Wistar rats and four male Wistar rats were each injected intraperitoneally with 1.0 ml. of this solution from day 1 to day 4 inclusively and again from day 49 to day 52.

The blood pressures of these animals were taken at intervals over

a period of eighty days.

b. Results (See fig. 5 and table 6.)

An immediate rise in the average blood pressure was observed within five days of the administration of the first injection of caffeine. This rise continued to increase until day 20 at which time the average blood pressure was 90 mm. Hg above the normal average value.

From day 20 there was a consistent decrease in blood pressure until day 34. At this time the average blood pressure was again back to normal levels.

During this period of hypertension individual elevations of blood pressure were observed to vary from 26 mm. Hg to 128 mm. Hg above their normal values.

There were no significant differences in blood pressure values between the two sexes.

Administration of the second set of injections failed to elicit any further rise in the blood pressure of the rats.

EXPERIMENT V.
THE HYPERTENSIVE EFFECT OF A CAFFEINE SOLUTION OF CONCENTRATION 0.01 MILLIGRAM PER MILLILITER

a. Method

100 mls. of a caffeine solution of concentration 0.01 mg. per ml. was prepared as described in experiment IV.

Each of four female Wistar rats and four male Wistar rats was injected intraperitoneally with 1.0 ml. of this solution from day 1 to day 4 and again from day 49 to day 52.

The blood pressures of these animals were taken at intervals over

a period of eighty days.

b. Results (See fig. 6 and table 7.)

An increase in the average blood pressure was found within seven days of the administration of the first injection of the caffeine solution. There was a continued increase until a maximum average value of 63 mm. Hg above the normal value was attained on day 18.

Following the attainment of this maximum increase there was a consistent decrease in blood pressure until the normal average value was again established. This occurred on day 28.

Individual variations in blood pressure values during this hypertensive state ranged from 22 mm. Hg to 76 mm. Hg above the normal values.

The only difference noted between the two sexes during this hypertensive state was the early re-establishment of the normal blood pressure of all the female rats on day 23 whereas this did not occur in the males until day 28.

No elevation in blood pressure resulted from the administration of the second set of injections of caffeine.

EXPERIMENT VI.
THE HYPERTENSIVE EFFECT OF A CAFFEINE SOLUTION OF CONCENTRATION 0.001 MILLIGRAM PER MILLILITER

a. Method

100 mls. of a caffeine solution of concentration 0.001 mg. per ml. was prepared as described in experiment IV.

Four female Wistar rats and four male Wistar rats were each injected intraperitoneally with 1.0 ml. of this solution daily from day 1

to day 4 and again from day 49 to day 52.

The blood pressures of these animals were taken at intervals over a period of eighty days.

b. Results (See fig. 7 and table 8.)

The average blood pressure was observed to increase within six days of the administration of the first injection of the caffeine solution. on day 11 the average blood pressure had attained a maximum value of 47 mm. Hg above the normal average value.

Following the attainment of this maximum increase in the average blood pressure there was an ensuing decrease which terminated at the re-establishment of the average normal value on day 21.

Variations of 20 mm. Hg to 70 mm. Hg above the normal values of blood pressure were observed in individual animals.

There were no significant differences in the degree or duration of hypertension between the two sexes.

Administration of the second set of injections did not produce any rise in the blood pressure of the animals.

DISCUSSION

Figure 8 depicts the changes in the average blood pressure of Wistar rats after receiving injections of three different concentrations of caffeine. Table 9 shows the differences found in the hypertensive state induced by each of these concentrations.

There was a marked increase in the average blood pressure with each of the concentrations used. This increase was greatest with the 0.1 mg./ml. and least with the 0.001 mg./ml., the .01 mg./ml. being intermediate.

The average maximum increase in blood pressure varied with each

of the concentrations used. There was a maximum increase of 90 mm. Hg above the normal average value in those animals which received injections of the 0.1 mg./ml. solution, whereas with the 0.01 mg./ml. solution and the 0.001 mg./ml. solution this value was 63 mm. Hg and 47 mm. Hg respectively.

The times at which the maximum increase in the average blood pressure occurred were different with each of the concentrations of caffeine. This increase occurred within 20 days of the administration of the first injection of the 0.1 mg./ml. solution, whereas with the 0.01 mg./ml. solution this occurred within 18 days, and with the 0.001 mg./ml. solution within 11 days.

During the hypertensive state induced by each of the concentrations of caffeine there were no significant differences in the degree or duration of hypertension between the two sexes to warrant any consideration in the differences in response.

Animals which were once rendered hypertensive by injections of caffeine and had re-established their normal levels of blood pressure did not show any increase in blood pressure to a subsequent treatment of an equal number of injections of caffeine. The concentration of caffeine used in the second set of injections was the same as that used in the first set of injections.

The response obtained with the different concentrations of caffeine parallels the results obtained by Grollman (53). This worker found that low concentrations of caffeine did not affect the blood pressure of humans whereas greater concentrations elicited an increase in their blood pressures.

The action of caffeine on the cardio-vascular system is unpredictable since it is believed that this drug elicits a diphasic action,

a central vasoconstriction together with a peripheral vasodilation (146).

Caffeine has been shown to be a central nervous system stimulant. Its action has been confined to the cerebral cortex, the medulla oblongata, and in very concentrated doses the spinal cord (49). In the medulla its action is specifically concerned with the vagal center, the respiratory center, and the vasomotor center. In addition to its action on the central nervous system caffeine has been shown to stimulate the myocardium of the heart thus causing an increase in cardiac output (49).

The increase in blood pressure after administration of caffeine has been attributed to numerous factors. It has been suggested that the increase in blood pressure may be due to the combination of increased cardiac output due to stimulation of the myocardium of the heart together with the increasedvasomotor tone which results from stimulation of the vasomotor center in the medulla (49).

Salter (117) has also suggested that the increase in blood pressure may be due to the initiation of a nervous state which causes an increase in heart rate.

Irrespective of the mechanisms involved in the initiation of a hypertensive state through the administration of caffeine it appears that this hypertensive state when induced in Wistar rats is dependent on the concentration of caffeine administered.

Fig. 5 The changes in the average blood pressure of four female Wistar rats and four male Wistar rats after receiving four injections of .100 mg. caffeine on days 1, 2, 3 and 4, and again on days 49, 50, 51 and 52.

Fig. 6 The changes in the average blood pressure of four female Wistar rats and four male Wistar rats after receiving four injections of .010 mg. caffeine on days 1, 2, 3 and 4, and again on days 49, 50, 51 and 52.

Fig. 7 The changes in the average blood pressure of four female Wistar rats and four male Wistar rats after receiving four injections of .001 mg. caffeine on days 1, 2, 3 and 4, and again on days 49, 50, 51 and 52.

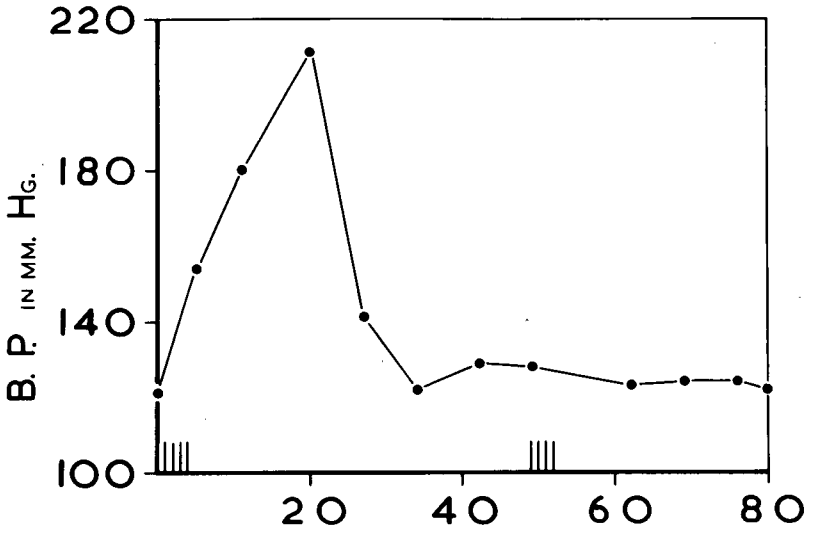


FIG. 5

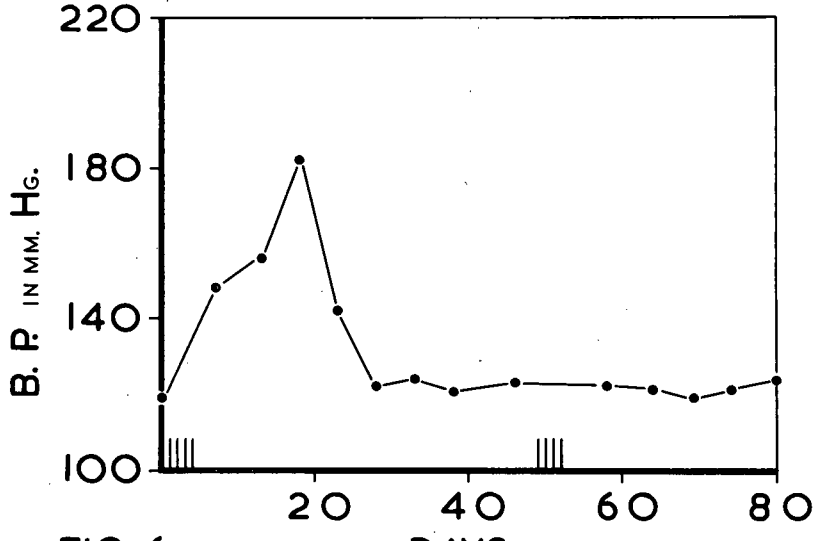


FIG. 6

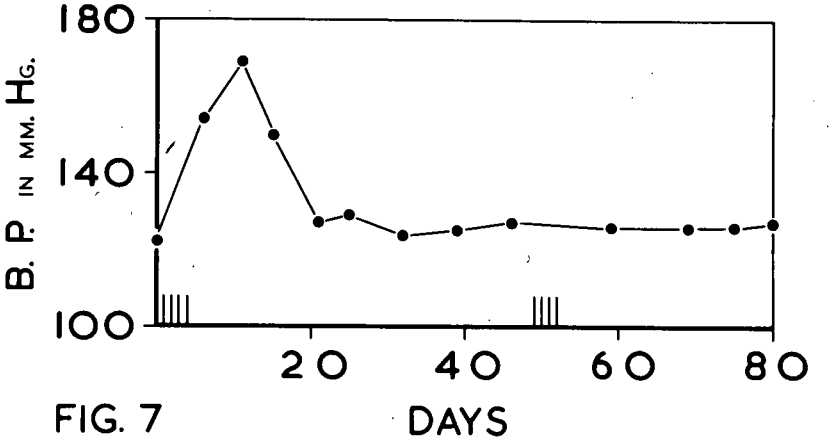


FIG. 7

SEX	RAT NO.	TIME IN DAYS													
		0	1--4	5	11	20	27	34	42	49	49-52	62	69	76	80
MALES	1	113	4 injections of a caffeine solution of concentration 0.1 mg./ml.	169	170	204	120	110	118	116	4 injections of a caffeine solution of concentration 0.1 mg./ml.	110	116	113	118
	2	116		153	230	210	122	140	140	130		132	124	120	122
	3	122		N.R.	205	225	158	132	131	136		132	130	128	124
	4	117		165	175	240	121	129	141	120		122	120	116	118
FEMALES	1	121		131	188	190	200	122	124	139		124	130	126	122
	2	129		160	165	196	131	114	139	138		125	129	130	125
	3	125		151	167	170	140	115	122	121		118	124	130	122
	4	122		150	140	250	138	116	119	125		122	121	127	126

Table 6. Showing the blood pressures of four female Wistar rats and four male Wistar rats after receiving four injections each, of a caffeine solution of concentration 0.1 mg./ml. and four subsequent injections of the same concentration 49 days after the administration of the first injection.

SEX	RAT NO.	TIME IN DAYS															
		0	1--4	7	13	18	23	28	33	38	46	49-52	58	64	69	74	80
MALES	1	120	4 injections of a caffeine solution of concentration 0.01 mg./ml.	172	169	173	164	113	126	120	124	4 injections of a caffeine solution of concentration 0.01 mg./ml.	119	122	127	125	125
	2	111		N.R.	116	173	172	121	124	123	127		116	113	104	116	120
	3	120		123	121	178	166	124	127	125	120		120	120	119	127	120
	4	110		142	166	175	164	126	113	110	120		112	115	120	120	125
FEMALES	1	116		140	144	192	124	116	114	118	119		120	120	118	114	118
	2	122		144	154	194	110	128	130	116	130		126	131	120	120	125
	3	126		150	154	180	120	120	120	130	126		130	126	131	120	130
	4	125		164	172	190	120	128	130	118	120		130	120	120	126	123

Table 7. Showing the blood pressures of four female Wistar rats and four male Wistar rats after receiving four injections each, of a caffeine solution of concentration 0.01 mg./ml. and four subsequent injections of the same concentration 49 days after administration of the first injection.

SEX	RAT NO.	TIME IN DAYS														
		0	1--4	6	11	15	21	25	32	39	46	49-52	59	69	75	80
MALES	1	126	4 injections of a caffeine solution of concentration 0.001 mg./ml.	130	166	155	130	130	126	131	130	4 injections of a caffeine solution of concentration 0.001 mg./ml.	130	128	130	124
	2	120		143	164	130	132	133	120	120	125		128	130	128	126
	3	112		142	166	182	130	135	120	118	120		118	114	120	120
	4	120		132	160	158	125	130	122	121	128		130	132	124	128
FEMALES	1	130		190	194	125	120	124	128	134	134		132	138	127	132
	2	122		162	172	158	131	132	121	120	118		121	119	128	120
	3	119		159	160	140	120	120	121	125	128		130	121	127	128
	4	125		174	168	155	125	125	131	128	128		128	125	120	133

Table 8. Showing the blood pressures of four female Wistar rats and four male Wistar rats after receiving four injections each of a caffeine solution of concentration 0.001 mg./ml. and four subsequent injections of the same concentration 49 days after the administration of the first injection.

Fig. 8 Changes in the average blood pressure of Wistar rats with three different concentrations of caffeine.

●——● Changes in the average blood pressure of four female, and four male Wistar rats after receiving injections of .100 mg. caffeine on days 1, 2, 3 and 4, and again on days 49, 50, 51 and 52.

O----O Changes in the average blood pressure of four female and four male Wistar rats after receiving injections of .010 mg. caffeine on days 1, 2, 3 and 4, and again on days 49, 50, 51 and 52.

▲——▲ Changes in the average blood pressure of four female and four male Wistar rats after receiving injections of .001 mg. caffeine on days 1, 2, 3 and 4, and again on days 49, 50, 51 and 52.

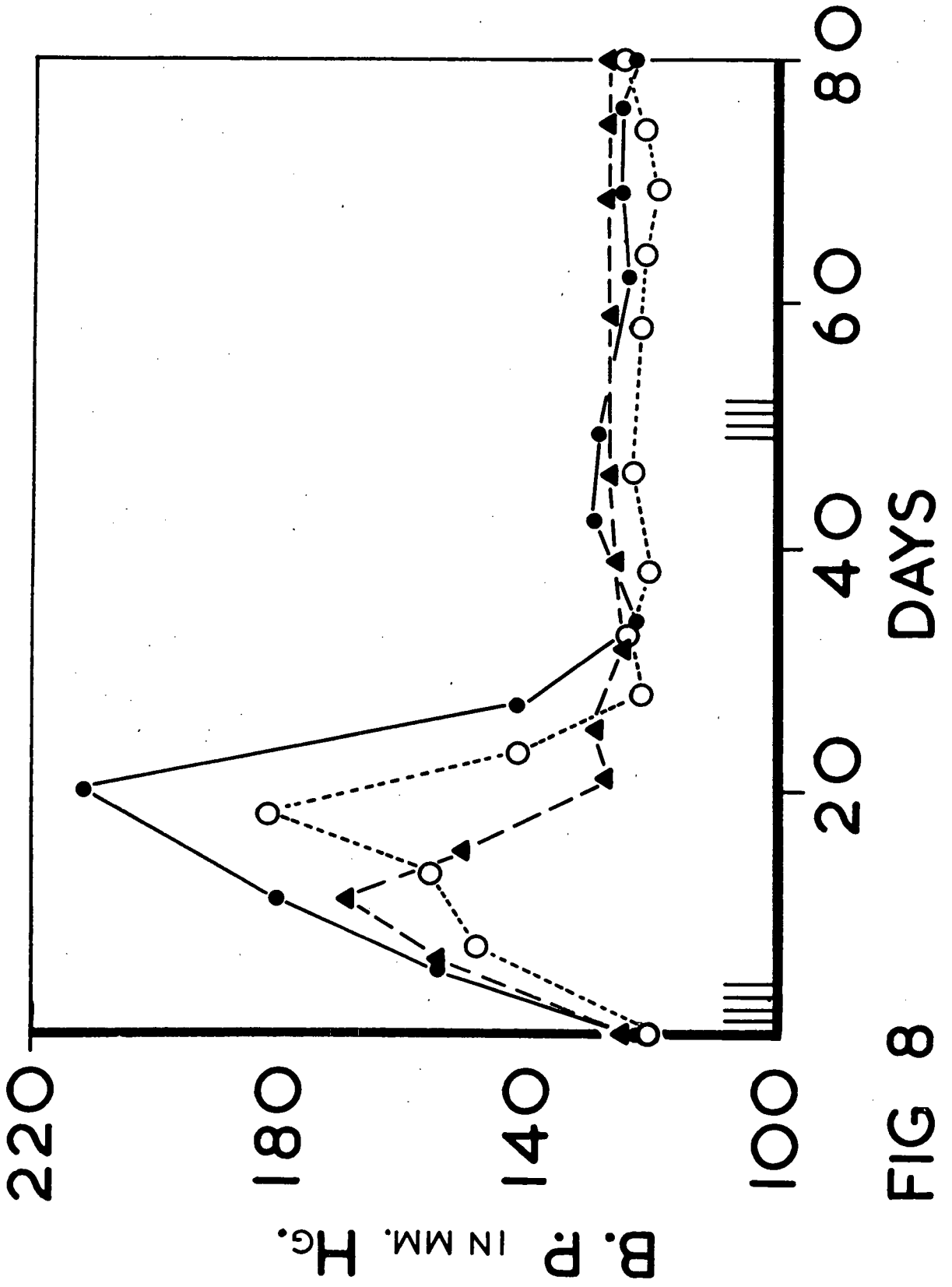


FIG 8

Concentration of caffeine used	Max. increase in blood pressure above normal average blood pressure	Time at which max. increase in blood pressure occurred	Duration of hypertension	Max. individual increase in blood pressure above normal blood pressure
0.100 mg./ml.	90 mm. Hg	Day 20	34 days	128 mm. Hg
0.010 mg./ml.	63 mm. Hg	Day 18	28 days	76 mm. Hg
0.001 mg./ml.	47 mm. Hg	Day 11	21 days	70 mm. Hg

Table 9. Showing the differences in the hypertensive state induced with three different concentrations of caffeine.

GENERAL DISCUSSION

From the results obtained in this investigation it would appear that caffeine exposed to negatively ionized air tends to lose its pressor activity. Also that in Wistar rats the degree and duration of caffeine-induced hypertension were dependent on the concentration of caffeine administered. It was also observed that once Wistar rats had been injected with caffeine and had re-established their normal levels of blood pressure subsequent administration of an equal number of injections of caffeine of the same concentration seemed to have no effect in causing a further rise in blood pressure.

Beutner (8) stated that every pharmacological action was ultimately due to a physical change which the drug brought about in the living tissue. Moreover, the electrical potential differences in tissue were of vital function and thus by changing the potential differences existing inside the tissue by introducing certain substances into the tissue some change was initiated which resulted in the stimulation of the tissue.

This worker found that certain alkaloids had the ability to change the electromotive force of an artificial cell-system. Of the alkaloids found to possess this ability, caffeine was found to have an ability which depended on the concentration of caffeine used. The higher the concentration of caffeine used the greater was the change in the electromotive force.

If Beutner's cell-system can be taken as representative of the tissue in vivo then an explanation of the action of caffeine as found in this investigation can be attempted.

Since negatively ionized air was found to depress the hypertensive effect of caffeine this loss in the pressor activity of this drug is

probably due to some interaction between negatively ionized air and the molecules of caffeine. Caffeine is a xanthine derivative, therefore molecules of caffeine, in solution, would be expected to possess a net positive charge due to the presence of the imidazolyl group in the molecule. This ability of caffeine to possess a net positive charge is probably the basis of Sjostrom and Nyakanen's report on the ability to seperate caffeine from phenacetin and antipyrine through the use of a resin column of ferric ions (124).

Caffeine molecules appear to possess a net positive charge and molecules of negatively ionized air carry a net negative charge. Any interaction between these two species of molecules will be one in which there is neutralization of the charges. Whether this results through the donation of an electron from the molecule of negatively ionized air to the positively charged caffeine molecule, or whether there is fusion of the two molecules through electrostatic attraction is unknown. However, regardless of the mechanism employed in the interaction between these two species of molecules the loss of the net positive charge on the caffeine molecule probably affects the ability of this substance to change the electromotive force of the tissues in the body, thereby resulting in the failure of exposed caffeine to induce a hypertensive state.

The tendency of the degree and duration of caffeine-induced hypertension to be reduced with decreasing concentrations of caffeine is probably due to the inability of low concentrations to cause a large change in the electromotive force of the tissue responsible for producing hypertension. The change in the electromotive force due to a low concentration of caffeine although strong enough to cause an increase in blood pressure is not strong enough to prolong or intensify this increase. On the other hand the change

obtained by a higher concentration might be strong enough to induce hypertension as well as to prolong and intensify this hypertensive state.

The manner in which Wistar rats, which have been rendered hypertensive with caffeine and have again re-established their normal blood pressure values, develop a negative response to subsequent administration of caffeine is still not fully understood. However, it can be suggested that since caffeine is reported to have a central vasoconstriction together with a peripheral vasodilatation this negative response might be due to the predominance of one of these actions over that of the other.

The hypertensive effect obtained with the initial injection of caffeine might be due to a predominance of central vasoconstriction over peripheral vasodilatation. With subsequent injections of caffeine this predominance is probably altered so that peripheral vasodilatation overcomes any increase in blood pressure that might have arisen from central vasoconstriction. On the other hand subsequent injections of caffeine might have facilitated peripheral vasodilatation to the degree at which the increase in vasodilatation balances the effect of central vasoconstriction.

SUMMARY

Caffeine exposed to negatively ionized air for a period of 168 hours was observed to undergo some change which resulted in a loss of its pressor activity in Wistar rats.

A complete loss in pressor activity was found when the caffeine was exposed in solution to the negatively ionized air, whereas when the caffeine was exposed in the crystalline state there was only a partial loss in pressor activity.

Animals which were once rendered hypertensive by an unexposed caffeine solution and by a caffeine solution prepared from caffeine crystals exposed to negatively ionized air, did not show any further increase in blood pressure with a subsequent treatment of an equal number of injections of unexposed caffeine of the same concentration. This subsequent treatment was administered after the animals had regained their normal levels of blood pressure. This negative response was also observed in animals which had not become hypertensive with injections of a caffeine solution which was exposed as such to negatively ionized air.

The degree and duration of caffeine-induced hypertension in Wistar rats were found to be dependent on the concentration of caffeine administered. With four 1.0 ml. injections of a 0.1 mg./ml. solution the hypertensive state lasted for 34 days, and the maximum increase in blood pressure above the normal average value was 90 mm. Hg. With equal injections of a 0.01 mg./ml. solution the duration of the hypertensive state was 28 days and the maximum increase in blood pressure was 63 mm. Hg above the normal average value. A similar treatment using a 0.001 mg./ml. solution produced a hypertensive state which lasted for 21 days and the maximum increase during this period was 47 mm. Hg above the normal average level of blood pressure.

There were no significant differences in the hypertensive states of male and female rats with the concentrations of caffeine used.

When once the hypertensive states were overcome and the animals had regained their normal levels of blood pressure an equal number of injections of caffeine of the same concentration as was initially administered failed to produce any further increase in the blood pressures.

CONCLUSIONS

1. Small intraperitoneal injections of caffeine will cause a transient state of hypertension in Wistar rats.

2. In caffeine-induced hypertension the height to which the blood pressure is elevated and the duration of the hypertensive state, are both dependent on the concentration of caffeine administered.

3. Wistar rats when once injected intraperitoneally with caffeine do not show any response in the elevation of blood pressure with subsequent injections of caffeine of the same concentration.

4. Negatively ionized air tends to have a marked effect in decreasing the pressor activity of caffeine in Wistar rats.

5. Caffeine exposed in solution to negatively ionized air tends to lose more of its pressor activity than caffeine exposed in the crystalline state.

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