THE ROLE OF THE VAGI AND CAROTID SINUS MECHANISMS IN RAUWOLFIA SERPENTINA HYPOTENSION IN DOGS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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*****

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INTRODUCTION

During the past few years the results of chemical, pharmacological, and clinical investigations of Rauwolfia serpentina and its related species have been forthcoming with ever increasing rapidity. These results, with but few minor exceptions, reveal a most promising future for the employment of Rauwolfia preparations in our armamentarium against hypertension.

The most obvious and presently valid pharmacological actions attributed to the Rauwolfia preparations used in hypertensive therapy are: a mild but definite sedative or tranquilizing effect; a mild but prolonged hypotensive action; and a remarkable freedom from toxicity (1, 2, 3, 4).

The hypotensive properties of Rauwolfia are mainly ascribed to a central action (5, 6, 7). Although by no means obscure, this central action has resisted most attempts at delimitation because of the extensive anatomical and physiological ramifications of the central nervous system. Some of the most common methods which were initially utilized to show this central manifestation and that have later been used to elucidate its mechanism have been: numerous sedation studies; direct stimulation of various central substrates, such as the hypothalamus before and after the administration of Rauwolfia; and the observation of a blocking of the usual pressor response to bilateral occlusion of the common carotid arteries, and to stimulation of the central vagus or femoral nerves following Rauwolfia therapy (8, 9, 10, 11).

The ability of Rauwolfia to block the pressor response to carotid occlusion or to central vagal stimulation is the particular pharmacological action which prompted the present study. More specifically,
this investigation was undertaken to ascertain the role of the vagi or afferent nerve endings at the carotid bifurcation play in the characteristic mild but prolonged hypotension elicited by Rauwolfia. In addition to the blood pressure blocking effect described above, the present study was both indicated and warranted for the following reasons:

1. The carotid sinuses and aortic arch pressoreceptors normally play a vital role in maintaining or striving to maintain a physiologically normal blood pressure level. In hypertensive individuals, however, with the possible exception of cases of pheochromocytoma, there is evidence that the pressoreceptors have been reset, possibly by adaptation, to operate at the elevated arterial pressure in a manner similar to that which they would ordinarily at normal levels of arterial pressure (12).

In this latter connection, it is interesting to note that Heymans and Heymans (13) have shown that the walls of arteries normally contain considerable amounts of adrenalin and noradrenalin which may maintain and regulate the intrinsic tone and resistance to stretch of the arteries where the pressoreceptors are located. They postulate that a decrease, not an increase, in tone and resistance to stretch of the walls of the arteries where the receptors to the moderator nerves of blood pressure are located (sino-aortic pressoreceptors) could be the primary mechanism of essential hypertension. In any event, it seems logical to assume that any drug which either lowers (e.g., Rauwolfia) or raises the arterial pressure must do so by blocking, or otherwise interrupting or antagonising the pressoreceptor reflex arc somewhere along its long pathway or at its ultimate effector organs, such as the heart and
arterioles.

2. Drugs which affect arterial pressure (epinephrine, NaNO₂) or which do not specifically alter the blood pressure (atropine, papaverine) are known to act on the carotid sinus or aortic arch pressoreceptors when injected intra-arterially in close proximity to the receptors or directly into the walls of the blood vessels where these receptors are located (13, 14, 15, 16). In this respect, some of the Veratrum alkaloids have been shown to produce hypotension when injected into the carotid sinus walls or intravenously in slightly larger doses (17, 18). When still larger doses of Veratrum alkaloids are injected or infused intravenously the carotid sinus reflex due to carotid occlusion is blocked (19). Since Rauwolfia preparations can also block the carotid sinus reflex when given orally or intravenously, a study of their effects when injected into the carotid sinus wall seemed to be indicated. Furthermore, these two drugs are frequently used clinically in combination and it would be interesting to learn if they both might act on the carotid sinus by the same or different mechanisms.
Hypertension

Hypertension is a disease of almost completely unknown etiology. In almost all cases it is due to an increased peripheral resistance rather than to an increased cardiac output. The increased peripheral resistance may be due to an increased tone of the vessels controlling blood flow, such as the terminal arteries, arterioles, and precapillary sphincters, or to structural changes resulting in narrowing of the lumen of these vessels. These latter changes may result from widespread thickening of the intima and hypertrophy of the muscle layer of the arterioles, or to migration of salts and water into the arteriole walls (12). The adrenal cortex also may be involved as a result of an absolute or relative increase in an adrenocortical salt-retainıng hormone, which in turn may be related to the migration of salts into the arteriole walls.

Vasoconstrictor substances produced by various glands or organs may also be responsible for certain forms of hypertension. Excess of sympathetic vasoconstrictor mediators, epinephrine and norepinephrine, may be present in hypertension associated with tumors of the adrenal medulla or when the sympathetic system is overactive for any number of reasons. Other vasoconstrictor substances which have been postulated to be causative or contributing agents in many forms of hypertension are: hypertensin, V.E.M. or vasoexcitator material, cerebral vasopressor principle, pherentasin, and nicotine-like bases. Most of these are found in association with, and several are known to be formed by partially ischemic kidneys (12). The role of the carotid sinus and aortic
arch pressoreceptors in normal individuals as well as the fact that
they may be reset to operate at elevated blood pressure levels in the
hypertensive patient has already been discussed.

Possible etiologic factors which may lead to temporary and finally
more permanent or malignant hypertension are: hereditary predisposi-
tion, age, environmental influences, emotional and personality factors,
and diet, as well as certain vascular deformities or pathologic condi-
tions, such as arteriosclerosis of the renal arteries.

From the preceding discussion it can readily be deduced that the
most troublesome nature of hypertension is that a vicious cycle can be
set up so that any one or a number of minor contributing factors can
result over a period of time in the development of a permanent hyper-
tension. For example, periodic or continuous emotional tension states
may cause an increased sympathetic outflow resulting in constriction of
the arterioles and an elevated blood pressure. The adrenal cortex may
elaborate more salt-retaining hormones, and this salt may then migrate
to the blood vessel walls causing organic changes and/or increased sen-
sitivity to even a normal outflow of sympathetic impulses. The blood
flow to certain vital organs, as the kidney, may accordingly be reduced
causing the elaboration of pressor substances from these organs. These
constrictor substances enter the general circulation and cause wide-
spread constriction and a further elevation of the blood pressure, pal-
pitation, headache, fatigue, blindness, etc. This turn of events
causes more nervous tension and worry in the individual and the whole
cycle of events is thereby reinforced. If this cycle is not broken,
angina pectoris, congestive heart failure, renal insufficiency, or
cerebral hemorrhage may ensue resulting finally in death of the patient. This is why pharmacological agents are so necessary. The patient must have relief from the ravages of the disease until and possibly even after the etiology of the disease, which is still so obscure, is fully elucidated. Furthermore, while lowering the systemic arterial pressure with drugs may impair the blood flow in some organs, this may be counterbalanced by a reduced load on the body as a whole, and the cycle of events maintaining the hypertension may be broken by restoring the arterial pressure to normal levels.

**General Antihypertensive Agents**

**Adrenergic Blocking Drugs.** - There are many synthetic, adrenergic, blocking agents now available commercially. Some of these are: Dibenzyline, Dibenamine, pentolamine (Regitine), tolazoline (Priscoline), azapetine (Ilidar), Piperoxan, Dibosane, and the natural or semi-synthetic yohimbine and lysergic acid (dihydroderivatives). All of these compounds have similar pharmacological effects (12). Minor differences are variations in the dose required to produce a given response, and the duration of the effects of the same doses. A chief difference is that some of these agents (Regitine and Benzodioxane) are adrenolytic only (block injected or circulating epinephrine only), while the others (dihydroergot derivatives, Priscoline, Dibenzyline) are both adrenolytic and sympatholytic (block both circulating epinephrine and sympathetic nerve stimulation) (3).

The chief use of this group of drugs is in detecting epinephrine secreting pheochromocytomas. In the presence of such tumors, small doses of these drugs will, by virtue of their epinephrine-reversing
effect, lower the hypertensive arterial pressure to hypotensive levels. In other forms of hypertension, such doses have little effect on the elevated blood pressure (12). If moderate doses of an appropriate drug from this group are given to a hypertensive individual, the vasoconstrictor responses to circulating epinephrine and to sympathetic constrictor impulses can be blocked and the blood pressure will be lowered. However, at this level of blockade, vasodilation occurs mainly in the skeletal musculature and splanchnic viscera, but doesn't occur in such vital regions as the heart, kidney, and cerebral vasculature. Side effects such as postural hypotension, nausea, dizziness, tachycardia, palpitation, and drug fever may also occur. For these reasons the adrenergic blocking agents have a limited use in the management of hypertension and at best are of value only in isolated and specifically selected cases, or in the diagnosis of pheochromocytomas.

1-Hydroslnophthalazine (Apresoline). — This drug has both a central and a peripheral action (adrenolytic and sympatholytic) (3) and blocks the effect of certain pressor substances which may play a role in the etiology of hypertension (20). The postulated chemical process for the inactivation of pressor substances by Apresoline is shown in Figure I (21).

Apresoline has a potent hypotensive effect when given parenterally and seems to be especially effective in treating the hypertension of toxemia of pregnancy. The antihypertensive response is somewhat less dramatic following oral administration. The undesirable decrease in cerebral and renal blood flow is not noted despite the fall in blood pressure when Apresoline is administered (22). Since there are other
more useful agents for the treatment of the milder types of hypertension (Rauwolfia), and because Apresoline rarely produces normotension in the more severe forms of the disease, its major contribution to hypertension therapy appears to be in the treatment of hypertension of toxemia of pregnancy and in combination with other drugs in the more severe cases of hypertension. Side effects of Apresoline are: headache, tachycardia, ankle and periorbital edema, and a relationship to collagen disease which is not yet definitely established (23).

Ganglionic Blocking Drugs. - These agents may be exemplified by the following compounds: pendiomide, hexamethonium, tetraethylammonium, pentamethonium, and pentolinium tartrate (Ansolysen). They are the most effective blocking agents and hypotensive drugs. When hexamethonium or Ansolysen are administered parenterally, blood pressure can be
lowered to any desired level. However, they must be administered with considerable caution lest severe hypotension ensue.

The principal pharmacologic action of these drugs is exerted in the autonomic ganglia both sympathetic and parasympathetic, at which site they block transmission at the synapse between the terminals of preganglionic fibers (white rami) and the dendrites of the ganglionic cells which give rise to the postganglionic fibers (grey rami). This blockade is a nicotine-like action (24).

Although the blood pressure can nearly always be lowered by these agents, their dual blocking action on both the sympathetic and parasympathetic impulses often leads to such severe and undesirable complications that they usually are used only when absolutely necessary and even then they may have to be withdrawn. Undesirable effects of these drugs are: blurring of vision, impotency, constipation which may be severe and persistent with paralytic ileus, postural hypotension, and poor absorption. Only 10-15% of the oral drug is absorbed (3). Patients given the drug parenterally must remain recumbent for at least 1 1/2 to 2 1/2 hours after the drug is given. Preliminary observations with Ansolysen indicate that it produces fewer instances of side reactions, especially constipation and consequently may prove to be the safest ganglionic blocking agent for the ambulatory patient.

Miscellaneous Drugs

The Veratrum alkaloids act centrally and/or peripherally by lowering the threshold of the "firing off" of the sino-aortic pressoreceptors. Most of these alkaloids or alkaloidal preparations can produce a moderate fall in blood pressure and a slowing of the pulse rate, but
the margin between the effective dose and the toxic dose is narrow. Tolerance to the hypotensive effect develops frequently. Used alone, these alkaloids are usually effective in hypertensive crises, especially those of pregnancy, but their greater future appears to rest in their use in combination therapy.

The nitrites, organic nitrates, thiocyanates, sodium nitroprusside and pyrogens probably have little use in the necessarily long-term management of most hypertensive patients. The nitrites and nitrates are erratic in action and tolerance is prone to develop. Thiocyanates may be useful for the relief of hypertensive headache, but a maintained effective blood level may cause serious reactions. Nitroprusside is converted to thiocyanate in the blood and therefore acts similarly. Pyrogens may be useful only in the fulminating type of malignant hypertension.

Rauwolfia Serpentina

Favorable reports on Rauwolfia indicate that it is more generally applicable to the treatment of hypertension than any other known drug. In some cases it is effective alone and in many cases it is an extremely valuable addition to other medications (1, 2, 3, 25).

History. - Rauwolfia serpentina Benth. Apocyanaceae (syn. Ophioxylon Serpentinum, Linn. Roxb.) is a small, erect glabrous shrub that is found fairly widely distributed in India, especially in the foothills of the Himalayas, and in other tropical and subtropical regions. It is about 1 1/2 to 3 feet in height and has crooked, tapering roots from 1/4 to 1/2 inch in diameter. Rauwolfia was named for Leonhard Rauwolf, a German physician and botanist who made a trip to Asia and Africa in 1773-5
to study medicinal plants. More than one hundred different species of Rauwolfia are known.

The use of Rauwolfia in the treatment of various forms of hypertension dates back to 1933 when Indian scientists first reported on its antihypertensive effect (26). In this respect, it is interesting to note that whereas in India the drug came into the hypertensive field from the psychiatric, it is now reversing that path in the United States where it was first introduced as a treatment for hypertension.

Chemistry. — The first important chemical investigation of Rauwolfia serpentina was conducted by the Siddiquis of India in 1931 (27). They isolated, identified, and partially characterized five different alkaloids. In 1939 they reported the isolation of two additional alkaloids (28). Since that time work in this field has increased, until today there are reported some 15 to 20 alkaloids. A list of these alkaloids is shown in Table I. Some duplication of these alkaloids may exist because of the large number of investigators who have reported them.

Many of the Rauwolfia alkaloids have been studied extensively but reserpine has perhaps been the most completely characterized. The structure of this ester alkaloid is shown in Figure II. It is closely related chemically to rescinnamine (trimethoxycinnamic ester of methylreserpate) which is also shown in Figure II for comparison (29). Both of these alkaloids have generally similar qualitative and quantitative pharmacological properties and are the most potent single alkaloids yet isolated from Rauwolfia serpentina (5).

Some of the alkaloids listed in Table I have also been found in other species of Rauwolfia, such as R. heterophylla, R. micrantha,
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<th>Alkaloid</th>
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<tr>
<td>Ajmaline</td>
<td>C\textsubscript{20}H\textsubscript{26}O\textsubscript{2}N\textsubscript{2}</td>
<td>158-160\degree C</td>
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<td>Ajmalanine</td>
<td>C\textsubscript{20}H\textsubscript{26}O\textsubscript{3}N\textsubscript{2}</td>
<td>180-181\degree C</td>
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<td>Ajmalicine</td>
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<td>250-252\degree C</td>
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<td>Serpentinine</td>
<td>C\textsubscript{21}H\textsubscript{22}O\textsubscript{2}N\textsubscript{2}</td>
<td>263-265\degree C</td>
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<tr>
<td>Serpentine</td>
<td>C\textsubscript{21}H\textsubscript{22}O\textsubscript{3}N\textsubscript{2}</td>
<td>157-158\degree C</td>
</tr>
<tr>
<td>Neoajmaline</td>
<td>C\textsubscript{20}H\textsubscript{26}O\textsubscript{2}N\textsubscript{2}</td>
<td>205-207\degree C</td>
</tr>
<tr>
<td>Isoajmaline</td>
<td>C\textsubscript{20}H\textsubscript{26}O\textsubscript{2}N\textsubscript{2}</td>
<td>264-265\degree C</td>
</tr>
<tr>
<td>Raupine</td>
<td>C\textsubscript{20}H\textsubscript{26}O\textsubscript{3}N\textsubscript{2}</td>
<td>325\degree C</td>
</tr>
<tr>
<td>Sarpaginine</td>
<td>C\textsubscript{19}H\textsubscript{22}O\textsubscript{2}N\textsubscript{2}</td>
<td>320\degree C</td>
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<tr>
<td>Rauwolfine</td>
<td>C\textsubscript{21}H\textsubscript{26}O\textsubscript{2}N\textsubscript{2}</td>
<td>160\degree C</td>
</tr>
<tr>
<td>Rauwolfinine</td>
<td>C\textsubscript{19}H\textsubscript{26}O\textsubscript{2}N\textsubscript{2}</td>
<td>235-236\degree C</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>C\textsubscript{21}H\textsubscript{26}O\textsubscript{3}N\textsubscript{2}</td>
<td>234\degree C</td>
</tr>
<tr>
<td>Reserpinine</td>
<td>C\textsubscript{33}H\textsubscript{40}O\textsubscript{9}N\textsubscript{2}</td>
<td>238-239\degree C</td>
</tr>
<tr>
<td>Reserpine</td>
<td>C\textsubscript{33}H\textsubscript{40}O\textsubscript{9}N\textsubscript{2}</td>
<td>277-278\degree C</td>
</tr>
<tr>
<td>Rescinnamine</td>
<td>----</td>
<td>238-239\degree C</td>
</tr>
<tr>
<td>Reserpiline</td>
<td>----</td>
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Figure II. - Similarity in Chemical Structure of the Two Most Potent Alkaloids of Rauwolfia, Reserpine and Rescinnamine

Reserpine

Rescinnamine

R. vomitoria, and R. canescens. Of special interest is the 11-desmethoxy derivative of reserpine which is reported to have the same range of pharmacological activity as reserpine (30). It has been isolated from R. canescens by three different laboratories and has been named recanescine, deserpidine, and canescine (30, 31, 32) by these different investigators. Since this alkaloid has the same order of sedative and hypotensive potencies as reserpine and rescinnamine, the methoxy group in position 11 of these latter molecules, which is absent in canescine, does not seem to be necessary for their action.

A number of analogues of reserpine have been prepared and screened pharmacologically. The trimethoxybenzoate radical has been replaced by veratratre, anisate, benzoate, furoate, nicotinate, phenylactate, acetate, etc. All of these changes caused, in general, a lowering of
activity while those most closely related to reserpine had the highest potency (33).

Various laboratories are presently at work trying to synthesise reserpine and its related alkaloids. At the moment their total synthesis seems none too imminent (34).

**Pharmacology.** - The pharmacological investigations of Rauwolfia have been quite prolific, especially since 1952. These studies have dealt for the most part with the various individual and purified alkaloids or with the alseroxylon fraction. Only part of these findings will be discussed here, while other portions of these investigations will of necessity be considered when the results of the present study are discussed.

The modern pharmacology, like the old, reveals two main effects produced by the drug, viz., a mild and prolonged hypotension accompanied by bradycardia and a mild, prolonged, and peculiar type of sedation or tranquilizing effect. This is true for the crude drug, for the alseroxylon fraction, or for the pure alkaloids reserpine and rescinnamine. Other known individual alkaloids of Rauwolfia serpentina apparently do not exert both of the above actions, i.e., they are mildly antihypertensive but without sedative qualities. Some of these latter alkaloids also possess a peripheral adrenolytic and/or sympatholytic action, while the alseroxylon fraction, reserpine, and rescinnamine are devoid of this property. Other striking features of the typical Rauwolfia effect are, depending upon the species of animal or the dose employed: relaxation of the nictitating membrane, diarrhea, ptosis, miosis, and hypothermia.
Although intravenous injections of the extracts of the crude drug (4) as well as larger doses of reserpine or the alseroxylon fraction can definitely produce an immediate and prolonged fall in the blood pressure (35, 36, 37), the usual clinical oral or intravenous doses of these preparations produce both clinically and in experimental animals a delayed (1/2 hour or more) and very prolonged fall in the systemic arterial pressure (5, 6, 7).

The final mode of action of the Rauwolfia alkaloids which accounts for their syndrome of effects and especially for their characteristic type of blood pressure lowering ability is still unknown. Much of the pharmacological and clinical evidence places the central nervous system in the foreground as their locus of action. This central action is made conspicuous by the well known sedation and prominent ability of the oral or intravenously administered alkaloids or crude drug to lower the blood pressure and produce bradycardia concomitantly with a blockage of certain experimental blood pressure elevating procedures such as by occluding the common carotid arteries, stimulation of the afferent vagus, and stimulation of the femoral nerve (7, 8, 9). Other direct or indirect evidence for a central site of action are: a blockage of the pressor response to hypoxia (6, 8); a rise in threshold for central excitation or a reduction in central vasomotor activity as shown by electrical stimulation of certain central nervous system substrates (10, 36); a pronounced depression of oxygen consumption of rat cerebral cortex slices in vitro by reserpine (38); the reduction of electroshock seizure latency; the blockade of the release of pituitary gonadotrophin, and the antagonism of morphine analgesia in mice, the latter....
three for reserpine (39, 40, 41).

Most all of the above studies point to interference with sympathetic activity somewhere in the brain stem. This idea is further augmented by eliminating adrenergic blockade and generalized ganglionic blockade as factors as exemplified by the ability of Rauwolfia preparations to increase the pressor response to epinephrine and to unalter the intact response to efferent vagal stimulation (6, 7, 8). Furthermore, reserpine does not inhibit pressor responses evoked by electrical stimulation of the splanchnic nerves (42), and the relaxation of the nictitating membrane by reserpine has been shown not to be due to ganglionic blocking or sympathicolytic activity (7). Reserpine has also been shown to augment the action potentials of synapses in the spinal cord (43).

Although the evidence for the central activity of Rauwolfia is quite convincing, there is considerable evidence to indicate that the peripheral effects should not be overlooked. This is especially true for the crude drug, for the alseroxylon fraction and to some extent for reserpine.

Some of the older known alkaloids such as ajmalinine and ajmaline have been shown to definitely possess peripheral adrenergic blocking activity and are without sedative action (44, 45). Serpine, an isomer of yohimbine and one of the most recently isolated alkaloids from Rauwolfia serpentina, also displays such an action (46). On the contrary, reserpine and the alseroxylon fraction are devoid of such activity, although the writer has seen no references where these latter, more potent alkaloids have been used in the high dosage levels that were
necessary to show adrenergic activity by the former, less potent agents.

The alkaloid serpine has also been shown to produce vasodilatation when injected into the circulation of the isolated hind limb of cats and monkeys. This action was independent of whether the limb was innervated or denervated. In this same preparation, serpine displayed an adrenalin-antagonistic action which was competitive (46). Again on the contrary, Tripoid and Meier who used the rabbit, hind-leg preparation concluded that reserpine exhibits no primary vasodilatation or vasodilator effect against adrenalin or noradrenalin, but that it has a strong antagonistic effect against BaCl₂ (47). Similar to reserpine and also opposed to serpine, the alseroxylon fraction has no direct vasodilator or vasoconstrictor actions as shown in the rat’s hindquarters preparation. In this latter preparation, the alseroxylon fraction was able to antagonize the vasodilator actions of acetylcholine (48).

In considering the above similarities in the action of the alseroxylon fraction and reserpine and their differences from some of the older alkaloids and serpine, several factors must be kept in mind. First, the alseroxylon fraction contains several known alkaloids but these account for only about 50% of the total alkaloids present (5). Consequently, the pharmacological effect of certain of these alkaloids may mask or antagonize the effects of others present. Secondly, the doses and specific experimental techniques employed by the various investigators may have led to different results; and thirdly, the species of animals used is important. This is borne out by the fact that McQueen, who employed the isolated innervated rabbits' hind leg preparation, found that reserpine when injected into the trunk of the rabbits
produced a fall in the systemic arterial pressure which was accompanied by an immediate rise in the limb perfusion pressure (37). Identical results were found for serpine when cats and monkeys were used (46).

Another peripheral action of certain of the Rauwolfia alkaloids is an anticholinergic effect. The alseroxylon fraction possesses anticholinergic properties on skeletal, smooth, and cardiac muscle as shown by its in vitro inhibition of acetylcholine on the frog rectus-abdominis muscle, the rabbit duodenum, and rabbit auricles, respectively (48). Reserpine also reduces the contractile response of the isolated rabbit ileum to acetylcholine. These findings tend to rule out a peripheral factor in the etiology of Rauwolfia on the autonomic regulation of G. I. functions (49), and may obscure any stimulant effect that is mediated via the parasympathetic system in certain types of experiments.

In concluding this pharmacological review, something must be said regarding the usual latency of action which is apparent whether the alseroxylon fraction or reserpine be given intravenously or orally, or whether the experiments are on isolated tissues. Several ideas have been conceived but so far the reason for this latency is unknown.

For isolated tissues, factors that have been considered are: a decreased permeability to acetylcholine or a stimulation of the production of cholinesterase (48). The latent period before hypotension or sedation in intact animals may be due to: partial inhibition of the production of some essential metabolite so that this metabolite must be used up before the effects of the drug are seen; dissolution of the drug when given intravenously with the time necessary for resolution being approximately equal to that required for oral absorption;
conversion of the drug in the body to an active form; and permeability factors associated with a possible intracellular site of action.

**Clinical.** - Probably no drug or group of drugs is today enjoying the widespread success and usefulness in the therapy of hypertension as are the various Rauwolfia preparations. Published reports indicate that Rauwolfia alone is most useful as a hypotensive agent in mild, labile, psychoneurotic hypertensive patients with tachycardia, headache, palpitation, nervousness, and irritability or anxiety. It will relieve the latter symptoms in most of these patients and will restore the blood pressure and pulse rate to normal in one-half of the cases (1, 2, 25).

Side effects of Rauwolfia are stuffy nose, diarrhea, sedation and bradycardia, although the latter two could easily be considered as useful in hypertensive therapy, and the sedation can often be obviated or controlled in a useful manner. For instance, if the patient wishes to reduce the daytime sedative effect, the whole dose of Rauwolfia may be taken at bedtime. However, if the patient has a particularly irritating job and wishes to have his reactivity to this lessened during the daytime, the total dose may be taken at breakfast (2). In hypertensive individuals with angina pectoris, the Rauwolfia-induced bradycardia may prevent or lessen anginal attacks (3).

As Wilkins has often pointed out (2), the best use of Rauwolfia is as an adjunct or background agent given in combination with the stronger antihypertensive agents. In this role, Rauwolfia may not only act additively but may also potentiate the stronger drugs to lower blood pressure. Rauwolfia in combination is also able to counteract some of the unfortunate side effects of the other drugs. For example, it will
lessen considerably the nausea and vomiting of Veratrum and at the same
time allow this drug to be used in larger doses than is possible alone.
Also, Rauwolfia plus Veratrum will cause a greater lowering of blood
pressure without nausea or vomiting than when Veratrum is used alone.
The dangerous constipating effect of hexamethonium is considerably re­
lieved by the bowel stimulating effect of Rauwolfia. The irregular blood
pressure effect of the former is also considerably smoothed by the latter
drug, and because of its bradycardic effect, Rauwolfia offsets the tachy­
cardia and palpitation ordinarily induced by hydralazine.

Another distinct advantage of Rauwolfia is as a continuing agent.
That is, when in combination therapy the blood pressure has been re­
duced to relatively normal levels, it is often possible to withdraw the
stronger agents and to maintain this lowered pressure with Rauwolfia
alone. This is an interesting action for frequently Rauwolfia will
hold the pressure at this lower level when it was not capable of ini­
tially lowering the pressure to that level without the addition of other
agents.

Clinical preparations of Rauwolfia that are commercially available
are: Tablets of the powdered root (Raudixin), the alseroxylon fraction
containing the active alkaloids in relatively pure form (Rauwiloid),
the pure, single alkaloid reserpine (Serpasil), and various combina­
tions of Rauwolfia with other antihypertensive drugs.

The action of these several preparations is clinically very simi­
lar provided equivalent dosages are used, i.e., 100 mgm. of the crude
root, 2 mgm. of the alseroxylon fraction, and 0.1 mg. of reserpine.
Wilkins (2) who has done a great deal of clinical experimenting with
the Rauwolfia preparations believes that there is no convincing evidence for a greater efficiency in hypertension for any one of these preparations over the others.

However, the crude drug, and possibly the alseroxylon fraction, have alkaloids which can lower the blood pressure but are without sedative effect (34, 35). This would mean that for equivalent hypotension, a correspondingly larger amount of reserpine would necessarily have to be administered, or, for the same degree of hypotension with reserpine, more sedation would be produced.

Another important clinical use of Rauwolfia, and most especially reserpine, is its widespread employment in the psychiatric field. In trials that are as yet more or less preliminary, this alkaloid shows some promise in the treatment of headache, irritable and hypertonic infants, organic convulsive states, head injuries, sickness caused by narcotic withdrawal, pre and post-operative sedation and in geriatrics.

The Sino-Aortic Pressoreceptors and the Chemoreceptors of the Aortic and Carotid Bodies.

Again, as in the pharmacological review, this discussion will be general and limited as more details on the subject will be brought out during the discussion of the present investigation. In addition, only the carotid pressoreceptors and bodies will be discussed because the mechanisms of the aortic receptors are generally similar, and because the vagi which are the afferent nervous pathways for the aortic receptors in the dog have usually been severed in the present study. Furthermore, an excellent review on this subject has recently been published (50).
The pressoreceptors or baroreceptors are located mainly in the walls of the internal carotid artery at the bifurcation of the common carotid artery or the carotid sinus. These afferent receptor nerve endings are located in the media and adventitia of the arterial wall of the carotid sinus, and are connected to or continuous with nerve fibers which join the intracarotid branch of the glossopharyngeal (IX cranial nerve). This branch of the glossopharyngeal is known as Hering's nerve or the carotid sinus nerve.

The aortic and carotid sinus nerves are the reflex buffer or modulator nerves of the systemic arterial pressure by virtue of the action of the arterial pressure itself on receptors in the vascular wall of the sino-aortic areas (13, 51).

Heymans, et al, have shown that arterial pressure itself does not act directly on the sino-aortic receptors, but acts indirectly by stretching the wall of the arteries where these receptors are located (13). They suggest that the state of contraction, tension, and distensibility of the arterial wall of the sino-aortic area could play a role in the mechanisms for the reflex regulation and homeostasis of the blood pressure. These facts have been borne out by the results of perfusion studies, studies with various drugs which are known or supposed to contract or relax arteries when applied locally, and with action potential studies on the carotid sinus nerve.

The sequence of events whereby the pressoreceptors reflexly regulate the blood pressure can best be shown by general examples. When the blood pressure becomes elevated, the intraluminal pressure in the carotid or aortic sinuses increases. The receptor nerve endings of the
sinus which are parallel with and/or in series with the muscle fibers of the sinus wall are stretched or squeezed or otherwise stimulated and the discharge of the carotid sinus nerve is increased. Through central connections, this increase in discharge brings about inhibition of cardioaccelerators, bradycardia, and inhibition of sympathetic vasoconstrictors. The systemic blood pressure is lowered and the discharge of the carotid sinus nerve then lessens.

A reversal of the above events takes place whenever the blood pressure is lowered so that in this situation the pressoreceptors lessen or stop "firing off" impulses entirely. Central sympathetic tone is relieved of its carotid sinus-induced inhibition and the blood pressure becomes elevated. This latter sequence of events accounts for the pressor effect from bilateral carotid occlusion.

The pharmacological actions of drugs on the carotid sinus pressoreceptors have been studied by utilizing several different experimental techniques: Drugs have been injected intravenously or intracarotidly via the superior thyroid, lingual, or common carotid arteries; injected into the conjunctival space surrounding the carotid sinus; applied with a cotton swab to the sinus area; or injected directly into the adventitia of the carotid sinus. The carotid sinus nerves have frequently been sectioned before or after the application of drugs. Action potentials from the carotid sinus nerve have also been recorded and studied.

Typical actions of drugs, especially when they are injected or applied locally to the sinus area, are described below: Epinephrine, norepinephrine, and vasopressin produce a local contraction of the sinus
wall and provoke a marked reflex fall of the systemic arterial pressure which is accompanied by a reduction or complete blocking of the hypertensive reflexes normally elicited by clamping the common carotid arteries (52, 53). These drugs also elicit a very marked increase in the impulse traffic of the pressoreceptor nerve. The effect of epinephrine or norepinephrine may be suppressed or reversed by the local action of adrenolytic agents, such as Dibenamine or Regitine (54, 55, 56). Drugs such as papaverine, Priscoline, and NaNO₂ which are known to relax smooth muscles produce, after local application or injection, a decrease in the intraluminal pressure in the sinus which reflexly induces a rise in the systemic arterial pressure (13, 53, 54). Certain of the Veratrum alkaloids owe at least part of their hypotensive activity to their ability to lower the threshold of the carotid sinus pressoreceptors (57, 58). In this case the carotid sinus nerves "fire off" more intensely than they would at the same blood pressure level without the influence of the Veratrum derivatives. Some drugs reflexly induce hypotension when injected into or applied to the carotid bifurcation by virtue of their local anesthetic action. The anesthetic effect of these drugs reduces the "firing off" of the pressoreceptor nerve fibers. Many other drugs have been shown to act on the carotid sinuses but the exact modes of their action have not always been clear or verified. Acetylcholine does not appear to be involved in the physiological stimulation of the carotid sinus pressoreceptors; instead, the experimental evidence suggests a pharmacological action (50).

The chemoreceptors of the carotid body are also located at the carotid bifurcation. These receptors respond to alterations in the
chemistry of the blood.

Much of the knowledge on the nervous organization within the carotid body is still in a state of flux, particularly in reference to whether or not there is a synapse in the body which is analogous to the ganglia of the autonomic nervous system. However, most investigators agree that the carotid bodies contain no chromaffine tissue, and are thus not intimately related to the sympathetic nervous system.

According to de Castro (59), the carotid body contains epithelioid or glomus cells which are directly in contact with the blood capillaries. An end-plate is present in each glomus cell and is connected with a myelinated nerve fiber. The nerve cells of these fibers are located in the petrosal ganglion. According to Heymans (50), de Koch has reinvestigated the histology of the carotid body of the cat, sheep, and rat. Besides ordinary glomus cells, he found a second type which was typically in closer association with the vascular supply than the glomus cells. Stellate and non-stellate interstitial cells occur. De Koch also traced the intra-glomular nervous supply. Fibers of the glossopharyngeal nerve were found to expand into an intra-glomular network in which interstitial cells were enmeshed. They then passed to the glomus cells, where they ended in an intracellular reticulum. The occurrence of solid end structures in the glomus cells were not found by de Koch.

In dogs, the carotid body is most often found astride the proximal part of the occipital artery. Occasionally it may be found on the ascending pharyngeal arteries. The blood supply to the carotid body comes mainly from branches from the occipital and external carotid
arteries, the ascending pharyngeal, and from muscle branches of the carotid. The veins from the body drain into the internal jugular, or, in its absence, the external jugular (60). Arterio-venous anastomoses are located at the origin of the arterial blood supply to the carotid body. The afferent arteries of these arterio-venous anastomoses have smooth muscles and a pressoreceptive innervation which regulates the blood supply through the body (59).

The nerve fibers from the carotid body, like the carotid sinus nerve fibers, enter the central nervous system via the carotid sinus and then the glossopharyngeal nerve.

The physiological properties of the carotid body chemoreceptors are quite complicated and as yet not firmly established. Many experimental observations have nevertheless been repeatedly confirmed. The aortic and carotid bodies are important as peripheral reflex mechanisms for regulating the activity of the respiratory and cardio-vascular centers. The receptors in these bodies are sensitive to increased CO₂ or decreased oxygen tension in the carotid blood. Stimulation of these receptors by increased CO₂ or decreased O₂ tension induces a reflex stimulation of the respiratory center and a rise in blood pressure. Decreased arterial CO₂ or O₂ tension reflexly provokes an inhibition of the respiratory center. The acidity or alkalinity of the blood affects the chemoreceptors also, and, of course, these are inter-related with the arterial CO₂ and O₂ tension (50). The relative potency or importance of the aortic and carotid chemoreceptors apparently varies with the species of animal being studied.

Numerous drugs have been found to modify the activity of the
chemoreceptors of the carotid body. Some of these stimulate or inhibit the receptors while others alter their excitability to the physiological stimulus.

Some of these drugs, such as cyanides, azides, and mono-iodo-acetic acid, interfere with normal metabolic processes, some interfere with the blood supply to the body (adrenalin and nitrites), while some are ganglionic stimulants (acetylcholine, nicotine and lobeline) (61). References on this subject are quite numerous with most of the observations coming from European investigators. The reader is referred especially to Heymans' recent review on this subject (50).

In concluding this review of the carotid and aortic presso-and chemoreceptors, it should be pointed out that the intracarotid branch of the glossopharyngeal nerve as well as the vagi contain fibers from both the presso- and the chemoreceptor areas. When the pressoreceptor fibers are stimulated, the blood pressure falls while the effects on respiration are not always similar. However, when the chemoreceptors are stimulated, the blood pressure rises and the respiration is stimulated. Consequently, whenever the sinus or vagi are cut, the effects on the blood pressure especially are the algebraic sum of the two different reflexes. Apparently the pressoreceptors are more important for blood pressure regulation since cutting of the vagi or sinus nerves always results in a sharp rise in blood pressure, especially if one of these sets of nerves has been cut previously, i.e., only the sinus nerves left intact.
EXPERIMENTAL

General Procedure

The laboratory animals employed in all the experiments involving blood pressure determinations were normotensive, mongrel dogs of mixed sex and varied weights. The blood pressure was always recorded from the femoral artery with a mercury manometer onto a smoked kymograph. An eight and one-half per cent sodium citrate solution was used as the anticoagulant but was found to be inadequate alone, especially in long experiments or in experiments in which the blood pressure became periodically elevated, such as from carotid occlusion. The addition of ten to fifteen mg. of heparin to the citrate in the arterial cannula and tubing prevented all but an occasional coagulation. If more heparin was used, some blood seepage invariably occurred from at least one of the multiple surgical areas despite precautionary measures. The ipsilateral femoral vein was cannulated and connected with rubber tubing to a burette filled with normal saline to facilitate the intravenous injection of drugs. The trachea was cannulated and the respiration recorded from a membrane tambour in the majority of experiments. In a few of the animals, the circumference of the trachea was such that a cannula slightly smaller than that desirable had to be used. In these animals the respiration was not recorded in order to assure a more free airway.

The blood pressure changes were measured from the pre-injection level to the nadir or summit of the mean arterial blood pressure tracings. The heart rate was determined with a stethoscope and/or by counting the oscillations of the mercury in the manometer. If time
permitted, three one-minute determinations were made and the average taken as the heart rate for that period. When the heart rate was irregular, counting the oscillations in the manometer was inaccurate as were the determinations with the stethoscope when the occasional animal had hyperpnea.

Various anesthetic agents were employed. Veterinary sodium pentobarbital or an extemporaneous six per cent aqueous solution of sodium pentobarbital was found adequate and was used in some of the experiments in which drugs were injected into the carotid sinus bifurcation. Small supplemental doses were occasionally necessary.

For the experiments which ran for six or more hours, or in the experiments in which the effects of bilateral carotid occlusion was particularly studied, sodium pentobarbital, sodium pentobarbital and urethane, urethane, sodium barbital, or chloralose alone were not satisfactory. Sodium pentobarbital or chloralose alone were too short acting. Supplementary doses were required which often changed the blood pressure in one direction as the animal became light from the previous dose, and in the other direction after the supplementary doses were injected. It was especially necessary to avoid this situation since the Rauwolfia alkaloids produce only gradual blood pressure changes of small magnitude and over a prolonged period of time. Sodium barbital, sodium pentobarbital, or sodium pentobarbital and urethane had the disadvantage of partially blocking the pressor effects of bilateral carotid occlusion so they could not be used advantageously when the blocking effects of Rauwolfia on this pressor response were studied. Urethane alone had the disadvantage of requiring the injection of large volumes
of solution because the anesthetic dose of this agent is relatively large. Chloralose does not modify the vasomotor responses to sinus or aortic nerve stimulation in dogs and rabbits (62). It appears to be the anesthetic of choice by numerous European investigators, especially when the carotid sinuses are being studied.

The anesthetic which was finally chosen for use in the long-running experiments and for most of the experiments in which drugs were injected into the carotid bifurcation was the triad: sodium pentothal 2 1/2% aqueous solution, 20% aqueous sodium barbital, and a 5% solution of chloralose in a 1-1 mixture of polyethylene glycol 400 and distilled water. Appropriate doses of each of the agents of this combination generally kept the animals under the desired degree of anesthesia for periods of ten to twelve hours or more. Only infrequently were additional, small doses of the anesthetic necessary. When they were required, small and equal amounts of chloralose and barbital, but not pentothal, were administered. These did not alter the blood pressure or respiration. A long-acting anesthetic was necessary because the operating procedure required anywhere from one to four hours, a lengthy, normal tracing was often obtained, and some of the animals were observed for seven to nine hours after certain doses of drugs were given.

The doses of the individual agents of this combination were: sodium pentothal, 10-15 mg./Kg.; sodium barbital, 140 mg./Kg.; and chloralose, 50 mg./Kg. The pentothal was injected intravenously in amounts just sufficient to enable the subsequent intraperitoneal injection of the other two agents without too much struggling by the animal. After one-half hour, or occasionally longer, the animal was in a
surgical state of anesthesia.

There are several advantages to this combination of anesthetic agents. The small, intravenous dose of pentothal assures a rapid and smooth induction of anesthesia for the animal and is convenient for the operator. It probably acts additively with the chloralose and barbital to hasten the onset of surgical anesthesia. The medium-acting chloralose and long-acting barbital combination appears to act in such a way that the carotid sinus pressor reflex remains strong while the duration of the barbital anesthesia remains quite lengthy. This is accomplished with the doses employed above, which were 50% of the usual intravenous dose of chloralose and 55% of the usual intraperitoneal dose of sodium barbital.

Many investigators who have done work on the carotid sinuses have had a great deal of success with a morphine-chloralose combination. One or two mg./Kg. of morphine is injected subcutaneously followed by 100 mg./Kg. of 1% aqueous chloralose intravenously. This combination was not used in the present work because there is some indication in the literature that Rauwolfia antagonizes morphine (63).

The Rauwolfia preparations used in this study were the alseroxylon fraction (Rauwiloid) and, to a much lesser extent, reserpine (Serpasil, Reserpid, Rau-sed). Accurately weighed amounts of these alkaloids were dissolved in 1.5 ml. of 25 to 50% citric acid and then diluted with distilled water until the final concentration of the citric acid was 0.375 or 0.75%, respectively. The final pH of the 0.375% solution was 2.3 and the 0.75% solution 2.2. The final concentration of the alseroxylon fraction was 4 or 5 mg./ml. and 0.5 mg./ml. for the less
soluble reserpine. The alseroxylon solution could be diluted with physiological saline if used immediately, but the reserpine solution could not be so diluted without causing precipitation. Fresh solutions of the alkaloids were prepared every two or three days. They were stored in the refrigerator in the meantime. Prior to injection, the solution was warmed by allowing it to stand in a dark area because some of the constituent alkaloids are known to be photosensitive (64).
Experiment I. - The Relative Importance of the Vagus Nerve and the Carotid Sinus Zone in the Hypotension Produced by the Alseroxylon Fraction of Rauwolfia Alkaloids.

Procedure

Eighteen dogs under the anesthetic triad (pentothal-chloralose-barbital) were used in this experiment. They can conveniently be divided into the following groups, with the number of animals in each group shown in parenthesis: normal animals (3), dogs with both vagi sectioned (5), animals with both carotid sinus zones denervated (5), and dogs with both vagi and carotid sinus zones denervated (5). Each of these dogs received a 1.0 mg./Kg. dose of the alseroxylon fraction by intravenous administration over a period of from one to four minutes, depending upon the volume of vehicle, which was 4.0 ml. in the largest animal.

In the intact (normal) animals, the blocking effect of Rauwolfia on the carotid sinus pressor responses was determined by occluding the common carotid arteries for approximately 45 seconds during the control period and at various intervals, usually every hour or sometimes more frequently, after injection of the drug. When the carotid sinus pressor response was considerably depressed due to the action of Rauwolfia, it was necessary to clamp the carotids for one minute or longer in order to elicit a response. The heart rates in all the animals were determined at various intervals, as previously described. The respiratory rates were either counted directly or recorded on the kymograph.

The vagotomies, tracheal cannulations, and carotid occlusions were performed at the mid-cervical level of the neck. At least one inch of each vagi was removed to lessen the chance for afferent or efferent vagal stimulation during the carotid clamping procedure. In the animals
in which the carotid sinus zones or the pressoreceptors were denervated, separate bilateral incisions were made directly over the areas of the carotid bifurcations. Major blood vessels of this surgical area, especially the large superficial veins, were left undisturbed. Surgical speed was sacrificed to avoid trauma and bleeding.

The carotid sinus area (chemoreceptors and pressoreceptors) was completely denervated either by dissection and sectioning of the freed carotid sinus nerve or by dissection and sectioning of the carotid sinus nerve together with the occipital and/or aberrant vessels along which the carotid sinus nerve runs.* These blood vessels were, of course, ligated before being sectioned. Thoroughness of complete carotid sinus area denervation was determined by abolition of the pressor response to carotid artery occlusion.

Results

Details of the results of the eighteen dogs of this experiment have been tabulated and are shown in Tables II, III and IV. While the blood pressure responses in several of the animals were observed for seven or more hours, the majority were followed for only six hours. Therefore the results in the tables for all the animals show the final blood pressure levels which were observed six hours after the administration of the alseroxylon fraction.

In order to eliminate the need for the inclusion of a large mass of figures from the protocols of the individual experiments, one

* The writer is grateful to Dr. S. C. Wang, Professor of Physiology, Columbia University, College of Physicians and Surgeons, who graciously supplied information relative to the method used for completely denervating the carotid area.
typical kymograph tracing with a detailed legend is shown for each of
the four groups of animals tested. These tracings adequately document
the findings while, at the same time, they exhibit the characteristic
effects of the drug over the complete six-hour observation period. Any
divergencies from the typical are mentioned in the account which follows.

Under pentothal, chloralose, barbital anesthesia, the average pre­
injection femoral arterial pressure for the four groups of animals was
164 mm. Hg. The range was from 130 mm. Hg to 224 mm. Hg as shown in
Table III. The dog with the pressure of 224 mm. Hg was doubtless hy­
pertensive because the normal tracing was 184 mm. The 224 mm. value
was the reading taken one hour post vagotomy and one and one-quarter
hours after the sinuses were denervated. The average value of 164 mm.
Hg agrees well with that reported by Wang, et al (160 mm. Hg average)
in vagotomized dogs which were anesthetized with a morphine chloralose
combination (65).

Intact Animals (Figure III)

The average blood pressure fall in these three animals at the end
of six hours was 36%. In two of the animals, the pressor effect from
bilateral carotid occlusion was considerably depressed, while in the
third animal it was almost completely blocked. In all three animals,
this pressor effect was somewhat delayed the closer the time ap­
proached the six hour period. In the second animal only, as shown in
Figure III, the last few carotid clampings produced a diphasic re­
sponse, first depressor and then pressor. The respiratory rate aver­
age 16/min. when the Rauwolfia was injected and 12/min. after six
hours. The depression of the heart rate for all the groups is shown
in Table II.

**Vagotomized Animals (Figure IV)**

After bilateral vagotomy the percentage hypotension was 35% or approximately the same as that observed in the normal animals. In this group as well as in the following two groups (but not in the normal group), the injection of the drug infrequently caused a slight rise in the mean arterial pressure which lasted for approximately ten to twenty minutes. After one-half hour the blood pressure had returned to normal or below, whereupon it continued to fall very gradually but persistently over most of the six-hour observation period. If the carotid arteries were clamped during the above post-injection hypertension, the pressor response was usually augmented. Toward the end of the six-hour period, the response to carotid occlusion was often delayed as it was in the normal animals.

In vagotomized animals #4 and #5, the blood pressure appeared still to be falling at the end of the observation time. In animals #6, #7 and #8, however, the nadirs of the blood pressure tracings were reached by the fourth or fifth hours of observation. The quantitative responses to bilateral carotid occlusion for the animals of these first two groups are shown in Table IV.

**Animals with Bilateral Denervation of Carotid Sinus Area (Figure V)**

Except for one animal in the following group, the pre-injection blood pressure levels in this group were higher than those of any other group. This was true despite relatively long periods of waiting for the blood pressure to level off. For instance, animal #10 had a blood pressure of 190 mm. Hg after the sinuses had been denervated and a
pre-injection level of 185 mm. Hg three hours later. Notwithstanding these higher pre-injection levels, the sixth hour blood pressure readings compared favorably with those of the first two animal groups. Consequently, the absolute and percentage differences in blood pressures were considerably larger in this than in the first two groups.

The respiration was somewhat slower but deeper in the vagotomized and in the sinus denervated animals than it was in the normal animals. An exception to this was animal #10 which had a respiratory rate of approximately 40/min. at the beginning and end of the experiment and hyperpnea of as high as 130 breaths/min. at about the third hour of the experiment.

Vagotomized Animals with Carotid Sinus Areas Denervated (Figure VI)

The transient hypertension following the injection of the alseroxylon fraction occurred not only in all of the animals of this group but was the most pronounced in this group, as can be seen from Figure VI.

The blood pressure after six hours was considerably lower in this group than in the other three groups. With the exception of the hypertensive dog which had a pre-injection level of 224 mm. Hg and a sixth-hour reading of 86 mm. Hg, all of the other animals had blood pressure readings which were in the seventies by the fourth or fifth hour. In the hypertensive animal, the blood pressure was 78 mm. at the end of seven hours of observation. As can be seen from Table III, the mean per cent reduction in blood pressure (54%) was also greatest in this group.

The respiration was slow and deep in all of the animals of this
The rate averaged approximately 12-14/min. before injection of the Rauwolfia and approximately 8/min. after six hours. In one dog (#16), the respiratory rate was 7/min. at both the beginning and end of the experiment.

As a sequence to the above four groups of animals, several additional animals were experimented with in an effort to shed more light onto the problem at hand. These experiments are explained and their results summarized below.

1. Because all of the animals of the fourth group (vagotomized plus carotid area denervation) showed an increase in blood pressure immediately following the injection of the Rauwolfia, one animal was run in which the appropriate volume of the vehicle alone (0.75% citric acid) was administered to this vagotomized, bilateral sinus denervated animal. In both cases the blood pressure rose 10 to 20 mm. After 15-20 minutes the blood pressure returned to its original level.

2. In an effort to determine if the delayed type of hypotension which is characteristic of the Rauwolfia alkaloids might be due to a conversion of the alkaloids to an active form by the action of certain constituents of the blood, 1.0 mg./Kg. of the alseroxylon fraction was incubated in 40 ml. of dog serum for four hours at 38°C. The serum did not precipitate the alkaloids from the citric acid vehicle nor did the vehicle or alkaloids precipitate any components of the serum. When the serum containing the drug was injected over a period of seven minutes, the same slowly developing and gradual fall in blood pressure, as seen in the other experiments, was observed.

3. In another dog which had been vagotomized and which had the
carotid presaoreceptors denervated leaving the carotid bodies intact, a dose of 1.0 mg./Kg. of the alseroxylon fraction was given intravenously. This animal had previously received two small doses of the alseroxylon alkaloids into the adventitia of the carotid bifurcation. Since a small amount of these alkaloids doubtless got into the blood stream, the intravenous dose was probably slightly larger, or perhaps 1.1 mg./Kg. In addition, the blood pressure was 122 mm. Hg at the time of the intravenous injection. At any rate, the blood pressure fell rather quickly after the intravenous injection and was only 78 mm. Hg after 2 1/2 hours. This further illustrates that in the vagotomized, sinus denervated animals the blood pressure falls to a lower level than it does in the other three groups of animals of Experiment I.

4. For a specific purpose which will be brought out later in this dissertation, a vagotomized animal was given 2.0 mg./Kg. of the alseroxylon fraction intravenously. When the carotid sinus pressor response was depressed but not blocked after five hours, an additional 1.0 mg./Kg. of the alseroxylon alkaloids was injected. One hour later, or after a total of six hours, the carotid sinus reflex response still was not blocked but remained similar in magnitude to the responses in the vagotomized animals shown in Table IV. The blood pressure level after six hours was 94 mm. Hg which also compares favorably with those of the vagotomized animals in Table III and indicates that 1.0 mg./Kg. of the alseroxylon alkaloids produces maximum blood pressure effects.

In concluding the results of Experiment I, some additional information which was obtained in two of the normal animals, but which was not included in Tables II, III, and IV is given. In animal #1 of
Tabla III, the blood pressure fell three more millimeters or to 107 mm. Hg by the end of seven hours. Both vagi were sectioned at this point. The blood pressure quickly rose to 120 mm. Hg and only slowly receded to 104 mm. Hg after two hours when the experiment was concluded. The heart rate remained the same, but the effects of carotid occlusion were somewhat augmented after the vagi were severed.

In normal animal #2, the vagi were sectioned after the sixth hour. After two more hours of observation, the blood pressure did not change, while the heart rate was slightly lower than before vagotony. The carotids were not occluded during the last two hours of this experiment.
<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Dog</th>
<th>Pre-inj &amp; Mean</th>
<th>After 6 hrs &amp; Mean</th>
<th>Absolute Diff &amp; Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
<td>165</td>
<td>132</td>
<td>-33</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>190</td>
<td>170 145</td>
<td>-20 -29</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>166</td>
<td>132</td>
<td>-34</td>
</tr>
<tr>
<td>Bilateral</td>
<td>4</td>
<td>170</td>
<td>145</td>
<td>-25</td>
</tr>
<tr>
<td>Cervical</td>
<td>5</td>
<td>160</td>
<td>150</td>
<td>-10</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>230</td>
<td>150 146</td>
<td>-80 -36</td>
</tr>
<tr>
<td>Vagotomy</td>
<td>7</td>
<td>174</td>
<td>140</td>
<td>-34</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>175</td>
<td>144</td>
<td>-31</td>
</tr>
<tr>
<td>Bilateral</td>
<td>9</td>
<td>184</td>
<td>160</td>
<td>-24</td>
</tr>
<tr>
<td>Carotid Sinus</td>
<td>10</td>
<td>190</td>
<td>160</td>
<td>-30</td>
</tr>
<tr>
<td>Denervation</td>
<td>11</td>
<td>200</td>
<td>148 153</td>
<td>-52 -31</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>186</td>
<td>160</td>
<td>-26</td>
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<td></td>
<td>13</td>
<td>160</td>
<td>138</td>
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<td>14</td>
<td>160</td>
<td>150</td>
<td>-10</td>
</tr>
<tr>
<td>Denervation</td>
<td>15</td>
<td>164</td>
<td>142</td>
<td>-22</td>
</tr>
<tr>
<td>Plus Vagotomy</td>
<td>16</td>
<td>200</td>
<td>145 142</td>
<td>-55 -28</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>158</td>
<td>126</td>
<td>-32</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>166</td>
<td>146</td>
<td>-20</td>
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Table III. - The Fall in Blood Pressure in Four Groups of Dogs after the Intravenous Administration of 1.0 mg./Kg. of the Alseroxylon Fraction of Rauwolfia Alkaloids

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Dog</th>
<th>Pre-inj &amp; Mean</th>
<th>After 6 hrs &amp; Mean</th>
<th>Absol Diff &amp; Mean</th>
<th>% Diff</th>
<th>Mean % Diff</th>
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<tbody>
<tr>
<td>Normal</td>
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<td>162</td>
<td>110</td>
<td>- 52</td>
<td>32</td>
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<tr>
<td></td>
<td>2</td>
<td>156</td>
<td>92</td>
<td>- 64</td>
<td>41</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>158</td>
<td>105</td>
<td>- 53</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>151</td>
<td>108</td>
<td>- 43</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td>5</td>
<td>145</td>
<td>84</td>
<td>- 61</td>
<td>42</td>
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<td></td>
<td>6</td>
<td>146</td>
<td>94</td>
<td>- 52</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>146</td>
<td>96</td>
<td>- 50</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>130</td>
<td>84</td>
<td>- 46</td>
<td>35</td>
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<tr>
<td>Cervical</td>
<td>9</td>
<td>194</td>
<td>96</td>
<td>- 98</td>
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<td>10</td>
<td>185</td>
<td>105</td>
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<td>43</td>
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<td></td>
<td>11</td>
<td>192</td>
<td>86</td>
<td>-106</td>
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<td>48</td>
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<tr>
<td></td>
<td>12</td>
<td>174</td>
<td>96</td>
<td>- 74</td>
<td>43</td>
<td></td>
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<tr>
<td></td>
<td>13</td>
<td>174</td>
<td>92</td>
<td>- 82</td>
<td>47</td>
<td></td>
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<tr>
<td>Vagotomy</td>
<td>14</td>
<td>224</td>
<td>86</td>
<td>-138</td>
<td>62</td>
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<tr>
<td></td>
<td>15</td>
<td>144</td>
<td>72</td>
<td>- 72</td>
<td>50</td>
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<tr>
<td></td>
<td>16</td>
<td>164</td>
<td>74</td>
<td>- 90</td>
<td>55</td>
<td>54</td>
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<td></td>
<td>17</td>
<td>154</td>
<td>74</td>
<td>- 80</td>
<td>52</td>
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<tr>
<td></td>
<td>18</td>
<td>164</td>
<td>78</td>
<td>- 86</td>
<td>52</td>
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Table IV. - The Blood Pressure Responses to Bilateral Carotid Artery Occlusion Following the Intravenous Injection of 1.0 mg./Kg. of Alseroxylon Alkaloids in Normal and Vagotomized Dogs.

<table>
<thead>
<tr>
<th>Before Inj.</th>
<th>After Injection of Alseroxylon</th>
<th>1 hour</th>
<th>2 hours</th>
<th>3 hours</th>
<th>4 hours</th>
<th>5 hours</th>
<th>6 hours</th>
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<tr>
<td>Normal Animal</td>
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<td>Bilateral</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>168-230</td>
<td>134-160</td>
<td>120-138</td>
<td>126-150</td>
<td>120-170</td>
<td>116-140</td>
<td>110-142</td>
</tr>
<tr>
<td>2</td>
<td>160-220</td>
<td>142-176</td>
<td>136-176</td>
<td>126-156</td>
<td>110-130*</td>
<td>105-82</td>
<td>92-90-95</td>
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<td>3</td>
<td>158-210</td>
<td>128-160</td>
<td>120-160</td>
<td>126-158</td>
<td>116-154</td>
<td>110-140</td>
<td>105-134</td>
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<tr>
<td>Carotid</td>
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<td></td>
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<td>Vagotomized Animal</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Responses,</td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>148-222</td>
<td>141-218</td>
<td>126-188</td>
<td>---</td>
<td>118-174</td>
<td>116-158</td>
<td>108-158</td>
</tr>
<tr>
<td>5</td>
<td>150-220</td>
<td>---</td>
<td>122-175</td>
<td>114-188</td>
<td>100-168</td>
<td>95-140</td>
<td>84-110</td>
</tr>
<tr>
<td>6</td>
<td>140-212</td>
<td>140-230</td>
<td>120-195</td>
<td>102-150</td>
<td>97-132</td>
<td>96-142</td>
<td>96-138</td>
</tr>
<tr>
<td>7</td>
<td>146-216</td>
<td>128-210</td>
<td>110-200</td>
<td>108-164</td>
<td>96-150</td>
<td>86-140</td>
<td>96-150</td>
</tr>
<tr>
<td>mm. Hg.</td>
<td>8</td>
<td>132-204</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>80-96</td>
<td>84-100</td>
</tr>
</tbody>
</table>

* The three values given correspond to the pre-occlusion pressure, a depressor phase, and then a pressor response.
Figure III. Hypotension in a Normal Dog after I.V. Injection of 1.0 mg./Kg. of Alseroxylon Alkaloids

1) Normal tracing—B.P. 160 mm. Hg; 2) Carotids clamped—B.P. 160 to 220 mm. Hg
3) 1.0 mg./Kg. alseroxylon alkaloids I.V.; 4, 5, 6, 7, 8, 9, 10) Carotid clampings
4) B.P. 142-176 mm. Hg; 5) B.P. 136-176 mm. Hg; 6) B.P. 126-156 mm. Hg; 7) B.P. 110 to 80 to 130 mm. Hg
8) B.P. 105 to 82 to 118 mm. Hg; 9) B.P. 94 to 72 to 112 mm. Hg; 10) B.P. 92 to 80 to 95 mm. Hg
Upper Tracing: respiration; Lower Tracing: time in minutes
Figure IV. - The Hypotension and Effects on Carotid Clamping Produced in a Vagotomized Dog by an Intravenous Injection of 1.0 mg./Kg. of the Alseroxylon Alkaloids

1) Normal Tracing: B.P. 150 mm. Hg; 2) Carotids clamped: B.P. 150 to 220 mm. Hg
3) Alseroxylon: 1.0 mg./Kg. I.V.; 4, 5, 6, 7, 8, 9, 10) Carotids clamped; 4) B.P. 150 to 220 mm. Hg
5) B.P. 122 to 175 mm. Hg; 6) B.P. 114 to 188 mm. Hg; 7) B.P. 100 to 168 mm. Hg
8) B.P. 95 to 140 mm. Hg; 9) B.P. 84 to 110 mm. Hg; 10) B.P. 80 to 88 mm. Hg
Note diphasic response from #5 through #10
Upper Tracing: Respiration; Lower Tracing: Time in minutes
Figure V. - Hypotension Following Intravenous Administration of 1.0 mg./Kg. of Alseroxylon Alkaloids in a Dog with Complete Carotid Area Denervation and Intact Vagi

1 & 2) Carotid clampings - No change in blood pressure
3) 1.0 mg./Kg. of alseroxylon alkaloids, B.P. 194 mm. Hg
4:00) B.P. 160 mm. Hg, 5:00) B.P. 134 mm. Hg
6:00) B.P. 112 mm. Hg, 7:00) B.P. 106 mm. Hg
8:00) B.P. 100 mm. Hg, 9:00) B.P. 96 mm. Hg, 9:30) B.P. 92 mm. Hg

Lower Tracing: Time in minutes
Figure VI. - Hypotension Following Intravenous Administration of 1.0 mg./Kg. of Alseroxylon Alkaloids in a Dog with the Vagi and Carotid Bifurcations Completely Denervated

1) Carotids clamped - No blood pressure change
2) Alseroxylon, 1.0 mg./Kg. I.V. at 1:30, B.P. 164 mm. Hg.

Between 1:30 and 2:30, B.P. 164 to 204 mm. Hg.
2:30) B.P. 120 mm. Hg., 3:30) B.P. 114 mm. Hg.
4:30) B.P. 98 mm. Hg.; 5:30) B.P. 80 mm. Hg.
7:30) B.P. 74 mm. Hg.

3) 2.6 cc. of 0.75% citric acid vehicle given I.V. - B.P. 74 to 95 mm. Hg.

Lower Tracing: Time in minutes
**Discussion**

In analyzing the results of any pharmacological study, and especially one of the type exemplified by Experiment I of the present investigation, great care must be taken not to overlook seemingly unimportant technicalities which singly or collectively may be of critical importance when subjected to more rigid scrutiny. Some of these technicalities which are often neglected in many pharmacological studies but which were considered in this investigation are: the anesthetic used, the dose of drug administered, the length of the experiment, and the surgical background of the animals of the various groups.

Some of the disadvantages of frequently used anesthetic agents as well as the advantages of the pentothal-chloralose-barbital combination have already been discussed. The advantages of this latter combination have been confirmed in this study, as evidenced by the maintenance of a potent carotid pressor reflex response and a prolonged anesthesia without necessitating, except infrequently, undesirable supplementary doses during the course of the experiment.

The generally higher pre-injection blood pressure levels which were evident in the animals of Experiment I are also doubtless due to the effects of the anesthetic used. As previously stated, these are in agreement with those found in the literature for a morphine-chloralose combination (65). The reason for this higher blood pressure level is not known and is difficult to analyze, especially since multiple agents were used. However, the sodium barbital is probably at least partially responsible, for according to Trapold, et al., Gellhorn has shown that barbiturate anesthesia induces a compensatory increase in
sympathetic activity (66). That chloralose may be likewise partly responsible is shown by again referring to the work of Wang, et al., who used the morphine-chloralose combination (65). It seems logical to assume that the chloralose and not the morphine caused the higher blood pressure level in their animals since morphine is generally known to depress the blood pressure when injected alone. Consequently, both agents of the combination, barbital and chloralose, may be responsible for the higher pre-injection blood pressure levels.

The intravenous dose of 1.0 mg./Kg. of the alseroxylon alkaloids which was used in this experiment is considerably larger than the usual clinical doses of this drug (2-4 mg./day orally). This higher dosage was necessary, however, for several reasons. First, numerous clinical and pharmacological reports indicate that the cardiovascular effects of the Rauwolfia alkaloids are delayed in onset whether they are injected by vein or taken orally (1, 5, 6, 12, 67). The exact reason for this delayed effect is not known but it is presumably due to an accumulative effect of repeated doses (12). Secondly, not much is known about the dose-response curve of the alseroxylon fraction. However, there are indications that it is probably rather flat so that any reasonable dosage level could be employed providing the typical results are produced without at the same time eliciting toxic reactions which may interfere with or mask the specific effects being studied. The well known low order of toxicity of all the Rauwolfia preparations, therefore, permits one to use a relatively high dose; and third, the time normally allotted for acute experiments is five to six hours (66). All of these factors made it necessary to use a dose (1.0 mg./Kg.)
that would produce the characteristic effects looked for without too much delay, that would not produce toxic manifestations, and that would also reveal the prolonged effects produced by the drug while staying within bounds of the acute experiment.

The reason for observing the animals for at least six hours was also due to the nature of the action of Rauwolfia, namely, a slow onset and long duration of action. The overall length of the experiment was also extended because of the rather extensive and time-consuming surgery involved, especially in the animals in which the carotid sinuses were denervated. These factors also attributed to or specifically dictated the type of anesthetic that was necessary, thus ruling out many of the shorter acting anesthetics.

The surgical background of the various groups of animals must also be considered when studies are made, such as in the experiment under discussion. Besides the time consumed in the various surgical procedures, there is also always a certain amount of trauma and disruption of the normal anatomy, some bleeding, and most especially, an interference with the physiology of the area, brought about by sectioning of nerves or ligation of blood vessels and the like. Thus, each of the groups of animals of the present experiment have different surgical backgrounds which in itself may account for certain hidden aspects of the results. This fact as well as the others previously mentioned are kept in mind during the discussion of the results of Experiment I which follows. However, one must not deny the fact that it is the numerical values themselves that must be relied upon and weighed or emphasized the most heavily inasmuch as many of the factors (other than the
specific action of the drug being studied) which may contribute to these values are undetectable or too little known to be recognized. Furthermore, the use of statistics is of little value in many situations like that posed by Experiment I because the extra time and expense involved in the number of animals necessary for a statistical analysis would be prohibitive.

All of the animals of Experiment I (normal, vagotomized, sinus denervated, sinus denervated plus vagotomy) exhibited the same general type of pharmacological pattern after being administered 1.0 mg./Kg. of the alseroxylon alkaloids intravenously. The blood pressure began falling slowly, usually within one-half hour, and continued to slowly but progressively fall to a final level which was characteristic for the particular group of animals being studied. This hypotension was always accompanied by bradycardia and usually by a detectable degree of respiratory depression. A few of the animals developed hyperpnea during the course of the experiment, especially toward the end of the six-hour period. This was probably associated with or could be attributed to a high external environmental temperature as these particular dogs were experimented upon on very warm days when the laboratory temperature was in the 90's.

From Table IV, page 43 and Figures III and IV, page 44 and 45, it can be seen that the pressure response to carotid artery occlusion was progressively more blocked as the hypotension became more pronounced. In the three normal animals, the average degree of this reflex blocking was somewhat more complete than in the five vagotomized animals. This could be expected for the following two interdependent reasons. The
absolute elevation in blood pressure from carotid occlusion before the alseroxylon was injected was also always greater in the vagotomized than in the normal dogs. Since the carotid pressor reflex was never blocked completely, the vagi in the normal animals could assume their normal role in a partial attempt to limit the rise in blood pressure. These results (hypotension, bradycardia, carotid reflex blocking and respiratory depression) are all in agreement with those found in the literature for the alseroxylon fraction as well as for reserpine (5, 6, 7, 8, 9).

With reference to the carotid occlusion response following alseroxylon alkaloids, there is one other aspect which needs an explanation. That is the diphasic, depressor then pressor response seen in some of the animals of groups one and two from approximately the third to the sixth hour. The first or depressor phase is likely due to the blocking effects of Rauwolfia on the central components of the carotid reflex arc which prevents an immediate pressor response, together with a carotid-occlusion-produced ischemia of the pressor vasomotor center. This ischemia is the ultimate cause of the fall in blood pressure. After ten to thirty seconds, the pressor phase would ensue because of a carotid afferent impulse breakthrough of the central Rauwolfia block, as well as to a back-flow of blood which would relieve the ischemia of the vasomotor center produced by the carotid occlusion. In the normal animals, the above events (depressor and pressor phase) may be fortified by the presence of the intact vagi. Winder (68) has shown in animals with intact vagi that tachycardia and hypertension normally created by carotid occlusion may be violently converted to bradycardia.
and hypotension. When the heart rate of animal #2 of the normal animals was checked, it was found to be higher than in any of the animals of the four groups at the end of the sixth hour. Accordingly, the pressor phase in this animal could be due to a drastic reduction in heart rate or to a conversion of the pressor to a depressor response mediated via the vagi nerves.

There appears to be little difference in the degree of bradycardia which is produced by the alseroxylon alkaloids in any of the four groups of animals. While the individual values in each group of animals vary considerably both before and after the drug was injected for six hours, the average values of each group approach each other rather closely. The average absolute differences for each group also agree very well with the exception of the vagotomized group. One animal in this group had a pre-injection heart rate of 230 and an absolute difference in heart rate of 80 beats/min. This raised the average value of this group considerably.

Although it was admitted in the experimental portion of this study that the method used for obtaining the heart rate was sometimes not exceptionally accurate, it was felt that by averaging the results of three readings, which were frequently obtained by two different methods, the results would definitely be of analytical value. Accordingly, the values obtained indicate that the vagi and carotid sinus nerves are responsible for little, if any, of the bradycardia elicited by the alseroxylon alkaloids. In addition to the values shown in Table II, the data obtained in the two normal dogs which were vagotomized after the sixth and seventh hours of observation, respectively, offer further
support to this viewpoint. Two hours after the vagotomy in these two
dogs, the heart rate was somewhat lower in one animal and remained the
same as before vagotomy in the other.

These observations leave the ultimate mechanism of the alseroxylon-
induced bradycardia to a direct cardiac depressant action or to a de-
creased sympathetic tone. The writer has been unable to find any in-
formation in the literature about the alseroxylon alkaloids in this re-
spect but there is some information pertinent to reserpine, one of the
constituents of the alseroxylon alkaloids. Reserpine has been shown to
produce a decrease of activity in the isolated hearts of cats and rab-
bits, but only after comparatively long duration of action (47). In
intact cats, oscillographic records show a diminution of the sympa-
thetic outflow in a sympathetic cardiac nerve occurring simultaneously
with bradycardia. In this same animal, excitation of the vagal system
was not likely since no component inhibitable by atropine could be dem-
onstrated, and division of the vagus had no influence on the effect
(7).

A careful analysis of the blood pressure values recorded in Exper-
iment I reveals that a considerably greater absolute difference in the
blood pressure readings before and six hours after the alseroxylon al-
kaloids were injected occurred in the last two groups of animals
(carotid sinus denervated and carotid sinus denervated plus vagotomy)
than in the first two groups (normal and vagotomized animals). The
mean percentage difference is accordingly also considerably larger
in the last two animal groups. Little significance can be drawn from
these values, however, because of the larger pre-injection blood
pressure readings which also prevailed in these latter two groups. These higher pre-injection levels are probably due to the extra surgical manipulations conducted in these animals, as well as to sectioning of the carotid sinus nerve which is so important in the maintenance of normal blood pressure levels.

Of greater importance than the absolute or mean percentage differences are the actual blood pressure readings which were recorded at the end of the six-hour observation period. The values so obtained differed little in the first three animal groups, being on the average 102, 93 and 96 mm. Hg for the normal, vagotomized, and sinus denervated groups, respectively, as shown in Table III. The average value similarly obtained for the vagotomized plus sinus denervated animals, however, was only 77 mm. Hg. Further, the blood pressure values in all of the animals in this group with but one exception were in the 70's after six hours. In addition, one other animal which had the same surgical preparation as those of the last group, but which was not included in the table for reasons already discussed, also exhibited a blood pressure level in the 70's (78 mm. Hg) after a few hours of observation.

There are probably several other factors which help to lend credence to the deduction that the lower blood pressure readings in the last group are real and not just due to chance. For instance, not one of the thirteen animals of the first three groups showed a final blood pressure reading that was below 84 mm. Hg, while most of these animals had pressures which were considerably higher than this value, as can be seen from the averages in Table III. In the two normal animals which
were vagotomized after the sixth and seventh hours, respectively, and which were then observed for two additional hours, the final blood pressure was still only down to 104 and 92 mm. Hg. Furthermore, the vagotomized dog which was given a total of 3.0 mg./kg. of the alseroxylon alkaloids intravenously exhibited a blood pressure of 94 mm. Hg after six hours.

The major significance that can be attached to the above observations is that neither the vagi nor the carotid sinus nerves alone are of any import in the inducement of the hypotension elicited by the intravenous administration of the alseroxylon alkaloids in dogs. When both of these nervous mechanisms are intact, however, they definitely do play a part, at least in the acute experiment, as evidenced by the lower blood pressure level exhibited when they are both denervated. This role which they play together when intact would not be one which contributes to the hypotension, however. Instead, it would be in the nature of a buffering action which is effective in keeping the blood pressure from falling to the lower levels which ensue when both of these nervous pathways are surgically interrupted.

This conclusion is in line with the observed blood pressure levels of the four groups of animals studied, and is also in harmony with the normal physiological functions purported for the carotid sinus and vagi nerves. Hence, in the normal animals, both buffer mechanisms were at work in an effort to prevent the alseroxylon hypotension. In the vagotomized animals, the carotid sinus nerve acted as the sole buffer, while in the carotid sinus denervated group, the vagi assumed the buffer role. But when both buffer mechanisms were denervated, the
alseroxylon-elicited hypotension no longer was held under so strict abeyance. Consequently, the blood pressure in this latter group of animals continued to fall until it was between 70 and 80 mm. Hg, this final level being solely determined by the hypotensive potency of the alseroxylon alkaloids themselves.

The blood pressure elevation which appeared for 15 to 20 minutes immediately following the injection of the alseroxylon and which slowly gave way to the hypotensive effects of this drug was not studied completely. This brief hypertension occurred in all of the completely denervated animals and in two or three of the partially denervated dogs. In one completely denervated dog, it was shown that the vehicle alone could account for this hypertension. The vehicle alone also produced this hypertension when injected after the six-hour observation period in another completely denervated dog (Figure VI).

A likely mechanism for this blood pressure elevation is the direct action of the acid vehicle on the central vasomotor centers resulting in vaso-constriction in some part of the animal. The reason it was seen only in the occasional, partially denervated dog was probably due to an over-riding effect of the remaining buffer system, i.e., the vagi or carotid sinus nerves. In the completely denervated dogs no buffering action was possible, while in the few partially denervated animals which did become hypertensive, the buffer mechanism remaining was probably not powerful enough to inhibit the direct central activity. That the buffering mechanisms vary in potency in different animals and in the same animal under slightly different circumstances is a well-known fact (68).
The fact that the alseroxylon alkaloids produced the same slowly developing hypotension even after being incubated in dog serum for four hours indicates that the drug is probably not converted by the blood to a more active form, but that some other explanation is needed for this characteristic type of response. This may be highly negative reasoning in view of the fact that only serum instead of whole blood was used, that the incubated serum-drug ratio was much smaller than the blood-drug ratio resulting from an intravenous injection of the same dose of drug, and that only one animal was used. However, further support of this conclusion can be found in the work of Glasko, et al., who studied the metabolism of reserpine in the rat. They found little 'total' reserpine in the blood serum, brain and body fat while higher concentrations were found in the lung, kidney, spleen, and liver (69). This information in itself does not necessarily relegate the blood to a passive role in the mode of Rauwolfia hypertension, but it does suggest that other avenues of approach to the problem might be more fruitful. Consequently, no more animals were run in this series after the above information appeared in the literature.
Experiment II. - The Action of the Alseroxylon Fraction and Reserpine on the Carotid Receptor Areas.

Procedure

Twenty dogs were utilized in this experiment. The femoral artery and vein were cannulated for recording blood pressure and for injecting drugs or saline when necessary. The trachea was cannulated in all the animals to assure a freer airway. The respiration was recorded in selected cases. Both carotid sinus areas were exposed by blunt dissection with great care being taken to avoid trauma and disturbance of the blood and nerve supply to the area.

In the majority of animals, both vagi were cut at the beginning of the experiment in order to limit the reflex regulation of the blood pressure to the carotid sinus mechanism. In a few dogs, the vagi were sectioned during the course of the experiment, so the action of drugs on the carotid receptors could be compared before and after vagotomy. In two or three other animals, both vagi and one carotid sinus nerve were sectioned at the beginning of the experiment, leaving the reflex blood pressure regulation to the other carotid sinus nerve. This procedure was carried out so the drugs would only have to be injected into the region of one carotid bifurcation, thus reducing the number of injections and assuring a faster administration of the drugs. However, it is the opinion of the writer that the extra time, effort, and trauma involved in further isolation and sectioning of the carotid sinus nerve renders this method impractical.

All the drugs which were studied in this experiment (some preliminary trials excepted) were injected into the adventitial layer of
the carotid arteries at the region of the bifurcation. With one or two exceptions, the volume injected into either side was 0.1 ml. The injections were made with a 1/4 ml. syringe using a 27 gauge needle which had been made considerably thinner by filing. The actual injection technique involved injecting one-half of the volume (0.05 ml.) into the adventitia of both the lateral and medial surfaces of each carotid sinus area, or a total of four injections for one administration of each drug. Extreme care was always necessary to avoid inserting the needle into or rupturing one of the many small blood vessels of the area.

Only two or three injections could be made into each carotid area. Further injections usually resulted in responses of considerably less magnitude due probably to trauma and leakage of the drug from holes made by the previous injections.

The anesthetic employed was either veterinary sodium pentobarbital or the triad (pentothal-chloralose-barbital). The latter was preferred and used in the majority of the animals.

The vehicle for the Rauwolfia alkaloids was an 0.375% or 0.75% aqueous solution of citric acid as previously described. Vehicles and doses of drugs other than Rauwolfia that were used in this experiment are stated during the discussion which follows.

Results

Several dogs under sodium pentobarbital anesthesia were utilized in preliminary trials using drugs which produce known and characteristic actions when injected into the carotid sinus area. These trials were conducted to give the writer some experience in the technique of
injection into the carotid bifurcation, experience with the physiological and pharmacological aspects of the carotid area, and a better knowledge of the oftentimes variable anatomy of the area. The results in these animals revealed the following facts:

1. When 1/2 ml. or 500 mcg. of epinephrine in normal saline was further diluted in additional normal saline to 1 1/2 ml. and injected into the external conjunctival space surrounding each carotid sinus, a fall in blood pressure of 60 or more mm. Hg usually was obtained. This fall in pressure was accompanied by a reduction or blocking of the pressor response due to carotid occlusion. The same but usually somewhat lesser responses were observed when 0.1 ml. or 2 units of vasopressin was administered in a similar fashion.

2. One-tenth ml. of a 0.2% solution of NaNO₂ given in the same manner produced entirely opposite effects on the arterial blood pressure, viz., a rise in pressure with the carotid sinus reflex pressor response being retained.

3. When the above three drugs were applied to the carotid bifurcation by painting using cotton swabs previously soaked in the solutions of the drugs, entirely analogous responses were obtained. However, these responses varied considerably and were usually of reduced magnitude.

4. The most predictable and seemingly reliable results were obtained when these three drugs were injected into the adventitia of the carotid artery at the region of the bifurcation. These responses were usually immediate, more pronounced, and definite in character. In all three methods, however, the qualitative blood pressure responses were
In complete agreement with those found in the literature (13, 52, 53, 54).

Disadvantages of the direct adventitial injections are the possibility of producing trauma in the artery, and the leakage of part of the dosage especially on repeated injections.

Application of drugs with cotton swabs did not always give reproducible results nor results which were of sufficient magnitude, especially if the blood pressure tracings were previously somewhat erratic. When the drugs were injected into the external conjunctival space surrounding the carotid area, larger doses had to be employed. The major objection to this method, however, is that the drug reaches other nervous structures which underlie the carotid bifurcations, such as the superior cervical ganglion and the nodose ganglion of the vagus, and the drugs have to be washed out and the area rinsed with several changes of saline before additional drugs can be tested. Since the direct injection of drugs into the adventitia of the arteries appeared to be the best overall method, this route of administration was employed in the remainder of the animals in this investigation.

5. In order to conserve the limited supply of reserpine and the alseroxylon alkaloids, the Rauwolfia preparation tested in the preliminary studies was an aqueous extract of Rauwolfia serpentina which was designated as HLP-765 by this laboratory. This aqueous extract had been prepared from the dried drug marc remaining after previous, successive extraction of the crude drug with acetic acid and alcohol, respectively. The aqueous extract had been previously shown to possess immediate, potent, and prolonged anti-hypertensive activity when
injected intravenously in dogs (4).

When 0.125 ml. of this extract was injected into the adventitia of
the carotid bifurcation, the arterial blood pressure rose immediately
and frequently to quite high levels. This extract was also capable of
suppressing the reflex fall in blood pressure induced by a previous
injection of epinephrine, or by epinephrine injected following a pre­
vious injection of the Rauwolfia extract. These findings indicated
that the action of the Rauwolfia alkaloid on the carotid mechanism
warranted further study and consideration.

After the preliminary tests were completed, the next step was to
determine whether the alseroxylon alkaloids and reserpine could cause
the same rise in arterial pressure as that demonstrated by the aqueous
Rauwolfia extract, and if so, at what dosage level. Approximately one­
half of the twenty animals of Experiment II were used in this phase of
the study which also included a comparison of the effects of the Rau­
wolfia alkaloids before and after vagotomy. The anesthetic used in
these animals was the pentothal-chloralose-barbital combination.

In three dogs which were bilaterally injected with the arbitrarily
chosen doses of 250 to 500 mcg. of the alseroxylon alkaloids, almost
immediate and rather large elevations in the arterial blood pressure
occurred. The blood pressure remained elevated for some time, usually
at least one hour, and in several instances for two hours or longer.
During the hypertensive period, the carotid sinus reflex response to
occlusion was not blocked but was considerably depressed percentage­
wise due to the high blood pressure level previously established by
the Rauwolfia alkaloids.
Hypertension was produced by the alseroxylon alkaloids both before and after vagotomy. The post-vagotomy responses were always equal to and usually of greater magnitude than those elicited before the vagi were sectioned. This is understandable since the buffering effect of the vagi on the blood pressure would be removed after they were sectioned. Accordingly, in the remainder of the animals of this experiment, the vagi were always sectioned in order to remove their buffer action and to limit the reflex hypertensive responses to the carotid sinus nerves.

In several other animals, graded doses of the alkaloids were injected into the adventitia of each carotid bifurcation. At least one hour elapsed between each injection. Doses of 1.0 mcg. of the alseroxylon alkaloids produced no changes in the blood pressure whatsoever. Doses of 10, 25, 50 and 100 mcg. of the alseroxylon alkaloids produced only slight elevations of the blood pressure (5 to 20 mm. Hg), which were very transient in nature. All of these doses were administered in a total volume of 0.1 ml. in each side.

Because of a difference in solubility, only 50 mcg. of reserpine could be dissolved in 0.1 ml. of the citric acid vehicle. When this dose was injected into each sinus region, either no change in blood pressure occurred, or, in one or two instances, a slight but transient hypertension was recorded. In another dog, 150 mcg. of reserpine dissolved in 0.3 ml. was injected into each sinus region. On the first dose, a 10 mm. Hg rise in pressure occurred, while after the second similar dose no change in the pressure was observed. For purposes of comparison, the same doses of reserpine and the alseroxylon alkaloids
(150 mcg. in 0.3 ml.) were given at different intervals in still another animal. In this dog, the reserpine raised the blood pressure only 6.0 mm. Hg while the same dose of the alseroxylon alkaloids produced a 22.0 mm. Hg elevation. It should be emphasized that the volume of vehicle (0.3 ml.) is too large to be easily injected into the carotid area and was used only to increase the dose of the reserpine.

The remaining ten animals of Experiment II were utilized for the following purposes: to study the effects of the vehicle alone on the carotid bifurcation; to determine if the blood pressure elevation caused by the alseroxylon alkaloids was due to a possible adrenolytic action; and to establish whether the elevation in blood pressure was due to an action of Rauwolfia on the carotid sinus, the carotid body, or both.

Although several doses of the vehicle alone (0.75% citric acid) had been injected without effect into the carotid bifurcation in some of the previously mentioned animals, it was thought advisable to test the action of the vehicle alone in a dog that had not previously been given any drugs in the sinus area.

The results in this animal verified the fact that the vehicle alone is without effect when injected into the adventitia of the carotid area. That the carotid sinuses were responsive in this animal was shown by the later injection of the alseroxylon alkaloids, and by occlusion of the carotid arteries. These results are shown in Figure VII.

The possible adrenolytic action of the alseroxylon alkaloids was studied by injecting the alkaloids both before and after the previous injection of epinephrine. In all of these animals, the doses of the
alkaloids were 500 mcg. and that of epinephrine 100 mcg., each of which was in a volume of 0.1 ml. The results in this study were all the same qualitatively but they varied quantitatively depending upon the exact sequence of dosage employed. For instance, when epinephrine was injected initially, a sharp and rather large fall in blood pressure always occurred (Figure VIII, Figure IX). When epinephrine was injected following a previous dose of the Rauwolfia alkaloids, the fall in blood pressure was usually less impressive. On the contrary, when Rauwolfia was given initially, a sharp and pronounced rise in blood pressure always occurred (Figure IX). When Rauwolfia followed a previous dose of epinephrine, however, the effects of epinephrine were reversed (blood pressure elevated to a level higher than the pre-injection norm) or the blood pressure was simply returned to its pre-epinephrine level (Figures IX, X, and XI).

In the two remaining vagotomized animals of this experiment, the carotid pressoreceptors were differentially denervated leaving the chemoreceptors intact by sharp dissection of the pressoreceptor nerve fibers between the carotid body and sinus. The wall of the common carotid artery where it approached the carotid bifurcation, as well as the internal carotid artery, were carefully pinched with small forceps in an effort to disrupt any straggling pressoreceptor nerve fibers. Great care was taken to protect the blood and nerve supply to the carotid body. Thoroughness of complete carotid sinus area denervation was determined by abolition of the pressor response to carotid artery occlusion. The integrity of the chemoreceptors was verified by the hyperpnea resulting from the intravenous injection of a freshly prepared
solution of NaCN at a dose of 0.5 or 1.0 mg./dog.

When 500 mcg. of the alseroxylon alkaloids were injected into the carotid bifurcation in these two dogs, no change whatsoever occurred in the arterial blood pressure, as can be seen in Figure XII. A total of four such doses of the alseroxylon alkaloids were injected into these two animals. A 100 mcg. dose of epinephrine which was injected into each side of one of the animals 1 1/2 hours after a Rauwolfia injection raised the blood pressure from 120 to 126 mm. Hg instead of producing a fall. The effects of carotid clamping and the hyperpnea induced by NaCN are also shown in Figure XII.
Figure VII. - Action of the Citric Acid Vehicle Following Injection into the Carotid Sinus Area

Male dog, 12 Kg.; Upper Tracing: Respiration; Lower Tracing: Time in minutes

1) 0.1 cc. of 0.75% citric acid in each carotid area, no change in blood pressure
2) Carotid clamping still effective; 3) Same dose of vehicle repeated one hour after 1, little alteration in blood pressure; 4) Effects of carotid clamping somewhat reduced
5) Alseroxylon, 250 mcg. into each carotid area, fall in blood pressure due to manipulation during injection, later blood pressure elevated.
Figure VIII. - Mean Arterial Blood Pressure Changes Following Injection of the Vehicle and Epinephrine into the Adventitia of Each Carotid Sinus

Dog - Female, 17.4 Kg.; Upper Tracing: Respiration; Lower Tracing: Time in minutes

3) Vagi sectioned at 4:25; 4) Carotids clamped at 4:36, B.P. 134 to 200 mm. Hg.
5) 0.1 ml. of citric acid vehicle injected into each carotid area at 4:50, No change in blood pressure
6) Carotids clamped at 5:43, B.P. 124 to 216 mm. Hg.; 7) at 5:47, 100 mcg. of epinephrine in 0.1 ml. inj. into each carotid area, B.P. 120 to 54 mm. Hg.; 8) Carotids clamped 7 min. later, no change in blood pressure;
9) 500 mcg. of alseroxylon inj. into each carotid area at 5:55, B.P. 62 to 142 mm. Hg.; 10) Effects of carotid clamping restored.
Figure IX. - Hypertension Elicited by Intercarotidly Injected Alseroxylon Alkaloids Before and After Similarly Injected Epinephrine in a Vagotomized Dog

Dog - Male, 13.8 Kg.; Lower Tracing: Time in minutes

Left side - 1) Carotids clamped at 3:45, B.P. 100 to 134 mm. Hg.
   2) Alseroxylon, 500 mcg. in each carotid area at 3:51, B.P. 102 to 150 mm. Hg.
   3) Carotid clamping still effective

Right side - 1) Carotids clamped at 6:00 or two hours post alseroxylon, B.P. 122 to 168 mm. Hg.
   2) 100 mcg. of epinephrine in each carotid area at 6:03, B.P. 122 to 96 mm. Hg.
   3) Same dose of alseroxylon repeated, B.P. 96 to 120 mm. Hg.
Figure X. - Effects of Intercarotid Epinephrine and Alseroxylon Alkaloids on the Blood Pressure of a Vagotomized Dog

Male dog, 16.6 Kg.; Lower Tracing: Time in minutes

2) 4:00, carotids clamped, B.P. 148 to 206 mm. Hg.;
3) 4:23, epinephrine, 100 mcg. into each carotid area, B.P. 144 to 96 mm. Hg.;
4) carotids clamped at 4:39, no change in B.P.
5) 4:40, 500 mcg. of alseroxylon in each carotid area, B.P. 96 to 208 mm. Hg.
6) Effects of carotid clamping restored, B.P. 208 to 228 mm. Hg.
Figure XI. - Effects of Intercarotid Epinephrine and Alseroxylon Alkaloids on the Blood Pressure of a Vagotomized Dog

Dog - same as in Figure X (record of Figure X continued)

7) Carotids clamped at 6:45 or two hours post alseroxylon, B.P. 130 to 204 mm. Hg.
8) 150 mcg. of epinephrine in .15 ml. inj. in each carotid area, B.P. 130 to 106 mm. Hg.
9) Carotids clamped, no blood pressure change; 10) 0.15 ml. of 0.75% citric acid vehicle in each carotid area, no blood pressure change; 11) 500 mcg. alseroxylon in each carotid area, B.P. 106 to 136 mm. Hg.; 12) Carotids clamped at 7:20, B.P. 138 to 160 mm. Hg.
Figure XII. - Lack of Blood Pressure Responses Following Intercarotidly Injected Alseroxylon and Epinephrine in a Vagotomized Dog with the Carotid Pressoreceptors Selectively Denervated, Leaving the Carotid Bodies Intact

Female dog, 9.3 Kg.; Upper Tracing: Respiration; Lower Tracing: Time in minutes

2 and 3) Carotids clamped, little change in blood pressure indicating pressoreceptors denervated
4) NaCN, 1.0 mg./dog I.V., hyperpnea indicating chemoreceptors intact
5) Alseroxylon, 500 mcg. in each carotid area, little change in blood pressure
6) Epinephrine, 100 mcg. in each carotid area 1 1/2 hrs after 5, little change in blood pressure
7) Alseroxylon repeated 20 minutes after 6, little change in pressure
8) Carotids clamped one hour after 7, little change in pressure
9) NaCN, 1.0 mg./dog I.V., hyperpnea indicating chemoreceptors still active and intact
Discussion

Several definite conclusions can be drawn from the results of Experiment II. In the first place, the injection of 250 to 500 mcg. of the alseroxylon alkaloids into the adventitia of the carotid sinuses always resulted in a marked elevation of the arterial blood pressure. Of special interest in this respect is the fact that this response occurred with very little delay which is in direct contrast to the latency which is characteristic of most of the pharmacological actions attributed to the alseroxylon alkaloids. On the other hand, the alseroxylon-induced blood pressure elevation was prolonged which is in agreement with the usual prolonged action of these alkaloids. The precise duration of this action on the carotid mechanism could not be definitely established. It often appeared to persist for upwards of two hours or more, while on occasion the pre-injection level would be reached within a half-hour.

There are probably several reasons for this variance in duration of action. The size of the carotid bifurcation differed in the various animals as well as in opposite sides of the same animal so that the same volume and dose doubtless penetrated a larger or smaller portion of the total carotid area in the various animals. Leakage of part of the dose sometimes occurred so that a dose sufficient to block all the receptor nerve endings may have been administered in some occasions, while in others only sufficient drug would be available to block part of these endings. Thirdly, adaptation of the receptor nerve endings in the carotid bifurcation or of central components upon which the carotid afferents impinge may have occurred sooner in some animals.
than in others.

During the hypertension induced by the alseroxylon alkaloids, the carotid occlusion response usually remained intact but was of lesser magnitude due to the higher pre-occlusion pressure established by the drug. In a few of the animals, carotid occlusion was without effect. In these instances, the carotid sinus mechanism was probably completely blocked by the previous dose of Rauwolfia.

Another interesting aspect of this hypertension was that carotid occlusion frequently produced a further elevation of the blood pressure. However, when the clamps were removed, the effects of the clamping instituted a fall in the blood pressure which had previously remained for some time at a constant hypertensive level. The factor of adaptation was probably involved here also, although this is just an assumption. Accordingly, it seems possible that the change in pressure in the sinus region brought about by carotid occlusion could serve as a 'shaking up' or 'awakening' for the receptors which had been temporarily reset at the higher pressure level by the drug.

The action of the alseroxylon alkaloids on the carotid bifurcation when injected before or after epinephrine resembled in many respects the actions produced by known adrenolytic agents such as Hydergin, ergotamine, dihydroergotamine, Dibenamine and Regitine (13, 54, 55, 56). Thus, when epinephrine was injected initially, a fall in blood pressure with complete or partial blocking of the carotid sinus reflex always occurred. When the alseroxylon alkaloids were injected at the nadir of this fall in pressure, a progressive return of the arterial pressure to normal or to above normal levels always occurred.
The carotid occlusion pressor reflex was also re-established after the pressure had returned toward normal or above.

If the Rauwolfia was injected initially, an elevation in blood pressure always occurred. This is also in accordance with an adrenolytic action which is purported to be due to a reversal or blocking of the adrenalin or noradrenalin which is normally found present in arterial walls for maintenance of tone and resistance to stretch. At this point in the results, however, divergencies appear in the action of the alseroxylon alkaloids which detract from the possibility of its possessing a characteristic adrenolytic action. The pressure rise following initial doses of Rauwolfia was always of considerable magnitude, while the known adrenolytic agents usually produced only a very slight and almost imperceptible hypertension when injected alone. Further, it seems logical to assume that any strictly adrenolytic agent could only produce a slight elevation in blood pressure when injected into the carotid sinus alone, for if any epinephrine or norepinephrine is present in the arterial walls, it most probably is present in almost infinitesimal amounts. This fact indicates that the alseroxylon alkaloids must possess an action on the carotid bifurcation which is different from or in addition to an adrenolytic action.

Another observation which indicates that alseroxylon may not possess an adrenolytic action is that when epinephrine was injected during the hypertension induced by the alseroxylon injection, either no change in the blood pressure level was observed or the blood pressure slowly began to recede. In contrast, epinephrine always causes hypertension (a reversal of its usual action) when injected following a
previous dose of an adrenolytic agent (56). If epinephrine was injected after a considerable period of time following a previous injection of the alseroxylon alkaloids, a fall in blood pressure was always observed which suggested, as the pre-epinephrine blood pressure level also indicated, that the effects of the Rauwolfia had mostly worn off and that the epinephrine was exerting its usual action unopposed.

Doses of 1.0 mcg. of the alseroxylon alkaloids had no effect whatsoever on the pre-injection blood pressure level by the end of a one-hour observation period. This is in direct contrast to the majority of Veratrum alkaloids which produce hypotension when injected into the carotid sinus in small doses (17). An exception to this is veratridine which produces a marked hypertension in doses of at least 1.0 to 1.5 mcg. Veratramine apparently has only a central site of action (17, 18). Doses of the alseroxylon alkaloids ranging from 10 to 100 mcg. produced only slight and transient elevations in the blood pressure. Similar doses of reserpine produced only small pressure changes which were in the same direction, or no change at all. One hundred and fifty mcg. doses of reserpine produced results identical with the smaller doses, while 150 mcg. of alseroxylon was slightly more effective in elevating the blood pressure. All of these results place the effective dose of these alkaloids at 250 to 500 mcg. in this particular type of acute experiment. The larger dose often produced complete or nearly complete blocking of the carotid sinus nerve as observed by the carotid occlusion response.

As stated previously, doses of reserpine larger than 150 mcg. could not be tested because of its relative insolubility. Hence, an estimate
of its potency compared to the alseroxylon alkaloids cannot be made with any reliability. At the 150 mcg. dose level, however, the alseroxylon alkaloids appeared to be somewhat more potent. Of course, 3.0 ml. of solvent had to be injected at this dosage level which no doubt interfered in one way or another with the ensuing results.

At doses of 0.1 ml., which was the volume usually injected throughout this experiment, the vehicle was without effect as was shown by the results when the vehicle was injected alone in several of the animals which also had received other drugs, and in one dog which was given two different doses of the vehicle alone. These observations were surprising in view of the fact that the pH of the vehicle was 2.3. However, there are reports in the literature which show that such acidity somehow may not affect the receptor nerve endings in the carotid area. Adrenalin which has a pH of 3-4 does not block the receptors, but, in fact, reduces the blood pressure by increasing the number and frequency of the impulses transversing the carotid sinus nerve. The injection of 0.25 ml. of an HCl solution (pH 2.7) also leads to no change in the normal impulse pattern of the carotid sinus nerve, as shown by action potential studies (70). However, Gernandt (71) has shown that the intracarotid injection of 0.1 to 0.15 ml. of 0.5 N acetic acid often blocks the chemoreceptor impulses which emit from the carotid body. Doses of acetic acid which are 2-3 times as large (0.2 to 0.45 ml.) may also block the baroreceptors temporarily (5-10 minutes) while the chemoreceptors are permanently blocked. This latter information does not appear to be of importance in the present study because well over an hour was always allowed when observing the effects due to the injection
of the vehicle, and, as will be shown presently and in Experiment III which follows, neither the alseroxylon alkaloids nor the vehicle alone have any action on the carotid body (71).

The results recorded after injection of the alseroxylon alkaloids into two animals in which the vagi were sectioned and the pressoreceptors selectively denervated leaving the carotid bodies intact demonstrated conclusively that the hypertension produced by these alkaloids was mediated solely through a blocking of the pressoreceptors. This surgical procedure was carried out in a total of four animals. The effects of the alseroxylon alkaloids were negative in all cases. However, the results of only two animals are presented in the present paper, as the writer was completely convinced that the pressoreceptors were completely denervated in only two of these dogs. In the other two, carotid occlusion still produced a slight elevation in blood pressure which indicated that a few straggling pressor fibers might still be intact. This rise could have been due to the chemoreceptors, however, as they may elevate the blood pressure upon stimulation introduced by carotid occlusion.
Experiment III. - Attempts to Elucidate the Mechanism of Action of the Alseroxylon Alkaloids on the Carotid Receptor Areas.

Part A

Procedure - The possible local anesthetic effect of the alseroxylon alkaloids, as well as the vehicle, was determined by the method of Bulbring and Wajda (72). Intracutaneous injections of 0.1 ml. of the solution of the alkaloids, their vehicle (0.75% citric acid), the vehicle plus 0.7% saline, and normal saline were made into the backs of four guinea pigs which had been shaved clean twenty-four hours prior to the test. The local anesthetic effect was then determined by lightly pricking the wheals produced by the injection with a teasing needle. Absence of cutaneous muscular twitchings which were quite evident when a normal area of the back was pricked indicated local anesthetic action. Injections of a 1/2% solution of procaine HCl in normal saline, which was the same as the alseroxylon concentrations used, were made for comparative purposes.

Results - Five minutes after the injections, pricking of the wheals produced by normal saline did not prevent muscular twitchings. The normal saline was slowly absorbed from the area which at all times remained sensitive to pricking. Local anesthetic effect was evident over the procaine wheals within five minutes and persisted for twenty minutes, whereupon the area again became sensitive to the needle pricks.

All the animals were completely insensitive to pricking of the wheals produced by the injections of the alseroxylon alkaloids in 0.75% citric acid, 0.75% citric acid alone, or 0.75% citric acid in 0.7% saline. This insensitivity was still evident after twenty-four
hours, thereby indicating a local anesthetic effect. However, the tissue of the wheals produced by these latter three injections was somewhat hardened or toughened as opposed to the more placid and natural texture of the wheals produced by the procaine or normal saline solutions. Furthermore, these areas remained hard for several days before eventually sloughing off in mass or as flaking scales. This indurating effect indicates an irritant action rather than a true local anesthetic effect.

Part B

Procedure - A total of nine dogs was employed in this experiment in an effort to gain a further understanding of the mechanism of the hypertension caused by the injection of the alseroxylon alkaloids into the carotid bifurcation as well as to clarify the blocking or reversing effect which these alkaloids displayed against similarly injected epinephrine.

Four of these animals were given 0.5 mg./Kg. of the alseroxylon alkaloids orally each day for a period of ten days. The tenth dose was given approximately two hours before the administration of the anesthetic on the day in which the animal was experimented upon. Two other dogs were given 50.0 mcg./kg./day of reserpine in an identical manner. In both cases, the drug was administered by placing the powdered drug or ground tablets in the center of a meat ball which was vigorously devoured by the animals.

On the tenth day, these animals were anesthetized and set up for blood pressure recording in the manner previously described. After sectioning the vagi nerves, various drugs such as the alseroxylon alkaloids, epinephrine, and vasopressin were injected into the carotid
bifurcation and their effects noted.

The experimental techniques used in the other three animals of this experiment are discussed along with the results of this experiment. These three dogs did not receive any drugs orally.

Results - All of the dogs which received the Rauwolfia alkaloids orally acquired the sedation, diarrhea, and nictitating membrane prominence which is characteristically produced by these alkaloids. The nictitating membrane prominence became evident after the first day, while the sedation and diarrhea became particularly noticeable after the second or third day and appeared to reach a peak by the fifth to seventh day. In one of the animals given the alseroxylon alkaloids, the sedation appeared on the second day and almost completely disappeared after the fifth day.

All but one of the animals lost considerable weight during the ten-day period. The four alseroxylon treated dogs lost 1.0, 2.0, 1.0 and 1.0 Kg., respectively. One reserpine treated animal lost 1.0 Kg., while the other neither gained nor lost weight during the ten-day period.

The blood pressures and heart rates of the six orally treated animals are tabulated and shown in Table V. These values were obtained before any drugs were injected into the carotid sinus regions of these animals.

The results in the first three orally treated animals (these three received the alseroxylon alkaloids) were generally similar. Among the most striking and interesting of these results are: (1) an almost complete blocking of the hypertensive response which is reflexly elicited
in normal animals by occlusion of the carotid arteries; (2) a very marked rise in the arterial blood pressure following the injection of 100 mcg. of epinephrine into the adventitia of the carotid bifurcation; (This is opposed to the depressor response elicited by epinephrine injected similarly in normal animals) (3) only a very slight rise in the arterial pressure following the injection of 500 mcg. of the alseroxylon alkaloids into the carotid bifurcation; (4) almost identical elevations in the blood pressure when epinephrine was injected into the carotid bifurcation either before or after complete carotid sinus denervation, or before or after carotid injections of the alseroxylon alkaloids; and (5) similar but not identical responses when 100 mcg. of epinephrine was injected into the carotid bifurcation as when 2-3 mcg. of epinephrine was administered intravenously. A record of one of these three animals which depicts most of these responses is shown in Figure XIII.

There are some other interesting aspects to this peculiar epinephrine-produced pressor effect which were not mentioned in the above account. For instance, the same qualitative blood pressure response, but one of considerably less magnitude, could be obtained by application of epinephrine to the carotid sinus region with a swab. If the carotid sinus areas were denervated with phenol or injected with 500 mcg. of the alseroxylon alkaloids at the onset of or during the fall in blood pressure which immediately followed the pressor aspect of the epinephrine response, the blood pressure kept right on falling just as it would have had these experimental techniques not been instituted. Clamping of the carotid arteries during the depressor phase of the
Table V - The Mean Arterial Blood Pressures and Heart Rates of Six Vagotomized Dogs Which Had Received Daily Oral Doses of the Rauwolfia Alkaloids for a Period of Ten Days

<table>
<thead>
<tr>
<th>Animal</th>
<th>Drug</th>
<th>Dose/day</th>
<th>M.A.P. (mm. Hg)</th>
<th>H.R. (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alseroxylon Alkaloids</td>
<td>0.5 mg./Kg.</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>2</td>
<td>Alseroxylon Alkaloids</td>
<td>0.5 mg./Kg.</td>
<td>90</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>Alseroxylon Alkaloids</td>
<td>0.5 mg./Kg.</td>
<td>96</td>
<td>104</td>
</tr>
<tr>
<td>4</td>
<td>Reserpine</td>
<td>50 mcg./Kg.</td>
<td>93</td>
<td>118</td>
</tr>
<tr>
<td>5</td>
<td>Alseroxylon Alkaloids</td>
<td>0.5 mg./Kg.</td>
<td>132</td>
<td>98</td>
</tr>
<tr>
<td>6</td>
<td>Reserpine</td>
<td>50 mcg./Kg.</td>
<td>131</td>
<td>110</td>
</tr>
</tbody>
</table>
epinephrine response changed the contour of the curve only slightly.

The fourth animal of this group received oral reserpine rather than the alseroxylon alkaloids. When 100 mcg. of epinephrine was injected into the carotid sinus region, a pressor response was obtained just as in the three alseroxylon treated animals. However, the elevation in blood pressure was not quite as great, while the fall in pressure after the initial rise was more prolonged in the reserpine animal. These responses are shown in Figure XIV. The differences in the contour of the epinephrine curves in the reserpine and alseroxylon treated animals can be seen by comparing Figures XIII and XIV.

After the responses to epinephrine and carotid clamping were recorded, 0.05 ml. of 2% procaine HCl was injected into the carotid bifurcation to block the receptors of the area by anesthetic action. One mg./dog of NaCN was injected intravenously before and after the procaine to prove that the procaine had blocked the chemoreceptors. The pressoreceptors could not be checked by carotid occlusion because this reflex was already blocked by the effects of the oral reserpine.

When epinephrine was injected into the carotid bifurcation after the receptors were blocked, the usual pressor response was elicited. Again, as before the block, the fall in pressure was more delayed than that seen in the alseroxylon-treated animals.

An hour or more after the procaine was injected, carotid clamping and the injection of 500 mcg. of the alseroxylon alkaloids had no effect on the arterial pressure. The intravenous injection of NaCN, however, produced hyperpnea indicating that the chemoreceptors were functioning and that the effects of the procaine had worn off. At this point,
another 100 mcg. of epinephrine was injected into the carotid bifurcation. A small rise in blood pressure ensued. One hour later the local application of a 10% aqueous solution of cocaine hydrochloride increased the blood pressure from 80 to 142 mm. Hg. Injection of 100 mcg. of epinephrine into the carotid area at the height of this pressor response produced a very marked elevation in the arterial pressure. The contour of the curve of this epinephrine-induced hypertensive response was so sharp and so high that it resembled in every respect the type of curve produced when epinephrine was given intravenously. Subsequent inspections of the carotid area revealed bleeding at one of the injection sites, indicating that an appreciable amount of this dose of epinephrine had indeed entered the blood stream.

The fifth, orally treated animal received ten daily doses of the alseroxylon alkaloids. On the tenth day, the carotid sinus reflex response was almost completely blocked, while epinephrine again elevated the blood pressure when injected into the carotid sinus region. Doses of vasopressin, 0.1 ml. (2 units) and ephedrine sulfate, 0.1 ml. (1% solution) when injected into the carotid areas also produced elevations in the blood pressure. These responses were of less magnitude than that induced by epinephrine, as can be seen in Figure XV.

The intercarotid injection of 0.1 ml. (0.2% solution) of NaNO₂, on the other hand, caused a fall in the arterial pressure. This fall and the elevation in pressure produced by the ephedrine sulfate were somewhat latent in character. As in all the other oral animals, the slight rises in pressure from carotid clampings were usually delayed. These pressure rises, on occasion, persisted after the clamps were
During the time that elapsed while the last orally-treated dog was being given daily doses of Rauwolfia, it was thought advisable to treat two, vagotomized dogs with the alseroxylon alkaloids intravenously with the following purposes in mind: (1) to block the reflex hypertension from carotid occlusion to approximately the same extent that this reflex was blocked in the oral animals; (2) to see if the hypertensive response from the intercarotid injection of the alseroxylon alkaloids could be blocked as it was in the orally-treated animals; and (3) to learn whether intercarotid epinephrine would produce the marked hypertension in these animals as it did in the oral dogs.

The first animal of this group was given 2.0 mg./Kg. of the alseroxylon fraction intravenously immediately after checking the carotid occlusion response which produced an elevation in the blood pressure from 142 to 268 mm. Hg. When after five hours the blood pressure was still 110 and carotid clamping produced a further rise in pressure to 140 mm. Hg, another mg./Kg. of the alseroxylon alkaloids was administered intravenously. One hour later the blood pressure level was 94 mm. Hg but carotid occlusion was still capable of raising the pressure 40 mm. or to 134 mm. Hg. Since it appeared certain that only a considerably longer period of waiting would result in a possible further depression of the carotid occlusion reflex, 100 mcg. of epinephrine were then injected into each carotid sinus area. The blood pressure rose only from 94 to 100 mm. Hg after five minutes, whereupon it fell to 92 mm. Hg and remained at that level for over one-half hour.

Because it was evident from the results of Experiment I (see
Table IV, page 43) that 1.0 mg./Kg. of the alseroxylon alkaloids was capable of producing the same degree of carotid reflex blocking as 3.0 mg./Kg., only 1.0 mg./Kg. was given intravenously to this second animal in this group. In this dog the pre-injection blood pressure level was 132 mm. Hg, while carotid occlusion raised the pressure to 204 mm. Hg. Six hours after the alseroxylon alkaloids were injected, the blood pressure was down to 84 mm. Hg, and carotid occlusion was only effective enough to produce a 16 mm. Hg rise.

The intercarotid injection of 100 mcg. of epinephrine at this point resulted in an elevation of the blood pressure of only 10 mm. after five minutes. The blood pressure then receded to 70 mm. Hg which was approximately 12 mm. below the pre-injection level. These results can be seen in Figure XVI. One hour after the epinephrine injection, 400 mcg. of the alseroxylon alkaloids were injected into each carotid bifurcation. This elevated the blood pressure from 70 to 82 mm. Hg or to the pre-epinephrine injection level. The administration of 150 mcg. of epinephrine to each side at this time elevated the pressure to 126 mm. Hg. One-half hour later the blood pressure was down to 86 mm. Hg.

One-tenth ml. or 2 units of vasopressin were then injected into each side. The blood pressure rose slowly from 86 to 112 mm. Hg and persisted at that level for some time (Figure XVI). Approximately one-half hour later the bilateral injection of 0.1 ml. of 0.2% solution of NaNO₂ caused a fall in blood pressure which became evident only after a seven-minute delay.

After analyzing and comparing the results of the five, orally-treated dogs with those of the above two animals which had received
the alseroxylon alkaloids intravenously, the conclusion was reached that Dibenamine could possibly be used in the remaining orally-treated dogs to help elucidate the mechanism of action of the alseroxylon alkaloids and epinephrine when they are injected into the carotid sinus region of these orally-treated animals. Before administering Dibenamine to the oral animal, and in order to give the writer some specific, first-hand information on the action of Dibenamine, one vagotomized animal was given several intravenous doses of Dibenamine until the classical epinephrine reversal was obtained.

The following is a summary of the results obtained in this animal:

(1) Occlusion of the carotid arteries and the intravenous injection of 3.0 mcg./Kg. of epinephrine before the Dibenamine was given elevated the blood pressure 56 mm. and 84 mm. Hg, respectively.

(2) After the intravenous administration of 15 mg./Kg. of Dibenamine over a period of 45 minutes and then waiting an additional hour, the intravenous injection of 3.0 mcg./Kg. of epinephrine lowered the blood pressure from 102 mm. to 50 mm. Hg. However, this fall was preceded by an increase in pressure which lasted only a fraction of a second, but which indicated that the sympathetic system and/or epinephrine had not yet been completely blocked. This fact was verified by a subsequent injection of the same dose of epinephrine and by carotid occlusion, the latter procedure resulting in a 26 mm. Hg elevation in the arterial pressure.

(3) After injecting 10.0 mg./Kg. more of Dibenamine and waiting 2 1/2 hours, 3.0 mcg./Kg. of epinephrine was now completely reversed and carotid occlusion only produced an 8.0 mm. Hg rise in pressure.
(4) Two doses of 0.1 ml. (2 units) of vasopressin injected into each carotid area approximately one hour apart each raised the blood pressure to 120 mm. Hg, one dose from 86 to 120 and the other from 102 to 120 mm. Hg.

The last animal utilized in the present study was oral dog No. 6 which had been treated with 50 mcg. of reserpine/day for ten days. The results of the previous eight animals of Experiment III lead to the experimental design and drugs used in this animal. In order to give the reader a clearer picture of these results, the major portions of the protocol of this experiment are reproduced in Table VI. The kymograph tracing of this experiment is shown in Figure XVII (page 96).
Table VI - Blood Pressure Responses in a Vagotomized Dog Following 50 mcg./Kg. of Reserpine a Day for Ten Days

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Time</th>
<th>Blood Pressure Levels (mm. Hg)</th>
<th>Heart Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal Blood Pressure Tracing</td>
<td>1:10</td>
<td>131</td>
<td>110</td>
</tr>
<tr>
<td>2. Bilateral Carotid Occlusion</td>
<td>1:13</td>
<td>131-154</td>
<td>110</td>
</tr>
<tr>
<td>3. 100 mcg. epinephrine HCl injected into each carotid bifurcation</td>
<td>1:20</td>
<td>130-112-196</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(at peak of rise)</td>
</tr>
<tr>
<td>4. 2.0 mcg./Kg. epinephrine HCl intravenously</td>
<td>1:40</td>
<td>110-205</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(at peak of rise)</td>
</tr>
<tr>
<td>5. Bilateral Carotid Occlusion</td>
<td>3:30</td>
<td>140-154</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>3:59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. 2.0 mcg./Kg. epinephrine HCl intravenously</td>
<td>4:00</td>
<td>140-50</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>4:40</td>
<td></td>
<td>(at nadir)</td>
</tr>
<tr>
<td>7. 100 mcg. epinephrine HCl injected into each carotid bifurcation</td>
<td>4:41</td>
<td>140-50</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>5:35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. 0.1 ml. (2 units) vasopressin injected into each carotid bifurcation</td>
<td>6:25</td>
<td>96-140</td>
<td>120</td>
</tr>
</tbody>
</table>
Figure XIII. - Blood Pressure Responses From 14-Day Varicenized Dog Following a Ten-Day Oral Alserox
Figure XV. - The Blood Pressure Responses Produced by the Intercarotid Injection of Vasoactive Drugs in a Vagotomized Dog Following Oral Alseroxylon Treatment for a Period of Ten Days at a Dose of 0.5 mg./kg./Day.

Female dog, 20.5 Kg.; Upper Tracing: respiration; Lower Tracing: Time in minutes

1) Normal tracing, B.P. 132 mm. Hg.; 2) Carotids clamped, carotid reflex almost completely blocked
3) Epinephrine, 100 mcg. into each carotid area at 2:15, B.P. 130 to 170 mm. Hg.
4) Vasopressin, 0.1 cc. or 2 units into each carotid area at 3:17, B.P. 130 to 154 mm. Hg. & prolonged
5) NaN0₂, 0.1 cc. of 0.2% soln, into each carotid area, B.P. 154 to 118 mm. after a seven-minute latent period; 6) One hour post NaN0₂, carotids clamped, B.P. 116 to 122 mm. Hg.; 7) Ephedrine sulfate, 0.1 cc. of 1% soln. into each carotid area, B.P. 112 mm. slowly rising to 130 mm. Hg. after 25 min.
Figure XV. Blood pressure responses by Intercarbo

For rats which had been administered 1.0 m

1) Formaldehyde: 0.175% at 3:45 p.m.,...slow, prolonged effect;...after Fomblin wash.
Intracardially Injected Vasoactive Drugs in a Vasomotorized 1.0 mg./kg. Alseroxylon I.V. Six Hours Previously
Intravenous and Intercarotid Epinephrine Both Section of Dibenzamine in a Varotomized Dog Which Had 0.5 ml. of Dibenamine for a Period of Ten Days
Discussion

The tests for local anesthetic activity show that the alseroxylon alkaloids in the citric acid vehicle have a very definite irritating property. This irritant action can no doubt be attributed to the pH of the vehicle (pH 2.3) since it was also observed in the wheals which were produced by the vehicle alone. It has been shown previously that this pH in itself apparently does not contribute to or adversely affect the results when it is injected into the carotid sinuses along with the dissolved drug.

The question of whether the alseroxylon alkaloids possess any anesthetic potency is left to conjecture in the above test due to the irritant nature of the vehicle. However, other methods for testing local anesthetic action would probably be interfered with by the acid to a larger degree than the guinea pig wheal method. Besides, the guinea pig wheal method was selected because it involved injection of the drug into the tissues which resembled the way the drug was administered in the carotid sinuses. Of course, there is a difference in the embryological derivation of the two tissues involved, the artery being derived from mesoderm and the guinea pig skin from ectoderm. The morphology of the two tissues differs also, which may be important when absorption from the area is considered. In fact, neither the vehicle nor the drug plus the vehicle appeared to be absorbed from the guinea pig wheals, while the drug did definitely disappear after injection into the carotid adventitia. This is understandable since the carotid bifurcation has a richer vascular supply and one that would be in closer proximity to the injected drug. In addition, drugs injected into the
carotid bifurcation are only a matter of a millimeter or two from the lumen of the artery. Some of the drug would definitely be carried away by this route as will be shown in some of the succeeding pages of this report.

While the above details may partially explain why the vehicle may not be so irritating to the carotid bifurcation, they still do not answer the real question at hand. However, two independent reports in the literature were found which indicate that the alseroxylon fraction and reserpine, one of its major constituents, do not possess anesthetic activity. In one of these articles, only the statement that the "local anesthetic action of reserpine has been ruled out" was made. No details about the method or concentrations of drug used were given (49). In the other article it was reported that no significant anesthetic activity could be demonstrated for the alseroxylon alkaloids using the method of Sollman as modified by Bulbring and Wajda (48). Again the concentrations of the drug which were used were not given. The authors did state elsewhere in their paper, however, that their stock solution was 0.5% of alkaloids in an appropriate saline solution. At any rate, the above information leads one to conclude that the alseroxylon alkaloids do not possess anesthetic action.

A review of the literature dealing with the actions of drugs on the carotid sinus area indicates that drugs may produce an elevation of the systemic arterial pressure by at least three entirely different mechanisms when they are injected into the carotid bifurcation. These are: (1) by local anesthetic action whereby the carotid receptors or nerve fibers are blocked; (2) by adrenolytic action whereby the
constrictor effect of locally liberated or administered epinephrine or
norepinephrine is blocked or reversed (3) by vasodilation action which
reduces the intrasinusal pressure and results in less "firing off" of
the carotid sinus stretch receptors. All of these methods involve a
reduced flow of impulses over the carotid sinus nerves. The adreno­
lytic action also results in vascular dilatation but by an indirect
method rather than by a primary mechanism such as the direct action of
NaNO₂.

While the local anesthetic effect of the alseroxylon alkaloids can
probably be ruled out as the possible mode of action of these alkaloids
on the carotid bifurcation, whether or not they possess any direct
vasodilatory activity could not be demonstrated in the present study.
During the course of this investigation, the carotid arteries from
eight different dogs were removed, sectioned into rings which were
approximately five millimeters long, and set up for recording in a
manner similar to the tracheal chain method. Six or eight of the caro­
tid artery rings were formed into a chain by tying with thread, or by
forming connecting links with wire or with thin brass rings which were
obtained from a local jeweler. The constant temperature bath was of
Tyrode's solution. The arteries from some of the dogs were tested
immediately, while those from others were tested after refrigeration
in Tyrode's solution at 4°C for 24 hours.

In none of these experiments could constrictions or relaxations
be recorded either before or after various concentrations of epineph­
rine, vasopressin, NaNO₂, Mecholyl, or the alseroxylon alkaloids were
added to the bath. Some of the possible reasons for these negative
results are the very small and weakly muscular nature of the carotid arteries of dogs, and the poor mechanics of the chain-recording apparatus. However, arteries from larger animals could not be used because of the species difference which has been shown in the literature. In addition, spiral strips cut from the arteries could not be used because the arteries were too small for this procedure.

As was stated previously in the literature review, the results from other investigators' work on whether the Rauwolfia alkaloids can produce non-neurogenic vasodilatation is rather conflicting and seems to depend upon the species of animal as well as the precise experimental technique employed. Some of these reports indicate that the alseroxylon alkaloids and reserpine do produce primary dilatation while others suggest a vasodilatory activity which is only secondary to a drug-induced vasoconstriction. Accordingly, it appears that the vasodilatory properties of the Rauwolfia alkaloids will have to await considerable study. In the meantime, one can only conclude that the vasodilatory properties of the Rauwolfia alkaloids have not as yet been ruled out and that such an action remains as a definite possibility as one of their modes of action. Indeed, it will be shown during the remainder of this discussion that the alseroxylon alkaloids exert no adrenolytic action on the carotid areas which leaves, by the process of elimination, a vasodilatory action as the only mechanism by which these alkaloids may produce hypertension when injected into the carotid bifurcations.

The pre-injection blood pressure levels in the vagotomized and orally-treated animals of this experiment agree favorably with those
obtained by Gourzis, et al., who administered 0.5 mg./Kg. of the alseroxylon alkaloids orally for a period of five days (6). The other direct effects of the Rauwolfia alkaloids such as sedation, nictitating membrane prominence, and diarrhea were also quite typical and need not be discussed further. The loss in weight in these oral dogs should be mentioned because a gain in weight is often seen in clinical use of the Rauwolfia alkaloids. This difference can doubtless be explained by the larger doses employed in the present study. These larger doses caused violent diarrhea in all but the last two dogs so that little of the food in these animals was absorbed. The loss of weight was probably due to malnutrition rather than any direct influence of the drug on the metabolism of the dogs.

Two of the most interesting effects which were produced by the orally administered alkaloids was the marked reduction or blocking of the hypertensive response and the very marked elevation in blood pressure following the intercarotid injection of the alseroxylon alkaloids and epinephrine, respectively. These observations were in sharp contrast to the marked hypertension produced by the alseroxylon alkaloids and the distinct hypotension induced by epinephrine when they were similarly injected into the carotid bifurcations of normal dogs.

A careful, comparative analysis of these results in the oral dogs indicated that the reduced responses following the intercarotid injection of the alseroxylon alkaloids closely paralleled the reduction in the pressor response to carotid occlusion in these same animals. For instance, in the earlier part of these experiments the effects of carotid occlusion were almost completely blocked or effective enough to
produce only a slight elevation in the blood pressure. In these same instances, the effects of intercarotid alseroxylon were also completely blocked or resulted in a hypertensive response which was identical in magnitude to the carotid occlusion hypertension. In the latter portions of these experiments, the results were similar but were more frequently completely blocked than they were during the initial stages. The only exception to these observations was that a slight fall in the blood pressure infrequently occurred after an alseroxylon injection. This was of no significance, however, as it only occurred after the blood pressure had remained slightly elevated for a prolonged period of time following a previous injection of epinephrine or vasopressin.

These results definitely confirm the conclusions of other investigators that the Rauwolfia alkaloids produce hypotension by a central blocking of sympathetic tone (6, 7, 8, 9, 43, 73). If this were not so, the effects of carotid occlusion and the effects of intercarotid injection of the alseroxylon alkaloids should be the same in the orally-treated dogs as in normal animals. Further, the hypertension produced by both of these mechanisms in normal dogs was also considerably reduced even in those dogs which received the alseroxylon fraction by intravenous administration in acute experiments. This conclusion is also in line with the well-known fact that the hypertension or hypotension induced by any manipulation of the carotid bifurcation must, of necessity, involve some central components of the sympathetic nervous system (50).

While the reduced degree of hypertension following the intercarotid injection of the alseroxylon alkaloids in the oral dogs was con-
sidered a definite possibility and was therefore somewhat anticipated, the marked hypertension following the intercarotid injection of epinephrine in these animals was a baffling surprise. The writer's first impression was that some of the epinephrine had accidentally been injected either into one of the small blood vessels of the bifurcation or directly into the lumen of the carotid area. A subsequent intravenous injection of a small dose of epinephrine somewhat supported this view. The tracings obtained by the two routes of administration were similar in most every respect with the exception that the blood pressure rise was sharper, steeper and less latent after the known intravenous injection. However, while repeated intercarotid and intravenous injections always produced these similar types of blood pressure tracings, the viewpoint that the intercarotid injections always resulted in the drug accidentally getting into the blood stream became less and less convincing. Further, the fact that this response to epinephrine occurred only in these orally treated animals and was never seen in any of the normal animals of Experiment II ruled out the accidental intravascular injections as being the causative factor and indicated that the oral effects of Rauwolfia must be responsible in some manner for this peculiar response.

The only two remaining mechanisms whereby intercarotid epinephrine could produce this potent hypertensive response were by direct vasodilatation or by an adrenolytic effect caused by the oral Rauwolfia. Since epinephrine was the drug being injected, these two mechanisms became one and the same. However, the evidence against an adrenolytic action produced by the Rauwolfia alkaloids was overwhelming and
is as follows: (1) The results in the normal animals of Experiment II indicated that the Rauwolfia alkaloids have no adrenolytic action; (2) The process of absorption of the oral drug, together with the longer time in which the alkaloids had to act in these animals, may have resulted in the conversion of the alkaloids into some form which had adrenolytic properties. However, if that were so, then the rise in pressure elicited by epinephrine in these possibly adrenolytic animals would have to be mediated through the central sympathetic centers. These centers were shown to be blocked or almost completely blocked as evidenced by the reduced effects to carotid occlusion and intercarotid alseroxylon in the oral animals; (3) If the effects of epinephrine were reversed in these animals, the typical rise in pressure would be expected to be reversed, viz., a slow not too potent and prolonged effect rather than a high and transient elevation similar to that following intravenous epinephrine.

After all the generally known mechanisms by which epinephrine could produce this potent elevation in the blood pressure had been considered, the only conclusion that could be drawn was that some of the injected drug must always enter the blood stream by diffusion or other methods. This was actually found to be the case and the oral Rauwolfia was found to be responsible for the fact that this peculiar and interesting result was observed in the first place. A more or less general summary of the facts upon which this conclusion was based are below. These facts also add weight to the conclusion mentioned previously that the Rauwolfia alkaloids exert their main action through an inhibition of the central sympathetic nervous system.
When epinephrine is injected intercarotidly in normal animals, the blood pressure falls and the effects of carotid occlusion are depressed or blocked completely. This is caused by an epinephrine-induced vascular contraction which results in an increased impulse traffic over the carotid sinus nerve, an inhibition of central, sympathetic efferent outflow, general vasodilatation and a fall in the blood pressure. In this situation, some small portion of the injected epinephrine diffuses or otherwise gets into the general circulation but does not produce hypertension because of the potent nature of the carotid sinus reflex. In normal animals, the intercarotid injection of Rauwolfia alkaloids converts this epinephrine response so that the blood pressure returns to normal or above. This is probably due to a direct vasodilatory property of the Rauwolfia alkaloids and thus to a simple antagonistic, pharmacological effect. Whether the Rauwolfia alkaloids can convert the epinephrine hypotension to normal levels or to hypertension depends upon the relative effects of the two drugs (how effective they were administered) or by the length of time which elapses between their administration. At any rate, the Rauwolfia effects are due to a decrease in the impulse traffic over the carotid sinus nerve which is in turn due to the vasodilatation and reduction of pressure in the lumen of the carotid sinus. These events result in a lessened central sympathetic inhibition, increased sympathetic efferent outflow, vasoconstriction in the body of the animal, and an elevation of the general arterial pressure.

When Rauwolfia was administered orally to dogs, central sympathetic impulse outflow was inhibited by some unknown mechanism. This
was shown in the present experiment by the reduced effects to intercarotid alseroxylon and occlusion of the common carotid arteries. When epinephrine was injected into the carotid bifurcation in these animals, only the afferent portion of the usual sequence of events transpired. That is, the impulse traffic over the carotid sinus nerve increased, central sympathetic outflow was possibly inhibited, but a fall in blood pressure did not occur. The fall in blood pressure did not occur for two specific reasons. First, central sympathetic outflow was already inhibited and the blood pressure already reduced by virtue of the oral effects of the Rauwolfia. However, that this lower pre-injection blood pressure level was not the only factor involved can be seen by comparing the results of Experiment II with those of Experiment III. In normal dogs, 100 mcg. of epinephrine often lowered the blood pressure to as low as 50 mm. Hg, while the blood pressure in the oral Rauwolfia dogs was seldom below 80 to 130 mm. Hg. Second, the central blocking effects of the oral Rauwolfia having been manifest for a period of ten days, sensitized the effector end organs to any injected epinephrine just as they become sensitive after surgical sympathectomy. Consequently, the small amount of epinephrine which diffused into the blood stream following intercarotid injections produced a marked rise in the blood pressure in the oral Rauwolfia dogs and no effect in normal animals. Further proof of this Rauwolfia-induced, epinephrine sensitization are the following observations: Intravenous injections of epinephrine have been repeatedly shown in the literature to produce greater elevations in the blood pressure in Rauwolfia-treated animals than in controls. The authors of these papers have not stated the reason for this increased
effect or have attributed it to the lower pre-injection level or to the
obtundent vasomotor reflexes, both of which were induced by a previous
dose of Rauwolfia. When epinephrine was injected into the carotid bi-
furcations of the two dogs of Experiment III which had received only
intravenous Rauwolfia in an acute experiment, the blood pressure rose
only from 94 to 100 mm. Hg in one animal and from 80 to 92 mm. Hg and
from 70 to 82 mm. Hg after two doses in the other animal. After the
first dose of epinephrine in the second dog, the blood pressure fell
after ten minutes to 70 mm. Hg or to 10 mm. below the pre-injection
level. These results indicate that in the acute experiment the inter-
carotid injection of epinephrine can produce only minor changes in the
blood pressure which may be in only one or both directions. Accord-
ingly, epinephrine can produce only a slight rise in the pressure in
the acute experiment because the effector end organs have not yet be-
come sensitive to the drug that diffuses into the blood stream. In
the same situations, epinephrine may produce only a small, secondary
fall in pressure because the Rauwolfia has already, almost completely
blocked the central sympathetic pathways through which the epinephrine-
induced hypotension is mediated.

The blood pressure changes which occurred when vasopressin and
NaNO2 were injected intercarotidly into the orally treated animals and
into the oral animal which also was given Dibenamine intravenously
offer further verification of the fact that drugs which are injected
into the carotid bifurcation may diffuse into the blood stream where
they exert their typical systemic effects. These blood pressure
changes also add further proof to the fact that the epinephrine-induced
hypertension is not due to an adrenolytic action exerted by the Rauwolafia.

When vasopressin is given intercarotidly in normal dogs, the blood pressure falls by the same mechanism by which epinephrine induces hypotension. In the orally treated animals, both of these agents produced hypertension which was similar to the tracings normally obtained when these agents are given intravenously. However, the effects of epinephrine can be reversed while those of vasopressin cannot. Thus, these results rule out an adrenolytic action and show that the drug in both cases enters the blood stream to exert its effects. NaNO₂, on the other hand, causes hypertension in the normal animal while in the orally treated dogs, only hypotension was observed.

In the dog which received Dibenamine but no oral or intravenous Rauwolfia, carotid clamping and intravenous epinephrine produced the usual potent pressor responses before the Dibenamine was administered. After the Dibenamine was given, however, carotid clamping produced only an 8.0 mm. Hg rise in pressure which indicated that the carotid occlusion reflex arc had been almost completely blocked. This blocking was due to a peripheral adrenolytic and sympatholytic action in contrast to the same effects which are produced by Rauwolfia by virtue of its central sympatholytic activity. Also, after the Dibenamine was given, intravenous as well as intercarotid epinephrine (3.0 mcg./Kg. and 100 mcg./dog, respectively) produced profound falls in the arterial pressure. Intercarotid vasopressin, however, produced a rise in pressure since it cannot be reversed by Dibenamine. These results all indicate that some of the drug reaches the blood stream to exert its typical,
systemic effects.

Before Dibenamine was administered to oral dog No. 6, carotid clamping raised the pressure only from 131 to 154 mm. Hg. After Dibenamine, carotid clamping produced a similar pressor response of from 140 to 154 mm. Hg. These similar results could be expected since the oral Rauwolfia blocks the carotid reflex arc centrally, while the Dibenamine gave this animal an additional peripheral block which was unnecessary in this instance. When epinephrine was injected intravenously and intercarotidly before the Dibenamine was given, the blood pressure was raised from 130 to 196 mm. Hg and from 110 to 205 mm. Hg, respectively. After Dibenamine was given to produce its peripheral epinephrine reversal effects, however, both the intravenous and intercarotid injections of epinephrine produced exceedingly large reductions in the arterial pressure, while intercarotid vasopressin still produced an elevation in pressure. These results add concrete proof to the previously mentioned conclusions that Rauwolfia blocks the central component of the carotid reflex arc and thus prevents intercarotid epinephrine from producing its typical hypotensive effect. In addition, when Rauwolfia is given over a period of time, the epinephrine activated effector end organs of the sympathetic nervous system are sensitized so any epinephrine that enters the blood stream following intercarotid injection produces a marked elevation in the blood pressure. This pressor rise is not contributed to by any adrenolytic action for if it were, hypotension rather than hypertension would ensue as it did after Dibenamine.
A study has been made to determine the importance of the vagi and afferent nerve endings at the carotid bifurcation in the hypotension elicited by the alseroxylon fraction of Rauwolfia serpentina alkaloids in dogs. Following the intravenous injection of 1.0 mg./Kg. of the alseroxylon alkaloids, the blood pressure fell after six hours to approximately the same level in normal, vagotomized, or carotid denervated animals, while a considerably lower nadir was obtained when both the vagi and carotid areas were denervated. Therefore, neither the vagi nor the carotid afferent nerves contribute to the Rauwolfia-induced hypotension, but assume a buffering action in an effort to limit the hypotension instead. The fall in blood pressure in each of the four groups of animals was slow in onset, but progressive and prolonged. Approximately the same degree of bradycardia occurred in all the animals, which suggests that the vagi are not necessary for this reduction in heart rate. The pressor response following bilateral carotid artery occlusion was blocked in the normal and vagotomized animals. This is in accord with the results of other investigators and confirms their conclusions that the Rauwolfia alkaloids exert a blocking action on certain central components of the carotid reflex arc.

This investigation was extended by injecting drugs, and most specifically the alseroxylon alkaloids, into the adventitia of the carotid bifurcation of normal dogs as well as dogs which had been given ten daily, oral doses of either the alseroxylon alkaloids or reserpine. In normal animals, 250 to 500 mcg. of the alseroxylon alkaloids...
produced profound hypertension by blocking the afferent nerves of the carotid sinus. The vehicle alone or the carotid chemoreceptors were not involved in this effect. In these same animals, 100 mcg. of intercarotid epinephrine produced its usual hypotensive response concomitantly with a partial or complete carotid occlusion reflex blocking. The alseroxylon alkaloids could block or reverse the effects of epinephrine by their overpowering and antagonistic vasodilatory action rather than by an adrenolytic effect.

In the dogs treated orally with Rauwolfia, carotid occlusion and the intercarotid injection of the alseroxylon alkaloids each resulted in only feeble or completely blocked pressor responses. On the contrary, intercarotid epinephrine resulted in a marked but transient hypertension which could not be prevented or altered by carotid occlusion, intercarotid alseroxylon, or by complete carotid area denervation. Vasopressin and NaNO₂ in these animals produced hypertension and hypotension, respectively, which is the exact opposite of their actions when injected intercarotidly in normal dogs. These results prove that the alseroxylon alkaloids block the afferent portion of the reflex arc as well as at least certain of its central components. The central blocking aspect predominates and produces hypotension while the carotid afferent blocking effect acts to buffer this depression of the blood pressure. The epinephrine pressor response in the oral dogs results from some of the drug leaking into the blood stream where it acts on the adrenergic effector end organs which have become sensitized by an alseroxylon-induced, pharmacological sympathectomy. The epinephrine pressor response is also aided or made more potent by the existing
central sympathetic blocking of the carotid reflex arc.

All of the results in the three separate experiments of this investigation indicate that Rauwolfia produces hypotension through a central mechanism and that this hypotension is buffered by the action of Rauwolfia on the carotid receptor areas.
CONCLUSIONS

1. Following the intravenous administration of 1.0 mg./Kg. of the alseroxylon alkaloids to normal, vagotomized, carotid denervated, or vagotomized plus carotid denervated dogs, hypotension, bradycardia, and usually respiratory depression occurs. The hypotensive curve in all four groups of dogs is similar, being slow in onset, progressive and very prolonged. The same degree of bradycardia also occurs in each of the four groups of animals.

2. The blood pressure levels at the end of six hours of observation are practically the same in the normal, vagotomized, and carotid denervated groups of animals. However, the blood pressure falls to a lower level when both the vagi and carotid sinus nerves are sectioned in the same animal.

3. Taken together, the above observations indicate that the sino-aortic receptor mechanisms do not contribute to the hypotension elicited by the intravenous administration of the alseroxylon alkaloids. Instead, both the vagi and carotid sinus nerves act as buffering mechanisms in an effort to limit the hypotension which is elicited by the alseroxylon alkaloids by a central blocking of sympathetic vasomotor outflow.

4. The intact vagi are not necessary for the alseroxylon-induced bradycardia. The bradycardia must, therefore, be due to a direct cardiac inhibitory action or to a decreased cardiac sympathetic activity. Reference to the literature implicates the latter as the most likely mechanism.

5. The blood pressure rise following bilateral carotid artery
occlusion was progressively more blocked and somewhat more delayed the
closer one approached the end of the six-hour observation period.

6. When 250 to 500 mcg. of the alseroxylon alkaloids were injec­
ted into the adventitia of the carotid bifurcation in normal or vagoto­
mized animals, a sharp and prolonged rise in the arterial blood pres­
sure always occurred. This response was eliminated when the pressore­
cptors were selectively denervated leaving the chemoreceptors intact.
The blood pressure rise also did not occur when the vehicle alone was
injected. These observations indicate that the blood pressure rise was
due to the blocking action of the alseroxylon alkaloids themselves on
the pressoreceptor fibers or their receptor nerve endings. The specific
mechanism which initiates the sequence of events which ultimately re­
sults in the blood pressure elevation is most probably a direct vaso­
dilatory action on the carotid artery musculature.

7. Smaller intercarotid doses of the alseroxylon alkaloids or re­
serpine produced either none or only slight and transient blood pres­
sure elevations. Whether 250 or 500 mcg. doses of reserpine could pro­
duce similar results as those observed with the alseroxylon alkaloids
could not be studied because of the less soluble nature of the reser­
pine molecule.

8. Sufficiently large, intercarotid doses of the alseroxylon
alkaloids can block or reverse the hypotensive effects of similarly
injected epinephrine. This is not due to an adrenolytic action but to
the direct and opposite action of the Rauwolfia alkaloids on the caro­
tid musculature.

9. When 0.5 mg./Kg./day of the alseroxylon alkaloids were
administered to dogs for a period of ten days, the hypertension induced by carotid occlusion or by intercarotid injections of the alseroxylon alkaloids was completely or almost completely blocked. Intercarotidly injected epinephrine in these animals no longer produced hypotension, but, instead, a potent hypertension of short duration occurred. These results make the following conclusions possible: (a) the alseroxylon alkaloids owe at least a major portion of their hypotensive activity to a blocking action on certain central components of the carotid reflex arc; (b) the adrenergic effector end organs of the sympathetic nervous system are sensitized to epinephrine as a result of an alseroxylon-induced, pharmacological sympathectomy; (c) any drugs which are injected into the adventitia of the carotid bifurcation will display an action which is the algebraic sum of the action of the drug on the carotid receptors and the action of a small portion of the drug which diffuses into the systemic circulation.

10. The alseroxylon alkaloids probably have no local anesthetic action. The citric acid vehicle used in this study has definite irritant properties.

11. The results of Experiments I, II, and III all indicate that the alseroxylon alkaloids exert their antihypertensive activity by a central nervous mechanism. These results also show that any action which the alseroxylon alkaloids have on the carotid receptors is directed toward elevating the blood pressure or buffering the hypotension produced by the central components of these alkaloids.

12. The present investigation revealed that there are several reasons why clinical and pharmacological reports show that the
Rauwolfia alkaloids are only mildly acting antihypertensive agents: (a) In the usual doses employed, the central blocking action by these alkaloids is only partial rather than complete; (b) Vasodilation produced through this central action is probably mediated only by inhibition of the sympathetic nervous system which at the effector end organs is a passive rather than an active phenomenon; (c) The buffering action of the sino-aortic pressoreceptors which limit this hypotension acts by directly opposing the central sympathetic inhibition. This occurs both by virtue of the normal physiological role of these receptors as well as by a pharmacological action imposed upon them by the Rauwolfia alkaloids; and (d) Any buffering effect displayed by the sino-aortic receptors is mediated by increased central sympathetic outflow which would result in an active vasoconstriction. In any event, the total hypothetical blood pressure reduction depends directly upon the completeness of the central blocking effects. The final and true blood pressure level also depends upon the completeness of this central blocking action for it determines the amount of sino-aortic afferent breakthrough which in turn determines the degree of buffering action that is possible.

13. The pharmacological actions of the Rauwolfia alkaloids as observed in the present study help to vindicate the clinical use of Rauwolfia in combination with other anti-hypertensive agents. The following example, using Veratrum, may make this more evident. Most of the Veratrum alkaloids owe a large portion of their hypotensive activity to their ability to lower the threshold of the carotid pressoreceptors. This would aid the Rauwolfia alkaloids in inhibiting the central sympathetic system while at the same time it would nullify the
buffering effect exerted by these receptors when Rauwolfia is administered alone. The effective doses of both of these alkaloids could thus be reduced or more anti-hypertensive activity could be exhibited by the same doses. The undesirable side effects of the Veratrum alkaloids could also probably be largely eliminated in the majority of cases using this combination.


64. Pamphlet, New Indications for Serpasil, Ciba Company Limited, Montreal, P. Q.


I, Carl Alvin Schlagel, was born in Meredian, Pennsylvania, December 14, 1923. I received my secondary school education in the public schools of Slippery Rock, Pennsylvania. My undergraduate training was obtained from the University of Pittsburgh, Pittsburgh, Pennsylvania, from which I received the degree Bachelor of Science in 1952. From The Ohio State University, Columbus, Ohio, I received the degree Master of Science in 1954. During the academic year 1952-1953 I received a fellowship from The Columbus Pharmacal Company, Columbus, Ohio, and for the years 1953-1954 and 1954-1955 I was an American Foundation for Pharmaceutical Education Fellow.