A PHARMACOLOGICAL COMPARISON OF ADRENOLYTIC DRUGS HAVING DIFFERENT EFFECTS ON CARBOHYDRATE PRODUCTION AND UTILIZATION

by

David Albert McClure, B.S., M.S.

A THESIS

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APPROVED:

[redacted]

(Professor in Charge of Thesis)

[redacted]

(Chairman, Graduate Council)

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INTRODUCTION

The functions of the autonomic nervous system are important factors not only for the maintenance of health but also the production of disease. In the normal individual, autonomic nerve activities are balanced to yield a homeostatic steady state resisting any change. On the other hand, in pathophysiological conditions the functions of this system may be so altered as to lead to situations far removed from a constant "normal" state. For example, the physiological responses of the autonomic nervous system to slight changes in blood pressure are vastly different from those occurring in hypovolemic shock. Also, in psychosomatic disorders the signs and symptoms of the disease may be due to malfunctioning on the part of the autonomic nervous system.

Both the normal and the abnormal behavior of the autonomic nervous system depend upon endogenously produced chemicals. Because of this fact, extensive pharmacological study has been carried out in this area. Through the use of chemicals (drugs), the effects of the autonomic nervous system activity may be simulated or mimicked and, therefore, provide a better understanding of the normal and the diseased mechanisms occurring within the body.

A drug may be considered a diagnostic or research tool when its use in the animal body allows conclusions to be made regarding physiologic or pathologic mechanisms of action. Drugs may be used to unlock certain physiological activities whose actions are otherwise too subtle to determine.

Or, they may mimic the action of neurohumors.* Some drugs may be like a caricature in nature while others, by way of contrast, may block the usual physiological responses. Thus, various drugs may be used in interpreting the activities of the autonomic nervous system in health and disease.

An interesting aspect of the normal activities of the autonomic nervous system, as well as the changes in its activity induced by drugs, is the phenomenon of biphasic activity. The biphasic response is not exclusive to the autonomic nervous system, but appears to be a general, universal phenomenon elicited by either endogenous or exogenous drug action. Through chemical action, the cells (or structures) first appear to be stimulated, then depressed. For example, the action of nicotine is predominantly upon the autonomic ganglia, first stimulating, then depressing it. The biphasic action of epinephrine on blood pressure can be demonstrated when small doses of this drug are used. With epinephrine a transient depressor response occurs first and is rapidly followed by a short-lasting pressor response. This is followed by another depressor response before the blood pressure returns to normal. Theoretically, this biphasic effect on blood pressure is brought about by the interplay of epinephrine on two different autonomic nervous system effector receptors; the <u>alpha</u> (stimulatory) and the <u>beta</u> (inhibitory) receptors.

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*A neurohumor may be defined as a chemical substance formed in a neuron and is able to activate neighboring neurons or muscle. Such materials are acetylcholine, epinephrine, nor-epinephrine, and, possibly, serotonin and isoproterenol.

While terms such as neurohormone and transmitter substance may be used to define these substances, the term neurohumor is used here as a more specific term to rule out any possible confusion with endocrine hormones.

The biphasic action elicited by epinephrine also may be shown by the changes in blood glucose occurring in the dog when epinephrine is infused after adrenergic blockade. When one type of an adrenergic blocking agent (alpha sympathetic receptor blocker) is injected prior to epinephrine infusion, a potentiated epinephrine-induced hyperglycemia results. Conversely, prior injection of another type of blocking agent (beta sympathetic receptor blocker) results in a distinct inhibition of the epinephrine-induced hyperglycemia. Apparently, epinephrine-induced hyperglycemia depends upon the integrity of beta receptor sites and when these are blocked, hyperglycemia is inhibited.

The interaction of the two adrenergic receptors in response to epinephrine infusion is quite complex. They may both be stimulated or depressed. Also, the effects mediated by epinephrine on the liver receptors may not necessarily be similar to the effects on the muscle receptors.

A further point of possible clarification of the nature of these receptor effects is offered. In experiments where epinephrine is used with either type of blocking agent, the response shown in the blood glucose level (either a potentiated or inhibited hyperglycemia) presumably results from the interaction of epinephrine on the liver and the muscles. That is, the hepatic production and peripheral utilization of blood sugar may be increased or, at other times, one function may be increased while the other is decreased.

By using sympathomimetic amines and adrenergic blocking agents as tools, experiments may be designed to delineate and evaluate the true

role played by these chemicals in altering carbohydrate production and utilisation. The problem, then, is to determine the effects of epinephrine, nor-epinephrine and isoproterenol, in conjunction with azapetine (an alpha receptor blocking agent) and dichloroisoproterenol (DCI) (a beta receptor blocking agent), on the hepatic production and on the peripheral utilisation of blood sugar.

HISTORY

The activities of the autonomic nervous system are mediated by chemical substances acting within the brain, in the ganglia and at the neuroeffector terminations of autonomic nerves. Release of these substances leads to stimulation or inhibition of activity of the smooth muscle, of the secretory cells and of the cardiac muscle. Administration of certain chemical compounds resembling the true neurohumor epinephrine (sympathomimetic) causes arteriolar smooth muscle contraction with blood pressure increase, a thick copious flow of saliva as well as stimulation of other secretory and endocrine cells, and myocardial stimulation.

The chemical compounds responsible for mediating the activities of the autonomic nervous system have been extensively studied. In 1921, Otto Loewi (27) reported his studies dealing with the effects of neuro-humors on specific organs of the body. In his classical experiment, Loewi isolated and perfused the hearts of two frogs. After stimulating the vagus nerve to one of the hearts and producing cardiac arrest he found that, by allowing this perfusion fluid to come in contact with the second heart, it also stopped beating. He assumed that a chemical

substance was liberated from the first heart after vagal stimulation which he called "Vagusstoff".

In 1929, Sir Henry Dale (12) demonstrated that this neurohumor was acetylcholine. It was finally shown, quite conclusively, that acetylcholine was the chemical mediator at both the sympathetic and at the parasympathetic division of the autonomic nervous system (12) (13).

One last area in the autonomic nervous system remained to have its function explained on a neurohumoral basis. Loewi (27) also had studied the neuroeffector junction of the sympathetic division of the autonomic nervous system and demonstrated that stimulation of the sympathetic fibers leading to the frog's heart resulted in an increased heart rate. Later, in 1937, Cannon and Rosenblueth (7) showed that a chemical material was liberated from the postganglionic sympathetic fiber when stimulated. They identified the liberated substance as a chemical related to epinephrine which they called "sympathin". Recently (1956), U. S. von Euler (18) pointed out that Cannon's "sympathin" is either a combination of epinephrine and nor-epinephrine or is nor-epinephrine* alone.

Other compounds discovered in the human and animal body have been identified as neurohumors. Because of its effective vasoconstrictive activity, serotonin (5-hydroxytryptamine) is considered as such a compound.

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*The prefix nor- is derived from the German, Nitrogen ohne Radikal. When, as in isoproterenol, the nitrogen is, in fact, substituted, the term nor-, as in isopropyl nor-epinephrine, constitutes a paradox. However, this term is useful in comparing structural similarities.

Serotonin is found in high concentrations in the hypothalamus. Based on this discovery, Page (38) postulated that serotonin is a neurohumoral agent playing a role in the transmission of nerve impulses within the brain in a manner analogous to that of epinephrine and acetylcholine.

Other materials have been isolated from brain or nervous tissue and proposed as having a neurohumoral action. GABA (gamma amino butyric acid) (23) possibly acts as a central nervous system neurohumor. Isoproterenol has been identified in the adrenal glands of dogs by Lockett (26) although this report has not been confirmed.

The responses of normal animals to the older neurohumors has been described many times. For over half a century it has been known that epinephrine administration to man or animals increases the blood pressure and heart rate, relaxes the intestinal musculature, increases blood sugar, and elicits other "alarm" reactions. These phenomena are thought to be due to the specific pharmacological effects of epinephrine acting upon particular target cells. The production of hyperglycemia by epinephrine is of extreme physiologic importance. It is one of the primary mechanisms concerned by Cannon's "flight or fright theory" (7). Yet, it is a response that is not well understood since interpretation of the pharmacological mechanism of action is hampered by, as yet, incomplete biochemical inquiry.*

One of the first investigators to demonstrate that hyperglycemia and glycosuria were produced by injection of epinephrine was Blum (50) in 1901. However, because the extraction of the adrenal gland material was crude and incomplete, it was questionable at that time whether it was epinephrine or some other material which had caused the hyperglycemia.

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* See appendix

Not until 1948 was it conclusively demonstrated by Schumann (43) that subcutaneous injections of purified extracts of the adrenal medulla increased the blood sugar as well as the blood pressure of rabbits and cats. Besides epinephrine, other sympathomimetic amines have been found to have hyperglycemic activity. Schumann (43) found that nor-epinephrine increased the blood sugar in cats although not to the same degree as an equivalent amount of epinephrine. In 1953, Trendelenberg (48) found epinephrine 5.4 times more active than nor-epinephrine as a hyperglycemic agent.

Other congeners of epinephrine produce hyperglycemia. Some investigators believe that isopropyl nor-epinephrine (isoproterenol) has greater hyperglycemic activity than epinephrine while others feel that this drug is mid-way in potency between epinephrine and nor-epinephrine (18) (43) (49) (31).

Biochemically, the effects of sympathomimetic amines on blood sugar have been traced to the cyclic interacting functions of the Cori cycle (Figure 1). When the Cori (10) cycle is activated by intravenous injection of a sympathomimetic amine, the following glycemic changes take place: (a) a rapid conversion of liver glycogen to sugar which is then liberated into the circulation; (b) the promotion of muscle glycogenesis; (c) the conversion of muscle glycogen to blood lactic acid which is then released into the circulation, and, (d) the conversion of blood lactic acid to liver glycogen (hepatic glycogenesis). It has been found that epinephrine especially augments reactions (a) and (c).

FIGURE 1

ACTIVATION OF THE CORI

CYCLE BY EPINEPHRINE

Adapted from Harper (7)

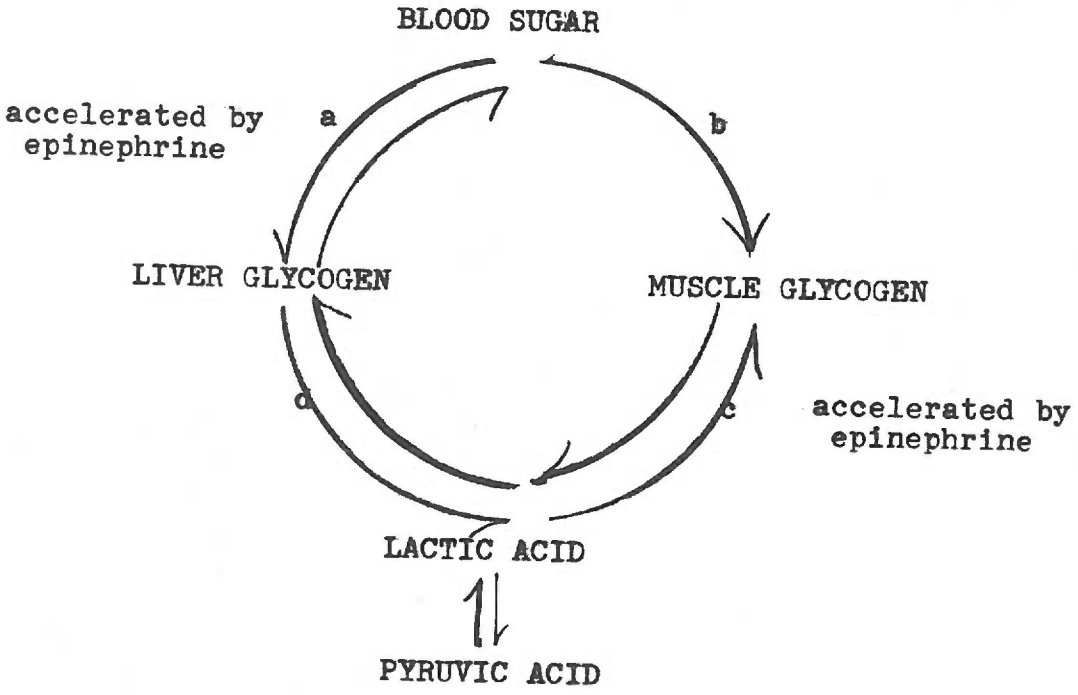

BLOOD SUGAR
accelerated by epinephrine
a
b
LIVER GLYCOGEN
MUSCLE GLYCOGEN
d
c
accelerated by epinephrine
LACTIC ACID
PYRUVIC ACID

Figure 1

The biochemical mechanism of action of epinephrine on carbohydrate metabolism has been elucidated by Cori and Cori (10) (11) and Sutherland and Cori (47). They observed that epinephrine increases the activity of the enzyme phosphorylase, thereby facilitating glycogen breakdown. The glucose thus produced passes into the circulation and is carried to those tissues where there is a need for increased activity and metabolism.

More recently von Euler (18), Vrij et at (12) and McClure (31) have compared the glycemic effects of the three closely allied sympathomimetic amines, epinephrine, nor-epinephrine and isoproterenol. These workers noted that the potency of the induced hyperglycemia was in the following order: isoproterenol > epinephrine > nor-epinephrine (18) (31) (49). But, when a comparison of the blood pressure activity of these amines was made, the order was reversed, i.e., nor-epinephrine > epinephrine > isoproterenol (18) (31) (49). It should also be pointed out that the effects of these three drugs on capillary smooth muscle differ. Nor-epinephrine causes gross vascular constriction while epinephrine and isoproterenol produce varying degrees of vasodilation (21). As yet, no explanation for these circulatory actions have been advanced.

Even more complicated is an interpretation of the effects elicited by adrenergic blocking agents on sympathomimetic amine-induced hyperglycemia. This problem was mentioned previously when it was pointed out that prior administration of adrenergic blocking agents altered epinephrine responses in no set uniform fashion. With respect to the mechanisms concerned when blocking agents interfere with epinephrine-

induced hyperglycemia, Drill (15) states, "The available evidence is not adequate to allow a definite statement that suppression of epinephrine-induced hyperglycemia is due to a specific adrenergic blockade comparable to that occurring in smooth muscle".

Many workers have attempted to explain the underlying pharmacologic mechanisms concerned when blockade of the effects of sympathomimetic amines occur. Probably the first to enter into this inquiry was Dale (12) in 1906. He found that prior administration of an ergot alkaloid not only blocked the blood pressure increase caused by epinephrine but also, in some cases, reversed it. From these findings, Dale (12) postulated that epinephrine has a dual role in its effect on blood pressure: one to increase blood pressure, and the other, to decrease it. The same type of response may occur when adrenergic blocking agents are used to block other actions brought about by sympathomimetic amines - including, in some instances, the glycogenolytic effect. The following table (Table 1) illustrates the confusion which has arisen as a result of various experimental studies on the effects of adrenergic blocking agents on sympathomimetic amine-induced hyperglycemia.

A comparison of the data in Table 1 with that in Table 2 reveals that most of the effects of adrenergic blocking compounds are a blockade of the induced response.

The effects, however, of adrenergic blocking agents on the increased blood glucose concentration brought about by sympathomimetic amines do not follow this regularly blocking pattern. While blockade of the induced

TABLE 1

EFFECTS OF ADRENERGIC BLOCKING AGENTS ON ADRENERGIC-INDUCED HYPERGLYCEMIA

BLOCKING AGENT	EFFECT ON ADRENERGIC-INDUCED HYPERGLYCEMIA
Dihydroergotamine	High degree of blockade (46)
Ergotamine	Low degree of blockade (46)
Ergonovine	No blockade or hyperglycemic enhancement (42)
Tolazoline	No blockade (24)
Yohimbine	High degree of blockade (22)
Phenoxybenzamine	Mild degree of blockade (25) (32)
Azapetine	Hyperglycemic enhancement (31)

TABLE 1

EFFECTS OF ADRENERGIC BLOCKING AGENTS ON ADRENERGIC-INDUCED HYPERGLYCEMIA

TABLE 2

EFFECTS OF ADRENERGIC BLOCKING AGENTS ON

VARIOUS ADRENERGIC-INDUCED PHENOMENON

TABLE 2

EFFECTS OF ADRENERGIC BLOCKING AGENTS ON VARIOUS ADRENERGIC-INDUCED PHENOMENON*

BLOCKING AGENT	EFFECT ON ADRENERGIC-INDUCED-				
	BLOOD PRESSURE INCREASE	GUT RELAXATION	MYDRIASIS	NICTITATING MEMBRANE	UTERINE CONTRACTION
Dihydroergotamine	Reversal	Blocked	?	Blocked	Blocked
Ergotamine	Reversal	Blocked	?	Blocked	Blocked
Ergonovine	Reversal	Blocked	?	Blocked	Blocked
Tolazoline	Reversal	?	Blocked	Blocked	Blocked
Yohimbine	Reversal	Blocked	?	Blocked	Blocked
Phenoxybenzamine	Reversal	Blocked	Blocked	Blocked	Blocked
Asapetine	Reversal	Blocked	?	Blocked	Blocked

*from Drill (15)

hyperglycemia occurs with dihydroergotamine and yohimbine, it does not occur with tolazoline. With ergonovine and azapetine, potentiation of the induced hyperglycemia takes place.

Returning to a consideration of the varying effects elicited by the sympathomimetic amines alone, only a few theories have been offered to explain these phenomena. Cannon and Rosenblueth (7) (1937) were the first to propose such an explanation. They postulated that the pressor and depressor effects of epinephrine occurred in the following manner: when epinephrine was either liberated from the postganglionic sympathetic neuron or injected into the animal, an elaboration of one of two substances from the effector cell resulted. This material was called Substance E or Substance I, and in conjunction with epinephrine caused the effect observed:

Epinephrine + E (excitatory substance) $\rightarrow$ Sympathin E

Epinephrine + I (inhibitory substance) $\rightarrow$ Sympathin I

One example of this action might be the response seen from the action of epinephrine on smooth muscle. According to Cannon and Rosenblueth (7) epinephrine acting on smooth muscle of arterioles would lead to the elaboration of Substance E which, in turn, would combine with epinephrine to form "Sympathin E". An excitatory action or contraction of the arteriole would then occur. On the other hand, the effect of epinephrine on the gut would lead to the elaboration of Substance I and the formation of "Sympathin I" resulting in relaxation of the gut. Although this theory was developed twenty-five years ago it still remains as a possible explanation of the mechanism of action of sympathomimetic amines.

Dale's (12) earlier use of ergot to reverse the pressor effect of epinephrine was, no doubt, very instrumental in the development of this theory. Cannon and Rosenblueth postulated that ergot either inactivates epinephrine or makes it unavailable to the effector cell so that Sympathin E could not be formed. As a result, the opposite effect, that is, the formation of Sympathin I, would take place with a resultant inhibitory action.

Objections to this theory are that both Substances E and I and Sympathin E and I have never been shown to exist. In fact, von Euler (18) has shown conclusively that the material liberated at the neuroeffector junction of the sympathetic division of the autonomic nervous system is nor-epinephrine along with, perhaps, a very small quantity of epinephrine. Furthermore, the time element necessary would be too long for epinephrine to cause the elaboration of one of the two "substances" and then for this substance to mix with epinephrine to form a "sympathin" to cause the response.

Recently, Ahlquist (1) modified Cannon and Rosenblueth's theory of the dual effect produced by epinephrine. Ahlquist's novel proposal is that endogenously produced or exogenously administered sympathomimetic amines act by impinging upon two types of cellular effector receptors. Ahlquist has named the receptor effecting an excitatory action the alpha and the one responding with an inhibitory action the beta receptor. Figure 2 (20) is a schematic illustration showing the postganglionic sympathetic nervous system innervation of an artery as proposed by Ahlquist.

FIGURE 2

SCHEMATIC DIAGRAM OF THE VARIOUS RECEPTORS IN THE SMOOTH MUSCLE OF THE WALL OF A BLOOD VESSEL AND OF THE SITES AT WHICH ADRENERGIC BLOCKING AGENTS ACT

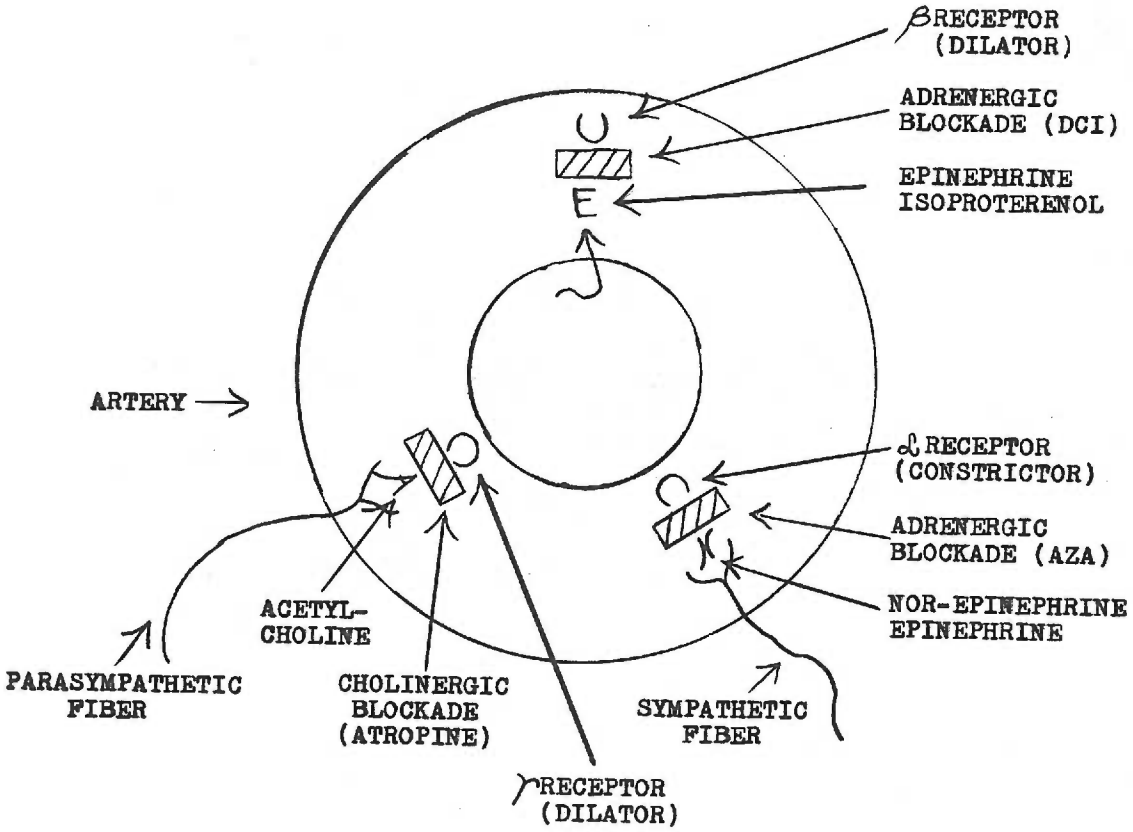

β RECEPTOR
(DILATOR)
ADRENERGIC
BLOCKADE (DCI)
EPINEPHRINE
ISOPROTERENOL
ARTERY
α RECEPTOR
(CONSTRICTOR)
ADRENERGIC
BLOCKADE (AZA)
NOR-EPINEPHRINE
EPINEPHRINE
ACETYL-
CHOLINE
PARASYMPATHETIC
FIBER
CHOLINERGIC
BLOCKADE
(ATROPINE)
SYMPATHETIC
FIBER
RECEPTOR
(DILATOR)

Figure 2

The now classical work of Ahlquist dealing with the alpha and beta receptors was first carried out on the blood pressure responses to sympathominetic amines. The following discussion is a detailed analysis of the present-day theory of Ahlquist dealing with the blood pressure.

When a sympathetic neuron leading to a smooth muscle of an arteriole is stimulated by a faradic current or an injection of epinephrine is given, simultaneous stimulation of both the alpha and the beta receptors in the area occurs. According to Ahlquist the beta receptor is more selective or sensitive to epinephrine than is the alpha receptor. However the alpha (stimulating) receptors exhibit the predominant effect on the arteriole, - contraction of its smooth muscle. This may be explained by referring to Figure 3.

After an intravenous injection of 5 mcg. per kilogram of epinephrine in the dog, a transient depressor effect first occurs. This is then followed by an abrupt rise in blood pressure, the magnitude being directly proportional to the dose. Finally, there occurs a short-lasting secondary fall in the blood pressure. Basing this response on the alpha-beta receptor theory, the following explanation is offered:

1. The immediate response on the blood pressure by epinephrine is a perceptible fall. This response might be due to central stimulation of the medullary areas by epinephrine, increase in vagal activity resulting in decreased heart rate, and the hypotension. However, in dogs, pretreatment with atropine failed to block this primary decrease in blood pressure. Apparently, epinephrine, per se causes this primary hypotensive

FIGURE 3

EFFECT OF EPINEPHRINE (5 mcg./kg.)

ON THE ARTERIAL BLOOD PRESSURE OF THE DOG

(Paper speed 0.5 mm. per second)

Figure 3

effect. Pharmacologically, the response is produced by the first few molecules of epinephrine arriving at the site and stimulating or activating the more sensitive receptors of the beta class. This results in an immediate arteriolar vasodilation with concomitant decrease in the blood pressure. In addition, further evidence was shown by Cobbold, Ginsburg and Paton (8) that the mean forearm blood flow in humans first increased and later decreased with an infusion of 2 mcg. per minute of epinephrine, the flow indicating vasodilation and vasoconstriction, respectively. Here the beta receptors take priority over the alpha receptors in the effect produced.

2. The alpha receptors now come into play as more epinephrine is made available. The stimulating action elicited by the alpha receptors overwhelms the beta depressor effect resulting in an increase in blood pressure. During the exhibition of the pressor effect, all the receptors, both alpha and beta, are being activated by epinephrine.

3. According to the alpha-beta receptor theory the secondary depressor response seen after an injection of epinephrine occurs because the activity of the alpha receptors decays at a more rapid rate than that of beta receptors. When the majority of the alpha receptors have lost their activity, the beta, or depressor, receptors still remain active and now cause vasodilation. That this hypotension is not due to changes in heart rate can be shown in electrocardiographic tracings. In addition, vagal block by atropine does not alter the response.

Further support of the alpha-beta receptor theory is provided by the intravenous injection of extremely small amounts of epinephrine (0.25 mcg. per kilogram) which results in only a depressor response. This may be

explained by the greater sensitivity of the beta receptor for epinephrine. As the dose of epinephrine increases the initial transitory depressor response is followed by a gradually increasing pressor response. Not only are the beta receptors more sensitive than the alpha receptors to epinephrine but they are less easily fatigued as demonstrated by the second depressor response which follows the alpha receptor pressor effect.

Still further support of Ahlquist's alpha and beta receptor theory may be seen when an adrenergic blocking agent (azapetine) is used prior to epinephrine injection in the experimental animal. In this case the response to epinephrine is a decreased blood pressure, i.e., a blood pressure reversal as shown in Figure 4. This response always occurs provided the dose of epinephrine is not excessively large. A pharmacologic explanation of the blood pressure reversal effect is that the adrenolytic agent, azapetine, blocks only the alpha receptors thus permitting the beta receptors to become fully activated. Moreover, as depicted in Figure 4, a potentiation of the depressor response results, indicating that epinephrine possesses the ability to exhibit an extensive beta receptor stimulation.

When the action of alpha adrenergic blocking agents on the pressor effects of other sympathomimetic amines is compared with that on the epinephrine response, a somewhat similar effect occurs. With nor-epinephrine and the blocking agent, the response shown in Figure 5 takes place. The differences in the responses shown in Figure 4 from those noted in Figure 5 may be due to the fact that nor-epinephrine exhibits neither a primary nor a secondary depressor response when given in dosages ranging from 0.025

FIGURE 4

EFFECT OF EPINEPHRINE (5 mcg./kg./min.) ON THE ARTERIAL BLOOD PRESSURE OF THE DOG BEFORE AND AFTER BLOCKADE OF THE ALPHA SYMPATHETIC RECEPTOR BY AZAPETINE (2 mg./kg.)

(Paper speed 0.5 mm. per second)

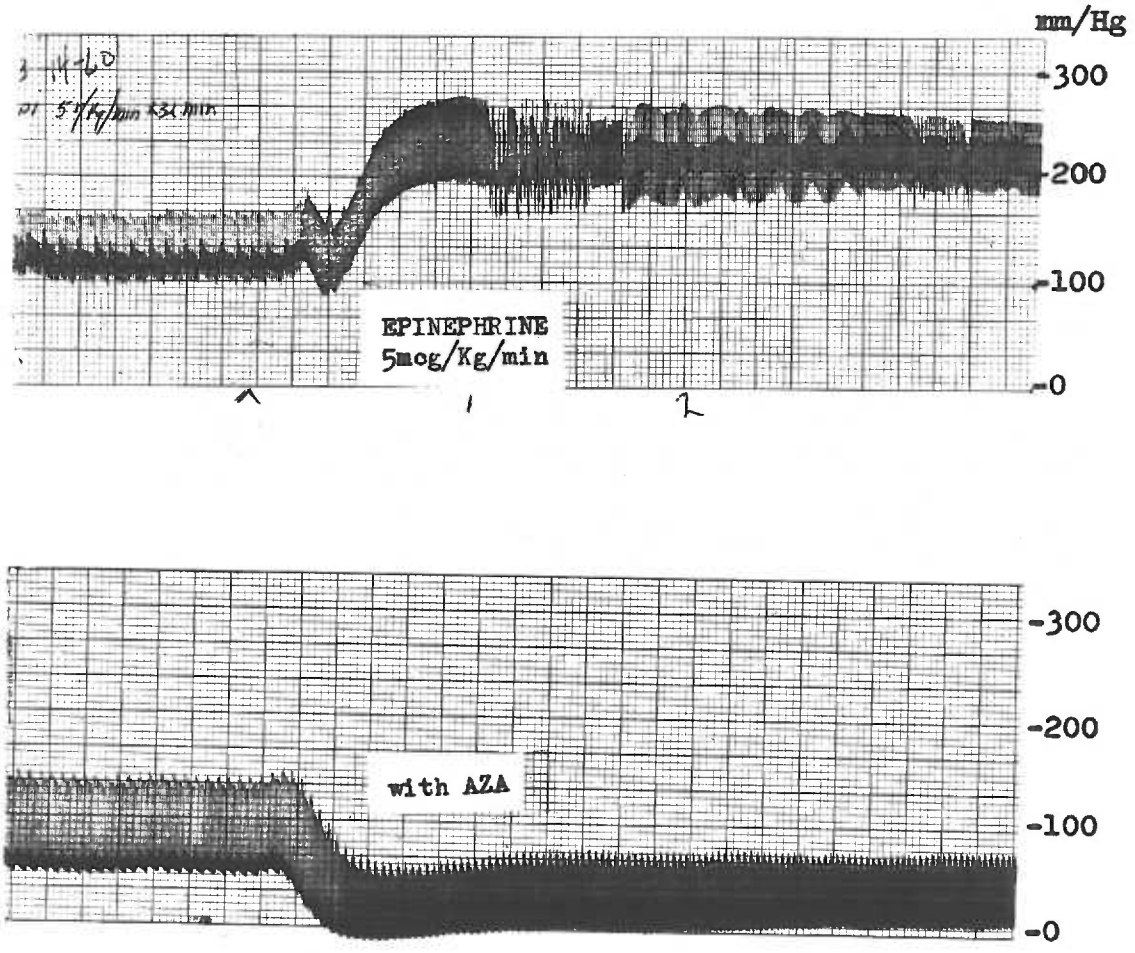

mm/Hg
300
200
100
0
EPINEPHRINE
5mcg/Kg/min
with AZA
300
200
100
0

Figure 4

FIGURE 5

EFFECT OF NOR-EPINEPHRINE (20 mcg./kg./min.)
ON THE ARTERIAL BLOOD PRESSURE OF THE DOG
BEFORE AND AFTER BLOCKADE OF THE ALPHA SYMPATHETIC
RECEPTOR BY AZAPETINE (2 mg./kg.)
(Paper speed 0.5 mm. per second)

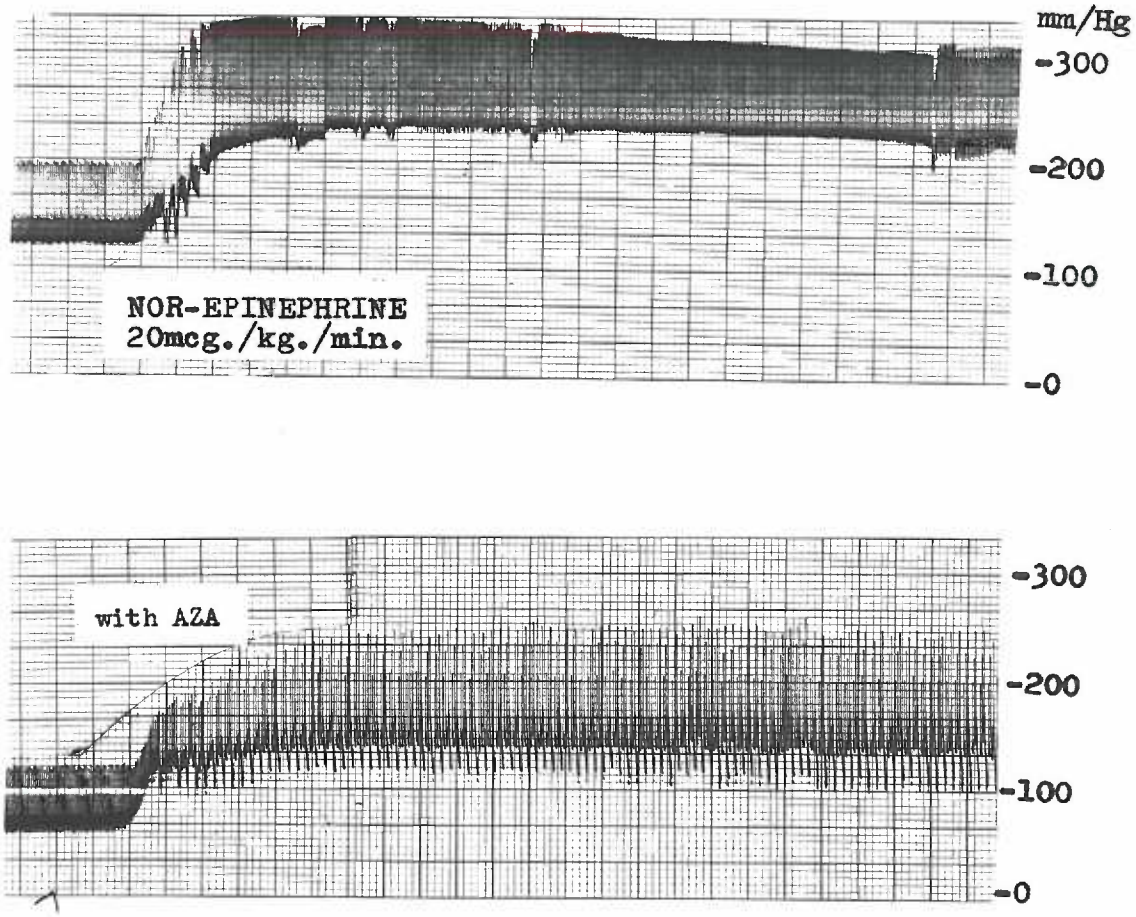

mm/Hg
-300
-200
-100
-0
NOR-EPINEPHRINE
20mcg./kg./min.
with AZA
-300
-200
-100
-0

Figure 5

to 1 mg. per kilogram. This indicates that nor-epinephrine predominantly stimulates alpha receptors and relatively few beta receptors. However, when the blocking agent is used, enough beta receptors are stimulated to bring about some decrease in the pressor effect of nor-epinephrine.

Another sympathomimetic amine, isoproterenol, shows only a depressor effect on the blood pressure when given alone. If an alpha receptor blocking agent (azapetine) precedes an injection of isoproterenol, the depressor response is further intensified as shown in Figure 6. Apparently, the predominant effect of isoproterenol is to stimulate the beta receptors. The potentiated depressor response taking place with isoproterenol after azapetine indicates that the latter drug acts only on the alpha receptor, allowing the beta receptors to come into full play. However, isoproterenol also has some alpha receptor activity since there is less depressor response when it is used alone than when it is used with azapetine.

It has been known for many years that some of the ergot alkaloids block the stimulating action (alpha response) of sympathomimetic amines on smooth muscle (Figure 7). Only recently, however, have compounds producing blockade of the inhibitory action (beta response) been available. In 1957, Powell and Slater (39) found, while working with the halogenated β-phenylethylamines, that the dichloro analogue of isoproterenol (dichloroisoproterenol or DCI*) (Figure 7) selectively blocked the depressor

*For brevity the symbol DCI hereafter used will indicate dichloroisoproterenol.

FIGURE 6

EFFECT OF ISOPROTERENOL (1 mcg./kg./min.) ON THE ARTERIAL BLOOD PRESSURE OF THE DOG BEFORE AND AFTER BLOCKADE OF THE ALPHA SYMPATHETIC RECEPTOR BY AZAPETINE (2 mg./kg.)

(Paper speed 0.5 mm. per second)

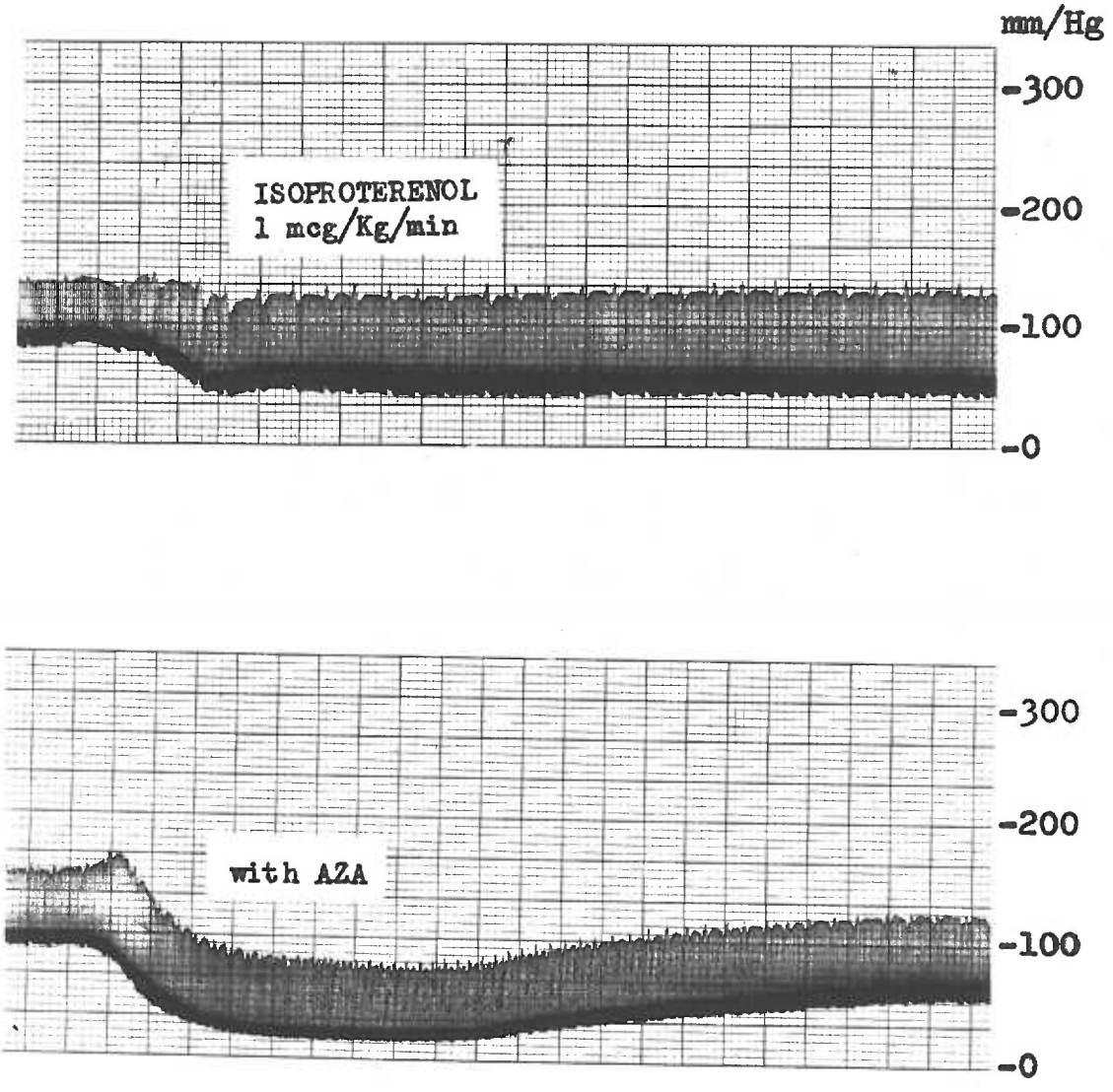

mm/Hg
-300
-200
-100
-0
ISOPROTERENOL
1 mcg/Kg/min
with AZA
-300
-200
-100
-0

Figure 6

FIGURE 7

CHEMICAL CONFIGURATION OF THE

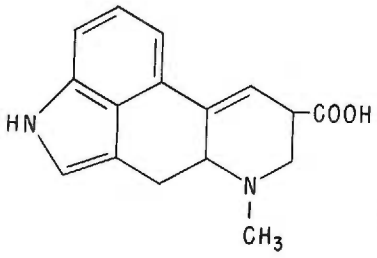
ALPHA AND BETA SYMPATHETIC

RECEPTOR BLOCKING AGENTS

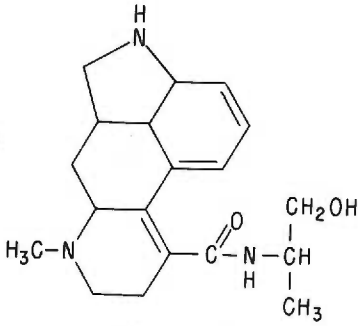
ADRENERGIC NERVOUS SYSTEM BLOCKING DRUGS

COMPOUNDS BLOCKING ALPHA RECEPTOR SITES

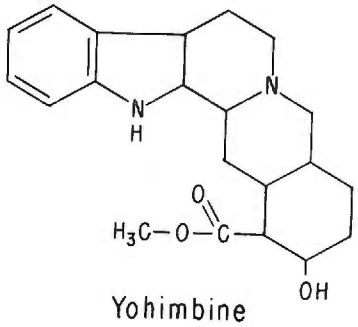
NATURAL COMPOUNDS



Lysergic Acid

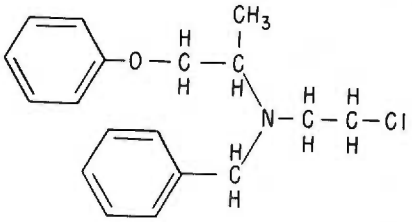


Ergonovine

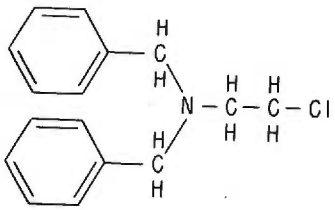


Yohimbine

β-HALOALKYLAMINES

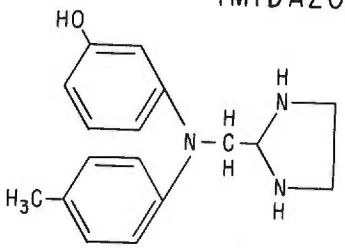


Phenoxybenzamine

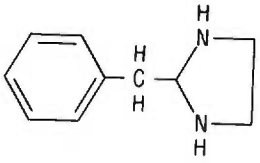


NN'-dibenzyl-β-chloroethylamine Cl

IMIDAZOLINES

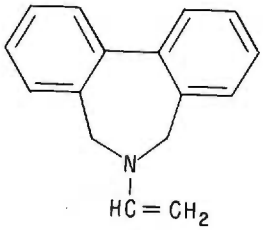


Phentolamine



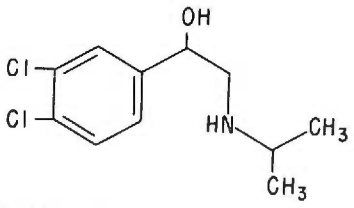
Tolazoline

DIBENZAZEPINE



Azapetine

COMPOUNDS BLOCKING BETA RECEPTOR SITES



Dichloroisoproterenol (DCI)

Figure 7

response of epinephrine or isoproterenol. This response was demonstrated in both dogs and cats in doses of DCI varying from 1 to 10 mg. per kilogram. For illustration, the effects of DCI on the blood pressure responses of epinephrine, nor-epinephrine, and isoproterenol are shown in Figure 8.

It is to be noted that changes in the epinephrine pressor responses after DCI administration are opposite to those which follow an alpha receptor blocking agent. (See Figures 4, and 8.) The alpha receptor blocking agent obliterates the pressor responses, while the beta receptor blocking agent effaces the depressor responses to the sympathomimetic amines. This is in accordance with Ahlquist's theory.

Other responses to sympathomimetic amines after pretreatment with DCI have been reported. Powell and Slater (39) also observed that the relaxation of the uterine muscle brought about by isoproterenol was either completely blocked or substantially reduced by DCI. They also showed that this beta receptor blocking compound completely blocked the inhibitory effect of epinephrine on rabbit intestinal strips. One other effect brought about by DCI may have some significance in the cardiovascular area. Moore and Swain (33) have demonstrated that DCI prevents ventricular fibrillation when epinephrine was administered after 1-phenyl-1-dimethylaminoethyl propriophenone hydrochloride. All of these responses have been discovered within the last three to four years.

In 1958, Moran and Perkins (35) showed that other compounds besides DCI possessed beta receptor blocking activity. They found that the dichloro analogues of epinephrine and nor-epinephrine have a similar

FIGURE 8

EFFECT OF EPINEPHRINE (5 mcg./kg./min.), NOR-EPINEPHRINE (20 mcg./kg./min.) AND ISOPRO-TERENOL (1 mcg./kg./min.) ON THE ARTERIAL BLOOD PRESSURE OF THE DOG BEFORE AND AFTER BLOCKADE OF THE BETA SYMPATHETIC RECEPTOR BY DICHLOROISOPROTERENOL (DCI) (10 mg./kg.)

(Paper speed 0.5 mm. per second)

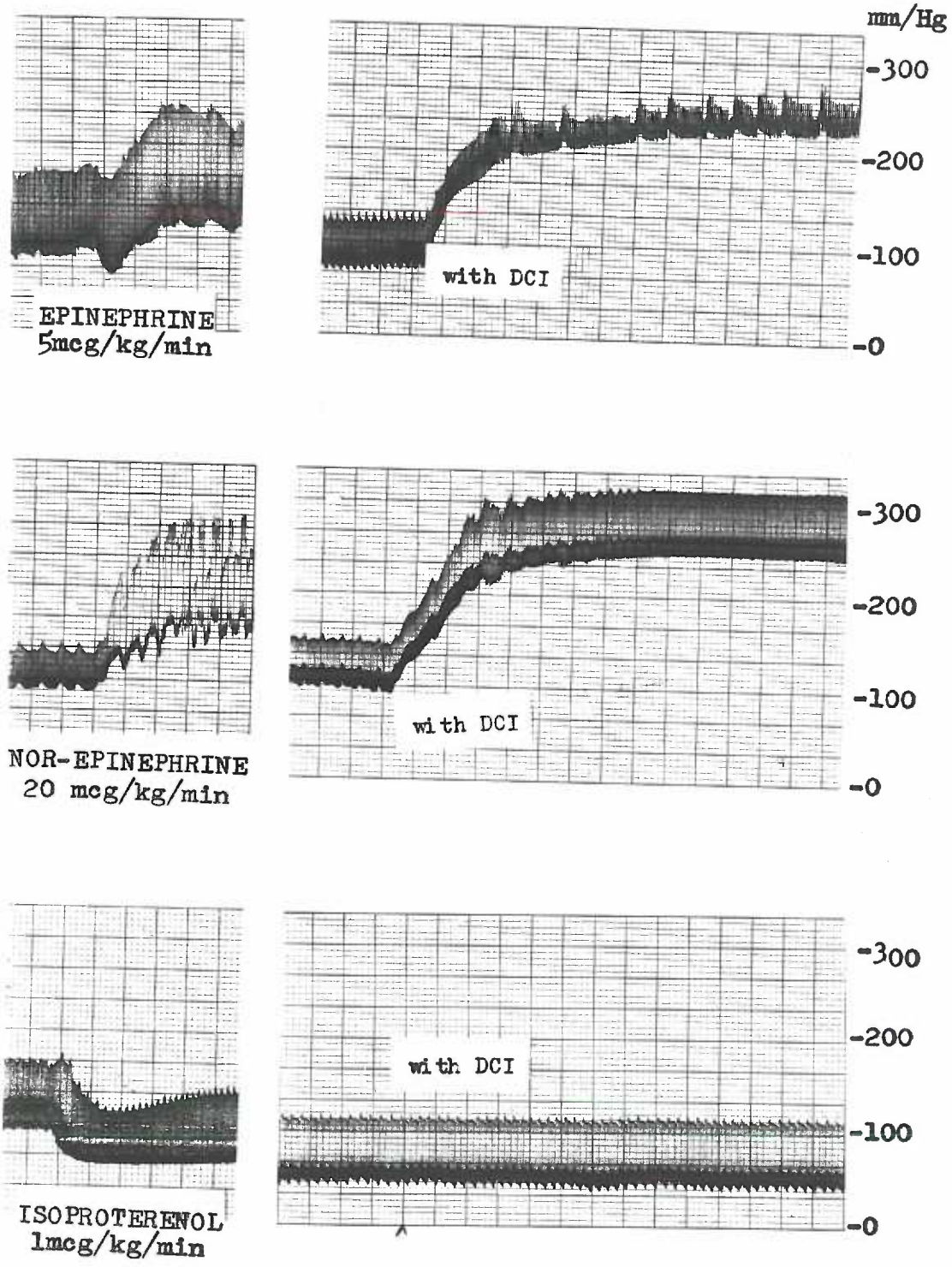

mm/Hg
-300
-200
-100
-0
EPINEPHRINE
5mcg/kg/min
with DCI
NOR-EPINEPHRINE
20 mcg/kg/min
with DCI
ISOPROTERENOL
1mcg/kg/min
with DCI

Figure 8

but less pronounced beta receptor blocking action than the isoproterenol analogue, DCI.

It was previously shown in Table 1 that the alpha receptor blocking agents produced varying effects on sympathomimetic amine-induced hyperglycemia. These variations may be due to a number of factors; the primary one being incomplete receptor blockade. Apparently, this occurred when only alpha receptor blocking agents were used. Therefore, it seemed to this author that more definite information as to the true effects of the alpha and beta receptors on sympathomimetic amine-induced hyperglycemia could be provided by using a beta receptor blocking agent. Accordingly, preliminary studies were undertaken by the author to determine the feasibility of this proposal. Using blood samples obtained from the femoral veins of dogs it was found that DCI consistently inhibited the hyperglycemia induced by epinephrine, nor-epinephrine or isoproterenol.

Mayer and Moran (28) have suggested a possible explanation for this inhibitory effect on DCI. They state that DCI, when given in doses of 7 mg. per kilogram prevents the activation of phosphorylase by epinephrine when the latter drug was infused at a rate of 1 mcg/Kg/min. Their proof stems from the fact that when epinephrine is infused intravenously at this dose for 15 minutes in dogs, the blood glucose level is elevated by 60 mg.per cent. After 7 mg/Kg of DCI the blood glucose response to the epinephrine infusion was reduced to 18 mg. per cent. These figures are in rough agreement with our preliminary study. The glucose values for each of these studies were obtained from venous samples. (29).

It was further shown that azapetine (an alpha receptor blocking agent) enhances the glycemic effect of the amines (31). It was felt that these two adrenergic blocking compounds could be used as tools in conjunction with epinephrine, nor-epinephrine and isoproterenol to reveal the true mechanism of action of sympathomimetic amine-induced hyperglycemia.

Since arterial blood samples might have a higher concentration of sugar, there is some doubt that the change elicited in the venous samples are representative of the changes in the arterial samples. The question thus arises: since phosphorylase activation probably plays a role in the hyperglycemic action of sympathomimetic amines, does dichloroisoproterenol (DCI) inhibit sympathomimetic amine-induced hyperglycemia and does azapetine potentiate the effect? If this were true then the responses produced would be mediated in the following way:

Sympathomimetic amine + azapetine:

action < alpha receptor blockade
beta receptor stimulation and activation

result < potentiated hyperglycemia

mechanism < increased hepatic output of blood sugar
decreased peripheral utilization of sugar
a combination of the two

Sympathomimetic amine + dichloroisoproterenol:

- action
  - beta receptor blockade
  - alpha receptor stimulation and activation
- result
  - inhibited hyperglycemia
- mechanism
  - decreased hepatic output of blood sugar
  - increased peripheral utilization of sugar
  - a combination of the two

The purpose of this research is, then, to determine the mechanism of action of sympathomimetic-asapetine potentiated hyperglycemia and of sympathomimetic-dichloroisoproterenol inhibited hyperglycemia. This then would lead to the answer of the question: to what degree do the alpha and beta receptors play in the production and utilization of blood sugar?

METHODS AND MATERIALS

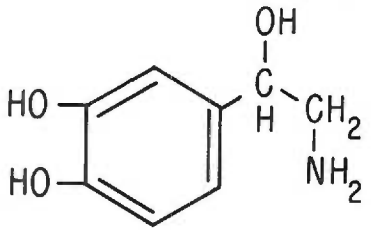
Forty-one male and female mongrel dogs weighing between 11 and 18 kilograms were used in this study. Each dog was fasted 18 to 24 hours before an experiment. The animals were anesthetized with pentobarbital sodium, 35 mg. per kilogram, intravenously. Supplemental doses were administered when needed. Each dog was used twice; once for the peripheral glucose utilization experiments and once for the hepatic glucose production experiments. The animals were allowed to rest, with water and food *ad libidum* in the interim of a week to ten days. After induction of anesthesia, the animals were tied in a supine position on a V-shaped dog board. The areas overlying the vessels to be catheteterized were shaved, washed and incised under non-sterile technique. After the first experiment had been completed the vessels were tied off, the area cleaned and the incision closed using 000 silk suture. The animals were then given an intramuscular injection of a tetracycline antibiotic and removed to the animal quarters.

To elucidate the mechanism of action of adrenergic blocking drugs in relation to glycogenic processes, epinephrine, nor-epinephrine or isoproterenol was infused intravenously at a constant rate. Similar experiments were run in the same dog after adrenergic blockade. The adrenergic blocking compounds used were azapetine (*alpha* adrenergic receptor blocking compound) and dichloroisoproterenol (*beta* adrenergic receptor blocking compound). The chemical configuration of the compounds are shown in Figure 9.

FIGURE 9

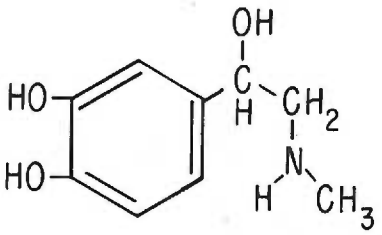
DOSES AND CHEMICAL CONFIGURATIONS OF THE SYMPATHOMIMETIC AMINES AND THE ADRENERGIC BLOCKING AGENTS USED IN THIS STUDY

Levarterenol



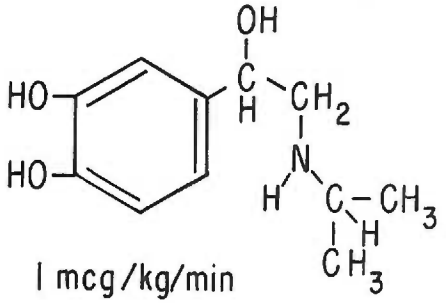
20 mcg/kg/min

Epinephrine



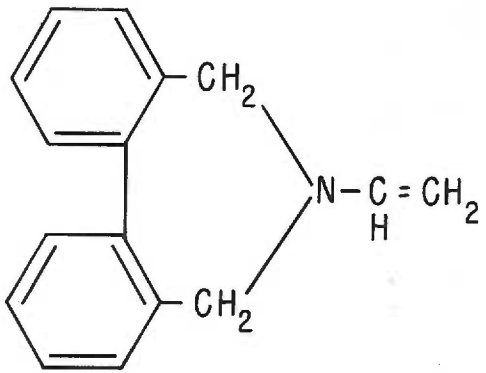
5 mcg/kg/min

Isoproterenol



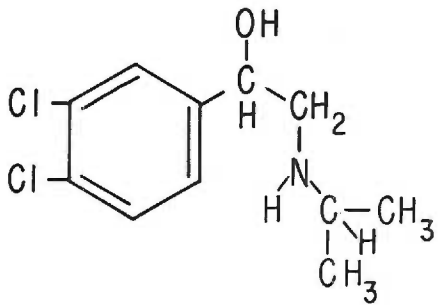
1 mcg/kg/min

Azapetine



2 mg/kg

DCI



15 mg/kg

Figure 9

Epinephrine, nor-epinephrine and isoproterenol were selected because these adrenergic agents may activate the alpha and beta receptors in the liver to different degrees. As previously mentioned, epinephrine activates both alpha and beta receptors, nor-epinephrine activates predominantly alpha receptors and isoproterenol activates predominantly beta receptors in relation to smooth muscle. It will be shown later how these compounds effect the hepatic receptors.

Table 3 shows the doses of azapetine and DCI which are effective when epinephrine, nor-epinephrine and isoproterenol are administered in the respective doses shown. The doses of the sympathomimetic amines were chosen primarily for their known effectiveness in producing an increase in blood sugar with secondary consideration to their effect on blood pressure. The variation in dosage for these three amines indicates that isoproterenol is an extremely potent hyperglycemic agent, epinephrine causes a moderate increase in the blood sugar while nor-epinephrine is a relatively poor hyperglycemic agent. These three naturally occurring adrenergic neurohumors were chosen for study because they exhibit their differences as hyperglycemic agents to a well defined and marked degree.

The dose of azapetine conforms to that used clinically. The dose of DCI is the same as that used by Moran and Perkins (34) who found that dosages between 7 and 15 mg. per kilogram lead to complete blockade of the beta receptors in the heart and smooth muscle.

In order to elucidate the hyperglycemic activity of sympathomimetic amines it was necessary to determine the production and utilization of carbohydrates under the experimental conditions. For determination of the

TABLE 3

DOSES AND RECEPTOR ACTIVITY OF

THE DRUGS USED IN THIS STUDY

TABLE 3

DRUG	DOSE	RECEPTOR ACTIVITY
Epinephrine	5 mcg./kg./min.	α-β stimulation
Nor-epinephrine	20 mcg./kg./min.	α stimulation
Isoproterenol	1 mcg./kg./min.	β stimulation
Azapetine	2 mg./kg.	α blockade
DCI	10 mg./kg.	β blockade

blood sugar uptake from muscles the blood samples were taken from the femoral artery and femoral vein of the left hind limb of the dog. For estimation of the amount of blood sugar production, samples were obtained from the hepatic vein and the aorta in the vicinity of the hepatic artery bifurcation. Also, the possibility existed that infusion of the adrenergic drugs might cause changes in the size of the blood vessels and this would have some effect on the blood sugar content in a particular sampling area. Thus, there arose the necessity for the measurement of blood flow in addition to blood sugar.

The amount of sugar entering the liver by way of the portal vein has been studied quite extensively by Sherlock (44). She states that under fasting conditions, the role played by the intestines in delivering carbohydrate to the liver is very small and that the difference between the blood sugar content found in the hepatic vein and in the hepatic artery can be taken to represent hepatic metabolism. Sherlock's work coincides with that of Bondy, James and Farrar (5). These workers kept their patients in a basal state in order to reduce the metabolism of the extra hepatic tissues and thus to achieve a close approximation of the activity of the liver itself. Since our animals were fasted from 18 to 24 hours, we assumed that there was little likelihood of any significant quantity of carbohydrate material entering the liver via the portal system from absorbed glucose. Therefore, we considered it unnecessary to determine the blood sugar content and the blood flow of the portal vein.

Determination of Peripheral Utilization of Blood Sugar

To determine the peripheral utilization of blood sugar, a left inguinal cut-down was performed on an anesthetized dog exposing both the femoral artery and vein. Following an intravenous injection of heparin sodium (2mg./kg.), the femoral artery was catheterized with a 4-inch piece of polyethylene tubing which approximated the diameter of the artery. Attached to the proximal end of the polyethylene tubing was a piece of rubber tubing, one inch in length, used to obtain blood samples for blood sugar determination and blood flow determination through the leg.

One cc. for blood sugar determination* was taken from both the femoral artery and vein at as nearly identical times as possible and coincident with a blood flow determination. The latter was carried out by allowing blood to drain into a glass graduate (marked in cubic centimeters) for five seconds. The blood flow per minute was calculated from this figure and finally the blood flow on a cc./kg./min. basis was computed. The blood that was removed from the animal for the blood flow determination was reintroduced into the left cephalic vein of the animal by way of a Murphy drip. The adrenergic blocking drug injections and sympathomimetic amine infusions were administered via the left external jugular vein. Figure 10 shows that set-up for the determination of the peripheral utilization of glucose from an infusion of a sympathomimetic amine.

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*See appendix.

FIGURE 10

METHOD OF PERIPHERAL BLOOD SUGAR
AND
BLOOD FLOW DETERMINATIONS

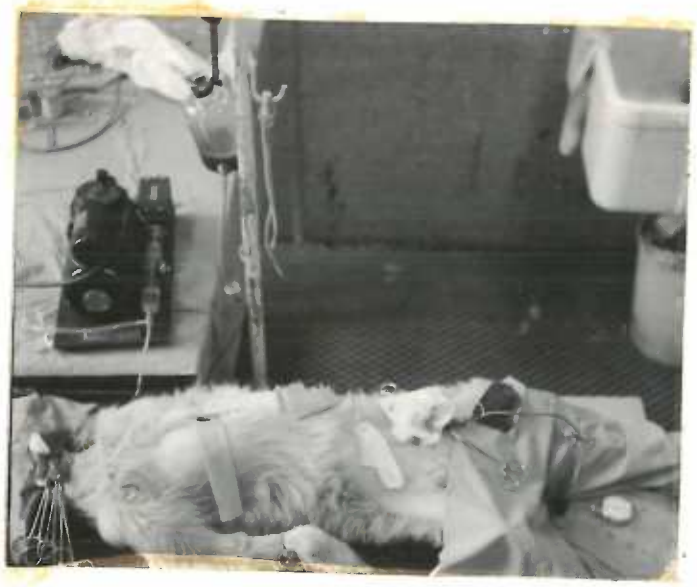

Figure 10

TABLE 3

DOSES AND RECEPTOR ACTIVITY OF
THE DRUGS USED IN THIS STUDY

Determination of Hepatic Production of Blood Sugar

The measurement of blood flow through the liver was necessary in order to determine the hepatic production of blood sugar. This was much more difficult technically than the determination of peripheral blood flow since the following procedures had to be undertaken:

1. Hepatic vein catheterization. This was performed by passing a radio-opaque French #10 Cournand type catheter under fluoroscopy into the right external jugular vein and down to the superior vena cava. The catheter tip was then pointed dorsally (44) and directed past the heart and into the inferior vena cava. It was then directed into the right hepatic vein. Three principal landmarks point to the accuracy with which the hepatic vein was catheterized:

a. Anatomically, two principal groups of vessels lead into the inferior vena cava; the hepatic veins and the renal veins. If, at the point where the catheter tip enters the inferior vena cava below the heart, the tip is directed to the right then slowly directed caudally, the first bifurcation that is met is that of the right hepatic vein. If the renal vessel is inadvertently catheterized, two visual points stand out under fluoroscopy: first, the catheter will be seen to move in a direction below the shadow of the diaphragm, and secondly, the catheter moves much further laterally, that is, the right hepatic vein does not extend as far laterally as does the right renal vein. Figure 11 shows a Cournand radio-opaque catheter inserted into the right hepatic vein.

The right hepatic vein was chosen for catheterization because it is the easiest of the hepatic veins to catheterize and is generally the

FIGURE 11

FLUOROSCOPIC CONFIRMATION OF POSITION OF COURNAND CATHETER FOR HEPATIC VENOUS BLOOD SAMPLES IN THE DOG

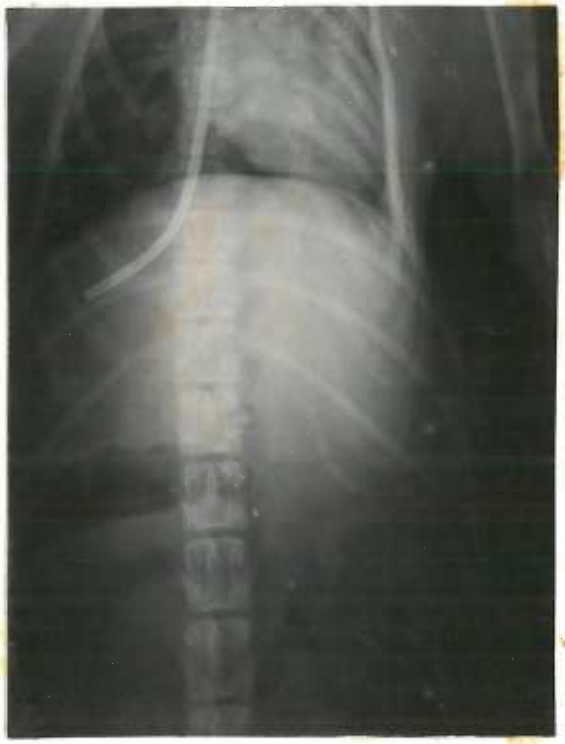

Figure 11

longest of the vessels. This latter point is of considerable importance when considering an infusion of a sympathomimetic amine. The drug-induced tachypnea and respiratory movements produced under these conditions has a tendency to move the catheter around in the vessel. If the tachypnea be severe, the catheter could be dislodged from the hepatic vein and would then lie in the inferior vena cava. Frequent fluoroscopic examinations were used to prevent such occurrences. It may also be mentioned that Shoemaker (45) found no significant difference between the right and the left hepatic venous blood flow in dogs.

b. The blood flow through the catheter does not occur at a steady regular pace but is affected by the respiration of the animal. During inspiration, the flow was much more rapid through the catheter than during expiration. If the hepatic vein were not catheterized or, if the right renal vein has been catheterized instead, this irregular blood flow through the catheter did not occur.

c. The results obtained were a definite indication of hepatic venous catheterization. The blood sugars obtained from the hepatic vein, when compared to simultaneously obtained hepatic arterial (see below) blood sugar samples are, quite naturally, higher in value. Also the dye (see below) which is removed solely by the liver would have a lesser concentration in the hepatic vein than in the arterial system. If a vessel other than the hepatic vein were catheterized, the concentrations of the dye in that vessel would be the same as the arterial dye concentration.

2. Hepatic artery catheterization. In order to obtain hepatic arterial blood samples, a second radio-opaque Cournand catheter was inserted into the right femoral artery. Under fluoroscopy the catheter was then directed rostrally to the area approximating the bifurcation of the hepatic artery and the aorta. It was felt that blood samples for blood sugar and dye determination obtained from this area were essentially equal to hepatic arterial blood samples.

Figure 12 demonstrates the complete set-up for hepatic blood sugar and blood flow determinations.

3. Dye technique. While bromsulfalein (BSP) has been one of the dyes used in determining blood flow through the liver, numerous authors have pointed out its inadequacies when used to measure the flow of blood through the liver. Cohn (9) found a 20 per cent disappearance BSP during the first two hours in post-hepatectomized dogs indicating a relatively large source of extra-hepatic removal of the dye. This would lead to incorrect determinations of hepatic blood flow. Shoemaker (45) lists the many errors of the BSP method in determining hepatic blood flow. Finally, Brauer, et al. (6) report that only 50 to 80 per cent of BSP is recoverable in the bile of normal dogs and that this excretion does not follow an exponential function.

Recently, a dye has been developed which allows great accuracy when used to measure hepatic blood flow. Indocyanine green (Cardio-Green^{R}*)

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*Cardio-Green^{R} is the trade name for indocyanine green made by Hynson, Westcott & Dunning, Inc. Baltimore, Maryland.

FIGURE 12

THE METHOD OF HEPATIC BLOOD SUGAR AND BLOOD FLOW DETERMINATION

(Figure 13) has been shown by Wheeler and associates (51) to be recovered to the extent of 97 per cent in the bile of dogs with chronic Thomas-type fistulas. They also observed that disappearance of the dye from the plasma follows a single exponential function. Rapaport (40) found no measurable extraction of the dye by the kidney, lungs or the extremities. Another very important advantage of indocyanine green over BSP is that hemolysis of the blood sample does not interfere with the spectrophotometric analysis of indocyanine green (36). This is because indocyanine green may be read in the spectrophotometer at its peak absorption of 800 millimicrons, that is, at the point where oxyhemoglobin and reduced hemoglobin transmit light equally.

Therefore, we decided to use indocyanine green for the determination of hepatic blood flow. A preliminary test carried out to obtain the average hepatic blood flow in the dog showed our results to be in close agreement with those in the literature (3) (45).

The technique of Banazak (3) was used for hepatic blood flow determination. This method utilizes a single intravenous injection of the dye followed by the collection of serial samples of blood from the efferent source (hepatic vein) and the afferent source (hepatic artery). Calculations for experimental hepatic blood flow are shown in Figure 14.

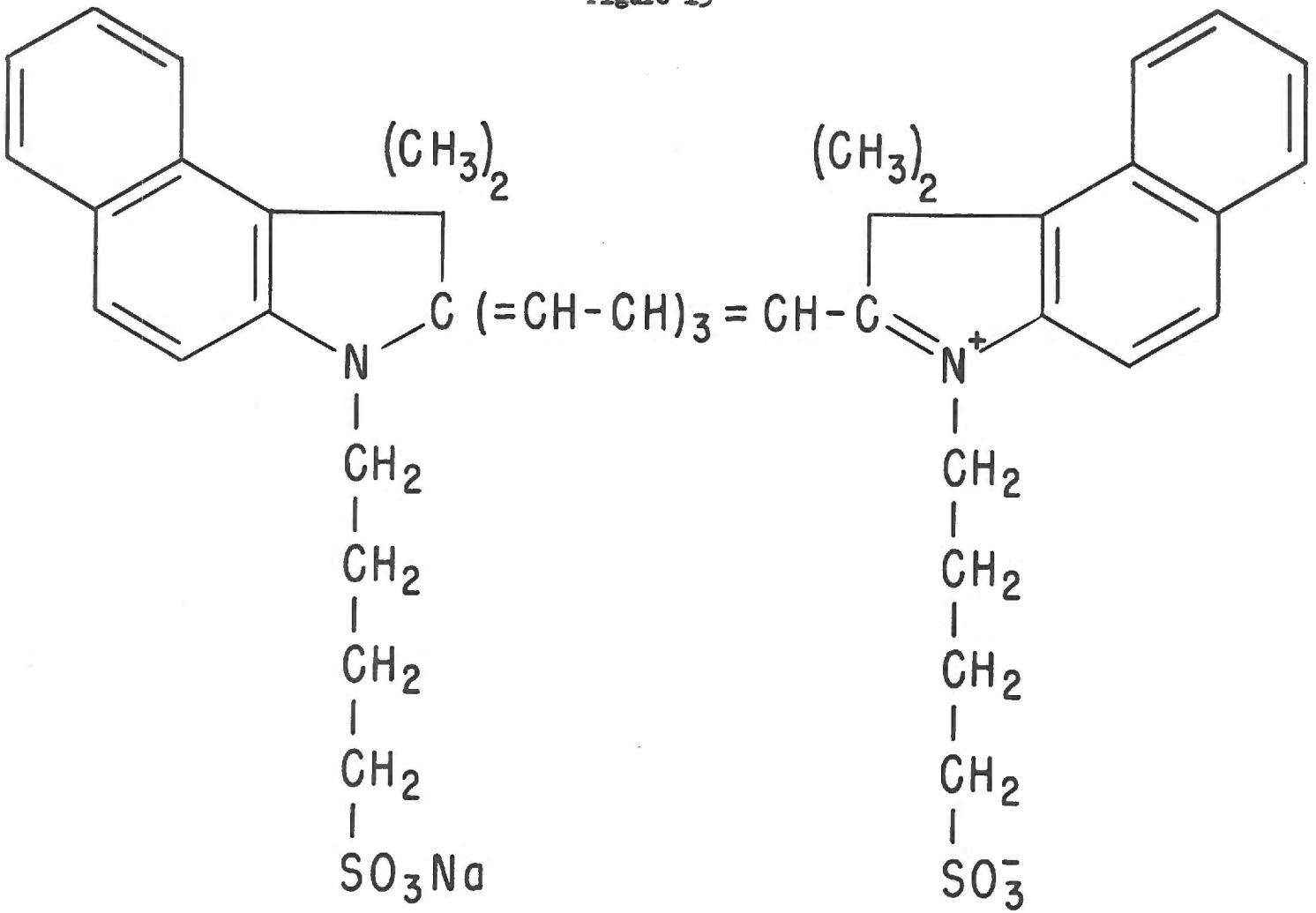
All the sympathomimetic amine infusions and all the adrenergic blocking agents were administered via either one of the external jugular veins. When a blocking agent was used it was administered not less than five nor more than ten minutes before the sympathomimetic amine infusion.

Figure 12

FIGURE 13

CHEMICAL STRUCTURE OF INDOCYANINE GREEN

Figure 13



Indocyanine Green

FIGURE 14

CALCULATIONS FOR EXPERIMENTAL HEPATIC BLOOD FLOW

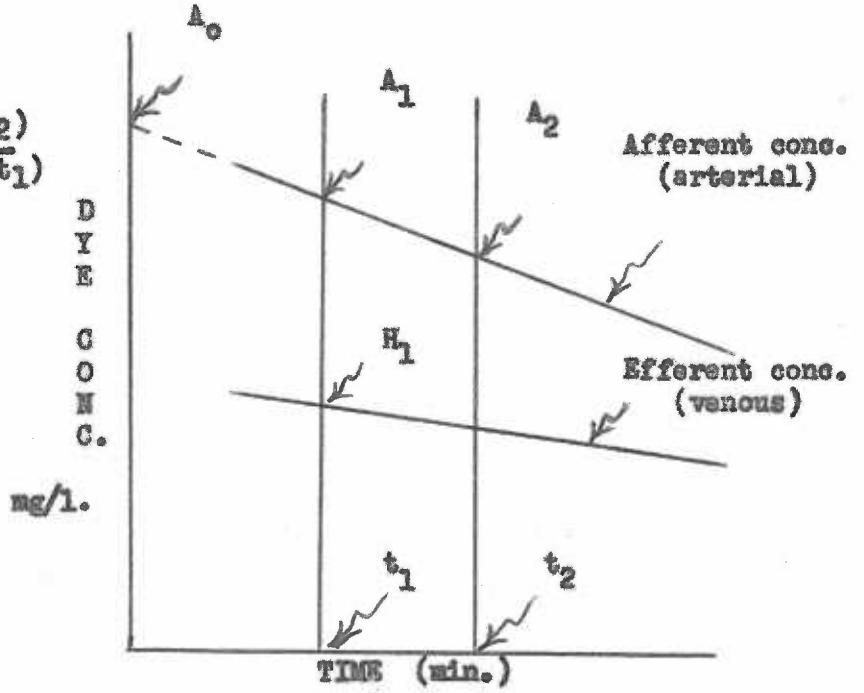
$$V_p = \frac{I}{A_o}$$

$$Cl = \frac{V_p \ (A_1 - A_2)}{A_1 \cdot A_2 \ (t_2 - t_1)}$$

$$E = \frac{(A_1 - H_1)}{A_1}$$

$$F_p = \frac{Cl}{E}$$

$$F_b = \frac{F_p}{1 - HCT}$$



V_p - plasma volume (l.)

I - amount of dye injected (mg.)

A_o - extrapolated concentration of dye at zero time (mg./l.)

Cl - clearance (l./min.)

A_1 - afferent concentration of dye at time t_1

A_2 - afferent concentration of dye at time t_2

E - extraction ratio

H_1 - efferent concentration of dye at time t_1

F_p - plasma flow (l./min.)

F_b - blood flow (l./min.)

HCT- hematocrit

Figure 14

Blood samples were taken at intervals of 1, 2, 4, 8, 16 and 32 minutes during the infusions. The samples for blood sugar determination were placed in seven cubic centimeters water in a test tube and assayed according to the method of Nelson (37). The blood samples for the dye determination were placed in small test tubes containing one evaporated drop of a solution of highly concentrated heparin anticoagulant ("Anticlot").

The Typical Protocol for an Experiment was as Follows:

A healthy male mongrel dog weighing 13.7 kg. was anesthetized with 35 mg./kg. of pentobarbital sodium, intravenously. The hepatic vein was catheterized via the right external jugular vein, care being taken not to wedge the catheter into the liver lobe. The area of the hepatic artery was catheterized via the right femoral artery. After catheterization, the animal was allowed to stabilize in a control state for one-half hour.

At the end of the stabilization period and also after fluoroscopic confirmation of the catheter positions and 2 mg./kg. heparin sodium, intravenously, five cc's of hepatic venous blood were taken for the hematocrit and for the spectrophotometic plasma-dye control analysis. Indocyanine-green (1 mg./kg.) was then injected into the femoral vein and blood samples (2-3cc's) were taken from the hepatic vein and hepatic artery at 2, 4, 6, and 8 minutes. This was done to determine the control blood flow through the liver. This procedure was carried out on all the animals to insure a liver blood flow within normal limits. At the ninth minute after the dye injection, the sympathomimetic amine (diluted to the

correct concentration with normal saline) was infused into the right femoral vein. In this case, epinephrine was infused at a rate of 5 mcg./kg./min. This material was diluted so that the infusion volume was 1 cc. per minute.

Again, samples of blood were obtained from the hepatic vein (1 cc. for blood sugar determination and 2-3 cc. for indocyanine-green determination) and from the hepatic artery (2-3 cc. for indocyanine-green determination). These samples were obtained at 1, 2, 4, 8, 16, and 32 minutes during the epinephrine infusion.

After completion of the experiment, the right external jugular vein and the right femoral artery were tied off with 000 silk suture and the incisions were closed using the same size suture. Tetracycline phosphate (250 mg.) was then given intramuscularly and the animal was removed to the animal quarters.

The blood sugar samples were assayed according to the method of Nelson (37)* and through the extrapolation from a standard curve of 60, 120, 180, 240, and 300 mg. per cent of dextrose (Figure 15) the concentration of blood sugar, in mg. per cent, was computed.

The blood samples (hepatic venous and hepatic artery) containing the indocyanine-green were treated as follows:

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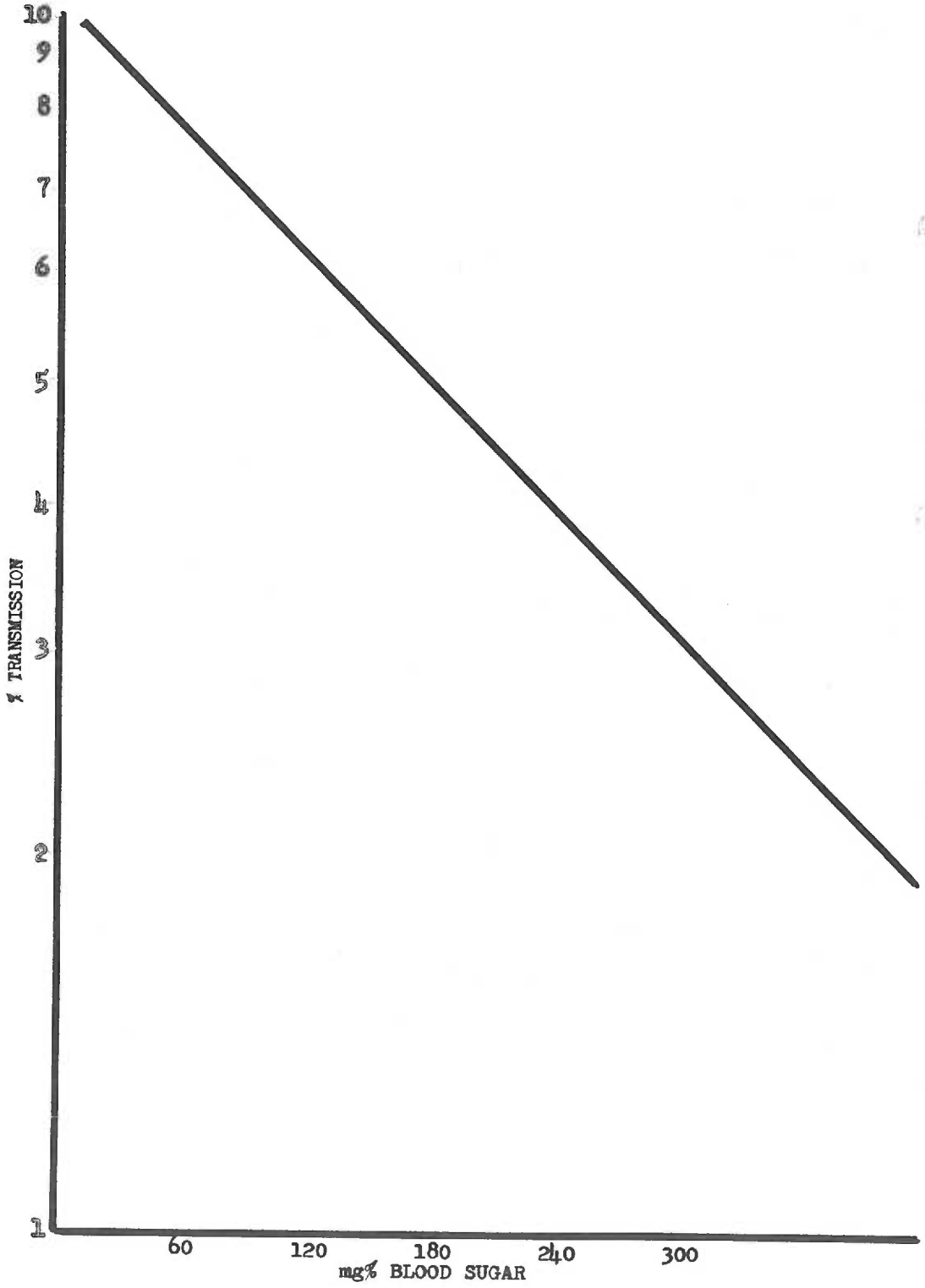
*See appendix.

FIGURE 15

STANDARD CURVE OF BLOOD SUGAR


10
9
8
7
6
5
4
3
2
1
% TRANSMISSION
60
120
180
240
300
mg% BLOOD SUGAR

1. Centrifugation of maximal speed (3600-3800 r.p.m.) in an Adams safety-head centrifuge.

2. One cc. of the plasma diluted to 6 cc. with physiological saline.

3. Optical density read in a Beckman DU Spectrophotometer at 800 mu.

These readings were then extrapolated from a standard curve made up of known solutions of indocyanine-green of 1.5, 3.0, 6.0 and 12.0 mg. pre liter (Figure 16).

To complete the experiment, this animal was used eight days later for the experiment concerning the peripheral utilization of glucose under the same conditions as previously described. The time element between this and other experiments varied from seven to ten days. This was done to make absolutely certain that any other previously administered sympathomimetic amine, adrenergic blocking agent or dye (indocyanine-green) was completely metabolized and excreted.*

After anesthetization and heparinization, the dog's left external jugular vein was exposed and isolated in preparation for the sympathomimetic amine infusion. In this case, the infusion solution was, again, epinephrine. The left femoral artery was catheterized and a slow infusion

---

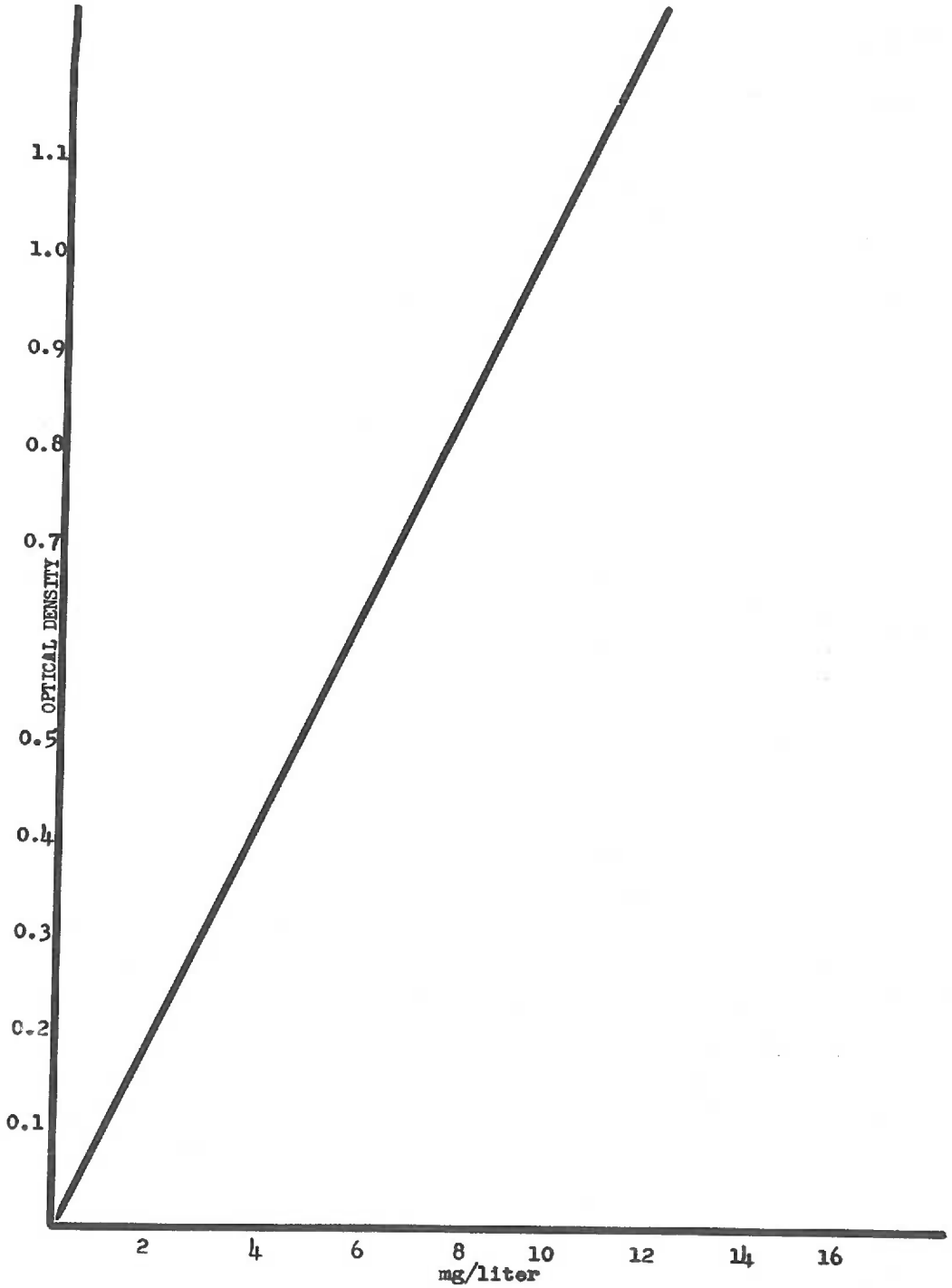
*Apparently this was ample time since the excretion rates for the compounds that were used are as follows:

1. Epinephrine	Less than 10 minutes	(15)
2. Nor-epinephrine	Less than 10 minutes	(15)
3. Isopropylarterenol	Less than 10 minutes	(15)
4. Dichloroisoproterenol	Less than 84 minutes	(30)
5. Azapetine	Less than 4 hours	(21)
6. Indocyanine green	Half life of 10 minutes	(17)

FIGURE 16

STANDARD CURVE OF

INDOCYANINE GREEN


OPTICAL DENSITY
1.1
1.0
0.9
0.8
0.7
0.5
0.4
0.3
0.2
0.1
2
4
6
8
10
12
14
16
mg/liter

of normal saline was started in the left cephalic vein via a Murphy drip. Femoral venous samples were obtained by direct puncture into and aspiration of blood from the vein.

The control femoral arterial blood flow was measured five times during a period of 25 minutes. The average was taken as direct blood flow. The blood, after each flow-test, was reinfused by way of the Murphy drip.

Control femoral arterial and femoral venous blood sugar samples were obtained and the sympathomimetic amine (epinephrine) was started.

Blood flows and blood sugars were taken at 1, 2, 4, 8, 16, and 32 minutes during the infusion, to correlate with the samples obtained in the hepatic production of glucose.

The following is a data sheet from an experiment using an infusion of epinephrine:

Studies Made: Peripheral and hepatic blood sugars and blood flows.

Animal : #15

Weight: 13.8 kg.

Sex: Male

Drugs: Epinephrine infusion (5 mcg./kg/min.)

Dose: 69.0 mcg./min.

Indocyanine green injection (1 mg/kg.)

Dose: 13.8 mg.

Blocking agent: none

Dose: none

The results of this one experiment are shown in the following tables and graph. Each experiment was tabulated in a similar manner.

Table 4 describes the hepatic vein, femoral artery and femoral vein blood sugar and blood flow values obtained from the experiment infusing epinephrine. The table shows both the spectrophotometric readings of indocyanine green and the colorimetric readings of the blood sugar samples as well as the actual extrapolated values.

Figure 17 is a graph depicting the change in the blood flow through the liver before and after the epinephrine infusion.

The actual calculations for the determination of hepatic blood flow is shown in Table 5.

FIGURE 17

EFFECT ON HEPATIC BLOOD FLOW

FROM AN INFUSION OF EPINEPHRINE

(5 mcg./kg./min.)

Figure 17

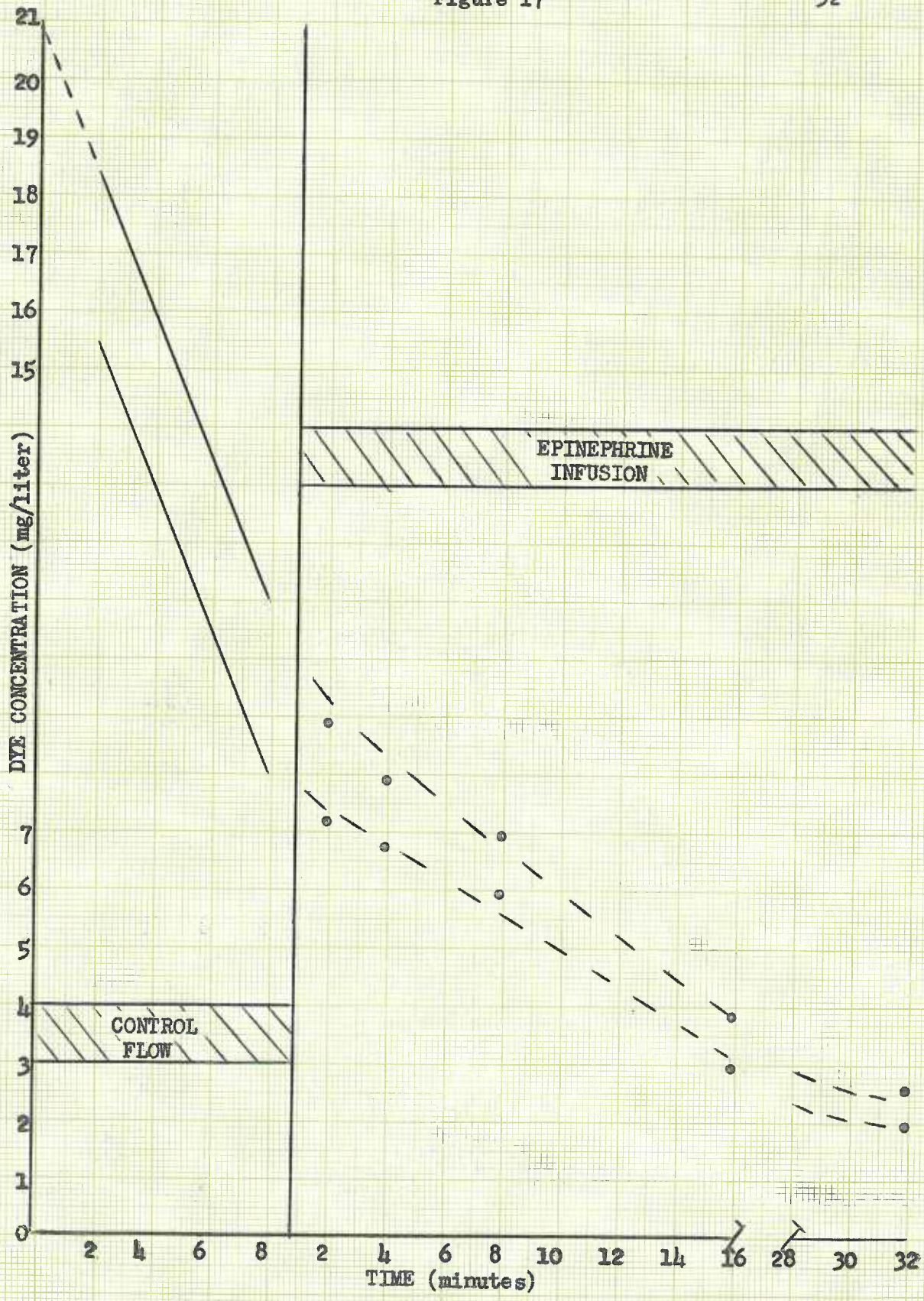

DYE CONCENTRATION (mg/liter)
21
20
19
18
17
16
15
7
6
5
4
3
2
1
0
EPINEPHRINE
INFUSION
CONTROL
FLOW
2
4
6
8
2
4
6
8
10
12
14
16
28
30
32
TIME (minutes)

TABLE 4

COMPLETE VALUES OBTAINED FROM AN EXPERIMENT

USING EPINEPHRINE

TABLE 4

TUBE NO.	TIME (MIN.)	% TRANS. HEP.VEIN	EXTRAP. VALUE (mg%)	% TRANS. FEM.ART.	EXTRAP. VALUE (mg%)	% TRANS. FEM.VEIN	EXTRAP. VALUE (mg%)
				CONTROL			
CONT	0	59.0	135	60.0	133	64.2	115
				EPINEPHRINE INFUSION			
1	1	58.0	136	57.2	144	68.2	100
2	2	50.0	178	54.3	157	63.5	117
3	4	43.6	222	50.8	173	64.2	115
4	8	37.5	257	47.5	193	58.0	136
5	16	25.0	350	35.7	260	54.0	158
6	32	27.0	342	31.6	290	44.0	210

TUBE NO.	TIME (MIN.)	EXTRAP.VENOUS VALUE (mg/l)	EXTRAP.ART. VALUE (mg/l)	HEPATIC BLOOD FLOW(cc/kg/min)	PERIPHERAL BLOOD FLOW (cc/kg/min)
			CONTROL		
1	2	15.55	18.35	40.4	25.5
2	4	13.10	15.90	-	26.2
3	6	10.60	13.40	-	25.8
4	8	8.10	11.00	-	25.4
			EPINEPHRINE INFUSION		
5	1	7.60	9.40	-	29.4
6	2	7.20	8.90	26.9	21.6
7	4	6.85	7.90	21.8	26.8
8	8	6.10	7.00	30.0	24.4
9	16	3.00	3.85	30.2	26.8
10	32	2.00	2.60	19.6	27.2

TABLE 5

CALCULATIONS FOR THE DETERMINATION OF HEPATIC BLOOD FLOW FROM AN EXPERIMENT USING EPINEPHRINE

TABLE 5

CONTROL HEPATIC BLOOD FLOW

$V_p = \frac{13.8 \text{ mg.}}{20.9 \text{ mg./liter}} = 0.662 \text{ liter}$

$Cl = \frac{0.662 \text{ liter } (2.45)}{34.24 \text{ min.}} = 0.0473 \text{ liter/min.}$

$E = \frac{2.80}{18.35} = 0.1526$

$F_p = 0.310 \text{ liter/min.}$

Blood Flow = 40.4 cc./kg./min.

BLOOD FLOW 2 min. AFTER EPINEPHRINE INFUSION

$Cl = \frac{0.662 \text{ liter}(1.05)}{17.74 \text{ min.}} = 0.0392 \text{ liter/min.}$

$E = \frac{1.80}{9.40} = 0.1915$

$F_p = 0.204 \text{ liter/min.}$

$F_b = 0.372 \text{ liter/min.}$

Blood Flow = 26.9 cc./kg./min.

BLOOD FLOW 4 min. AFTER EPINEPHRINE INFUSION

$Cl = \frac{0.662 \text{ liter } (0.65)}{16.06 \text{ min.}} = 0.0268 \text{ liter/min.}$

$E = \frac{1.35}{8.35} = 0.1619$

$F_p = 0.166 \text{ liter/min.}$

$F_b = 0.302 \text{ liter/min.}$

Blood Flow = 21.8 cc./kg./min.

TABLE 5

BLOOD FLOW 8 min. AFTER EPINEPHRINE INFUSION

$Cl = \frac{0.662 \text{ liter } (0.60)}{13.8 \text{ min.}} = 0.0288 \text{ liter/min.}$

$E = \frac{0.91}{7.20} = 0.1264$

$F_p = 0.227$ liter/min.

$F_b = 0.414$ liter/min.

Blood flow = 30.0 cc./kg./min.

BLOOD FLOW 16 min. AFTER EPINEPHRINE INFUSION

$Cl = \frac{0.662 \text{ liter } (0.55)}{7.94 \text{ min.}} = 0.0458 \text{ liter/min.}$

$E = \frac{0.85}{4.25} = 0.200$

$F_p = 0.229$ liter/min.

$F_b = 0.416$ liter/min.

Blood flow = 30.2 cc./kg./min.

BLOOD FLOW 32 min. AFTER EPINEPHRINE INFUSION

$Cl = \frac{0.662 \text{ liter } (0.30)}{6.10 \text{ min.}} = 0.0325 \text{ liter/min.}$

$E = \frac{0.70}{3.20} = 0.219$

$F_p = 0.149$ liter/min.

$F_b = 0.271$ liter/min.

Blood flow = 19.6 cc./kg./min.

The hepatic production and peripheral utilization of blood sugar from an experiment infusing epinephrine was determined as follows:

$$\text{Hepatic Production} = (\text{H.V.}-\text{H.A.})_{\text{mg/cc}} \times (\text{H.B.F.})_{\text{cc/kg/min}}$$

$$\text{Peripheral Utilization} = (\text{F.A.}-\text{F.V.})_{\text{mg/cc}} \times (\text{F.B.F.})_{\text{cc/kg/min}}$$

where:

H.V. = Hepatic Vein

H.A. = Hepatic Artery

F.A. = Femoral Artery

F.V. = Femoral Vein

H.B.F. = Hepatic Blood Flow

F.B.F. = Femoral Blood Flow

The foregoing experimental values were computed for hepatic production and peripheral utilization and may be seen in Tables 6 & 7.

The results are the effects of an infusion of epinephrine (5mg/kg/min) and were treated statistically with the results from epinephrine in conjunction with the blocking agents. The standard deviation of the hepatic production and peripheral utilization values were calculated and a "t" test calculated for significance*.

Each experiment was carried out in a similar manner; that is to say, each dog was tested for both the hepatic production and the peripheral utilization of sugar under the experimental conditions.

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*See appendix

TABLE 6

HEPATIC PRODUCTION OF BLOOD SUGAR

FROM AN EXPERIMENT USING

EPINEPHRINE

TABLE 7

PERIPHERAL UTILIZATION OF BLOOD

SUGAR FROM AN EXPERIMENT

USING EPINEPHRINE

TABLE 6

	HEP.VEIN (mg/cc)	HEP.ART. (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	HEPATIC OUTPUT OF BLOOD SUGAR (mg/kg/min)
CONTROL	1.35	1.33	0.02	40.4	0.808
16th MIN	3.50	2.60	0.90	30.2	27.180
32nd MIN	3.42	2.90	0.52	19.6	10.192

TABLE 7

	FEM.ART. (mg/cc)	FEM.VEIN (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	PERIPH. UPTAKE OF BLOOD SUGAR (mg/kg/min)
CONTROL	1.33	1.15	0.18	25.7	4.626
16th MIN	2.60	1.58	1.02	26.8	27.030
32nd MIN	2.90	2.10	0.80	27.2	21.760

RESULTS

Epinephrine, nor-epinephrine and isoproterenol may be expected to provoke different blood sugar responses both qualitatively and quantitatively. Therefore, the experimental results for each of these drugs are discussed separately before being compared. Likewise the individual results for liver and muscle glucose utilization are first described and then compared. Table 8 shows the type and number of experiments performed.

Due to the transient nature of the various responses obtained with epinephrine, nor-epinephrine and isoproterenol, these drugs were administered by constant intravenous infusion. Blood samples were taken at 1, 2, 4, 8 as well as at 16 and 32 minutes of the catechol amine infusion. The blood sugar values determined at the 16th and 32nd minute of the infusion were selected for detailed examination and analysis since, under the conditions of the experiment, the blood sugar responses may be presumed to be a constant process. With infusions of smaller doses of the catechol amines it was anticipated that the quantitative differences in blood sugar values would be lower in magnitude.

With respect to measurement of hepatic blood flows, Banazak (3) has shown that with the use of indocyanine green under controlled conditions in the anesthetised dog, the average blood flow is 42 cc./kg./min. Shoemaker (45) reports a similar value of 41 cc./kg./min. The mean hepatic blood flow obtained in the present study (mean value of 41 dogs) was 42.7 ± 2.89 cc./kg./min. (Table 9).

TABLE 8

SUMMARY OF THE TYPE AND NUMBER OF EXPERIMENTS

CARRIED OUT IN THIS STUDY

TABLE 8

SUMMARY OF THE TYPE AND NUMBER OF EXPERIMENTS CARRIED OUT IN ORDER TO COMPARE THE EFFECTS OF ADRENOLYTIC AGENTS ON THE HEPATIC PRODUCTION AND PERIPHERAL UTILIZATION OF BLOOD SUGAR FROM EPINEPHRINE, NOR-EPINEPHRINE AND ISOPROTERENOL

HEPATIC PRODUCTION OF BLOOD SUGAR NO. OF EXPERIMENTS	COMPOUND INFUSED PLUS BLOCKING AGENT	PERIPHERAL UTILIZATION OF BLOOD SUGAR NO. OF EXPERIMENTS
5	Epinephrine	5
5	Epinephrine & Azapetine	5
5	Epinephrine & DCI	5
3	Nor-epinephrine	3
3	Nor-epinephrine & Azapetine	3
3	Nor-epinephrine & DCI	3
3	Isoproterenol	3
3	Isoproterenol & Azapetine	3
3	Isoproterenol & DCI	3
2	Saline	2
3	Saline & Azapetine	3
3	Saline & DCI	3
TOTAL 41		TOTAL 41

This is well within the limits for the normal hepatic blood flow reported by other workers.

In our studies the mean hepatic output of blood sugar under controlled conditions and with saline infusion was found to be 1.202 ± 0.088 mg./kg./min.*

EPINEPHRINE

A. Hepatic Production of Blood Sugar

The intravenous infusion of epinephrine (5 mcg./kg./min.) after 16 minutes produced a vasoconstriction of the hepatic vessels as is evidenced by the diminution of the hepatic blood flow from 42.7 cc./kg./min. to 25.6 cc./kg./min. (Table 10). This decrease is in agreement with Grab (19) and also Dobsen and Jones (14). The latter workers state that when large doses of epinephrine are given, which elevate the systemic blood pressure, the blood flow through the liver is greatly reduced. However, as shown by Sherlock (4) very small doses of epinephrine (0.20 mcg./kg./min.) infused intravenously in human subjects increases the hepatic blood flow by as much as 33 per cent. These results, using either large or small doses of epinephrine, may be explained by the alpha-beta receptor theory of Ahlquist (1). According to this theory, the beta response is brought

*All values of blood sugar production or blood sugar utilization are reported on a per kilogram of body weight basis. Complete tabulation of all blood sugar determinations may be found in the appendix.

TABLE 9

THE MEAN AND STANDARD DEVIATION OF EXPERIMENTAL HEPATIC BLOOD FLOWS IN FORTY-ONE DOGS

TABLE 9

CANINE HEPATIC BLOOD FLOWS (cc./kg./min.)

44.3	45.0	43.7	38.9	44.8
43.0	43.6	42.7	40.0	40.2
42.3	48.8	41.9	41.0	48.5
42.0	39.8	45.7	41.0	42.4
40.6	42.0	44.0	48.6	41.6
33.7	43.8	41.8	39.0	44.0
42.4	46.0	42.8	40.2	48.0
39.1	43.0	40.6	42.4	43.0
				43.6

MEAN	S.D.
42.7	±2.89

about by very small doses of epinephrine which produce a vasodilation and a subsequent increase in blood flow. As the quantity of epinephrine is increased the vasodilation is replaced by a vasoconstriction due to the drug's now more prominent action on the alpha receptors. This, then, decreases the blood flow.

Epinephrine infused at a rate of 5 mcg./kg./min. in anesthetized dogs regularly produces hyperglycemia. This is believed to be mediated by the action of epinephrine on the enzyme phosphorylase, whose activity increases and brings about glycogenolysis (31) (48)(49). Our results after 16 minutes of an infusion of this dose of epinephrine are shown in Table 10. Although the blood flow through the liver was decreased by almost 38 per cent of the control value, there was an increase in the hepatic venous-hepatic arterial blood sugar difference by a factor greater than ten. The net result was an increase in hepatic blood sugar output from 1.202 $\pm$ 0.088 mg./kg./min. to 8.960 $\pm$ 1.080 mg./kg./min. Further, the direct effect of epinephrine did not change the hepatic blood flow to any great extent from the 16th to the 32nd minute of the infusion (Tables 10 and 11). This presumes that there was maximal vasoconstriction of the hepatic vessels under these conditions. Also, comparison of the hepatic blood sugar output for the 16th minute with that of the 32nd minute, shows the difference to be negligible and not statistically significant*.

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*Unless otherwise indicated, the use in this section of the term significant for the results is limited to a probability of 0.05 (5 per cent level) or greater. See appendix for complete tabulation of statistical analyses.

TABLE 10

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 16th MINUTE OF AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 10

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 16th MINUTE OF AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	HEPATIC VEIN BLOOD SUGAR (mg/cc)	HEPATIC ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	HEPATIC OUTPUT OF BLOOD SUGAR** (mg/kg/min)	
CONTROL	1.328	1.302	0.026	42.4	1.202±0.088	
EPI	2.880	2.530	0.350	25.6	8.960±1.080	
DCI	1.360	1.195	0.165	40.8	6.732	6.168±0.301
EPI-DCI	2.712	2.112	0.600	21.5	12.900	
AZA	1.323	1.265	0.058	37.3	2.163	15.623±1.680
EPI-AZA	3.090	2.642	0.448	39.7	17.786	

*Mean values of four experiments (See appendix)

**Hepatic output = $\left[(HV-HA)_{mg/cc} \times HBF_{cc/kg/min}\right]$

where: HV = hepatic vein
HA = hepatic artery
HBF = hepatic blood flow

EPI = Epinephrine
DCI = Dichloroisoproterenol
AZA = Azapetine

TABLE 11

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 32nd MINUTE OF AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 11

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 32nd MINUTE OF AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	HEPATIC VEIN BLOOD SUGAR (mg/cc)	HEPATIC ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	HEPATIC OUTPUT OF BLOOD SUGAR** (mg/kg/min)	
CONTROL	1.328	1.302	0.026	42.4	1.202±0.088	
EPI	3.255	2.900	0.355	24.8	8.804±0.940	
DCI	1.420	1.200	0.220	46.0	10.120	6.580±0.873
EPI-DCI	3.086	2.472	0.614	27.2	16.700	
AZA	1.320	1.245	0.075	39.6	2.970	38.180±2.390
EPI-AZA	4.150	3.327	0.823	50.0	41.150	

*Mean values of four experiments (See appendix)

**Hepatic output = $\left[(HV-HA)_{mg/cc} \times (HBF)_{cc/kg/min}\right]$

where: HV= hepatic vein
HA= hepatic artery
HBF= hepatic blood flow

EPI = Epinephrine
DCI = Dichloroisoproterenol
AZA = Azapetine

Apparently, the epinephrine-induced hyperglycemia and hepatic blood flow have reached their maximum effect at least by the 16th minute of the infusion.

It is hypothesized that sympathomimetic amine-induced hyperglycemia is mediated through the beta receptors. Therefore, when epinephrine is infused after dichloroisoproterenol (a beta receptor blocking agent) there should be an inhibition in the induced hyperglycemia. As shown in Tables 10 and 11 such was the case in our studies. It is also important to note that DCI, per se, caused a slight hyperglycemia as seen by the differences in the hepatic A-V blood sugar level concentrations. This is in agreement with the recent work (1961) of Root (41) who stated that a slight transient hyperglycemia occurs in dogs when DCI is given in doses ranging from 2 to 5 mg./kg. Therefore, the true effect - solely due to epinephrine with beta receptor blockade - would be the difference between the hyperglycemia produced by dichloroisoproterenol and that produced by the combination of epinephrine and dichloroisoproterenol.

The hepatic output of glucose in both the 16th and 32nd minute samples of the epinephrine infusion in conjunction with beta receptor blockade were less than those with epinephrine alone at similar times. The difference in mean hepatic blood sugar output at the 16th minute of the epinephrine injusion (8.960 $\pm$ 1.080 mg./kg./min.) and of epinephrine with beta receptor blockade (6.168 $\pm$ 0.301 mg./kg./min.) is significant. Also, at 32 minutes of the infusion, a similar inhibition

occurs (8.804 ± 0.940 mg./kg./min. as compared to 6.580 ± 0.873 mg./kg./min.). That is, the inhibition that DCI affords to epinephrine-induced hyperglycemia is a true blockade of the effect. Further, when comparing the 16th minute sample to the 32nd minute sample of epinephrine with beta receptor blockade, the results indicate there is no significant change in the hepatic output of blood sugar. The same is true for epinephrine without blockade. Therefore, DCI inhibits the hepatic output of blood sugar from epinephrine infusion to a similar degree at the 16th and 32nd minute.

At the present time dichloroisoproterenol is the only compound to be classified as a beta receptor blocking agent. Since DCI also acts to inhibit epinephrine-induced hyperglycemia, it was felt that azapetine, classified as an alpha receptor blocking agent, should act in opposition to DCI and produce, in fact, a potentiation in the hyperglycemia when used in conjunction with epinephrine. When the hepatic output of sugar was determined at the 16th minute of epinephrine infusion, the value of 8.960 ± 1.080 mg./kg./min. was obtained. With epinephrine plus alpha receptor blockade the hepatic output of sugar was 15.623 ± 1.680 mg./kg./min. These results indicate a highly significant potentiation when an alpha receptor blocking agent was used. The same degree of significance also occurred for the 32nd minute sample (8.804 ± 0.940 mg./kg./min. as compared to 38.180 ± 2.390 mg./kg./min., respectively) (Tables 10 and 11).

In contradistinction to the observation that between the 16th

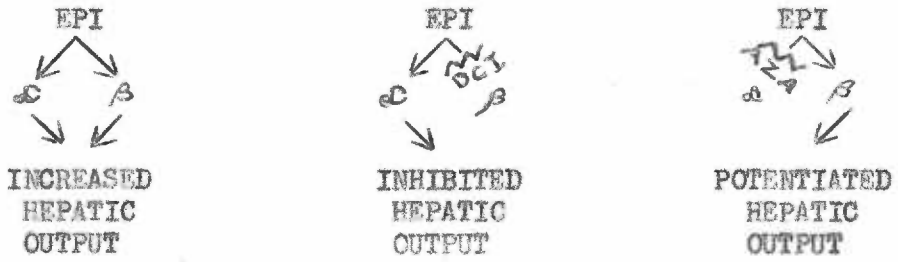
and the 32nd minute of the infusion there was no appreciable change in hepatic output of blood sugar either with epinephrine alone or with epinephrine used in combination with the beta receptor blocking agent, DCI. There was a significant change between the 16th and 32nd minute sample with epinephrine plus azapetine, the alpha receptor blocking agent. As seen in Tables 10 and 11, the glucose output increased from 15.623 ± 1.680 mg./kg./min. in the 16th minute sample to 38.180 ± 2.390 mg./kg./min. in the 32nd minute sample. This increase may be attributed not only to the fact that a much larger flow of blood was traversing the liver at this time but also a much greater hepatic A-V difference occurred indicating a high degree of glycogenolysis.

The results indicate, therefore, that blockade of the beta adrenergic receptor in the liver by DCI (permitting unhindered activity of the alpha receptors) produce a significant inhibition in the induced hepatic output of blood sugar when an infusion of 5 mcg./kg./min. of epinephrine was used. On the other hand when blockade of the alpha adrenergic receptors in the liver are effected by azapetine (permitting unhindered beta receptor activity) there was produced, under an equivalent infusion of epinephrine, a significant potentiation in the induced hepatic output of blood sugar. On this basis it would appear that the beta receptors present in the liver are predominantly responsible for epinephrine-induced hyperglycemia. However, the alpha receptors cannot be ruled out altogether in producing an increased hepatic output of blood sugar. For when the alpha receptors were permitted to act unhindered under the influence of epinephrine and DCI a slight hyperglycemia still occurred.

This hyperglycemia was less than that produced by epinephrine alone but significantly more than that produced under control conditions. Table 12 summarizes the hepatic production of blood sugar from an infusion of epinephrine before and after adrenergic blockade.

The results discussed above may be shown more clearly in the graphic presentation in Figure 18. The large degree of potentiated hepatic output of blood sugar is to be noted when azapetine was used before epinephrine. Also, an interesting fact noted in the graph is the steady, uniform inhibition by DCI in epinephrine-induced hyperglycemia.

The following schema represents the over-all effect of epinephrine activation and adrenolytic blockade of sympathetic receptors in the liver with the corresponding results.



B. Peripheral Utilization of Blood Sugar

The rate and volume of blood flow through the femoral artery and vein may be said to be equal. This was determined in the following ways:

1. The mean value of five experiments using infusions of epinephrine showed no significant difference between the femoral arterial and femoral venous blood flow*.

---

*See appendix

TABLE 12

SUMMARY OF HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 16th AND 32nd MINUTE OF AN INFUSION OF EPINEPHRINE BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 12

SUMMARY OF HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 16th AND 32nd MINUTE OF AN INFUSION OF EPINEPHRINE BEFORE AND AFTER ADRENERGIC BLOCKADE*

	CONTROL	EPINEPHRINE	EPINEPHRINE-BETA RECEPTOR BLOCKADE	EPINEPHRINE-ALPHA RECEPTOR BLOCKADE
16MINUTE HEPATIC OUTPUT OF BLOOD SUGAR	1.202 ±0.088	8.960 ±1.080	6.168±0.301 p 0.01	15.623±1.680 p 0.01
32MINUTE HEPATIC OUTPUT OF BLOOD SUGAR	1.202 ±0.088	8.804 ±0.940	6.580±0.873 p 0.02	38.180±2.390 p 0.001

*All values in mg./kg./min.

FIGURE 18

HEPATIC PRODUCTION OF BLOOD SUGAR FROM AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

HEPATIC PRODUCTION OF BLOOD SUGAR FROM AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

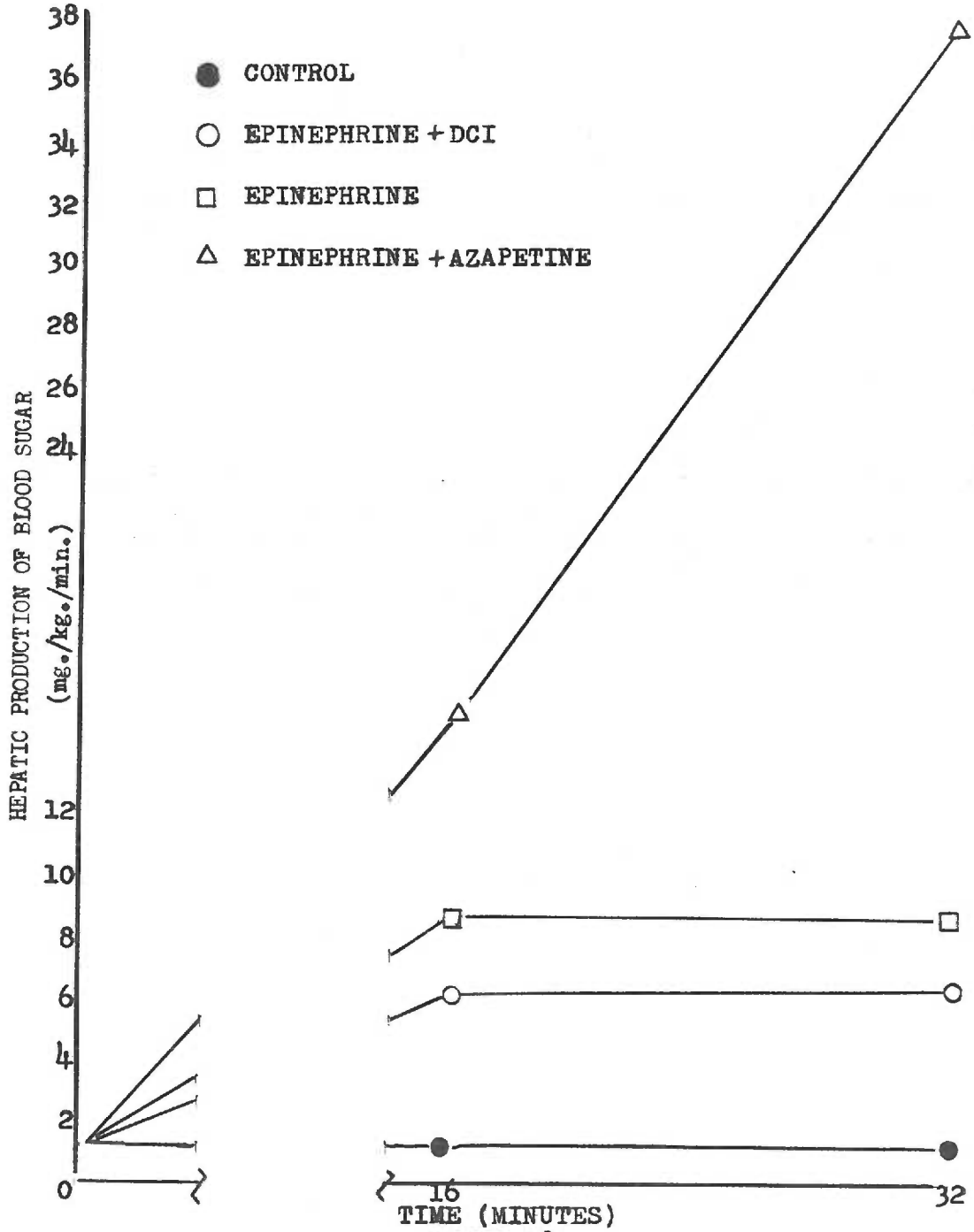


Figure 18

2. If any great difference had existed between the two flows, either an increase in the volume (i.e., a swelling) or a diminution in the volume of the hind leg would have occurred. Although not measured plethysmographically, neither of these changes appeared to occur under the experimental conditions. The results of the peripheral utilization of blood sugar for the 16th minute and 32nd minute of the epinephrine infusion are shown in Tables 13 and 14, respectively.

An infusion of 5 mcg./kg./min. of epinephrine was found to increase the blood flow through the hind limb of the dog from 23.0 cc./kg./min. to 26.0 cc./kg./min. for the 16th minute and from 23.0 cc./kg./min. to 27.0 cc./kg./min. on the 32nd minute. This is in agreement with Duff and Swan (16) and with Allen, Barcroft and Edholm (2) who found, independently, that epinephrine causes a significant increase in the blood flow through the arm and leg of the human. Also, these results indicate that in both the 16th and 32nd minute samples there was an increase in peripheral utilization of blood sugar from the epinephrine infusion by a factor roughly of five (Tables 13 and 14).

When compared to the effects of epinephrine alone, the use of epinephrine with a beta receptor blocker led to an inhibition of the hepatic output of sugar. The same was true with respect to the peripheral utilization of blood sugar. These results are shown in Tables 13 and 14. The peripheral utilization of blood sugar was 20.490 ± 1.084 mg./kg./min. at the 16th minute of the epinephrine infusion alone. With the combination of epinephrine and DCI (beta

TABLE 13

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 16th MINUTE OF AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 13

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 16th MINUTE OF AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	FEMORAL VEIN BLOOD SUGAR (mg/cc)	FEMORAL ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	PERIPHERAL UPTAKE OF BLOOD SUGAR** (mg/kg/min)	
CONTROL	1.117	1.302	0.185	23.0	4.260±0.184	
EPI	1.742	2.530	0.788	26.0	20.490±1.084	
DCI	1.122	1.195	0.073	19.4	1.146	16.792±1.282
EPI-DCI	1.487	2.112	0.625	28.7	17.938	
AZA	1.120	1.265	0.145	19.7	2.866	
EPI-AZA	1.880	2.642	0.762	17.7	13.496	10.630±0.543

*Mean values of four experiments (See appendix)

**Peripheral uptake = $\left[(FA-FV)_{mg/cc} \times (FBF)_{cc/kg/min}\right]$

where: FA = femoral artery
FV = femoral vein
FBF = femoral blood flow

EPI = Epinephrine
DCI = Dichloroisoproterenol
AZA = Azapetine

TABLE 14

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 32nd MINUTE OF AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 14

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 32nd MINUTE OF AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	FEMORAL VEIN BLOOD SUGAR (mg/cc)	FEMORAL ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	PERIPHERAL UPTAKE OF BLOOD SUGAR** (mg/kg/min)	
CONTROL	1.117	1.302	0.185	23.0	4.260±0.18#	
EPI	1.952	2.900	0.948	27.0	25.596±2.141	
DCI	1.139	1.200	0.061	20.3	1.243	25.690±1.904
EPI-DCI	1.527	2.472	0.954	28.5	26.933	
AZA	1.130	1.245	0.115	20.9	2.404	20.295±1.382
EPI-AZA	2.120	3.327	1.207	18.8	22.692	

*Mean values of four experiments (See appendix)

**Peripheral uptake = $\left[(FA-FV)_{mg/cc} \times (FBF)_{cc/kg/min}\right]$

where: FA = femoral artery
FV = femoral vein
FBF = femoral blood flow

EPI = Epinephrine
DCI = Dichloroisoproterenol
AZA = Azapetine

receptor blockade) the peripheral utilization of blood sugar was decreased to 16.792 ± 1.282 mg./kg./min. This decrease, at the one per cent level of probability, was significant. However, in the 32nd minute sample no change in the peripheral utilization of glucose occurred. For epinephrine alone it was 25.596 ± 2.141 mg./kg./min. With epinephrine and the beta receptor blocker, DCI, it was 25.690 ± 1.904 mg./kg./min. This would suggest that the blockade of the peripheral glucose receptors by DCI is short lasting so that by the 32nd minute the blockade has all but disappeared.

When the alpha receptor blocking agent, azapetine, was used prior to epinephrine it was presumed that potentiation of the peripheral utilization of blood sugar would occur. This presumption was based on the previously mentioned studies where the use of azapetine with epinephrine led to increased hepatic production of blood sugar. The data in Tables 13 and 14 disproves this. Instead, azapetine caused inhibition of the peripheral utilization of glucose. This effect was greater than the inhibition produced by DCI and epinephrine at the 16th minute period and about equal to that at the 32nd minute determination time.

In order to consider the role played by the various biochemical processes concerned with the peripheral utilization of blood sugar several propositions are reviewed. First, is it possible that no adrenergic receptors exist in the muscle to participate in glucose uptake and activity? This seems unlikely since our studies showed a definite and significant inhibition in the peripheral utilization of

blood sugar when the two adrenergic blocking agents, dichloro-isoproterenol and azapetine, were used with epinephrine.

Second, is the effect of blood sugar utilization dependent solely upon the flow of blood through the muscle? Again, this does not seem to be the case, as shown by the results in Tables 13 and 14. With azapetine and epinephrine the flow decreased from 27.0 cc./kg./min. to 18.8 cc./kg./min. But when DCI was used with epinephrine the flow increased from 27.0 cc./kg./min. to 28.5 cc./kg./min. Apparently, while alterations may occur in the blood flow when these drugs are given, the blood sugar is not changed in the same direction.

Third, do both the alpha and beta peripheral receptors responsible for glucose activity work in the same direction? This seems to be true. When each of the blocking agents was used with epinephrine peripheral utilization of glucose was less than when epinephrine was used alone. The purpose of the peripheral glucose adrenergic receptors may be to maintain a high rate of glucose utilization in the muscle. Our studies demonstrated that when either receptor is blocked a decrease in muscle metabolism occurs as evidenced by the decreased peripheral utilization of blood sugar.

Table 15 illustrates the changes in the peripheral utilization of blood sugar under various experimental conditions at the 16th and the 32nd minute when epinephrine and adrenergic blocking drugs are used. These results are shown graphically in Figure 19.

The results of this study concerned with the peripheral utilization of sugar reveal that no matter which peripheral adrenergic

receptor (alpha or beta) is blocked in the muscle, a significant inhibition of blood sugar utilization occurs at the 16th minute of the epinephrine infusion. These results are the converse of those obtained when epinephrine is used alone. However, it appears that the intensity of action of glucose receptor blockade begins to break down after 32 minutes as shown in Table 15 and Figure 19. The blood sugar values for epinephrine in conjunction with either blocking agent approach the value for epinephrine alone for the 32nd minute samples. This may indicate the actual length of receptor blockade by the drugs or it may indicate "receptor tolerance" (i.e., a tachyphylaxis due to continual bombardment of the receptors by the molecules of the blocking agents).

The following schema represents the overall effect of epinephrine activation and adrenolytic blockade of the sympathetic receptors in the muscle with the corresponding results.

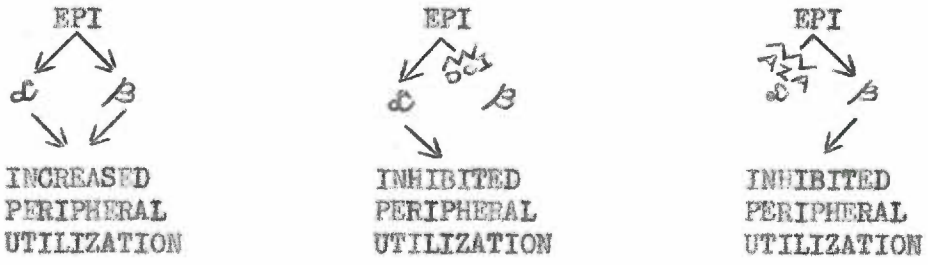


TABLE 15

SUMMARY OF THE PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 16th AND 32nd MINUTE OF AN INFUSION OF EPINEPHRINE BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 15

SUMMARY OF PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 16th AND 32nd MINUTE OF AN INFUSION OF EPINEPHRINE BEFORE AND AFTER ADRENERGIC BLOCKADE*

	CONTROL	EPINEPHRINE	EPINEPHRINE-BETA RECEPTOR BLOCKADE	EPINEPHRINE-ALPHA RECEPTOR BLOCKADE
16 MINUTE PERIPHERAL UPTAKE OF BLOOD SUGAR	4.260 ±0.184	20.490 ±1.084	16.792±1.282 p 0.01	10.630±0.543 p 0.001
32 MINUTE PERIPHERAL UPTAKE OF BLOOD SUGAR	4.260 ±0.184	25.596 ±2.141	25.690±1.904 NOT SIG	20.295±1.382 p 0.02

*All values in mg./kg./min.

FIGURE 19

PERIPHERAL UTILIZATION OF BLOOD SUGAR FROM AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

PERIPHERAL UTILIZATION OF BLOOD SUGAR FROM AN INFUSION OF EPINEPHRINE (5 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

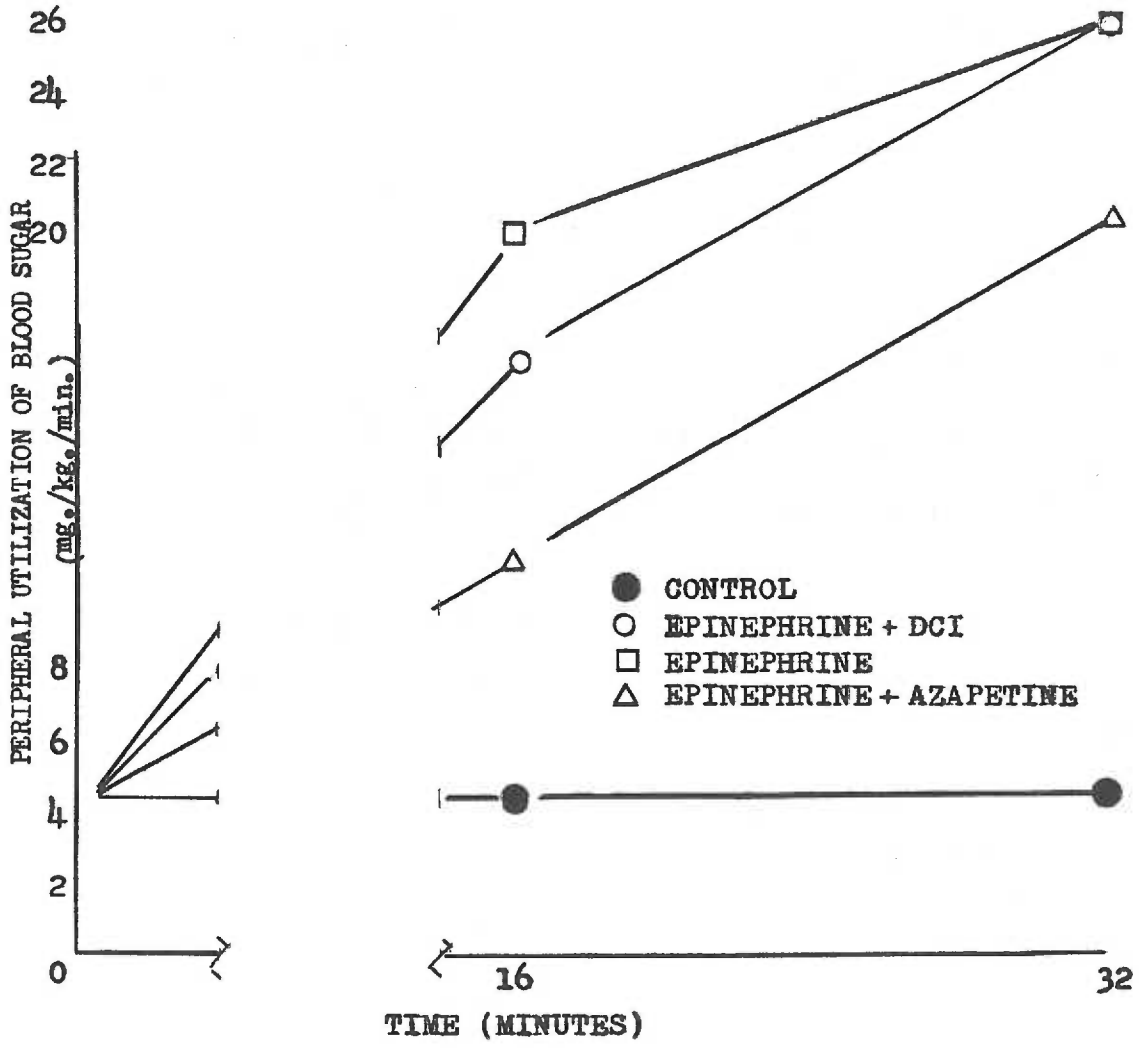


Figure 19

ISOPROTERENOL

A. Hepatic Production of Blood Sugar

When isoproterenol was infused at a rate of 1 mcg./kg./min. glycogenolysis occurred since there was an increase in A-V difference in blood sugar concentrations of the hepatic vessels. Also a significant increase in the hepatic blood flow (Tables 16 and 17) took place. The marked hyperglycemia produced by isoproterenol at this dose demonstrates its potency in this respect. Isoproterenol is predominantly a beta receptor stimulating agent according to Ahlquist(1). Thus, the observed increase in hepatic blood flow from 80 to 100 per cent was due to vasodilation as a result of beta receptor stimulation. A rather pronounced tachycardia (chronotropism) also was observed.

If the beta receptors in the liver are responsible for the glycemic increase after sympathomimetic amine infusion, blockade by DCI should decrease hyperglycemia. Both Tables 16 and 17 show that such a decrease occurs at the 16th and 32nd minute when DCI precedes isoproterenol infusion. At the 16th minute a significant inhibition of the hepatic output of blood was noted. For example, the values of 20.933 $\pm$ 1.463 mg./kg./min sugar output for isoproterenol alone compared to 0.931 $\pm$ 0.082 mg./kg./min. for DCI - isoproterenol show this highly significant difference. In fact, when comparing the effects of the DCI - isoproterenol combination with that of the control value (no drug given) there was no statistically significant difference. Thus, DCI completely blocked the large degree of hyperglycemia produced by isoproterenol.

TABLE 16

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 16th MINUTE
OF AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.)
BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 16

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 16th MINUTE OF AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	HEPATIC VEIN BLOOD SUGAR (mg/cc)	HEPATIC ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	HEPATIC OUTPUT OF BLOOD SUGAR** (mg/kg/min)	
CONTROL	1.328	1.302	0.026	42.4	1.202±0.088	
ISO	2.467	2.200	0.267	78.4	20.933±1.463	
DCI	1.360	1.195	0.165	40.8	6.732	0.931±0.082
ISO-DCI	1.502	1.308	0.194	29.9	5.801	
AZA	1.323	1.265	0.158	37.3	2.163	23.503±0.791
ISO-AZA	2.524	2.230	0.294	87.3	25.666	

*Mean value of three experiments (See appendix)

**Hepatic output = $\left[(HV-HA)_{mg/cc} \times (HBF)_{cc/kg/min}\right]$

where: HV = hepatic vein
HA = hepatic artery
HBF = hepatic blood flow

ISO = Isoproterenol
DCI = Dichloroisoproterenol
AZA = Azapetine

TABLE 17

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 32nd MINUTE
OF AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.)
BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 17

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 32nd MINUTE OF AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	HEPATIC VEIN BLOOD SUGAR (mg/cc)	HEPATIC ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	HEPATIC OUTPUT OF BLOOD SUGAR** (mg/kg/min)
CONTROL	1.328	1.302	0.026	42.4	1.202±0.088
ISO	2.597	2.258	0.339	85.5	28.985±0.517
DCI	1.420	1.200	0.220	46.0	10.120
ISO-DCI	1.563	1.377	0.186	22.0	4.092 } 6.028±0.128
AZA	1.320	1.245	0.075	39.6	2.970
ISO-AZA	2.643	2.433	0.344	88.5	30.332 } 27.364±1.591

*Mean values of three experiments (See appendix)

**Hepatic output = $\left[(HV-HA)_{mg/cc} \times (HBF)_{cc/kg/min}\right]$

where: HV = hepatic vein
HA = hepatic artery
HBF = hepatic blood flow

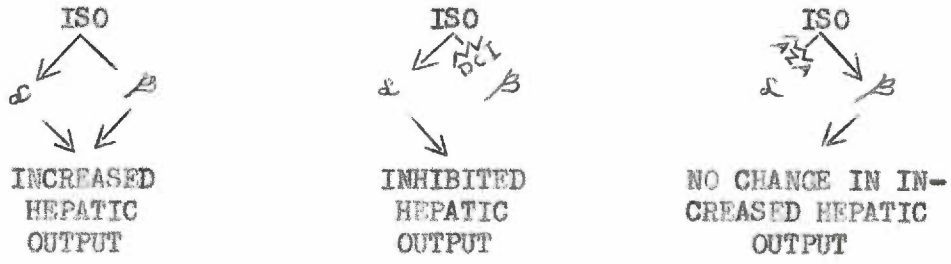
ISO = Isoproterenol
DCI = Dichloroisoproterenol
AZA = Asapetine

At the 32nd minute period it is of interest to note that the blockade of the isoproterenol-induced hyperglycemia by dichloro-isoproterenol was less than at the 16th minute of the infusion. However, there was still a significant inhibition of hyperglycemia. The data in Tables 16 and 17 show that the isoproterenol-DCI combination increased the hepatic output of sugar from 0.931 ± 0.082 mg./kg./min. at the 16th minute to 6.028 ± 0.128 mg./kg./min. at the 32nd minute period. This may have been due to detoxification of dichloro-isoproterenol releasing more beta receptors for stimulation by iso-proterenol.

Azapetine (alpha receptor blockade) does not seem to influence isoproterenol-induced hyperglycemia at the 16th and 32nd minute periods. Tables 16 and 17 show that there was no significant change in the hepatic output of blood sugar when azapetine was used prior to isopro-terenol. This observation lends further support to Alquist's theory categorizing isoproterenol as a beta receptor effector. The beta stimulating effect of isoproterenol is evident, particularly, at the 16th minute observation period. In the previous experiment using dichloroisoproterenol to effectively block the beta receptors, it was shown that isoproterenol had lost its ability to produce hyperglycemia at the 16th minute period. On the other hand, in this experiment when the alpha receptors were blocked by azapetine, no significant change occurred in the hepatic output of blood sugar. This strongly suggests that the beta receptors are responsible for isoproterenol-induced hyperglycemia.

Table 18 summarizes the hepatic output of blood sugar at the 16th and 32nd minute of an infusion of isoproterenol before and after adrenergic blockade with dichloroisoproterenol or azapetine. A graphic presentation of these results are shown in Figure 20. When azapetine is used in combination with isoproterenol the blood sugar curve is similar to that of isoproterenol alone. Similarly, the large degree of DCI inhibition of isoproterenol-induced hyperglycemia is apparent.

The following schema represents the overall effect of isoproterenol on the hepatic blood sugar before and after adrenergic blockade:



B. Peripheral Utilization of Blood Sugar

Isoproterenol causes very little increase in the peripheral utilization of blood sugar. Table 19 (16th minute sample) reveals a slight increase in peripheral utilization from the control value of 4.260 ± 0.182 mg./kg./min. to an experimental value using isoproterenol of 6.583 ± 0.513 mg./kg./min. This slight increase is completely nullified at the 32nd minute period as shown in Table 20. The control value at this period is 4.260 ± 0.182 mg./kg./min. and the experimental value is 4.212 ± 0.611 mg./kg./min. These low peripheral utilization values of glucose, together with the relatively

TABLE 18

SUMMARY OF THE HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 16th AND 32nd MINUTE OF AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 18

SUMMARY OF HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 16th AND 32nd MINUTE OF AN INFUSION OF ISOPROTERENOL BEFORE AND AFTER ADRENERGIC BLOCKADE*

	CONTROL	ISOPROTERENOL	ISOPROTERENOL-BETA RECEPTOR BLOCKADE	ISOPROTERENOL-ALPHA RECEPTOR BLOCKADE
16 MINUTE HEPATIC OUTPUT OF BLOOD SUGAR	1.202 ±0.088	20.933 ±1.463	0.931±0.082 p 0.001	23.503±0.791 NOT SIG
32 MINUTE HEPATIC OUTPUT OF BLOOD SUGAR	1.202 ±0.088	28.985 ±0.517	6.028±0.128 p 0.001	27.364±1.591 NOT SIG

*All values in mg./kg./min.

FIGURE 20

HEPATIC PRODUCTION OF BLOOD SUGAR FROM AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

HEPATIC PRODUCTION OF BLOOD SUGAR FROM AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

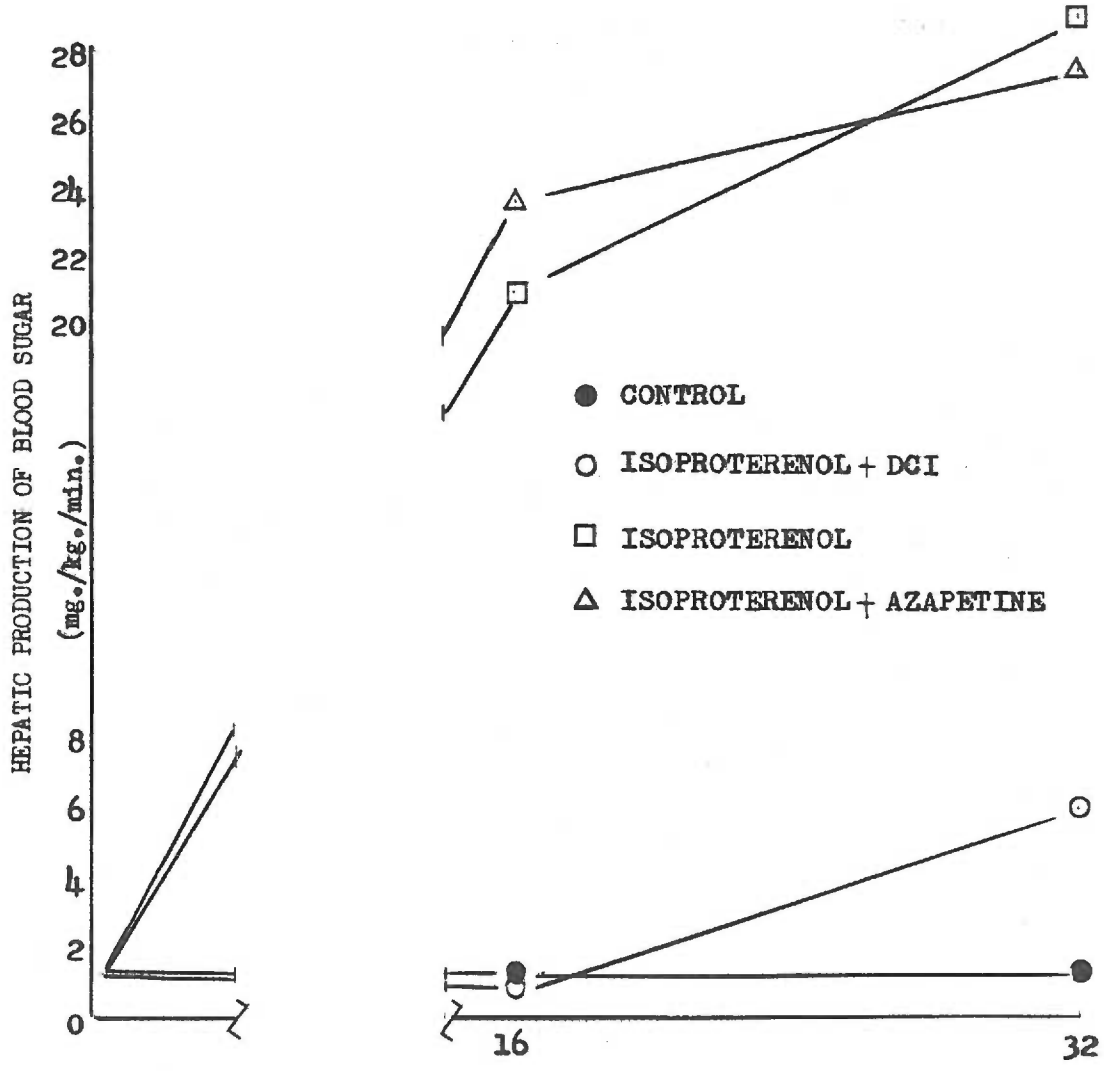


Figure 20

TABLE 19

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 16th MINUTE OF AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 19

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 16th MINUTE OF AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	FEMORAL VEIN BLOOD SUGAR (mg/cc)	FEMORAL ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	PERIPHERAL UTILIZATION OF BLOOD SUGAR** (mg/kg/min)
CONTROL	1.117	1.302	0.185	23.0	4.260±0.184
ISO	1.882	2.200	0.318	20.7	6.583±0.513
DCI	1.122	1.195	0.073	19.4	1.146
ISO-DCI	1.204	1.308	0.104	18.8	1.966 — 0.809±0.124
AZA	1.120	1.265	0.145	19.7	2.860
ISO-AZA	1.973	2.199	0.226	17.8	4.023 — 1.163±0.118

*Mean values of three experiments (See appendix)

**Peripheral uptake = $\left[(FA-FV)_{mg/cc} \times (FBF)_{cc/kg/min}\right]$

where: FA = femoral artery
FV = femoral vein
FBF = femoral blood flow

ISO = Isoproterenol
DCI = Dichloroisoproterenol
AZA = Azapetine

high hepatic output values of glucose, explain why isoproterenol produces such high systemic blood sugar levels.

When DCI is used before isoproterenol a highly significant inhibition in the induced peripheral utilization of blood sugar takes place. This occurs at the 16th minute of the infusion and to a slightly lesser degree at the 32nd minute. Both of these values are less than the corresponding control value.

When azapetine was given before isoproterenol considerable inhibition of peripherally utilized blood sugar occurred at the 16th minute period of the infusion (Table 19). The effect was similar when DCI, the beta receptor blocking agent, was used. However, at the 32nd minute, the azapetine inhibitory effect decreased to a point where no significant difference existed between the peripheral utilization of glucose effected by isoproterenol alone or by isoproterenol in conjunction with azapetine. Table 21 illustrates the peripheral utilization of blood sugar from isoproterenol before and after use of the two adrenergic blocking drugs. These results are shown graphically in Figure 21.

TABLE 20

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 32nd MINUTE OF AN INFUSION IF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 20

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 32nd MINUTE OF AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	FEMORAL VEIN BLOOD SUGAR (mg/cc)	FEMORAL ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	PERIPHERAL UTILIZATION OF BLOOD SUGAR** (mg/kg/min)
CONTROL	1.117	1.302	0.185	23.0	4.260±0.184
ISO	2.042	2.258	0.216	19.5	4.212±0.611
DCI	1.139	1.200	0.061	20.3	1.240
ISO-DCI	1.211	1.377	0.166	19.0	3.154 — 1.916±0.022
AZA	1.130	1.245	0.115	20.9	2.400
ISO-AZA	2.100	2.433	0.333	18.7	6.227 — 3.823±0.439

*Mean values of three experiments (See appendix)

**Peripheral uptake = $\left[(FA-FV)_{mg/cc} \times (FBF)_{cc/kg/min}\right]$

where: FA = femoral artery
FV = femoral vein
FBF = femoral blood flow

ISO = Isoproterenol
DCI = Dichloroisoproterenol
AZA = Azapetine

TABLE 21

SUMMARY OF THE PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 16th AND 32nd MINUTE OF AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 21

SUMMARY OF PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 16th AND 32nd MINUTE OF AN INFUSION OF ISOPROTERENOL BEFORE AND AFTER ADRENERGIC BLOCKADE*

	CONTROL	ISOPROTERENOL	ISOPROTERENOL-BETA RECEPTOR BLOCKADE	ISOPROTERENOL-ALPHA RECEPTOR BLOCKADE
16 MINUTE PERIPHERAL UPTAKE OF BLOOD SUGAR	4.260 ±0.182	6.583 ±0.513	0.809±0.124 p 0.001	1.163±0.118 p 0.001
32 MINUTE PERIPHERAL UPTAKE OF BLOOD SUGAR	4.260 ±0.182	4.212 ±0.611	1.916±0.022 p 0.01	3.823±0.432 NOT SIG

*All values in mg./kg./min.

FIGURE 21

PERIPHERAL UTILIZATION OF BLOOD SUGAR FROM AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

PERIPHERAL UTILIZATION OF BLOOD SUGAR FROM AN INFUSION OF ISOPROTERENOL (1 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

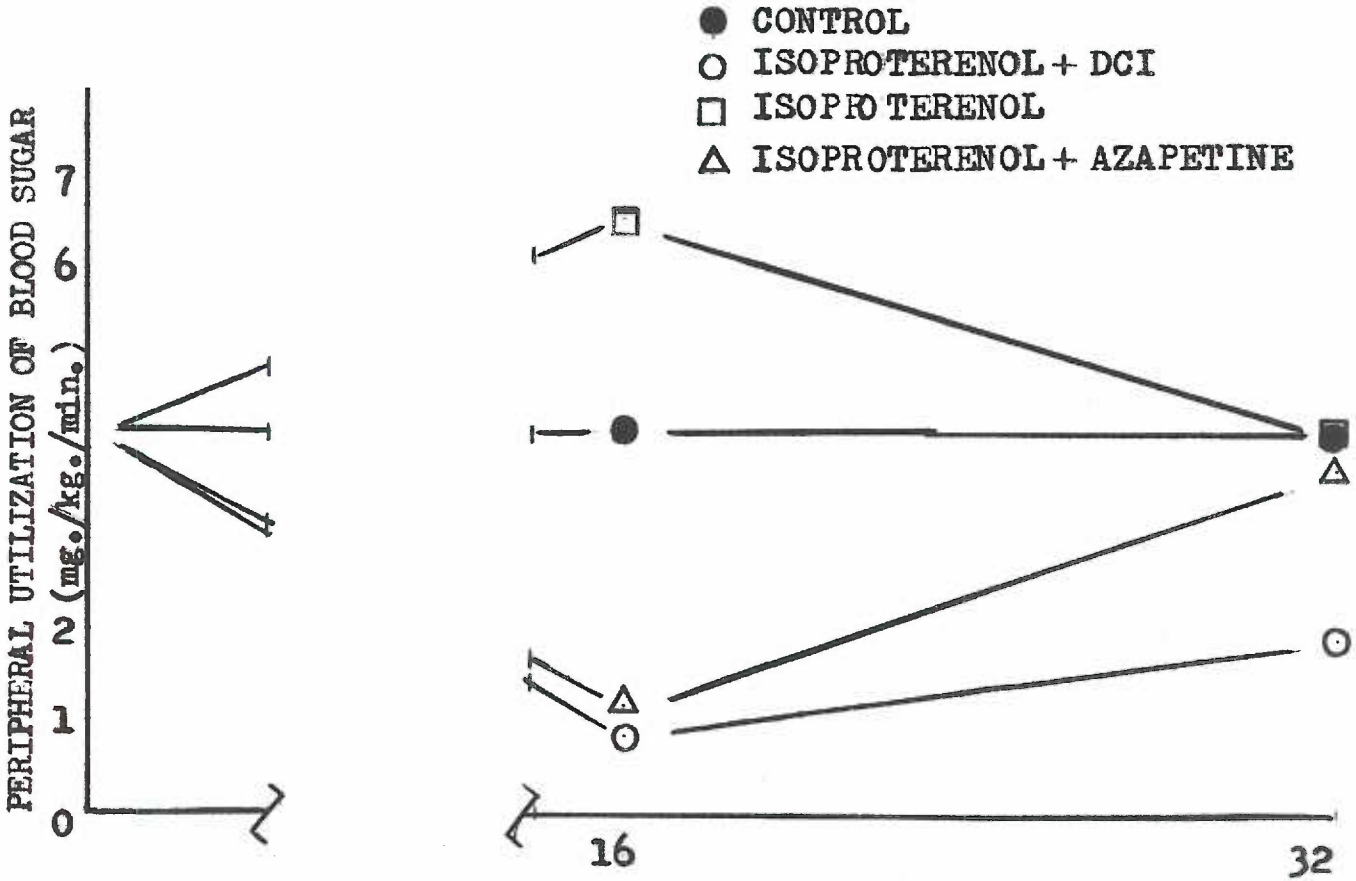
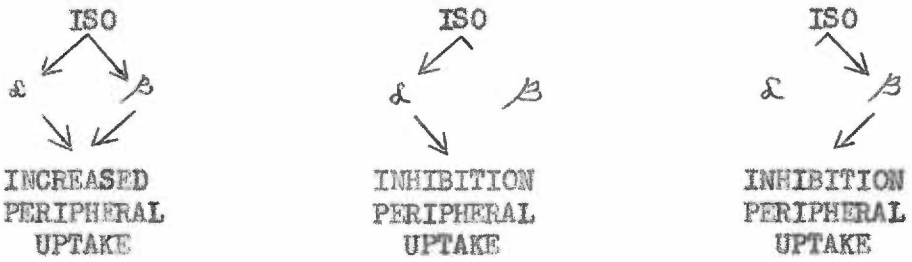


Figure 21

Figure 21 clearly shows the inhibitory effect of DCI and azapetine on isoproterenol-induced peripheral utilization of blood sugar. Both the maximal uptake and the greatest inhibition in uptake of sugar occurred at the 16th minute. The reason for this can only be speculative. It may be that the receptors are exhibiting tachyphylaxis or, the compounds are being detoxified at a more rapid rate at this particular time.

The following schema illustrates the overall responses to the effects of an infusion of isoproterenol in conjunction with adrenergic receptor blockade:



NOR-EPINEPHRINE

A. Hepatic Production of Blood Sugar

One of the most interesting facts arising from an intravenous infusion of 20 mcg./kg./min. of nor-epinephrine was the decrease in blood flow through the liver. As can be seen in Table 22 the flow of blood after 16 minutes of the infusion decreases from a control value of 42.4 cc./kg./min. to the experimental value of 9.7 cc./kg./min. This reduction in flow is in agreement with the findings of Beorn, Billing, and Sherlock (4) and also with Ahlquist's theory of alpha-beta sympathetic receptors. According to Ahlquist (1) nor-epinephrine is a powerful alpha receptor stimulator. Since alpha receptors are responsible for smooth muscle contraction, nor-epinephrine produces a large degree of vasoconstriction leading to the decrease in hepatic blood flow.

In spite of this decreased blood flow in the liver a concomitant increase in the A-V difference for the blood sugar occurs. The net result is an increased hepatic output of blood sugar. After 16 minutes of an infusion of nor-epinephrine the hepatic blood sugar output increased from a control value of 1.202 $\pm$ 0.088 mg./kg./min. to an experimental value of 6.848 $\pm$ 0.133 mg./kg./min. (Table 22). This increase in hepatic production of blood sugar by nor-epinephrine continues through the 32nd minute period of the infursion (Table 23).

When considering the effects of beta receptor blockade by DCI in conjunction with nor-epinephrine, the results indicate that after 16

TABLE 22

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 16th MINUTE OF AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 22

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 16th MINUTE OF AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	HEPATIC VEIN BLOOD SUGAR (mg/cc)	HEPATIC ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	HEPATIC OUTPUT OF BLOOD SUGAR** (mg/kg/min)	
CONTROL	1.328	1.302	0.026	42.4	1.202±0.088	
NOR	2.443	1.737	0.706	9.7	6.848±0.133	
DCI	1.360	1.195	0.165	40.8	6.732	8.812±0.971
NOR-DCI	2.000	1.464	0.536	29.0	15.544	
AZA	1.323	1.265	0.058	37.3	2.163	24.457±1.749
NOR-AZA	2.558	1.053	0.605	44.0	26.620	

*Mean values of three experiments (See appendix)

**Hepatic output = $\left[(HV-HA)_{mg/cc} \times (HBF)_{cc/kg/min}\right]$

where: HV = hepatic vein
HA = hepatic artery
HBF = hepatic blood flow

NOR = Nor-epinephrine
DCI = Dichloroisoproterenol
AZA = Azapetine

minutes of the infusion there was a slightly significant change in hepatic output of sugar (significant at the 5 per cent level). Then, at the 32nd minute period the degree of inhibition of the induced hyperglycemia was highly significant (0.10 per cent level). This decreased hepatic output of sugar produced by beta receptor blockade, is still significantly higher than the control value. Again, as previously noted when epinephrine and isoproterenol were studied these results indicate that the beta receptor is responsible for sympathomimetic-induced hyperglycemia.

The effects of an infusion of nor-epinephrine in conjunction with azapetine (alpha receptor blocker) are also shown in Tables 22 and 23. Again, as with epinephrine and isoproterenal under similar conditions, there was a highly significant (0.1 per cent level) potentiation in the induced hyperglycemia. These results illustrate that while nor-epinephrine is classified as predominantly an alpha receptor stimulator, and blockade of these receptors leaves unhindered the stimulation of the beta receptors, nor-epinephrine in itself also possesses considerable beta receptor stimulation. This contention is supported by the large degree of hyperglycemia produced when azapetine was used with nor-epinephrine. The potentiated hepatic output of blood sugar occurred at both the 16th and 32nd minute of the nor-epinephrine infusion.

Table 24 summarizes the hepatic output of blood sugar from an infusion of nor-epinephrine before and after adrenergic blockade.

TABLE 23

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 32nd MINUTE OF AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 23

HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 32nd MINUTE OF AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	HEPATIC VEIN BLOOD SUGAR (mg/cc)	HEPATIC ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	HEPATIC OUTPUT OF BLOOD SUGAR** (mg/kg/min)	
CONTROL	1.328	1.302	0.026	42.4	1.202±0.088	
NOR	2.633	1.850	0.783	12.1	9.474±0.699	
DCI	1.420	1.200	0.220	46.0	10.120	2.247±0.224
NOR-DCI	1.486	1.243	0.243	32.4	7.873	
AZA	1.320	1.245	0.075	39.6	2.970	22.039±0.815
NOR-AZA	2.790	2.233	0.557	44.9	25.009	

*Mean values of three experiments (See appendix)

**Hepatic output = $\left[(HV-HA)_{mg/cc} \times (HBF)_{cc/kg/min}\right]$

where: HV = hepatic vein
HA = hepatic artery
HBF = hepatic blood flow

NOR = Nor-epinephrine
DCI = Dichloroisoproterenol
AZA = Azapetine

These results also are shown graphically in Figure 22. Again, the high degree of potentiation of hepatic output of blood sugar can be seen when the alpha receptors are blocked by azapetine. Also, a high degree of inhibition of sugar output from the liver occurs when DCI is used (32 minute sample).

The consistent blocking and potentiating effect of the adrenergic blocking agents on hepatic production of blood sugar by nor-epinephrine may be schematically presented as follows:

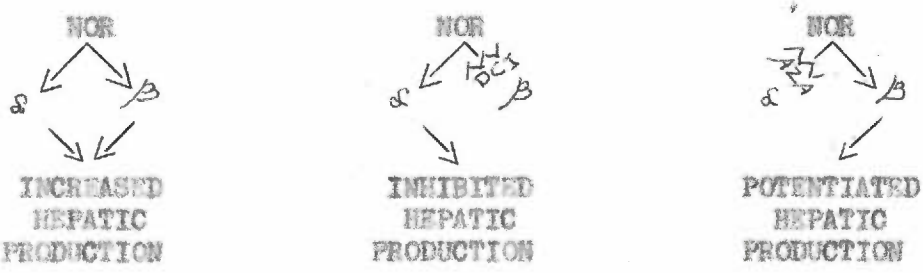


TABLE 24

SUMMARY OF THE HEPATIC PRODUCTION OF BLOOD SUGAR FROM AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENEROTC BLOCKADE

TABLE 24

SUMMARY OF HEPATIC PRODUCTION OF BLOOD SUGAR AT THE 16th AND 32nd MINUTE OF AN INFUSION OF NOR-EPINEPHRINE BEFORE AND AFTER ADRENERGIC BLOCKADE*

	CONTROL	NOR-EPINEPHRINE	NOR-EPINEPHRINE-BETA RECEPTOR BLOCKADE	NOR-EPINEPHRINE-ALPHA RECEPTOR BLOCKADE
16 MINUTE HEPATIC OUTPUT OF BLOOD SUGAR	1.202 ±0.088	6.848 ±0.133	8.832±0.971 p 0.05	24.457±1.749 p 0.001
32 MINUTE HEPATIC OUTPUT OF BLOOD SUGAR	1.202 ±0.088	9.474 ±0.699	2.247±0.224 p 0.001	22.039±0.815 p 0.001

*All values in mg./kg./min.

FIGURE 22

HEPATIC PRODUCTION OF BLOOD SUGAR FROM AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

HEPATIC PRODUCTION OF BLOOD SUGAR FROM AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

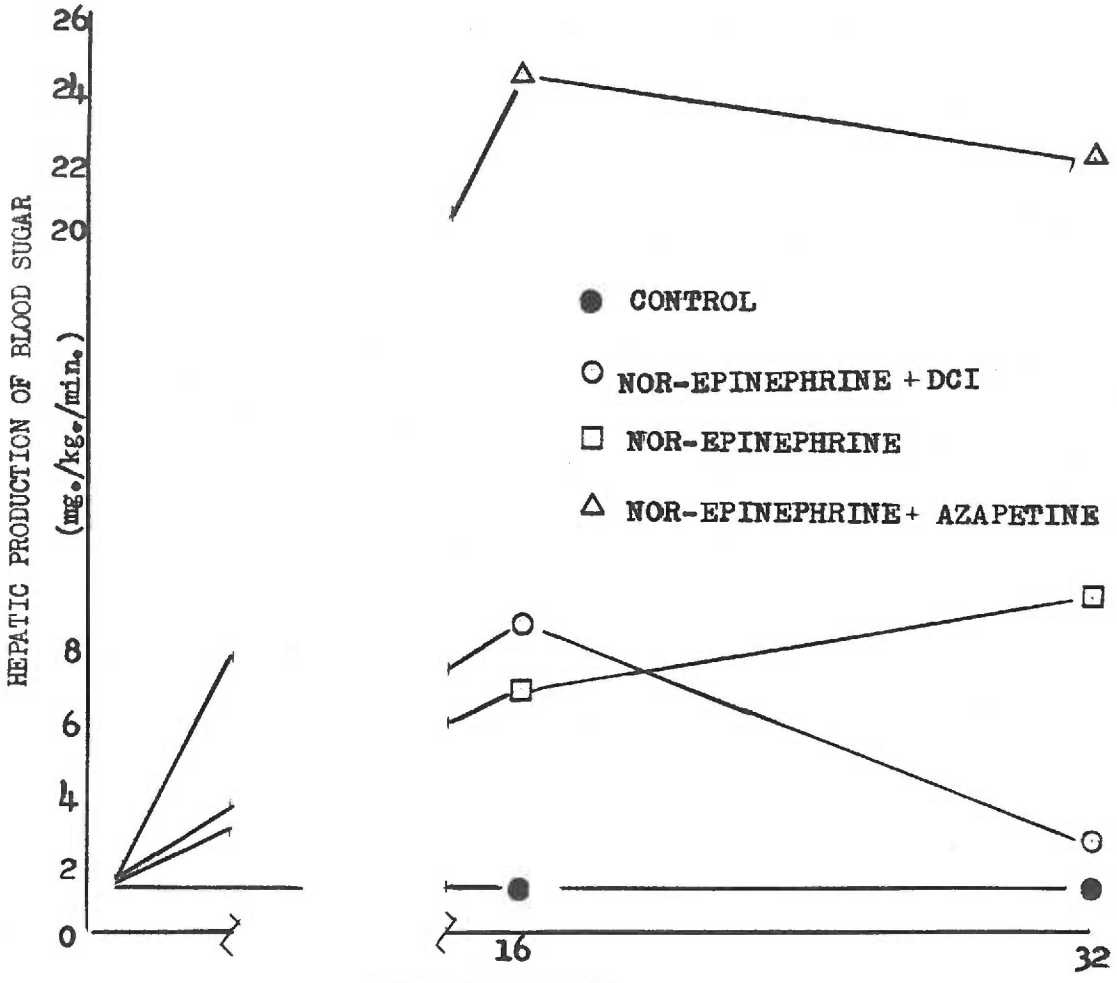


Figure 22

B. Peripheral Utilization of Blood Sugar

Nor-epinephrine, when infused at a rate of 20 mcg./kg./min. into dogs increases the peripheral utilization of blood sugar. This increase is less than that produced by epinephrine but greater than with isoproterenol.

Table 25 illustrates the results obtained after 16 minutes of nor-epinephrine infusion. As can be seen, when DCI was used in conjunction with nor-epinephrine only a very slight rise in blood sugar was observed compared to the results obtained with nor-epinephrine alone (10.922 $\pm$ 0.683 mg./kg./min. as compared to 12.071 $\pm$ 0.089 mg./kg./min.). This is not statistically significant. However, when comparing the 32nd minute samples under the same conditions (Table 26) a significant decrease in the induced hyperglycemia occurred (13.072 $\pm$ 0.72 mg./kg./min. of sugar for nor-epinephrine alone and 11.684 $\pm$ 0.517 mg./kg./min. for DCI-nor-epinephrine combination).

With blockade of the alpha receptor by azapetine there also occurred an inhibition of the nor-epinephrine-induced hyperglycemia.

It can be seen in Table 25 that this blockade by azapetine at the 16th minute of the infusion produced results that were not significantly different than the control values (no drug). This indicates, that at the 16th minute of the nor-epinephrine infusion azapetine was effectively and completely blocking the peripheral utilization of blood sugar. At the 32nd minute (Table 26) the blockade was slightly less forceful as seen by the slight, but significant, rise in peripheral utilization

TABLE 25

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 16th MINUTE OF AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 25

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 16th MINUTE OF AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	FEMORAL VEIN BLOOD SUGAR (mg/cc)	FEMORAL ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	PERIPHERAL UTILIZATION OF BLOOD SUGAR** (mg/kg/min)	
CONTROL	1.117	1.302	0.185	23.0	4.260±0.184	
NOR	1.403	1.737	0.334	32.7	10.922±0.683	
DCI	1.122	1.195	0.073	19.4	1.146	12.071±0.089
NOR-DCI	1.167	1.464	0.297	44.5	13.217	
AZA	1.120	1.265	0.145	19.7	2.860	3.880±0.316
NOR-AZA	1.547	1.953	0.406	16.6	6.740	

*Mean values of three experiments (See appendix)

**Peripheral utilization = $\left[(FA\text{-}FV)_{mg/cc} \times (FBF)_{cc/kg/min}\right]$

where: FA = femoral artery
FV = femoral vein
FBF = femoral blood flow

NOR = Nor-epinephrine
DCI = Dichloroisoproterenol
AZA = Azapetine

TABLE 26

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 32nd MINUTE OF AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 26

PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 32nd MINUTE OF AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE*

DRUG	FEMORAL VEIN BLOOD SUGAR (mg/cc)	FEMORAL ARTERY BLOOD SUGAR (mg/cc)	BLOOD SUGAR A-V DIFF. (mg/cc)	BLOOD FLOW (cc/kg/min)	PERIPHERAL UTILIZATION OF BLOOD SUGAR** (mg/kg/min)	
CONTROL	1.117	1.302	0.185	23.0	4.269±0.184	
NOR	1.420	1.850	0.430	30.4	13.072±0.197	
DCI	1.139	1.200	0.061	20.3	1.240	11.684±0.517
NOR-DCI	1.170	1.486	0.316	40.9	12.924	
AZA	1.130	1.245	0.115	20.9	2.400	5.708±0.369
NOR-AZA	1.767	2.233	0.466	17.4	8.108	

*Mean values of three experiments (See appendix)

**Peripheral utilization = $\left[(FA-FV)_{mg/cc} \times (FBF)_{cc/kg/min}\right]$

where: FA = femoral artery
FV = femoral vein
FBF = femoral blood flow

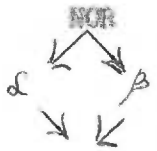
NOR = Nor-epinephrine
DCI = Dichloroisoproterenol
AZA = Asapetine

of blood sugar (3.880 ± 0.316 mg./kg./min. at the 16th minute level increasing to 5.708 ± 0.369 mg./kg./min. at the 32nd minute).

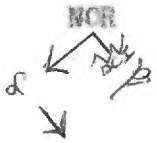
Table 27 summarizes the peripheral utilization of blood sugar under the experimental conditions of nor-epinephrine and the adrenergic blocking compounds.

These results are shown graphically in Figure 23. The inhibition in the peripheral uptake of glucose brought about by azapetine can readily be seen. However, the inhibition brought about by DCI is not as clear cut. It can be seen in Figure 23 that the inhibition of nor-epinephrine-induced hyperglycemia by DCI does not begin until after the 16th minute of the infusion.

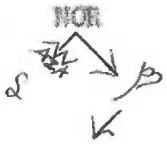
The following is a schematic representation of the results:



INCREASED
PERIPHERAL
UTILIZATION



DECREASED
PERIPHERAL
UTILIZATION



DECREASED
PERIPHERAL
UTILIZATION

TABLE 27

SUMMARY OF THE PERIPHERAL UTILIZATION OF BLOOD SUGAR FROM AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 27

SUMMARY OF PERIPHERAL UTILIZATION OF BLOOD SUGAR AT THE 16th AND 32nd MINUTE OF AN INFUSION OF NOR-EPINEPHRINE BEFORE AND AFTER ADRENERGIC BLOCKADE*

	CONTROL	NOR-EPINEPHRINE	NOR-EPINEPHRINE-BETA RECEPTOR BLOCKADE	NOR-EPINEPHRINE-ALPHA RECEPTOR BLOCKADE
16 MINUTE PERIPHERAL UPTAKE OF BLOOD SUGAR	4.260 ±0.184	10.922 ±0.683	12.071±0.089 NOT SIG	3.880±0.316 p 0.001
32 MINUTE PERIPHERAL UPTAKE OF BLOOD SUGAR	4.260 ±0.184	13.072 ±0.197	11.684±0.517 p 0.05	5.708±0.369 p 0.001

*All values in mg./kg./min.

FIGURE 23

PERIPHERAL UTILIZATION OF BLOOD SUGAR FROM AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

PERIPHERAL UTILIZATION OF BLOOD SUGAR FROM AN INFUSION OF NOR-EPINEPHRINE (20 mcg./kg./min.) BEFORE AND AFTER ADRENERGIC BLOCKADE

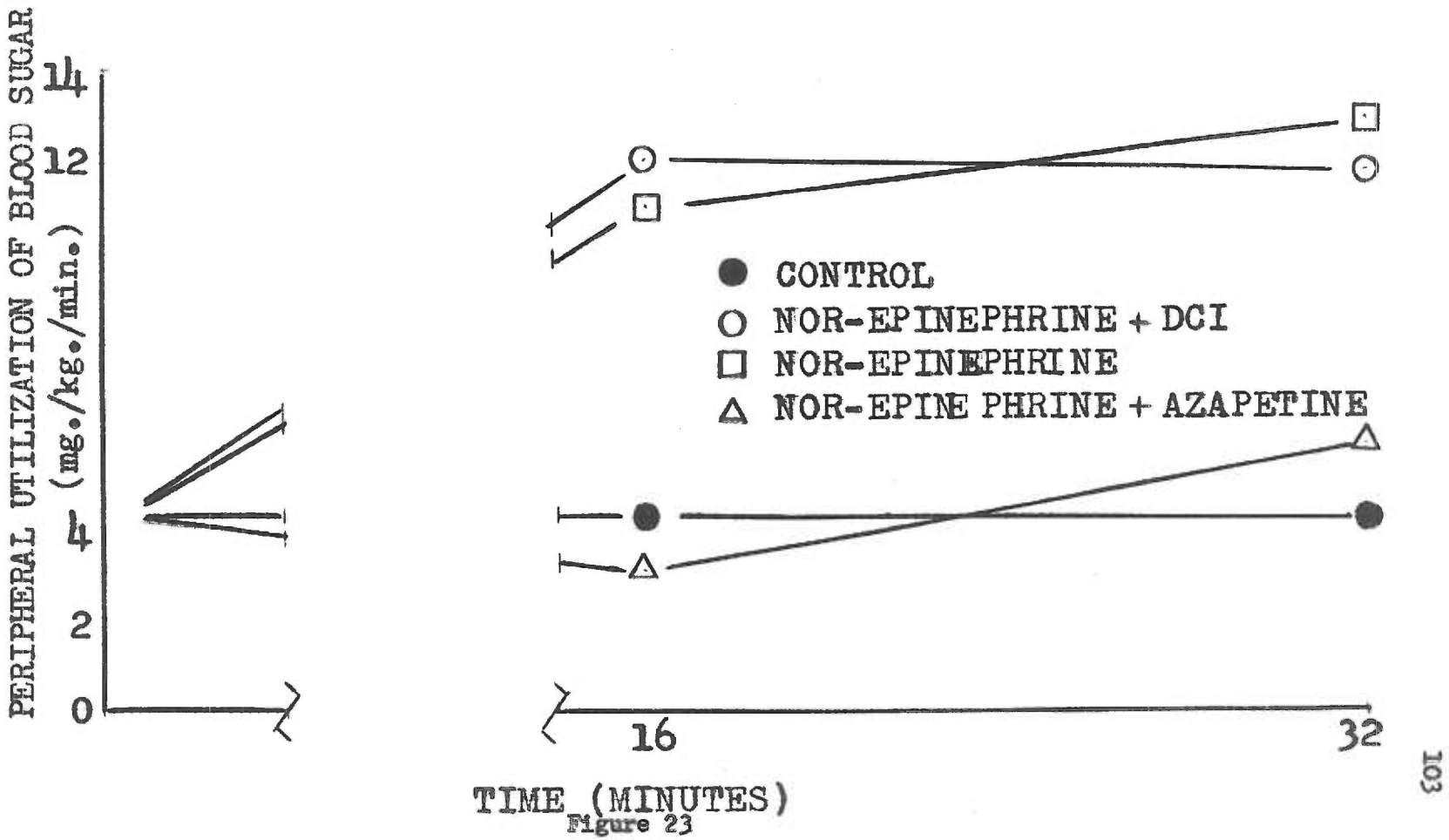


Figure 23

DISCUSSION

Ahlquist (1) recently proposed a new theory to explain the pharmacologic actions of epinephrine and related sympathomimetic amine drugs. Ahlquist's theory proposes that there are alpha sympathetic receptors which are stimulatory in activity and beta sympathetic receptors which are inhibitory in response to the adrenergic drugs. In support of this theory are the responses shown by the blood vessels, the blood pressure, splenic contraction, gut relaxation and other "fight or flight" mechanisms, including the changes in blood sugar associated with sympathomimetic amine introduction into the body.

The administration of a sympathomimetic amine produces hyperglycemia. This supplies the tissues with a large amount of sugar which can be broken down and utilized in the form of energy. This energy is expended in the muscles with a consequential conversion of the sugar into lactic acid.

The overall response of blood sugar release and utilization from the administration of sympathomimetic amines appears to be a combination of effects on the liver and muscle. Therefore, this study was an attempt to bring out the actual mechanisms at the receptor level in sympathomimetic amine-induced hyperglycemia.

It has been stated above that sympathomimetic amines produce their effect by stimulation of one of two types of adrenergic receptors. These receptors are thought to be present at the

neuro-effector junctions of the sympathetic nervous system. Blockade of either of these receptors, which is now possible, in conjunction with sympathomimetic amine administration, will produce responses pertinent to the non-blocked receptors. Applying this technique we were able to ascertain whether blockade or potentiation of sympathomimetic amine-induced hyperglycemia would take place and whether the response was due to an increased or decreased hepatic production of blood sugar, an increased or decreased peripheral utilization of blood sugar or a combination of the two.

Since the sympathomimetic amines produce a large outpouring of sugar from the liver, one might conclude that this response is mediated solely through the alpha (stimulatory) sympathetic receptors. However, as has been shown in this report nor-epinephrine, the most powerful alpha receptor stimulating agent, actually produces a very mild degree of hepatic glycogenolysis. Epinephrine, classified by Ahlquist as having predominantly alpha receptor stimulating activity and a relatively mild degree of beta receptor stimulation, produces a greater hepatic production of blood sugar than does nor-epinephrine. Furthermore, isoproterenol, the most powerful beta receptor stimulating agent, produces an outflow of sugar from the liver greater than either epinephrine or nor-epinephrine.

It can be presumed then, that the beta receptors, in response to their stimulation by sympathomimetic amines, appear to be responsible for hepatic glycogenolysis.

This fact can further be elucidated by using adrenergic blocking agents in conjunction with sympathomimetic amines.

That certain drugs produce a blockade of the stimulatory (alpha receptors) activity of sympathomimetic amines has been known for quite some time. These compounds cause a blood pressure reversal when used before epinephrine; they tend to block the vasoconstrictive properties of nor-epinephrine; and they potentiate the depressor response of isoproterenol. A typical example of an alpha sympathetic receptor blocking agent is azapetine.

Compounds with the ability to block the inhibitory (beta receptor) activity of sympathomimetic amines have not been known until very recently (1958). Indeed, there is, at the present, but one such compound, dichloroisoproterenol (DCI). This compound has the ability to block the mild depressor activity exhibited by epinephrine; it has a tendency to potentiate the pressor response of nor-epinephrine; and it completely obliterates the depressant action on the blood pressure that isoproterenol exhibits.

Using either of these compounds, then, it is possible to block one portion of the sympathetic nervous system. With this blockade, the effect of sympathomimetic amines on the other non-blocked portion of the sympathetic nervous system may be demonstrated.

By using a technique to block either receptor system, the receptor responses in the liver to sympathomimetic amines were elucidated. In order to obtain blood samples to measure the output of sugar from the liver, the blood sugar content of the arterial and venous supply of the liver were studied. A double catheter technique was used. This

procedure requires one radio-opaque Cournand catheter being directed, under fluoroscopy, up the femoral artery and into the lower aorta. Then, the catheter is directed upward to a point approximating the hepatic artery. A second catheter is passed down the right external jugular vein, around the heart and into the right hepatic vein. Thus, with these two areas being sampled it was possible to obtain not only blood sugar entering and leaving the liver but also the blood flow through the liver.

Peripheral utilization of blood sugar was ascertained from the femoral artery and vein.

The above two methods were carried out to obtain the hepatic production and the peripheral utilization of blood sugar under the influence of sympathomimetic amines before and after adrenergic blockade.

The results obtained under these conditions suggest that the hepatic production of blood sugar from sympathomimetic amine administration is brought about by beta receptor stimulation. When DCI was injected before epinephrine, nor-epinephrine or isoproterenol inhibition in the induced hepatic production of blood sugar was effected. Conversely, when the alpha receptor blocking agent, azapetine, was administered before epinephrine or nor-epinephrine, a potentiation was produced in the induced hepatic production of blood sugar. However, azapetine did not significantly potentiate isoproterenol-induced hepatic output of blood sugar. This would

suggest that isoproterenol is totally and complete a beta receptor effector. The effect of the sympathomimetic amines on the output of blood sugar from the liver was the same whether the alpha receptors were blocked or not. Also, when the beta receptors were blocked there was complete inhibition in the response brought about by isoproterenol when measured at the 16th minute of the infusion.

The substantiating facts indicate then, that the beta receptors are responsible for sympathomimetic amine induced hepatic production of blood sugar.

The effect of sympathomimetic amines and adrenergic blocking agents on the peripheral utilization of blood sugar do not follow the same pattern as their effects on hepatic production. While all three amines, epinephrine, nor-epinephrine and isoproterenol increase the hepatic production of blood sugar, only two of the three, epinephrine and nor-epinephrine, produce a significant degree of increased peripheral utilization. Isoproterenol, perhaps because it is solely a beta receptor stimulator, did not significantly increase the peripheral utilization of blood sugar.

Further, regardless of the adrenergic blocking agent (azapetine or DCI) used before the sympathomimetic amines, there occurred an inhibition in the amine-induced peripheral utilization of blood sugar, except in a few instances where there was no significant difference.

Table 28 on the following page summarizes the compiled results of the several studies done to compare the effects of adrenergic blocking agents on the sympathomimetic amine induced hepatic output of sugar and the peripheral utilization of sugar.

The clear-cut results obtained for the studies dealing with the hepatic production of blood sugar conform to Ahlquist's theory of the function of alpha and beta receptors. However, the variable results obtained for the peripheral utilization of blood sugar when the adrenergic blockers and sympathomimetic drugs were given need explanation. Two possibilities exist. The first is that the receptors for glucose activity in the liver are not the same as those receptors present in the muscle. If the receptors were of the same nature then one would expect the changes in sugar concentration to be relatively in the same direction. This did not occur. It has been shown that when DCI is used before the sympathomimetic amine infusion both the hepatic production and the peripheral utilization of blood sugar are inhibited. When the amines were given after azapetine there was potentiation of the hepatic production and, as with DCI, an inhibition of the peripheral utilization of blood sugar. Therefore, the receptors do not appear to have the same function in the liver as in the muscle. The other possiblity concerning the peripheral receptors alone is that they may be dissimilar anatomically but similar physiologically. That is, the blocking agents, azapetine and DCI, are blocking their respective receptors, alpha and beta. However, the physiological function of both types of receptors in the muscle appear

TABLE 28

SUMMARY OF HEPATIC PRODUCTION AND PERIPHERAL UTILIZATION OF BLOOD SUGAR UNDER THE INFLUENCE OF SYMPATHOMIMETIC AMINES BEFORE AND AFTER ADRENERGIC BLOCKADE

TABLE 28

SUMMARY OF HEPATIC PRODUCTION AND PERIPHERAL UTILIZATION OF BLOOD SUGAR UNDER THE INFLUENCE OF SYMPATHOMIMETIC AMINES BEFORE AND AFTER ADRENERGIC BLOCKADE

		HEPATIC PRODUCTION* (CONTROL = 1.20 ±0.08)			PERIPHERAL UTILIZATION* (CONTROL = 4.26 ±0.18		
DRUG	TIME	AMINE	AMINE PLUS DCI	AMINE PLUS AZA	AMINE	AMINE PLUS DCI	AMINE PLUS AZA
EPI	16 min	8.96 ±1.08	6.17 ±0.30 SIG	15.62 ±1.68 SIG	20.49 ±1.08	16.79 ±1.28 SIG	10.63 ±0.54 SIG
	32 min	8.80 ±0.94	6.58 ±0.87 SIG	38.18 ±2.39 SIG	25.60 ±2.14	25.69 ±1.90 NOT SIG	20.29 ±1.38 SIG
ISO	16 min	20.93 ±1.46	0.93 ±0.08 SIG	23.50 ±0.79 NOT SIG	6.58 ±0.51	0.81 ±0.12 SIG	1.16 ±0.12 SIG
	32 min	28.99 ±0.52	6.03 ±0.13 SIG	27.36 ±1.59 NOT SIG	4.21 ±0.61	1.92 ±0.02 SIG	3.82 ±0.43 NOT SIG
NOR	16 min	6.85 ±0.13	8.81 ±0.97 SIG	24.46 ±1.75 SIG	10.92 ±0.68	12.07 ±0.09 SIG	3.88 ±0.32 SIG
	32 min	9.47 ±0.70	2.25 ±0.22 SIG	22.04 ±0.82 SIG	13.07 ±0.20	11.68 ±0.52 SIG	5.71 ±0.37 SIG

*All values in mg./kg./min.

EPI = Epinephrine NOR = Nor-epinephrine AZA = Azapetine
ISO = Isoproterenol DCI = Dichloroisoproterenol

to be to aid in the peripheral utilization of circulating blood sugar. It appears from the results of this study that both receptors are necessary for the maintainance of peripheral utilization of glucose under sympathomimetic amine administration. When either peripheral receptor types are blocked, the net result is a decrease in the amount of sugar being utilized.

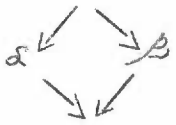
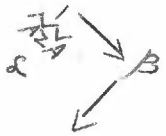
This latter theory may, perhaps, be more accurate. Stressful situations, where the bodily responses may be compared to the giving of infusions of sympathomimetic amines, create the need for an increase in energy, especially in the muscles. It, therefore, seems highly probable, that any material interfering with the glucose receptors at the peripheral level would tend to decrease the amount of blood sugar utilized by the muscle.

Possibly the glucose receptors in the muscle are present specifically for the uptake and utilization of blood sugar. On the other hand the main function of the glucose receptors in the liver is for the regulating the production of blood sugar.

It can be seen then, that the response of the sympathetic glucose receptors in the liver conform to and aid in the proof of Ahlquist's theory. An explanation of the function and the responses of the peripheral glucose sympathetic receptors may be better demonstrated in non-resting muscle. Probably under the conditions of an active muscle mass (i.e., muscle stress), the peripheral uptake of glucose would conform closely to Ahlquist's theory. However, with the lesser metabolic demands of a resting muscle mass, both sympathetic receptors

function for the normal peripheral utilization of blood sugar under adrenergic stimulation.

An overall view of the effects of adrenergic amines in relation to sympathetic receptor blockade may be seen in the following schema:

ADRENERGIC AMINE	ADRENERGIC AMINE	ADRENERGIC AMINE
		
INCREASED HEPATIC OUTPUT OF BLOOD SUGAR	INHIBITED HEPATIC OUTPUT OF BLOOD SUGAR	POTENTIATED HEPATIC OUTPUT OF BLOOD SUGAR
INCREASED PERIPHERAL UPTAKE OF BLOOD SUGAR	INHIBITED PERIPHERAL UPTAKE OF BLOOD SUGAR	INHIBITED PERIPHERAL UPTAKE OF BLOOD SUGAR

SUMMARY

Hepatic Production of Blood Sugar

1. Infusions of epinephrine, nor-epinephrine and isoproterenol significantly increased the hepatic production of blood sugar.

2. Pretreatment with dichloroisoproterenol (DCI), a beta sympathetic receptor blocking agent, produced a significant inhibition in the induced hepatic output of blood sugar by all three sympathomimetic amines.

3. Pretreatment with azapetine, an alpha sympathetic receptor blocking agent, significantly potentiated epinephrine and nor-epinephrine induced hepatic output of blood sugar.

4. Alpha receptor blockade in conjunction with an infusion of isoproterenol produced no change in hepatic production of blood sugar when comparing the response to isoproterenol with no receptor blockade.

5. The results indicate that isoproterenol is practically completely beta receptor stimulatory in nature and epinephrine and nor-epinephrine possess varied degrees of both alpha and beta stimulating activity in relation to the hepatic production of blood sugar.

6. Sympathomimetic amine induced hepatic production of blood sugar is mediated through the beta receptors in the liver.

Peripheral Utilization of Blood Sugar

1. Infusions of epinephrine and nor-epinephrine produced significant increases in the peripheral utilization of blood sugar. There was a very slight increase in peripheral utilization with isoproterenol mid-way through the infusion (16 minutes). By the end of the infusion (32 minutes) no significant difference was determined between the control values and the experimental values.

2. Pretreatment with DCI showed, in the majority of cases, an inhibition in the peripheral utilization of blood sugar.

3. Pretreatment with azapetine also demonstrated significant inhibition in the peripheral utilization of blood sugar in the majority of cases.

4. These results indicate that both alpha and beta receptors must be present and active in order to have normal peripheral utilization of blood sugar from sympathomimetic amines.

APPENDIX

TABLE 29

COMPARISON OF FEMORAL ARTERIAL AND VENOUS BLOOD FLOW DURING EPINEPHRINE INFUSION*

TIME (min)	FEMORAL ARTERY (cc./kg./min.)	FEMORAL VEIN (cc./kg./min.)
CONT	23.0	22.8
1	29.4	30.0
2	27.2	28.0
4	28.1	28.0
8	26.8	27.0
16	26.0	26.2
32	27.0	26.9
$\Sigma x =$	187.5	189.2
$\bar{x} =$	26.79	27.03
S.D. =	±1.84	±1.87
	t = 0.935 NOT SIG	

*Mean values of five experiments.

STANDARD DEVIATION CALCULATED FROM:

$$\sqrt{\frac{\sum x^2 - \frac{(\Sigma x)^2}{n}}{n}}$$

SIGNIFICANCE TEST CALCULATED FROM:

$$\sqrt{\frac{\sum x_1^2 - \frac{(\Sigma x_1)^2}{n} + \sum x_2^2 + \frac{(\Sigma x_2)^2}{n}}{n(n-1)}}$$

Further study is necessary for the evaluation of the interaction of endogenously released hormones with sympathomimetic amines in relation to carbohydrate metabolism.

BLOOD SUGAR DETERMINATION
See Nelson, N. (37)

1. 1 part blood plus 7 parts water.
   Add 1 part 7% $CuSO_4 \cdot 5H_2O$.
   Shake
   Add 1 part 10% $Na_2WO_4 \cdot 2H_2O$.
   Shake

2. Filter (#42 paper)

3. Take 1 cc filtrate and add to sugar tube.

4. Add 1 cc of a mixture of 25 parts A and 1 part B.
   A is 25 Gms Na_2CO_3 (anhyd.)
   25 Gms NaK tartrate
   20 Gms $NaHCO_3$
   200 Gms Na_2SO_4 (anhyd.)
   Make up to 800 cc. Filter in 48 hrs. and make to 1 liter.

   B is 15% $CuSO_4 \cdot 5H_2O$
   1 or 2 drops H_2SO_4

5. Make standard and blank.

6. Mix and heat on boiling water bath for 20 minutes.

7. Cool in cold water.

8. Add 1 cc of color reagent.
   Arsenomolybdate solution:
   25 Gms ammonium molybdate in 450 cc water
   21 cc conc. H_2SO_4
   Mix
   3 Gms $Na_2HAsO_4 \cdot 7H_2O$ in 25 cc water
   Make to 500 cc
   Incubate at 37 degrees for 36-48 hours

9. Make to mark (25 cc) and read in colorimeter (500 mu).
   100% transmission = blank
   Color is stable.

TABLE 30

HEPATIC PRODUCTION OF BLOOD SUGAR

DRUG	TIME (min.)	MEAN BLOOD SUGAR (mg./kg./min.)	S.D. (±)	t	p
SALINE	-	1.20	0.09	-	-
EPI	16	8.96	1.08	-	-
	32	8.80	0.94	-	-
EPI-DCI	16	6.17	0.30	4.29	0.01
	32	6.58	0.87	3.33	0.02
EPI-AZA	16	15.62	1.68	5.74	0.01
	32	38.18	2.39	19.72	0.001
ISO	16	20.93	1.46	-	-
	32	28.98	0.52	-	-
ISO-DCI	16	0.93	0.08	19.29	0.001
	32	6.03	0.13	61.27	0.001
ISO-AZA	16	23.50	0.79	2.19	NOT SIG
	32	27.36	1.59	1.37	NOT SIG
NOR	16	6.85	0.13	-	-
	32	9.47	0.70	-	-
NOR-DCI	16	8.81	0.97	2.83	0.05
	32	2.25	0.22	13.92	0.001
NOR-AZA	16	24.46	1.75	14.19	0.001
	32	22.04	0.82	16.53	0.001

S.D. = Standard deviation
t = t test value
p = Probability
EPI = Epinephrine
ISO = Isoproterenol
NOR = Nor-epinephrine
DCI = Dichloroisoproterenol
AZA = Azapetine

TABLE 31

PERIPHERAL UTILIZATION OF BLOOD SUGAR

DRUG	TIME (min.)	MEAN BLOOD SUGAR (mg./kg./min.)	S.D. (±)	t	p
SALINE	-	4.26	0.18	-	-
EPI	16	20.49	1.08	-	-
	32	25.60	2.14	-	-
EPI-DCI	16	16.79	1.28	3.84	0.01
	32	25.69	1.90	0.05	NOT SIG
EPI-AZA	16	10.63	0.54	14.19	0.001
	32	20.29	1.38	3.61	0.02
ISO	16	6.85	0.51	-	-
	32	4.21	0.61	-	-
ISO-DCI	16	0.81	0.12	15.48	0.001
	32	1.92	0.02	5.28	0.01
ISO-AZA	16	1.16	0.12	14.53	0.001
	32	3.82	0.44	0.73	NOT SIG
NOR	16	10.92	0.68	-	-
	32	13.07	0.20	-	-
NOR-DCI	16	12.07	0.09	2.36	NOT SIG
	32	11.68	0.52	3.55	0.05
NOR-AZA	16	3.88	0.32	13.24	0.001
	32	5.71	0.37	24.96	0.001

S.D. = Standard deviation
t = t test value
p = Probability
EPI = Epinephrine
ISO = Isoproterenol
NOR = Nor-epinephrine
DCI = Dichloroisoproterenol
AZA = Azapetine

BIBLIOGRAPHY

1. Ahlquist, R.P. A study of adrenotropic receptors. Am. J. Physiol., 1948. 153, 586-600.

2. Allen, W.J., Barcroft, H. & Edholm, O.G. Action of adrenaline on blood vessels in human skeletal muscles. J. Physiol., 1946. 105, 255-267.

3. Banasak, E.F. Estimation of hepatic blood flow using a single injection dye clearance method. Am. J. Physiol., 1960. 198, 877-880.

4. Bearn, A.G., Bill, B. & Sherlock, S. The effect of adrenaline and noradrenaline on hepatic blood flow and splanchnic carbohydrate metabolism in man. J. Physiol., 1951. 115, 430-441.

5. Bondy, P.K., James, D.F. & Farrar, B.W. Studies of the role of the liver in human carbohydrate metabolism by the venous catheter technic. J. Clin. Invest., 1949. 28, 238-244.

6. Brauer, R.W., Pessoti, R.L. & Krebs, J.S. The distribution of S 35 labelled sulpheromophthalic-sodium administered to dogs by continuous infusion. J. Clin. Invest., 1955. 34, 35-43.

7. Cannon, W.B. & Rosenblueth, A. Autonomic neuro-effector systems. New York: The Macmillian Co., 1937.

8. Cobbold, A.F. Ginsburg, J. & Paton, A. Circulatory, respiratory and metabolic responses to isopropylarterenol in man. J. Physiol., 1960. 151, 539-550.

9. Cohn, C., Levine, R. & Streicher, D. The rate of removal of intravenously injected bromsulphalein by the liver and extra hepatic tissues of the dog. Am. J. Physiol., 1947. 150, 299-303.

10. Cori, C.A. & Cori, G.T. The fate of sugar in the animal body. J. Biol. Chem., 1927. 74, 473-494.

11. Cori, C.A. & Cori, G.T. The mechanism of epinephrine action. J. Biol. Chem., 1928. 79, 309-319.

12. Dale, H.H. & Dudley, H.W. The presence of histamine and acetylcholine in the spleen of the ox and the horse. J. Physiol., 1929. 68, 97-123.

13. Dale, H.H. & Gaddum, J.H. Reactions of denervated voluntary muscle, and their bearing on the mode of action of parasympathetic and related nerves. J. Physiol., 1930. 70, 109-144.

14. Dobsen, E.L. & Jones, H.B. The behavior of intravenously injected particulate material. ACTA Medica Scan., 1952. Supp. 272-275.

15. Drill, V.A. Pharmacology in medicine. (2nd Ed.) New York: McGraw-Hill Book Co., 1958.

16. Duff, R.S. & Swan, H.J.C. Further observation on the effect of adrenaline on the blood flow through human skeletal muscle. J. Physiol., 1951. 144, 41-55.

17. Edwards, A.W.T., Bassingthwaight, J.B., Sutterer, W.K. & Wood, E. H. Blood levels of indocyanine green in the dog during multiply dye curves and its effect on instrumental calibration. Pro. Staff Meet. of Mayo Clin., 1961. 35, 745-751.

18. von Euler, U.S. Noradrenaline. Springfield, Illinois: C.C. Thomas, 1956.

19. Greb, W., Janssen, S. & Rein, H. Uber die Grösse der Leberdurchblutung. Zeit. fur Biol., 1929. 89, 324.

20. Green, H.D. & Kepchar J.H. Control of peripheral resistance in major systemic vascular beds. Physiol. Rev., 1959. 39, 617-686.

21. Grollman, A. Pharmacology and therapeutics. (4th Ed.) Philadelphia: Febiger, 1960.

22. Hanson, S. Action de la yohimbine sue les effets de l'adrenaline a l'egard du sucre du sang. Comp. Rend. Soc. de Biol., 1929. 101, 603-605.

23. Harper, H.A. Review of physiological chemistry. (7th Ed.) Los Altos, California: Lange Medical Publications, 1959.

24. Hermann, H. & Vial, J. Sur quelques actions pharmacodynamiques du 2-benzyl-imidazoline. Comp. Rend. Soc. de Biol., 1942. 136, 803-805.

25. Levy, J. & Ditz, E. Sur quelques a fonction ether oxyde phenolique inversant certained actions pharmacodynamiques de l'adrenaline. Arch. Internal. de Pharmacodyn. et de The., 1934. 47, 136-208.

26. Lockett, M.F. Identification of an isoprenaline-like substance in extracts of adrenal glands. Brit. J. Pharmacol., 1954. 9, 498-505.

27. Loewi, O. Uber humorale Ubertragbarkeit der Herznerven wirkung. Arch. ges. Physiol., 1921. 189, 239-242.

28. Mayer, S.E. & Moran, N.C. Blockade of the hyperglycemia action of epinephrine by DCI. Fed. Proc., 1960. 19, 296.

29. Mayer, S.E., Moran, N.C. & Fain, S.J. The effect of adrenergic blocking agents on some metabolic actions of catecholamines. J. Pharmacol. & Exp. The., 1961. 134, 18-27.

30. Mayer, S.E. The physiological deposition of H^3-DCI. J. Pharmacol. & Exp. Ther., 1962. 135, 204

31. McClure, D.A. Dichloroisoproterenol (DCI) inhibition of sympathomimetic-induced hyperglycemia. The Pharmacologist, 1960. 2, 94.

32. McCutcheon, R.S. Canine blood sugar and lactic acid responses to adrenergic agents after adrenergic blockade. Fed. Proc., 1961. 20, 315.

33. Moore, M.E. & Swain, J.N. Potentiation of epinephrine arrhythmias by 1-phenyl-1-dimethylaminoethyl proprionone HCl. Fed. Proc., 1958. 17, 396.

34. Moran, N.C. & Perkins, M.E. Blockade of adrenergic stimulation of the heart by 1(3'-dichlorophenyl)-2-isopropylaminoethanol HCl. J. Pharmacol. & Exp. The., 1958. 122, 55A.

35. Moran, N. C. & Perkins, M.E. Adrenergic blockade of the mammalian heart by a dichloro analoque of isoproterenol. J. Pharmacol. & Exp. The., 1958. 124, 223-237.

36. Nicholson, J.W., III & Wood, E.H. Estimation of cardiac output and evans blue space in man, using an oximeter. J. Lab. & Clin Med., 1951. 38, 588-603.

37. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 1944. 153, 375-380.

38. Page, I.H. Serotonin (5-hydroxytryptamine). Physiol. Rev., 1954. 34, 563-588.

39. Powell, C.E. & Slater, I.W. Blockade of the inhibitory receptor by a dichloro analogue of isoproterenol. J. Pharmacol. & Exp. The., 1958. 122, 480-488.

40. Rapaport, E., Wetterer, S.C. & Weigand, B.D. Hepatic clearance of indocyanine green. Clin. Resh., 1959. 7, 289-290.

41. Root, Mary. Dichloroisoproterenol and blood glucose in several species. Fed. Proc., 1961. 20, 168.

42. Rothlin, E. Sur les proprietes pharmacologiques d'un nouvel alcaloid de l'ergot de Seigle, l'ergobasine. Comp. Rend. Soc. de Biol., 1935. 119, 1302-1304.

43. Schumann, H.J. Arterenol in Nebennierenmark. Klin. Wschr., 1948. 26, 604.

44. Sherlock, Sheila. Diseases of the liver and biliary system. Springfield, Illinois: C.C. Thomas, 1955.

45. Shoemaker, W.C. Measurement of hepatic blood flow in the anesthetized dog by a modified BSP method. J. Appl. Physiol., 1960. 15, 472-478.

46. Spuhler, O. Die experimentelle Untersuchung eines neuen Sympathicolyticum, des Dihydroergotamins. Schweiz. Med. Wchnschr., 1947. 77, 28-32.

47. Sutherland, E.W. & Cori, C.F. Effect of hyperglycemia-glycogenolytic factor and epinephrine on liver phosphorylase. J. Biol. Chem., 1951. 188, 531-543.

48. Trendelenberg, U. Thyroid and hyperglycemia produced by adrenaline and nor-adrenaline. Brit. J. Pharmacol., 1953. 8, 454-460.

49. Vrij, C., Cho, B.F., deGroot, C.A. & Weber, J.F. The effects of isopropyl nor-adrenaline on the glycogen content of skeletal muscle and liver in the rat. ACTA Physiol. Pharmacol. Neerl., 1956. 4, 547-554.

50. West, E.S. & Todd, W.R. Textbook of biochemistry. (2nd Ed.) New York: The Macmillian Co., 1957.

51. Wheeler, H.O., Cranston, W.I. & Neltzer, J.I. Hepatic uptake and biliary excretion of indocyanine green in the dog. Pro. Soc. Exp. Biol. & Med., 1958. 99, 11-14.

AN ABSTRACT OF THE THESIS OF

David Albert McClure for the Ph. D. in PHARMACOLOGY

Date of receiving this degree _____

Title: A Pharmacological Comparison of Adrenolytic Drugs Having Different Effects on Carbohydrate Production and Utilization

Approved: [redacted]

The effects on carbohydrate production and peripheral utilization from administration of sympathomimetic amines in conjunction with sympathetic blocking agents was studied. This work was undertaken to further prove the theory of R. P. Ahlquist. This theory explains the biphasic action of sympathomimetic amines on the adrenergic nervous system. Briefly, it states that there are present at the neuro-effector junctions of the sympathetic nervous system two types of receptors, alpha and beta. The alpha receptors are responsible for the stimulatory (Pressor, vasoconstriction, etc.) responses and the beta receptors are responsible for the inhibitory (depressor, vasodilatory, etc.) responses of sympathomimetic amines. Blockade of the alpha receptor response (stimulatory) by certain drugs has been known for some time. However, it has been only very recently that beta receptor blockade has become possible.

The drugs and doses chosen for this study were as follows: epinephrine (5 mcg./kg./ min), nor-epinephrine (20 mcg./kg./min.),

isoproterenol (1 mcg./kg./min.), azapetine (alpha receptor blocking agent, 2 mg./kg.), and dichloroisoproterenol (DCI) (beta receptor blocking agent, 10 mg./kg.). The sympathomimetic amines were infused at a constant rate before and after the blocking agents. The doses of the amines were chosen from their effectiveness in producing hyperglycemia. Both the sympathomimetic amines and the blocking agents were administered intravenously into dogs.

In order to determine hepatic production of blood sugar the hepatic vein was catheterized under fluoroscopy with a French # 10 Cournand type catheter via the right external jugular vein. The hepatic artery was approximated by placing a similar catheter in the aorta via the femoral artery opposite the hepatic artery. Samples of blood were then obtained from each area simultaneously and assayed for blood sugar and a dye, indocyanine green, used for the determination of hepatic blood flow.

The determination of the peripheral utilization of blood sugar was carried by using the hind limb of the dog. Blood sugar determinations were obtained from the femoral artery and vein simultaneously with the determination of femoral arterial blood flow.

Blood samples were taken at 1, 2, 4, 8, 16 and 32 minutes during the infusions. Each dog was used twice; once for the determination of the hepatic production of blood sugar and once for the determination of the peripheral utilization of the blood sugar. Control blood sugars and blood flows were obtained from each dog and the same drugs were used in the same dog. A total of forty-one experiments were performed.

The results indicate that the alpha sympathetic receptor blocking agent (azapetine) potentiated the hepatic production of blood sugar caused by each of the sympathomimetic amines. DCI, the beta sympathetic receptor blocking agent, blocked the induced hepatic production of blood sugar.

This demonstrates quite clearly that the hepatic production of blood sugar results from beta sympathetic receptor stimulation by sympathomimetic amines.

Both adrenergic blocking agents blocked the sympathomimetic amine induced peripheral utilization of blood sugar. Explanation of this response is that both types of peripheral receptors (alpha and beta) are necessary for the uptake and utilization of blood sugar resulting from sympathomimetic amine infusion.