1949

Functional tests of the adrenal cortex.

BOSTON UNIVERSITY

GRADUATE SCHOOL

Dissertation

THE INFLUENCE OF BLOCKING DRUGS ON EPINEPHRINE-INDUCED EFFECTS ON CARBOHYDRATE METABOLISM

by

Eugene Leslie Komrad
S.B., Long Island University, 1948
A.M., Boston University, 1949

Submitted in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

1953

Approved by:

First Reader .....Earl R. Loew.....
Professor of Physiology

Second Reader .....A. H. Hegnauer.....
Associate Professor of Physiology

TABLE OF CONTENTS

TABLE OF CONTENTS - continued

TABLES AND FIGURES

TABLES AND FIGURES - continued

INTRODUCTION

In the course of the battle between the living mammalian organism and the external environment, the animal body must make constant adjustments to meet stress situations and to maintain the homeostasis of the milieu interieur. The glands of internal secretion play an important role in these adjustments. Of the endocrine secretions, the hormones of the adrenal gland, both the cortex and the medulla are of paramount importance.

In an evaluation of the metabolic alterations to stress situations, it is necessary to realize that the endocrine glands are intricately regulated by a system of checks and balances. This system is most readily seen in the endocrine regulation of carbohydrate metabolism. It is obvious that the forces involved in maintaining a homeostatic level of carbohydrate metabolism can act in only two dimensions, increase or decrease, and only when these forces are at equilibrium, i.e., rate of production equals rate of destruction or immobilization, can a homeostatic situation occur. It is interesting to note that the individual endocrine glands not only affect carbohydrate metabolism by the direct action of their respective hormones, but in addition they exert an effect by influencing either directly or reflexly the activity of the other

endocrine glands. Thus, the adrenal gland, the pituitary, and the thyroid act synergistically to increase the blood glucose while the islets of Langerhans act to decrease the blood glucose. It should be understood that the effect of artificial stimulation or injection of an individual hormone will, in the intact organism, be governed by the existing endocrine balance in that organism at the time of stimulation.

In the course of the past century, a great deal of emphasis has been placed upon the evaluation of the role of the adrenal gland in the control of body metabolism in the normal individual as well as in the individual during a stress situation. This gland is interesting in that it is regarded anatomically as a single gross structure, yet physiologically and histologically there are two units, each capable of functioning quite independently of the other and affecting each other only by way of another gland, the anterior pituitary, or by changes in tissue metabolism (103a). Therefore, when considering, for example, the effects in an intact animal of epinephrine administration of stimulation, it is necessary to remember that in addition to the actions of epinephrine, one is bound to reveal some of the effects of the adrenal cortical hormone, since the former will stimulate secretion of the latter by stimulating the anterior pituitary to secrete adrenocorticotropin. The beauty of the

synergism of activity between the adrenal cortex and the adrenal medulla is readily exemplified in their effects on carbohydrate metabolism.

Under conditions of general body stress, the adrenal medulla is stimulated, either directly or reflexly to secrete its hormone, epinephrine. It is the purpose of this study to evaluate the role of epinephrine and its action on carbohydrate metabolism. The initial body adjustment to stress sacrifices the homeostasis of carbohydrate metabolism in order to meet the higher energy requirements of the organism. However, this interruption in the homeostasis is quite transient, and a new adjustment occurs at a higher level if stress is continued and its former level if it ceases. Ever since Blum's experiment in 1901 (11) showing that epinephrine injected into a dog produced glycosuria, there has been an overwhelming number of reports in the literature which have offered theories and proofs of the mechanism of action of epinephrine in its effect on carbohydrate metabolism.

It would be well, in this introduction, to consider some of the fundamentals of carbohydrate metabolism and to define a few of the terms which will be used in the course of this study; for a misuse of terms is one of the reasons for the confusing results which are seen.

The available evidence makes it rather clear that

in the postabsorptive mammalian individual the only source of blood glucose is the liver. The level of blood glucose is basically regulated much the same as the level of water in a sink with a faucet and a sewer drain. One could cause the level of water to rise by four methods. First, the inflow from the faucet could be increased without increasing the outflow from the sewer drain; second, the outflow from the sewer drain could be decreased without increasing the rate of inflow from the faucet; third, both the inflow and the outflow could be increased with the inflow increasing faster than the outflow; and finally the inflow could increase and the outflow decrease in all proportions to each other. Exactly the opposite of the above four methods would cause the level of the water to decrease and only when the inflow from the faucet is in dynamic equilibrium with the outflow from the sewer drain will the level of the water remain constant. The analogy to the level of blood sugar will be realized if one substitutes the liver supply of glucose for the faucet and the uptake of glucose by the tissues as the sewer drain (108a).

The liver stores its glucose as glycogen. Under a given stimulus, this glycogen is hydrolyzed and phosphorylated forming a hexosemonophosphate ester, glucose-1-phosphate which is isomerized to glucose-6-phosphate, the Cori ester. Under the action of a hexosephosphorylase,

this ester is split into phosphoric acid and glucose. It is generally considered that this reaction occurs within the liver cell, and the process is known as glycogenolysis. It is important to remember that this process is quite reversible, and as such it provides a means for glucose to enter the liver cell, and under the action of hexokinase be esterified with phosphate and undergo the transformation to glycogen. The liver glycogen can be formed, in addition, from carbohydrate metabolities, such as lactic acid, by an enzymatic conversion of two and three carbon fragments to glycogen. This formation of glycogen from glucose and carbohydrate metabolities is known as glycogenesis. However, liver glycogen can also be formed from non-carbohydrate precursors, a process known as glyconeogenesis. The main precursors for this process are proteins, which in the course of their metabolism are broken down to two and three carbon fragments. These fragments entering the liver cell are exposed to a number of enzyme systems, and are finally converted to glycogen (26, 43a, 61a, 108a).

The muscle glycogen, which comprises the greatest part of the tissue glycogen is unable to form blood glucose. Like the liver glycogen, it is esterified with phosphate, then isomerized to form the Cori ester, the beginning of the process of glycolysis. However, following this, the ester undergoes a series of transformations, passing through a

number of acid forms, finally ending as pyruvic acid. This entire process is anaerobic, and if it continues in an anaerobic state, lactic acid will be formed. In the presence of oxygen, the organic acid enters an oxidative cycle, which is known as the Krebs' cycle, and finally is broken down to carbon dioxide and water. This entire process, the transformation of glycogen to its final end products, lactic acid, carbon dioxide, and water is an exothermic reaction, and is believed by most physiologists to provide the ultimate energy for muscular contraction (26, 43a, 61a). This process of the degradation of glucose to carbon dioxide, water, and lactic acid, together with the process of glycogenesis in both liver and muscle, we shall define as glucose utilization.

To complete the carbohydrate cycle, the lactic acid is carried by the blood stream to the liver where it is converted, as described above, to liver glycogen. In the mammalian organism, the muscle is unable to convert lactic acid to muscle glycogen. How then does the muscle replenish its glycogen? A process exactly the same as occurs in the liver also occurs in the muscle; i.e., muscle glucose and phosphoric acid are esterified under the action of hexokinase to form the Cori ester, glucose-6-phosphate. This phase of glucose metabolism, the removal of glucose from the interstitial fluids by the tissues, and its

phosphorylation, from now on will be referred to as the uptake of blood sugar to distinguish it from glucose utilization, which refers to the combustion and catabolism of glucose as well as glycogen formation from hexosemonophosphate. The Cori ester can undergo two main transformations. It can either be isomerized to glucose-1-phosphate, and then be converted to glycogen, or it can be broken down to the various acids finally forming carbon dioxide and water. In the literature, the amount of glucose uptake is called glucose utilization. If this were true, in isolated tissue studies, all of the glucose removed from the medium should be accounted for in terms of glycogen synthesis and carbon dioxide and lactic acid formation. However, the results of previous studies (64, 115, 121) as well as this study have been unable to support the validity of this hypothesis. Actually, it has been shown that it is possible for the isolated muscle to store hexosemonophosphate under certain conditions, i.e., while under the influence of epinephrine (64, 115, 151). Therefore, it is believed to be of paramount importance to separate the two phases, glucose uptake and glucose utilization, in order to interpret the mechanism of epinephrine activity in carbohydrate metabolism.

THE EFFECT OF ADRENERGIC BLOCKING DRUGS ON EPINEPHRINE-INDUCED HYPERGLYCEMIA IN RABBITS

INTRODUCTION

A majority of the available adrenergic blocking drugs are known to diminish or block many of the excitatory effects of epinephrine, although the positive chronotropic effect of epinephrine upon the myocardium is not known to be blocked by any adrenergic blocking drugs. Since an excellent extensive review on the pharmacology of adrenergic blockade has been published by Nickerson (92), no attempt will be made to review this subject in this thesis. In view of the ability of the adrenergic blocking drugs to inhibit so many of the physiologic effects of epinephrine, it seems important to know the effects of these drugs on hyperglycemia produced by exogenous epinephrine. Data concerning the effect of these drugs upon the metabolic effect of epinephrine are limited in quantity and scope. If adrenergic blocking drugs could be proved to block some specific phase in carbohydrate metabolism, they would be useful research tools for investigating the mechanism of epinephrine-induced hyperglycemia as well as other problems in carbohydrate metabolism. Consequently, the following experiments were made to determine whether or not some of the potent adrenergic blocking drugs recently synthesized are capable of blocking epinephrine-induced hyperglycemia.

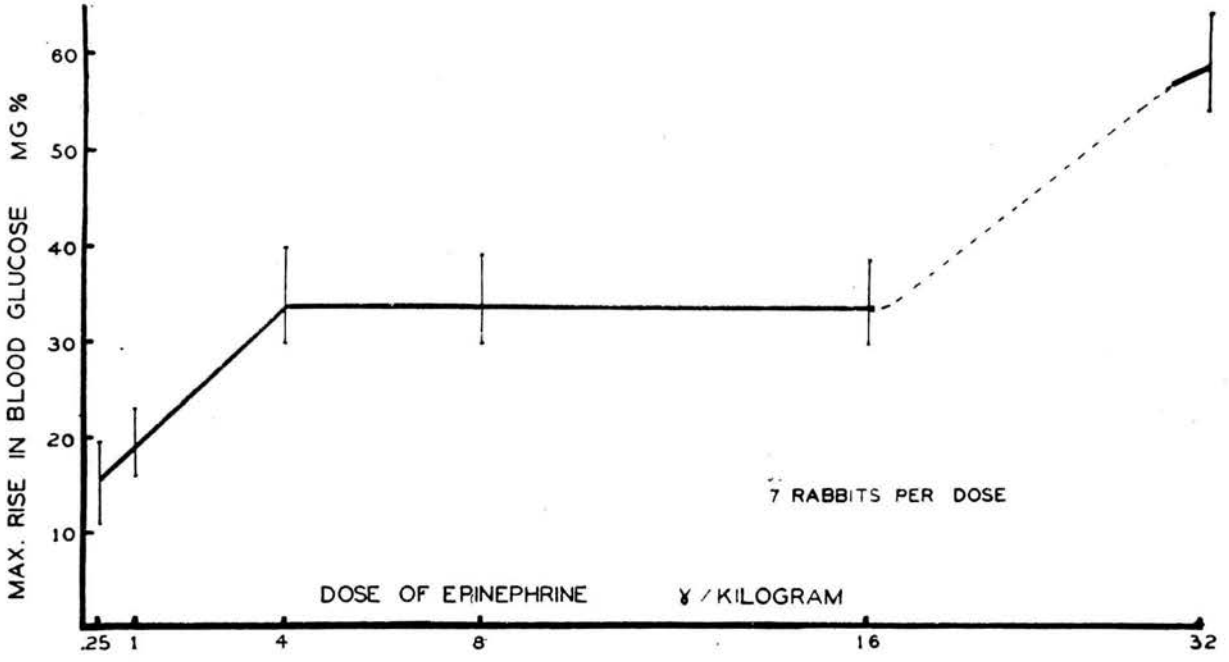
METHODS

Adrenergic blocking drugs were injected into male albino rabbits in order to determine their influence on hyperglycemia induced by epinephrine injected 30 minutes after the test drug. All agents were injected intravenously in order to remove the influence of the rate of absorption.

All experiments were performed on a series of 35 rabbits which when not in use were kept in individual wire cages, and fed Purina Rabbit Pellets and cabbage. The animals were fasted for 18 hours prior to the test, and water was supplied ad libitum. They were acclimatized to the experimental procedures (sitting in rabbit boxes, injection procedures, and withdrawal of blood) at least twice before any data were tabulated. No agent was tested more than once in the same rabbit, and the experiments were so designed that on any one day a number of compounds and saline controls were tested. The animals were placed in individual rabbit boxes which were uncovered except when injections or blood withdrawals were made. Blood samples were taken by cutting the ear vein and collecting a sample of freely flowing blood.

The procedure consisted of injecting the agent to be tested over a one minute period. Thirty minutes later, a blood sample was taken and epinephrine was injected.

FIGURE 1



DOSE-RESPONSE CURVE of epinephrine-induced hyperglycemia in rabbits.

Blood samples were collected at 5, 10, 15, 20, and 40 minutes following the injection. The blood sugars were then determined by the Folin-Malmros micromethod (41).

A standard dose of 4 gamma/kg. of pure l-epinephrine* in a volume of 0.5 ml. was injected at a steady rate during 30 seconds. This dose was selected on the basis of the dose-response curve shown in Figure 1. Six doses of epinephrine were injected into a series of rabbits, and the blood sugar was measured at five minute intervals following the injection. A total of seven rabbits were tested with each dose. There was no significant difference between 4, 8, and 16 gamma/kg. while with 32 gamma the curve rose sharply, suggesting that the dosage level produced its effect by means of another mechanism. This curve is in close agreement with the one described by Eadie (34). The dose of 4 gamma/kg. was reported (15) to be just within physiologic range. Furthermore, doses of a similar magnitude have been reported to induce a moderate hyperglycemia in rabbits (34), and cats (55). Although a saline injection did not have any effect on the fasting blood sugar level over a period of 180 minutes, the injection of a standard dose of epinephrine induced a prompt rise which reached its maximum at 15 minutes and returned to normal in 40 minutes. The magnitude of the rise in blood sugar with this dose was surprisingly consistent, since it varied only from 27 to 40 mg./100 ml. of blood in 60 untreated rabbits.

---

*Throughout this dissertation the dose of epinephrine is given in terms of pure l-epinephrine base.

TABLE 1.

EFFECT OF ADRENERGIC BLOCKING DRUGS ON EPINEPHRINE-INDUCED HYPERGLYCEMIA

COMP.	PREPARATION TESTED[1]	DOSE I.V.	NO. OF RABBITS	MEAN LEVEL 30 MINUTES AFTER DRUG	MEAN LEVEL 15 MINUTES AFTER EPI.[2]	MEAN RISE[3]	% OF CONTROL RISE
		mg/kg.		*mg %*	*mg. %*	*mg. %*	
	Saline, control for drugs *1* to *9*	1 ml.	11	103±7	140±11	37±4	100
	Saline, control for drugs *10*, *11*	1 ml.	14	101±3	132±4	31±4	100
1	Dihydroergocornine (Sandoz—DHO-180)	0.1	6	107±3	110±2	3±2	8
2	Ethyl-2-chloroethyl-2-ortho-benzylphenoxyethylamine HCl (Eli Lilly—04679)	2	6	112±3	114±5	3±3	8
3	1-Phenyl-2N-methylbenzyl-aminoethylchloride HCl (Eli Lilly—08353)	2.5	6	108±2	110±3	2±2	5
4	N-9-Fluorenyl-N-ethyl-2 chlorethylamine HCl (Parke, Davis SY-21)	2	6	107±7	121±9	14±4	38
5	1-Naphthylmethylethyl-2-bromoethylamine HBr (Parke, Davis SY28)	2	6	110±5	124±5	14±3	38
6	3-(N-Ethyl-N-2-chloro-ethyl) aminoethyl thianaphthene HCl (Eli Lilly—08125)	5	6	112±6	126±8	14±4	38
7	2-(2-Biphenylyloxy)-2'-chloro-triethylamine HCl (Parke, Davis SY-8)	5	6	112±4	129±6	17±3	46
8	N-Ethyl-N-(2-chloroethyl) benzhydrylamine HCl (Parke, Davis SY-2)	10	4	108±6	145±9	37±5	100
9	3(N-Methyl-N-2-chloroethyl)-aminomethyl thianaphthene HCl (Eli Lilly–08124)	5	4	110±7	149±6	39±4	105
10	1-Naphthylmethylethyl-2 hydroxyethylamine HCl (Parke, Davis SY-73)	4	5	102±3	132±2	30±1	98
11	1-Phenyl-2N-methyl-benzyl-aminoethanol HCl (Eli Lilly—02074)	5	5	103±3	134±3	31±4	100

[1] *Drugs 1 to 9* were tested in a group of 20 rabbits. *Drugs 10* and *11*, in 10 rabbits.

[2] Epinephrine was injected i.v. in a dose of 4 μg/kg. over 30 seconds 30 minutes after the i.v. administration of the blocking agent.

[3] Statistical comparison of group data for each of the *compounds* no. *1* to *7* with control data yields a P value <0.01.

RESULTS

The effects of drugs on epinephrine-induced hyperglycemia are tabulated in Table 1. The administration of an adrenergic blocking drug did not change the time of maximum rise in the hyperglycemic curve. Therefore, only the rise occurring at 15 minutes after the injection of epinephrine is herein reported, although as mentioned above blood samples at 5, 10, 15, 20, and 40 minutes were obtained. Each agent was tested for its effect on the fasting blood sugar level in 3 rabbits and in no case was there any significant change over a period of 150 minutes.

In most cases the dose of adrenergic blocking drug selected for use was several times the dose required to reverse epinephrine-induced hypertension, since in these experiments we are probably dealing with only the excitatory phase of adrenergic activity, while in the blood pressure response both the excitatory and inhibitory phases are present, and a slight reduction in the excitatory phase is exaggerated by the presence of the inhibitory phase.

Dihydroergocornine. The pharmacology of dihydroergocornine (DHO-180) has been studied by Rothlin (102), who found this agent to be a potent blocking agent as measured by the effect on hypertension induced with epinephrine. In his experiments to test the effect of the drug

on epinephrine-induced hyperglycemia, both the DHO-180 and epinephrine were injected subcutaneously. His results show that hyperglycemia was diminished or blocked. In this study his experiment has been repeated, using the same dose of DHO-180, but injecting intravenously as described above. It is interesting to note that in each of the six rabbits tested this agent produced an effective blockade of the blood sugar rise in a dose which is smaller than the one required to consistently reverse epinephrine hypertension. One wonders if this means that the compound has a selective action on the liver cells. As shall be seen in a subsequent part of this study there is further evidence in support of the validity of this suggestion.

The remainder of the compounds tested belong to a series of 2-haloalkylamines. It has been found (94) that the ethyl homologues of this series have a greater adrenergic blocking activity than the methyl homologues, and if the halogen is replaced by an hydroxyl group the resulting compound loses its blocking ability (78, 79, 80, 94). Several ethyl homologues have here been tested for their effect on epinephrine hyperglycemia.

2-Orthobenzylphenoxyethyl. Compound 2(Table 1) was previously reported (65) to be a potent adrenergic blocking drug. In the present experiments a dose somewhat larger than that required to reverse epinephrine hypertension

in dogs produced a complete blockade of induced hyperglycemia in rabbits.

1-Phenyl-2-N-Methylbenzyl. Preliminary experiments (20) have shown that compound 3 will reverse epinephrine hypertension in the dog in a dose of 2 mg./kg. Following injection of 2.5 mg./kg. of this agent, there was no change in the fasting blood sugar level in response to epinephrine. As will be shown in the next series of experiments in this study, this compound also reduced the hyperglycemia resulting from an infusion of epinephrine in dogs. When the halogen of this compound was replaced by an hydroxyl group (Compound 11) the resulting agent even in twice that dose did not affect the hyperglycemia in rabbits, indicating that the presence of the halogenated ethylamine group is required for the blockade of hyperglycemia as well as the pressor response of epinephrine.

9-Fluorenyl. Compound 4 has been found to be among the most potent of adrenergic blocking drugs (71, 78). It is a specific agent, antagonizing only the effects of epinephrine and some of its congeners. This compound in a dose of 2 mg./kg. effectively reduced the induced hyperglycemia in rabbits and as we shall see later, in dogs, although a complete blockade did not occur.

1-Naphthylmethyl. Previous reports have shown that Compound 5 is a potent epinephrine antagonist (79, 113),

although not specific in its adrenergic blocking activity, since it is a potent antihistaminic (78, 79, 113). This compound in a dose of 2 mg./kg. produced the same reduction in hyperglycemia as Compound 4. When the halogen was replaced by an hydroxyl radical (Compound 10) the resulting agent lost all of its effect in antagonizing the epinephrine toxicity in mice (79). This gives further support to the belief that the ability to inhibit the hyperglycemic effect of epinephrine is related to the ability to inhibit the other effects of epinephrine.

Methylthionaphthalene. Compound 6 is not as potent as the above compounds in its adrenergic antagonism, as measured by reversal of the pressor response to epinephrine (20). It required a dose of 5 mg./kg. to effect a reduction of the induced hyperglycemia equivalent to that obtained by Compounds 4 and 5.

The methyl homologue of this compound (Compound 9) was weakly active in causing reversal of epinephrine hypertension (20), and had no effect on the induced hyperglycemia. Thus, it is again noted that an agent which loses its adrenergic blocking activity also loses its ability to reduce epinephrine-induced hyperglycemia.

2-Biphenylyloxy. It has been reported that Compound 7 is less potent than Compounds 2 to 5 when its blocking of adrenergic activity is measured (80). This

agent, like Compound 5, is not only an adrenergic blocking drug, but also shows antihistaminic activity. Like Compound 6, a dose of 5 mg./kg. only reduced the hyperglycemia. Thus again there is evidence that adrenergic blocking drugs of low potency are not highly effective in diminishing epinephrine-induced hyperglycemia.

Benzhydryl. Compound 8 has been described as being of the same order of adrenergic blocking potency as Dibenamine (78). Dibenamine, itself, has been tested (93) (although the injections were made subcutaneously), and found to be without effect in reducing induced hyperglycemia. However, further experiments (49) demonstrated that if the numbers of animals were increased, and a cross-over type of experiment was employed, a statistically significant decrease in the epinephrine-induced hyperglycemia could be demonstrated. The dose of test drug employed in those experiments was large. When Compound 8 was used in a dose of 10 mg./kg., it failed to elicit a significant reduction in hyperglycemia.

Thus, Compounds 2 to 5 which are the most potent members of the ethyl homologues of the 2-haloalkylamine series succeeded in blocking or markedly reducing the hyperglycemic response to 4 gamma/kg. of epinephrine. It required almost twice the dose of the less potent homologues (Compounds 6 and 7) to effect an equivalent blockade, and the weakest member of the series, (Compound 8) was without effect.

The methyl homologue (Compound 9) and the non-halogenated derivatives (Compounds 10 and 11) which are either devoid of the ability to reverse epinephrine hypertension or show a very weak adrenergic antagonism, were also without effect in reducing epinephrine-hyperglycemia.

DISCUSSION

As early as 1912, Miculicich (88) demonstrated that ergotoxin reduced the hyperglycemia and glycosuria due to subcutaneous injection of epinephrine in the rabbit. However, since that time, other adrenergic blocking drugs have been shown to be effective when used in the rabbit.

In 1929, Hanson (58) studied the effect of yohimbine on epinephrine-induced hyperglycemia. The rise in blood sugar was elicited by a subcutaneous injection of 0.1 mg./kg. in rabbits. This rise was completely suppressed by an intravenous injection of 1.5 mg. of yohimbine per kg. of body weight. Whereas the control blood sugar rose from 115 to 315 mg. % in 1.5 hours after epinephrine injection, the rise in yohimbine treated rabbits was only from 115 to 135 mg. %. This slight rise in the yohimbine treated rabbits could not be inhibited, even if the dose of yohimbine was increased to 25 mg./kg. This study was a confirmation of the previous work of Nitzescu (95).

The influence of the benzodioxane derivatives was tested by Blancher in 1934 (10). Working with rabbits, he injected epinephrine in a dose of 0.2 mg./kg. subcutaneously, and obtained exactly the same result as Hanson did using half of that dose, namely from 115 to 314 mg. %. This rise, however, was said to occur three hours after the injection

rather than 1.5 hours reported by Hanson. It was shown that diethyl-amino-methyl-3-benzodioxane (883F) and piperido-methyl-3-benzodioxane (933F) injected in doses of 50 mg./kg., subcutaneously, blocked this hyperglycemic response.

Early experiments with Dibenamine, the first in the aryl-2-haloalkylamine series, indicated that this agent was ineffective in diminishing the hyperglycemic effects of epinephrine. Nickerson and Goodman (93) in one of their first reports of the pharmacologic activity of this compound, reported their experiences in the attempt to block epinephrine-induced hyperglycemia in rabbits. They injected their fasted rabbits subcutaneously with 0.2 mg./kg. of epinephrine, and followed the blood sugar rise periodically. They noted an increase of from 100 to 250 mg. % in 180 minutes. That this rise is less than that reported by previous workers may be accounted for on the basis of more sensitive methods for blood sugar analysis. If the rabbits were pretreated with 50 mg./kg. of Dibenamine given subcutaneously, the blood sugar rose from 125 to 240 mg. % in 40 minutes. It was claimed that there was no significant difference in the two curves except for the earlier peak following Dibenamine. This latter effect was said to be due to a hastened absorption of the epinephrine under the influence of the blocking drug. However, the authors failed to take into account the fact that the hyperglycemic effect started from a higher basal level,

125 mg. % as opposed to 100 mg. %, and hence a reduction in the peak response of 25 mg. % could be shown. In addition, due to the hastened peak of response with a rather rapid return to normal, the area, i.e., the combination of magnitude and duration of hyperglycemia, was certainly curtailed following treatment with Dibenamine.

That these small differences could be magnified into statistical significance was shown by Grant (49) who employed the cross-over type of experiment with 12 rabbits, and using the same dosage as Nickerson and Goodman, demonstrated a statistically significant decrease in the hyperglycemia induced by epinephrine in those animals pretreated with Dibenamine.

Hecht and Anderson (62) have found that Dibenamine was ineffective in the blockade of epinephrine-induced hyperglycemia in man.

In 1951 Chen and Clarke (18) attempted to block epinephrine-induced hyperglycemia in rats using another of the aryl-2-haloalkylamine series, N-(2-chloroethyl)-N-ethyl-1-naphthaline methylamine. HCl (SY-14). They were unable to inhibit the effect of epinephrine given subcutaneously in a dose of 0.5 mg./kg. with SY-14 given in doses of 6 and 12.5 mg./kg. However, 25 mg./kg. of SY-14 given 1/2 hour before the epinephrine caused a decrease in the blood sugar while 50 mg./kg. blocked it completely. It

is noteworthy that if 25 mg./kg. of SY-14 were given 24 hours prior to the injection of epinephrine, there was a complete blockade of the hyperglycemia expected.

Recently, Harvey, Wang, and Nickerson (61) have reported on the influence of a series of 15 compounds of this 2-haloalkylamine series on epinephrine-induced hyperglycemia. Their experiments differed from our own in that the blocking drug as well as the epinephrine were injected subcutaneously. The blocking drug was allowed to act for 4 hours at which time epinephrine in a dose of 0.1 mg./kg. was administered. All 15 compounds tested manifested some degree of suppression of hyperglycemia. It was noted that higher doses of blocking drug were required to block the hyperglycemia effect than to block the blood pressure effect in many cases. However, it should be borne in mind that the pressor effect of epinephrine is only an algebraic summation of pressor and depressor effects, and only a slight inhibition in the pressor effect would cause a change in the balance to allow the depressor effect to manifest itself in what appeared to be a partial or complete blockade. Hence in measuring pressor responses, one has the depressor effect of epinephrine aiding the action of the blocking agent, while in the hyperglycemic response, the blocking agent must act alone. These workers did not find any correlation between the ability to suppress the hyperglycemia and the ability

to block pressor responses. Thus, these authors felt that the conclusions reported for this study (73a) were unjustified in view of their data and review of the literature. However, upon the examination of the data reported in their paper, it appeared to this author that there was a rough approximation of relative dose required for blockade of hyperglycemia and blockade of pressor responses, at least in the rabbit. There are several exceptions in that some compounds affecting the blood pressure response do not affect the hyperglycemia and vice versa. However, as will be shown in the next series of experiments herein reported, the *in vivo* blockade of epinephrine-induced hyperglycemia, at least in rabbits, is by no means limited to the adrenergic blocking drugs. As regards the review of the literature, Harvey *et al.* have gleaned their evidence from work done in several animal species. As will be pointed out in the next series of experiments, it is necessary to confine one's self to a given species in speaking about this effect, since the response has some degree of species specificity. It is still this author's contention that there appears to be a rough correlation between the potency of blocking drug in its effect on blood sugar increase and pressor effect due to epinephrine.

Ellis and Anderson (36) reported a suppression of epinephrine-induced hyperglycemia in the rat with Dibenamine,

SY-28, dihydroergotamine, and Priscoline. Priscoline had previously been reported to be ineffective in blocking the hyperglycemia (60, 66) but this was probably due to an ineffective dosage level. The imidazoline type of alkylamine, C-7337 (Regitine), has been reported to suppress epinephrine-induced hyperglycemia in man (63).

The natural and dihydrogenated ergot alkaloids have been studied more thoroughly than the other adrenergic blocking drugs in this response, probably because of their earlier appearance in the laboratories. As was mentioned earlier in this discussion, Miculicich described this property of ergotoxin in 1912 (88). Barger (7) in 1938 has extensively reviewed the older literature on the metabolic actions of the natural ergot alkaloids, and the reader is referred to that reference for the earlier literature on the subject. Recently, Rothlin (101, 102) has shown that the dihydrogenated derivatives of the ergot alkaloids are increased in potency when measured against the hyperglycemic actions of epinephrine. However, this potency was not increased quite as much as against the pressor effects of epinephrine. However, the doses required to block the hyperglycemia were much smaller than those necessary to inhibit the hypertensive action of epinephrine. Dihydroergotamine (112), and dihydroergokryptine (42) have also been shown to be active in preventing epinephrine-induced hyperglycemia in man. In addition, it

has been shown that ergonovine (60), with no known adrenergic blocking activity, in large doses blocked hyperglycemia induced by epinephrine. It is this author's belief that this is due to some indirect effect since it will be shown in the next section there are other agents without adrenergic blocking activity which will inhibit the hyperglycemia.

A discussion of the possible mechanisms of action of some of these agents will be found in the last section of this thesis.

SUMMARY

A number of adrenergic blocking drugs were tested in rabbits for their effect on epinephrine-induced hyperglycemia. With the exception of dihydroergocornine, all the agents tested belonged to a series of 2-haloalkylamines. It is suggested that the effectiveness of 2-haloalkylamines in diminishing or blocking epinephrine-induced hyperglycemia in rabbits is roughly related to their potenty as measured by antagonism of other effects of epinephrine. The basis for this suggestion is that the most potent drugs block or diminish the blood sugar rise in a dose of 2 mg./kg., while the less potent homologues require a dose of 5 mg./kg. to obtain the same effect. The least potent of these agents did not reduce the hyperglycemia even in a dose of 10 mg./kg. In addition, the methyl homologue of one of these adrenergic blocking drugs known to have a markedly reduced epinephrine antagonism did not have any effect on hyperglycemia. Likewise, when the halogen is replaced by an hydroxyl group, and the resulting agent loses its epinephrine reversal action, it also loses its ability to block epinephrine-induced hyperglycemia. A review of the literature together with the data obtained seems to indicate that it is a general property of the adrenergic blocking drugs to inhibit the hyperglycemic response to epinephrine in the

rabbit. However, this effect may have some species specificity. In addition, some other agents without adrenergic blocking activity may elicit this response.

THE EFFECT OF CERTAIN ANTIHISTAMINES ON EPINEPHRINE-INDUCED HYPERGLYCEMIA IN THE RABBIT. ALSO, THE INFLUENCE OF SEVERAL ANTIHISTAMINES AND ADRENERGIC BLOCKING DRUGS ON EPINEPHRINE-INDUCED HYPERGLYCEMIA AND LACTICACIDEMIA IN THE DOG.

INTRODUCTION

Attention has been drawn previously to the fact that several synthetic drugs possessing antihistaminic properties were also capable of diminishing or blocking some excitatory effects of epinephrine (76, 77). The most striking example was the discovery that 1-naphthylmethyl-2-bromoethylamine HBr (SY-28) was a potent adrenergic blocking drug of the 2-haloalkylamine type, and, in addition, this drug exerted a strong antihistaminic action. Thus a single compound pharmacologically antagonizes the effects of epinephrine and histamine, substances which are in themselves antagonists in many respects. Nevertheless, the majority of antihistamines are fairly specific with respect to histamine blockade, although many of them are known to increase the pressor responses to epinephrine in anesthetized dogs (77). As indicated previously (76, 77), the ability of antihistamines either to increase or decrease certain effects of epinephrine suggests that there may be some similarity in the ultimate site or mechanism of action of histamine and epinephrine. The evidence cited certainly suggests that the antihistamines which heretofore have been regarded as rather specific might influence some effects of epinephrine other than those revealed in previous studies.

Accordingly, several of the available antihistamines have been tested for their ability to influence epinephrine-induced hyperglycemia in rabbits. In addition, the question of species specificity as well as the ability of agents without blocking activity to influence this response has been examined.

METHODS

Rabbits. Male albino rabbits maintained on a diet of rabbit pellets and greens as previously described were fasted for 18 hours prior to an experiment. Following the withdrawal of fasting blood samples from the marginal ear vein, the agent to be tested was administered intravenously into the other ear in a volume of 1.0 ml./kg. The injection was made slowly at the rate of 4 ml. in 2 to 3 minutes. Either 15 or 30 minutes later another blood sample was withdrawn, and pure l-epinephrine base in a dose of 4.0 gamma/kg. in a volume of 0.1 ml./kg. was injected intravenously during a period of 30 seconds. The reasons for this choice of dosage of epinephrine were mentioned in a previous portion of this treatise. Blood samples were obtained from the marginal ear vein at 10, 15, 20, and 30 minutes following the epinephrine injection. The experiments were arranged so that no rabbit was tested more than once with a given drug, and at least two agents with a saline control were tested on any given day.

Dogs. Six female mongrel dogs weighing 13-17 kg. were trained to stand in dog stocks and to submit to experimental procedure without excitement. These animals were fasted for 24 hours before an experiment. After withdrawal of a fasting blood sample from the vein of a forelimb,

the agent to be tested was injected intravenously in a concentration of 10 mg./ml. over a 3 minute period. One half hour later another blood sample was withdrawn, and a solution prepared from pure l-epinephrine base was infused at the rate of 1 gamma/kg./minute for 15 minutes. Following this infusion, samples of venous blood were obtained at 0, 10, 20, 30, 45, 60, 75, and 105 minutes. In another series following injection of 4 gamma/kg. of epinephrine during a 30 second period, venous blood samples were obtained at 0, 5, 10, 15, 20, 40, and 60 minutes.

All blood samples of both rabbits and dogs were tested for their blood sugar content by the micromethod of Folin and Malmros (41). The blood samples were tested for their lactic acid content by the method of Barker and Summerson (8).

TABLE 2.

Effect of antihistaminics on epinephrine-induced hyperglycemia

DRUG TESTED*	DOSE USED I.V.	MEAN LEVEL 30′ AFTER TREATMENT	MEAN LEVEL 15′ AFTER EPI.	MEAN RISE	% OF CONTROL†
	mgm./kgm.	*mgm. %* ± *S.D.*	*mgm. %* ± *S.D.*	*mgm. %* ± *S.D.*	
Saline (14 rabbits)	1 ml.	101 ± 3	132 ± 4	31 ± 2	100
N,N-Dimethyl-N′-(α-pyridyl)-N′-(α-thionyl) ethylene diamine·HCl (Histadyl)	5	115 ± 2	117 ± 3	3 ± 3	10
	3	105 ± 9	110 ± 13	3 ± 3	10
	1	102 ± 3	119 ± 4	17 ± 1	55
N′-Pyridyl-N′-5-chlorothenyl-N-dimethylethylenediamine citrate (Chlorothen)	3	112 ± 5	116 ± 3	3 ± 1	10
	1	102 ± 2	125 ± 5	23 ± 4	74
N′-p-Methoxybenzyl-N′,N′-dimethyl-α-pyridylethylenediamine maleate (Neoantergan)	3	106 ± 5	111 ± 9	5 ± 2	16
N,N-Dimethyl-N′-benzyl-N′-(α-pyridyl)-ethylenediamine·HCl (Pyribenzamine)	3	103 ± 5	106 ± 4	3 ± 3	10
N′-β-Dimethylaminopropylthiodiphenylamine·HCl (Phenergan)	3	101 ± 3	107 ± 4	5 ± 5	16
1-(p-Chlorophenyl)-1-(2-pyridyl) 3 N,N-dimethylpropylamine maleate (Chlortrimeton)	3	103 ± 5	114 ± 9	9 ± 4	29
	1	103 ± 3	127 ± 4	24 ± 2	77
1-(10-Acridyl)-2-dimethylaminoethane·HCl (Lilly 01798)	3	106 ± 8	109 ± 8	3 ± 3	10
1-Dimethylamino-2-(2′-benzyl-4′-chlorphenoxy) ethane·HCl (Lilly 01780)	3	108 ± 4	122 ± 5	16 ± 2	52
N,N-Dimethyl-N′-phenyl-N′-(2-thienylmethyl) ethylenediamine·HCl (Diatrin)	5	107 ± 5	124 ± 6	18 ± 2	58
β-Dimethylaminoethyl benzhydryl ether·HCl (Benadryl)	5	104 ± 5	131 ± 6	27 ± 3	87
2-Methyl-9-phenyl 2,3,4,9-tetrahydro-1-pyridindene tartrate (Thephorin)	5	104 ± 5	131 ± 6	27 ± 2	87
N,N-Dimethyl-N′-(p-methoxybenzyl) N′-(2-pyrimidyl) ethylenediamine·HCl (Neohetramine)	5	106 ± 6	128 ± 9	22 ± 4	71
2[α-(2-Dimethylaminoethoxy)-α-methylbenzyl] pyridine succinate (Decapryn)	5	103 ± 4	126 ± 3	23 ± 2	74
N-Methyl-N′-(4-chlorobenzhydryl) piperazine·2 HCl (Perazil)	5	102 ± 3	127 ± 3	24 ± 1	77

Epinephrine (4.0 microgm./kgm.) was injected I.V. 30 minutes after the test drug or saline was injected.

* Each drug and each dose were tested in 5 rabbits from a pool of 15 rabbits.

† The "p" value of all agents when compared with saline by group means equals <0.01.

RESULTS

A. Hyperglycemia in Rabbits:

The effects of antihistaminic agents on epinephrine-induced hyperglycemia in rabbits are listed in Table 2. It was found that when antihistamines were injected intravenously and slowly, as they were in this series, they did not produce any rise in the fasting blood sugar level. Thenylpyramine (Histadyl), N-pyridyl-N'-5-chlorothenyl-N-dimethylethylenediamine citrate (Chlorothen), Pyranisamine (Neoantergan), Tripelenamine (Pyribenzamine), N'-2-dimethylaminopropyl thiodiphenylamine. HCl (Phenergan), Chlorprophenpyridamine (Chlortrimeton) and 1-(10-acridyl)-2-dimethylaminoethane. HCl (Lilly 01798), have been described as being among the most potent antihistaminics (20, 75, 76, 122).

These were found to be the most effective in diminishing epinephrine-hyperglycemia in rabbits. Such activity cannot be due solely to the ethylenediamine structure contained in the five compounds listed in Table 2, since this structure is absent in the two active compounds, Chlortrimeton and Lilly 01798. Furthermore, aminophylline proved ineffective even though the amount injected represents at least twice as much ethylenediamine as contained in an effective dose of antihistamines of the

TABLE 3.

Effect of various agents on epinephrine-induced hyperglycemia in rabbits

NO.	DRUG TESTED[1]	DOSE I.V.	MAXIMUM RISE 15 MIN. AFTER EPI.	"P" VALUE OF DRUG VS. CONTROL[2]
		mgm./kgm.	*mgm. % ± S.D.*	
	Saline—14 rabbits (for #1–3)	1 ml.	31 ± 2	
	Saline—9 rabbits (for #4–8)	1 ml.	31 ± 3	
1	Aminophylline	10	30 ± 1	>0.05
2	Sodium pentobarbital (Nembutal)	30	30 ± 1	>0.05
3	Procaine HCl	5	31 ± 2	>0.05
4	Cocaine HCl	2	32 ± 2	>0.05
5	Atropine Sulfate	3	31 ± 4	>0.05
6	Pitressin	0.2 u.	4 ± 3	<0.01
7	Pitocin	0.2 u	20 ± 4	<0.01
8	Ephedrine Sulfate	3	6 ± 6	<0.01

Epinephrine (4.0 microgm./kgm.) was injected I.V. either 30 min. (#1–3) or 15 min. (#4–8) after the test drug or saline was injected.

[1] Each drug was tested in 5 rabbits; number 1–3 from a pool of 15 rabbits; number 4–8 from pool of 12 rabbits.

[2] 'P' value determined by comparison of group means.

ethylenediamine type. In addition, appreciable doses of Methapheneline (Diatrin), and Thonzylamine (Neohetramine) which do contain the ethylenediamine structure did not markedly inhibit the epinephrine-induced hyperglycemia. The last seven compounds listed in Table 2 are not highly potent antagonists of histamine. These compounds only slightly reduced the hyperglycemia induced by epinephrine, even though the dosage employed was usually 5.0 mg./kg. It is suggested, in view of these data, that the ability of the antihistaminic drugs to block epinephrine-hyperglycemia in rabbits is related to the potency of these agents as measured by the antagonism of the effects of histamine on the bronchioles and on the ileum of guinea pigs.

Since the antihistamines have been regarded as fairly specific agents, it was of interest to determine whether any agents in addition to adrenergic blocking drugs and antihistamines would produce an inhibition of epinephrine-hyperglycemia. The agents tested and the results are listed in Table 3. Since neither cocaine nor procaine altered the blood sugar rise, it is unlikely that the effectiveness of the synthetic antihistamines is related to their local anesthetic properties. In addition, the fact that atropine was ineffective indicates that the atropine-like activity of some of these agents is not

TABLE 4.

The effect of antihistamine and adrenergic blocking drugs on epinephrine-induced hyperglycemia and lacticacidemia in dogs

Treatment[a]	BLOOD GLUCOSE: Mean Max. rise mgm. % ± S. D.	P[b]	Mean area[c] of hyperglycemia mgm. min. ± S. D.	P[b]	BLOOD LACTIC ACID: Mean max. rise mgm. % ± S. D.	P[b]	Mean area[c] of lactic-acidemia mgm. min. ± S. D.	P[b]
a) Epinephrine infusion of 1 microgm./kgm./min. for 15 min.								
Epinephrine infusion Control	34.7 ± 1.3		1600 ± 432		27.2 ± 2.2		1086 ± 376	
Histadyl 1.5 mgm./kgm.	45.3 ± 3.1	<0.01	1904 ± 501	0.2 <p<0.3	28.3 ± 2.3	0.02<p<0.05	1029 ± 139	>0.5
Chlortrimeton 3 mgm./kgm.	44.0 ± 1.5	<0.01	1596 ± 282	>0.5	28.2 ± 1.6	>0.5	1092 ± 158	>0.5
Chlorothen 5 mgm./kgm.	28.2 ± 1.1	<0.01	936 ± 242	0.01<p<0.02	28.5 ± 2.1	0.1 <p<0.2	1192 ± 204	>0.5
N-9-Fluorenyl-n-ethyl-2 chloroethylamine HCl. (Parke, Davis SY-21) 2 mgm./kgm.	7.3 ± 2.3	<0.01	146 ± 66	<0.01	34.5 ± 2.5	<0.01	1175 ± 229	0.01<p<0.02
1-Phenyl-2N-methyl-benzyl-aminoethyl chloride HCl. (Lilly 08353) 2 mgm./kgm.	14.7 ± 1.4	<0.01	359 ± 140	<0.01	29.5 ± 2.6	<0.01	1094 ± 138	>0.5
Dihydroergocornine[d] (Sandoz DHO-180) 0.05 -0.1 mgm./kgm.	34.6 ± 2.6	>0.5	1061 ± 625	0.3 <p<0.4	28.2 ± 1.9	0.2 <p<0.3	1227 ± 102	>0.5
b) Epinephrine injection of 4.0 microgm./kgm. over a 30-second period								
Epinephrine injection (Control)	26.0 ± 3.5		604 ± 150		20.3 ± 1.7		326 ± 114	
Chlorothen 5 mgm./kgm.	15.5 ± 2.4	<0.01	466 ± 88	0.1 <p<0.2	21.3 ± 1.1	>0.5	331 ± 95	<0.5

[a] Drugs injected 30 min. before epinephrine administration.

[b] Determined by 'paired data' analysis with controls.

[c] Determined by 'Trapezoid Rule'.

[d] Combined results of 2 dogs with 0.05 mgm./kgm. and 3 dogs with 0.1 mgm./kgm. since responses are of same magnitude. Remainder of drugs tested on 6 dogs each.

responsible for their activity. It is noteworthy that Pitressin, Pitocin and ephedrine were all effective in reducing the hyperglycemia. These three agents are all known to produce vasoconstriction and other circulatory changes. One wonders if these agents could be interfering with the activity of epinephrine by a redistribution of blood, and a resulting interference with the distribution to all parts of the body of any hyperglycemia produced.

B. Hyperglycemia and Lacticacidemia in Dogs.

It was important to know whether there were any species specificity with regard to the inhibition of epinephrine-hyperglycemia. To this end, epinephrine was infused into trained dogs at a physiologic rate (15), and the blood sugar was measured at intervals in control and drug-treated animals. It was also of interest to know whether the lacticacidemia known to occur after epinephrine injection would be affected, thereby reflecting an action in muscle as well as liver glycogen. The data are tabulated in Table 4. Each dog served as its own control. The peaks of the hyperglycemia and lacticacidemia have been tabulated in addition to the respective areas under the curves from onset to completion. It is suggested that the maximum rise most clearly demonstrates the effect of epinephrine itself, since the duration (reflected by the area) may be influenced by a number of other factors such as insulin

and adrenocortical hormones.

The peak response occurred from ten to twenty minutes after the end of the epinephrine infusion, and in control animals reached a height of about 35 mg.%. Interestingly, Histadyl and Chlortrimeton, which were among the most potent of the antihistaminics tested in rabbits, did not decrease the hyperglycemia in dogs; on the contrary, they potentiated the maximum rise, although they did not alter the fasting blood sugar level. However, the total area of the hyperglycemic curve was not different from the controls. Histadyl produced a significant increase in the peak level of lacticacidemia without altering the total area of response, while Chlortrimeton produced no alteration in the lactic acid rise following epinephrine. It will be noted that while the dose of Histadyl used in rabbits to produce complete blockade of the hyperglycemic effect of epinephrine was 3.0 mg./kg., the dogs received only 1.5 mg./kg. Doses higher than this were found to be convulsive to dogs, and 1.5 mg./kg. was found to be approximately the maximum tolerated dose.

Chlorothen was well tolerated by the dogs, and a dose of 5.0 mg./kg. significantly decreased the hyperglycemia both in height and duration without altering the lacticacidemia or the fasting blood sugar.

It will be noted in the experiment on dogs, that

the experimental design was altered from that of the rabbit experiments in that the epinephrine was infused instead of injected. It was important to determine if this change in experimental design was responsible for the results which differed from those obtained with rabbits. Accordingly, epinephrine was injected intravenously in a dose of 4.0 gamma/kg. over a 30 second period into dogs which had been injected intravenously with 5.0 mg./kg. of Chlorothen 30 minutes earlier. The results as shown in the bottom of Table 4 demonstrate that Chlorothen reduced the hyperglycemic effects of epinephrine injected in this manner more than when epinephrine was infused. Interestingly, the area of hyperglycemia following the infusion of epinephrine was diminished by Chlorothen, while the area of the hyperglycemia following the injection of epinephrine was unaffected. The findings of Susina and Unna (114), using an epinephrine injection of 5.0 gamma/kg. were similar to those reported herein. They found that diphenhydramine (Benadryl) and Chlortrimeton potentiated the hyperglycemia while Thephorin diminished it. Their measurements of hyperglycemia were exclusively by areas.

It is felt that these experiments with varied methods of administration of epinephrine add weight to the suggestion that the influence of the antihistamines on the response measured is possibly a hemodynamic one.

In view of the negative results with the antihistaminics in their ability to block the effects of epinephrine in inducing hyperglycemia in dogs, a series of experiments were included using the adrenergic blocking drugs, N-9-fluorenyl-N-ethyl-2-chloroethylamine. HCl (SY-21) and 1-phenyl-2-N-methyl-benzylaminoethylchloride. HCl (Lilly 08353) to see if a marked inhibition of epinephrine-induced hyperglycemia was limited to rabbits. These agents were injected into dogs in a dose of 2 mg./kg., and the hyperglycemia following the infusion of epinephrine was markedly reduced from the control value both in magnitude and duration. Both of these agents caused an increase over the control values in the lactic acid rise, but only SY-21 produced an increase in the total area of lacticacidemia.

The adrenergic blocking drug, dihydroergocornine (DHO-180) was injected intravenously into dogs, but the dose used in rabbits (0.1 mg./kg.) was found to be emetic in dogs. It did not produce any change in hyperglycemia or lacticacidemia in the 3 dogs in which it was tested. The dose was reduced to 0.05 mg./kg. and retching without vomiting was noted in 2 dogs tested. Again there was no alteration in the blood sugar or lactic acid changes produced by epinephrine infusion.

DISCUSSION

The reduction of an excitatory effect of epinephrine by compounds possessing antihistamine properties has been previously reported. 2-Isopropyl-5-methylphenoxyethyl diethylamine (929F) (13), 1-naphthylmethyl-ethyl-2-bromoethylamine (SY-28) (79), and N-2 (2-biphenylyloxy)-2'-chlorotriethylamine (SY-8) (80), have been shown to inhibit the pressor response to injected epinephrine in dogs. On the other hand, several of the available antihistamines have been shown to potentiate the effect of epinephrine in dogs (77). Furthermore, evidence is available which indicates that at least one of the antihistamines, Benadryl, is capable of reversing epinephrine reversal (19). The mechanism of action of the antihistamines in their diverse effect on the action of epinephrine is unknown. It has led to the suggestion that the ultimate site or mechanism of action of epinephrine and histamine on certain tissues is similar (76). It is possible that the antihistaminic agents are effective, therefore, by blocking such a common site of action at the liver. It is more likely, however, that the antihistamines act in some non-specific manner, such as circulatory changes in which carbohydrate metabolism may be only incidentally involved. The finding that agents such as Pitocin, Pitressin, and ephedrine, which induce

circulatory changes, also inhibited epinephrine-induced hyperglycemia in the rabbit, makes this hypothesis likely.

The failure of some of the potent antihistamines to block the hyperglycemia in dogs may reveal a species difference, which, as was pointed out in the previous section, proved to be an important consideration in evaluating the effects of drugs on epinephrine-induced hyperglycemia. With at least two antihistaminic agents, Histadyl and Chlortrimeton, there was, in the dog, a potentiation of the hyperglycemia similar to the potentiation of the pressor response in this animal.

To this author's knowledge, there has been only one other attempt to study the effects of the antihistamines on epinephrine-induced hyperglycemia in the dog. Susina and Unna, in 1951 (114), working with dogs and using techniques very similar to the ones used in this investigation, found a potentiation with Benadryl and Chlortrimeton. They found, however, that Thephorin produced a reduction in the hyperglycemia. The only drug that we used in common was Chlortrimeton which we also found potentiated the hyperglycemia induced by epinephrine. It will be noted, that Chlorothen was the only one of the drugs we tested that reduced the hyperglycemic response to infused epinephrine significantly both in magnitude and in area. In response to injected epinephrine, the dogs pretreated with Chlorothen

did not show a significantly lower area of hyperglycemia but did not show any evidence of a potentiation. One also wonders if the failure of the antihistamines to have any effect might not be due to a dosage effect. It will be noted that the dose of Histadyl could not be raised above the 1.5 mg./kg., while the Chlorothen was injected in a dose of 5 mg./kg.

Chen and Clarke (17) studied the effect of Benadryl on epinephrine-induced hyperglycemia in rabbits. Epinephrine was injected in a dose of 0.1 to 0.2 mg./kg. and Benadryl in a dose of 20 mg./kg. Both drugs were injected subcutaneously. It was found that both agents were hyperglycemic and that their effects were additive. The effect of the Benadryl could be removed with pentobarbital anesthesia. Although Benadryl given intravenously, as we used it, did not cause a significant hyperglycemia, our results are essentially the same as those reported by Chen and Clarke. If the hyperglycemic action of the Benadryl was removed in their experiments by the use of pentobarbital, the epinephrine-induced hyperglycemia was reduced but this reduction was not statistically significant. It can be seen that in our experiments, the reduction of only 4 mg.% was statistically significant, because it was all in the same direction. However, this minute reduction does not suggest any definite biologic significance.

It is important again to point out that ephedrine, Pitressin, and Pitocin were all effective in reducing the hyperglycemia. This is in agreement with the previous work of Laurin (74) and Ellis (35). These agents all produce some circulatory change. Further evidence that the blockade of epinephrine-induced hyperglycemia is not limited to adrenergic blocking drugs is the report that ergonovine (61) will inhibit the response.

Regarding the effects of the adrenergic blocking drugs in dogs, the SY-21 and Lilly 08353 inhibited the hyperglycemia in dogs just as it did in rabbits. The report of a potentiation of the hyperglycemia by Susina and Unna (114) using SY-28 in the dog may reflect a species difference since we found that this compound was effective in the rabbit. However, in personal communication with Susina, I learned that the SY-28 was injected quite rapidly (about 30 seconds), and precipitated a rise in blood sugar by its own action. Our technique was to inject the drug over a 3 minute period. One wonders if this difference in technique was not responsible for their failure to elicit a blockade of the secondary hyperglycemia due to epinephrine. A slight initial hyperglycemia might sum with a reduced epinephrine-induced hyperglycemia resulting in an apparent potentiation. However, the work of Harvey et. al. (61) which was discussed in the last section seems to indicate that the efficiency of the

blocking drugs may be species specific. The absence of any effect with DHO-180 may be due to a dosage effect. It was used in a dose of 0.05 mg./kg. as opposed to a dose of 0.1 mg./kg. used in rabbits but which was toxic in dogs. Susina and Unna (114) were unable to inhibit epinephrine-induced hyperglycemia in dogs with dihydroergotamine injected in doses of 0.1 to 0.25 mg./kg. However, when they increased the dose to 0.5 mg./kg., they did demonstrate a blocking of the blood sugar rise due to epinephrine. They reported that these doses also caused retching.

The failure to reduce the lacticacidemia following epinephrine in dogs may be a circulatory effect. It could, of course, also be due to a failure to block at the muscle. In addition we should consider the possibility that it reflects an increased utilization of blood sugar or a decreased removal of lactic acid from the blood stream by the liver. These various possibilities will be considered in the discussion of the next section.

SUMMARY

Although a majority of adrenergic blocking drugs have been shown to diminish epinephrine-induced hyperglycemia in rabbits, it has been observed that potent antihistamines and several pressor agents are likewise effective. A number of the available synthetic antihistamines have been tested for their effects on epinephrine-induced hyperglycemia and lacticacidemia in dogs. It was found that those compounds which have been known to be the most potent in antagonizing the effects of histamine on the bronchioles and intestine of guinea pigs, are also the most effective in reducing the epinephrine-hyperglycemia in rabbits. The effectiveness of the antihistaminic agents is not related to their ethylenediamine structure, their atropine-like, or their local anesthetic activity. Pressor agents such as Pitocin, Pitressin, and ephedrine diminished the induced hyperglycemia in rabbits. It is suggested that all these agents work by some non-specific effect, influencing the carbohydrate response to epinephrine only incidentally. The evidence suggests that antihistamines inhibit epinephrine-induced hyperglycemia more readily in the rabbit than in the dog, which may reveal an important species difference. None of the antihistamines or adrenergic blocking drugs tested in the dog decreased epinephrine-induced lacticacidemia.

THE INFLUENCE OF ADRENERGIC BLOCKING DRUGS AND NEOANTERGAN ON EPINEPHRINE-INDUCED EFFECTS ON GLYCOGEN, GLUCOSE UPTAKE, AND OXYGEN CONSUMPTION OF THE ISOLATED HEMIDIPHRAGM AND LIVER SLICE OF THE RAT

It is felt that before going into the experimental results of the next and last section of this series of studies, it would be advantageous briefly to review the highlights of the literature on the mechanism of the epinephrine-induced hyperglycemia. That epinephrine causes a liver glycogenolysis is incontrovertable. The question is what part in this carbohydrate plethora is played by a decreased utilization and/or uptake of glucose from the blood stream.

This has been the source of a great deal of confusion in the literature. The main difficulty is that in the intact animal it is difficult to measure separately the glucose uptake and the glucose utilization, and the terms have become linked, when actually they should not be so.

Cori (26) has marshalled evidence from which he concludes that epinephrine-induced hyperglycemia is in part due to a decreased utilization of blood sugar by muscle tissue. This conclusion is based on three main theses.

1. The amount of glucose which he had supplied to normal rats in order to produce a hyperglycemia of similar magnitude and duration as that caused by certain doses of epinephrine, was such that it could not possibly be derived from liver glycogenolysis.

2. Simultaneous determinations of A-V blood sugar levels in man and rabbits have shown that for the same degree of hyperglycemia, the A-V blood sugar difference was greater when the hyperglycemia was caused by sugar administration than when it was due to epinephrine injection. This, however, was a measurement of glucose uptake, as has been defined in an earlier part of this dissertation.

3. Colwell and Bright (25) have reported that when epinephrine and glucose were continuously injected into amytalized cats, the non-protein R.Q. (instead of rising progressively as under identical conditions without epinephrine) dropped to the fat level within 3-5 hours, and remained there as long as the epinephrine administration persisted. These animals excreted an increasingly greater proportion of sugar administered. The authors concluded that epinephrine was capable of suppressing glucose utilization even in the presence of an increased supply.

This theory of decreased utilization of blood glucose has been a very provocative one, and many workers have attempted experiments to verify or deny its validity (cf. Griffith (50) for extended references). Most of the work has been performed on intact animals, and the results obtained have been most confusing, for the utilization of blood glucose has been shown to be increased, decreased,

or unaltered by epinephrine. Each investigator is quite certain that his own view is correct. Leading among those investigators supporting the view that the utilization is decreased is Cori, and among those advocating increased utilization are Soskin and Griffith. Soskin (111) has been of the opinion that the method employed by Cori and others of comparing the hyperglycemia of injected glucose with that induced by epinephrine is unwarranted. They cite the experiments of Mann and Magath (84) who claimed that the liver itself must use large quantities of injected glucose since the hepatectomized rats required only about 1/10 the amount of glucose used by Cori to simulate the blood sugar rise produced by epinephrine. It becomes a problem of whether there is any essential difference between the metabolism of exogenous or endogenous circulating sugar.

Soskin and his co-workers (111) claimed that one could not consider measurements of arterio-venous differences a valid estimate of blood sugar utilization unless, concomitant measures of changes of blood flow were made. Here again we have an example of the source for confusion in which glucose uptake is called glucose utilization. They reasoned that an increase in blood flow could account for a decrease in utilization, and proceeded to measure the blood glucose A-V differences taking into account blood flow and hydration. They removed samples from the carotid

artery, femoral, portal, and hepatic veins. Epinephrine was injected intravenously at the rate of 0.12 mg./kg./hour into amytalized dogs and their results indicated that there was no evidence that epinephrine decreases the utilization of blood sugar by the muscles of the hind limb.

Cori, Fisher, and Cori (32) upon revising their experiments criticized Soskin's group in two main aspects. They claimed that Soskin's solution of epinephrine was not protected by antioxidants, and injected over such a long period of time that the solutions may have spoiled. They further noted that the blood flow gradually declined to extremely low levels in each experiment, indicating a poor physiological condition of the dogs. The method of recording the blood flow (one which removed the blood from the dog's body and then reinjected it) was one which might have led to a gradual deterioration of the condition of the dogs and the circulation of the blood.

Three years later, the Soskin group (109) repeated their studies using a different method for recording the blood flow. Again, they could not demonstrate an increase in glucose uptake due to epinephrine when they considered the changes in blood flow in their calculations. The increases in blood flow were substantial, and tended to increase the glucose uptake rather than decrease it.

Cori and his co-workers (32) then decided to

investigate the matter again using measurements of blood flow. In amytalized dogs they noted that only 0.015 mg. of epinephrine/kg./hour caused a hyperglycemia of about the same magnitude as that obtained by Soskin with eight times that dose. In addition, they noted that this rate of injection not only produced a hyperglycemia without any change in blood flow through the leg, but also produced a steady hyperglycemia instead of the erratic changes noted by Soskin. Instead of measuring the A-V differences as compared to injected glucose, they measured the differences as compared to insulin, and noted that while epinephrine caused an increase of only 1-2 mg. %, insulin caused an increase of 10-25 mg. %. This is a rather peculiar comparison since the amount of increase of utilization caused by insulin would probably vary with the dosage. The experiments, however, are significant in that they afford evidence that hyperglycemia could be induced without alteration in blood flow.

It was Cori's contention that the measurements of the blood flow are not essential in evaluation of A-V differences. He showed that the error of $\pm$ 1 mg. % for the determinations of blood sugar was so great that it is useless to calculate with the appropriate changes in flow. For example, a set of values of 143 and 138 for the arterial and venous blood glucose levels, respectively, would give a difference

of 5mg. %. Assuming an error of ± 1 mg. %, then a difference of 144-137 = 7 or 142-139 = 3 mg. % might also be found. With a blood flow of 4 cc/ minute through the leg, the sugar retention in the 3 cases would be 120, 168, and 72 mg./ hour respectively. Hence with such a large error, the utilization could be shown to change even if it was exactly the same for three consecutive measurements. The best that can be hoped for by measurements of A-V differences may be a qualitative index.

There have since been many investigators who have duplicated the experiments and results of both Soskin and Cori. There are two outstanding experiments which should be cited for their significance. Soga (106) showed that continuous administration of glucose inhibited the fall in blood glucose of hepatectomized dogs. If epinephrine was infused along with the glucose into such dogs, there was an increase in blood sugar and a decrease in muscle glycogen. In the hepatectomized dog, such a hyperglycemia could only be due to a decrease in glucose uptake and/or a decreased utilization. The finding that the muscle glycogen decreased does not aid in the interpretation, since it may be due to either or both of these possibilities. However, since epinephrine did not prevent the fall in blood sugar in an hepatectomized dog which was not treated with glucose, there was the indication that epinephrine could only act in the

face of a given critical level of blood sugar.

Another experiment of significance was performed in unanesthetized humans by Somogyi (107). He employed a dose of epinephrine of 0.2 mg. subcutaneously, a dose so small that it did not alter the pulse rate or blood pressure, and produced a rise in blood sugar of only 13 mg.%, an hyperglycemia equivalent to the ingestion of 5.0 gms. of glucose. A subject was first fed 50.0 gms. of glucose without any epinephrine administered, and the A-V differences in blood glucose following the alimentary hyperglycemia were recorded. A few days later the subject went through a second test which differed from the first only in that the subject received 0.2 mg. of epinephrine, subcutaneously, ten minutes before the ingestion of the 50.0 gms. of glucose. It was noted that the hyperglycemia resulting from the combined epinephrine and glucose was greater than that due to glucose alone. At the same time the A-V difference was less in the hyperglycemia due to epinephrine and glucose than in the hyperglycemia due to glucose alone. This latter finding indicated that the greater hyperglycemia when epinephrine was given in addition to glucose was due to a decreased glucose uptake and/or utilization by the tissues.

These data revealed another important bit of information. It was noted that with epinephrine plus glucose, the blood sugar rose to higher peaks and then dropped much

more steeply than the glucose controls. This was an indication of a marked increase in insulin secretion, and the ever-present dynamic balance between insulin and epinephrine. The resulting hyperglycemia and glucose utilization is nothing more than the result of an antagonistic action of these two substances. As indicated several times above, it may be that the results of some workers showing an increase in utilization was due to an increase in insulin secretion. On the other hand, the results of some investigators indicating a decrease in utilization could be caused by an additional output of adrenocortical hormone (69), for it should be clear that epinephrine will act in the intact animal upon the existing endocrine state of that individual. Indeed, some of Soskin's (109) graphs indicate that there was some degree of hypoglycemia at the start of their experiments, suggesting that there may have been a good deal of circulating insulin. In addition, in Soskin's experiments, the experimental design was such that glucose was infused, and after the return to normal the epinephrine was injected. The hyperglycemia induced by glucose infusion might have caused large amounts of insulin to be released, which may not have been entirely destroyed by the time epinephrine was administered, and the primary action of insulin could counteract the effects of epinephrine, as shown in the isolated muscle study of Riesser (100).

In as much as the results are so variable regarding the effects of epinephrine on glucose utilization, it appears to be almost impossible to predict the effect of a given injection. However, it is of interest to evaluate the various factors contributing to the end result. One of the ways of evaluating these factors is to place the tissue in isolation, and thus remove the various hormonal influences.

There have been a good many studies on isolated tissue, but comparatively few studies on the isolated skeletal muscles alone, which in the opinion of many, contributes the greatest area of the utilization of carbohydrate.

Most of these experiments can be criticized in that the tissues have been severely injured in removing them, mincing and homogenizing. In order to minimize the trauma of preparation, the rat diaphragm has been suggested by Gemmill (44) for these studies. Two Israeli workers, Tuerkescher and Wertheimer (117) presented an interesting study. They used rats that were first anesthetized with Nembutal. The animals were bled, the diaphragm was removed and placed in a Warburg apparatus. As a measure of glycogen synthesis, the difference in glycogen content between one test sample incubated for 6 minutes and the other for 3 hours were used. Difference in glycogen following the addition of

epinephrine was measured by the difference in the glycogen content of 2 samples after 3 hours of incubation; one sample being incubated in the usual manner, and the other with epinephrine added. Glucose was present in the medium in a concentration of 100 mg.%, and the experiment was conducted in an atmosphere of pure nitrogen. Glycogenolysis was measured by the decrease in glycogen in 3 hours of incubation in a glucose free medium. The addition of epinephrine HCl at a concentration of 0.33 gamma/ml. regularly decreased glycogen synthesis by about 44%; at 0.0033 gamma/ml., the effect disappeared, and at 0.033 gamma/ml. the effect was irregular. The decreased glycogen synethsis in the diaphragms with epinephrine added could be observed after one hour. The effect was blocked by ergotamine tartrate (8.3-10 gamma/ml.) which itself reduced the rate of muscle glycogenesis. Therefore, the effect of ergotamine must have been due to a blockade of the action of epinephrine. It was of interest to note that at a concentration of epinephrine of 0.33 gamma/ml. there was an increase in glycogenolysis of 47%. This is to be compared with the decreased glycogen synthesis with epinephrine of 44%. This finding indicates that the apparent decrease in glycogen synthesis was probably a reflection of an increased glycogenolysis due to epinephrine.

Cohen (22) reported a series of experiments on the

effect of epinephrine on glucose utilization in the isolated rat diaphragm. He was unable to demonstrate any effect of epinephrine added in doses of 50 - 100 gamma *in vitro*. Therefore, he injected epinephrine in a dose of 1 mg./kg., subcutaneously into rats 40 to 70 minutes before removing their diaphragms. The tissues were incubated in an atmosphere of nitrogen containing 5% carbon dioxide to equalize the bicarbonate solution. The medium contained 0.2% glucose, and he measured the rate of anaerobic glycolysis. Since the lactic acid produced as a result of anaerobic glycolysis would release carbon dioxide from the bicarbonate in the medium, a measure of the carbon dioxide released was a measure of the anaerobic glycolysis occurring within the muscle.

The glucose entering the tissue would presumably be phosphorylated, and then either converted to glycogen, broken down to lactic acid, or stored as hexosemonophosphate. Although Cohen found an accumulation of phosphate in both the control and epinephrine-treated diaphragms after incubation, he was unable to demonstrate any difference in the amount of accumulation of phosphates between these two groups. While the glycogen content of the untreated diaphragms decreased after incubation with glucose, the glycogen content of the epinephrine-treated diaphragms did not change after incubation with glucose. Oddly, the glycogen value of

epinephrine-treated diaphragms did not change even when they were incubated in the absence of glucose, although lactic acid was still produced!! One wonders what could have been the precursor for lactic acid. The lactic acid (measured by the carbon dioxide released and checked by chemical determination) produced by the epinephrine-treated diaphragms was significantly less than that produced by the control diaphragms. From this, the author concluded that there was a decreased glycolysis due to epinephrine. He claimed, since there was no change in the glycogen content of the epinephrine-treated diaphragm before and after incubation, that the decreased rate of glycolysis was akin to a decreased rate of utilization of the glucose entering the tissue. Since there was no difference in the phosphate intermediates content between the control and epinephrine-treated tissue, the decreased glycolysis was also an indication of a decreased rate of glucose uptake.

However, Cohen did not measure the rate of disappearance of glucose from the medium. Since the glycogen value of the epinephrine-treated tissue did not change from the initial value following incubation, while it decreased in the control diaphragms, some of the glucose taken up from the medium must have been converted to glycogen. Therefore, it is possible that the rate of glucose uptake may not have decreased even though the rate of glycolysis decreased.

An interpretation of these experiments is difficult to make. The observation that epinephrine added *in vitro* in doses as high as 50 - 500 gamma produced no effect on any of the facets of glycolysis measured is most surprising. It appears that when epinephrine was injected into a rat before the diaphragm was removed and incubated, that an inhibition in anaerobic glycolysis did occur. At least in these experiments, it does not seem that the inhibition was due to an accumulation of intermediates, since they were not appreciably different from the controls. Cohen did demonstrate that there was no reduction of glycolysis in epinephrine-treated diaphragms if they were incubated in hexose diphosphate, which indicates that there was some interference before that particular step in the glycolysis chain.

The finding that epinephrine added to diaphragms *in vitro* had no effect, led Cohen to another series of experiments (23) in an attempt to find the possible mechanism of action. He reasoned that either the epinephrine was transformed into a more active compound somewhere in the body, or else in view of the existing knowledge of hormonal balance, the epinephrine stimulated the hypophysis or adrenal cortex to activity, and this was the actual agent involved. He repeated his experiments using hypophysectomized and adrenalectomized rats, and his results

are quite interesting. It was observed that epinephrine injected into an hypophysectomized rat did not produce inhibition of glucose utilization, indicating that the indirect action of epinephrine was probably mediated through the hypophysis. Moreover, this indirect action of epinephrine is independent of the adrenal cortex, since epinephrine injected into adrenalectomized rats still exhibited the inhibition.

A Dutch investigator, Riesser (100), reported a study of the effect of epinephrine on the isolated rat diaphragm. This study was not conducted in the Warburg, but in a flask kept at constant temperature. In addition, the preparation was a rib-diaphragm preparation, rather than a diaphragm alone. This type of preparation involved less cutting of the muscle fibers of the diaphragm, and presumably the presence of bone would not appreciably influence the glycogen content of the muscle during incubation. After incubating the tissue in oxygenated solution of phosphate-buffered Ringer's solution containing 0.5% glucose for 3 hours in the presence of such massive doses of epinephrine as 50-100 gamma, the glycogen level was seen to fall. However, since no measurement of glucose or lactic acid were made, there is no way of telling whether this action was due to an increased glycogenolysis or an inhibition of glucose uptake. The author was of the opinion

that both explanations applied. He showed that the muscle glycogen decreased even in the presence of insulin, and took this to mean that there was an antagonism of insulin by epinephrine, since the action of insulin is generally accepted to be to increase glycogenesis and to prevent glycogenolysis.

As can be seen from the foregoing, the existing experiments with the intact animal dealing with the effect of epinephrine on glucose uptake and utilization presents a rather confusing picture. If the reader will recall the water analogy given in the Introduction, the level of water (blood sugar) in a sink (blood stream) could rise not only by increasing the rate of water inflow from the faucet (liver glycogenolysis) but also by decreasing the rate of outflow from the sewer-drain (glucose uptake and utilization by the tissues). It has already been pointed out that the rate of glucose uptake and glucose utilization, if defined individually, can vary independently of each other because of the ability of muscle and liver tissue to store hexosemonophosphate. In the intact animal it is quite difficult to measure both the glucose uptake and the glucose utilization independently, and the best one can hope for is to measure the rate of glucose uptake from the blood stream by the tissues. The technique for this measurement is the determination of the A-V difference in blood sugar. However, when

this is done, the glucose uptake can be shown to either increase or decrease following epinephrine administration. Some attempt has been made to correct for the changes in blood flow due to epinephrine on the theory that glucose spends less time at the cell membrane, and, therefore, less is absorbed, and the glucose uptake appears to decrease. On the other hand, one may consider that an increase in blood flow presents more glucose to the cell membrane per minute. In such a case, an increase in uptake by dint of mass action effects rather than any enzymatic facilitation by epinephrine may be the responsible factor. However, despite correction for blood flow different laboratories have shown the glucose uptake to increase or decrease following epinephrine injection.

It has been pointed out by Cori's group (32) that the error in the determination of the A-V differences is such, that it is difficult to rely upon such measurements as quantitative indicators of glucose uptake.

It should be remembered that in the intact animal the influences of other hormones might play a role in the final observed effect. Thus epinephrine could increase the rate of glucose uptake by the tissues and at the same time stimulate the secretion of adrenocortical hormones by way of the anterior pituitary. The adrenocortical hormones are known to decrease glucose uptake. Thus, in Cori's experiments

in which small doses of epinephrine were used, the action of the adrenocortical hormones could overshadow the action of epinephrine. On the other hand, in Soskin's experiments, the doses of epinephrine used were relatively large, in which case the action of epinephrine could still be dominant despite the presence of adrenocortical hormone.

Studies on the isolated muscle have done little to clarify the situation, primarily because the rate of glucose disappearance from the medium was not measured. Most of the isolated studies which do not use the diaphragm are generally difficult to interpret because of the possible injury done to the tissues by mincing and homogenization. Most of the studies with the rat diaphragm give some indication that the glucose utilization is decreased, but the available information is so scant that no definite conclusions can be reached.

A portion of the experimental data in this section is based on the effects of epinephrine on the oxygen consumption of isolated liver and muscle. It is believed appropriate, at this point, to discuss some of the outstanding papers on the calorigenic effects of epinephrine in the intact animal. This discussion is by no means meant to be exhaustive, but is only meant to point out the highlights, and to place some type of order in a subject which is in a rather chaotic state. The reader is referred to the recent

review article by Griffith (50) for an excellent detailed bibliography.

Administration of epinephrine in physiological dosage to the intact animal leads to an increased respiratory metabolism--an increased O_2 utilization which is interpreted to be an increase in caloric production by the body. Thus one describes a "calorigenic" action to epinephrine. Such a calorigenic activity is an intricate part of the "emergency theory" which Cannon has introduced to explain the diverse activities of the adrenal medullary hormone.

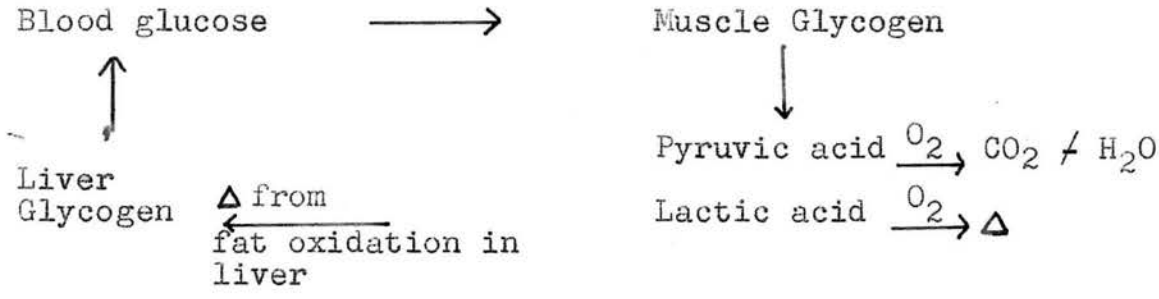
Attempts to demonstrate a calorigenic action on isolated tissues have led to results so contradictory as to be completely inconclusive. In an attempt to determine whether the hormone is a specific or general stimulant to cellular metabolism, nearly all organs and tissues of the mammalian body, and some others have been tested in vivo or in vitro. These include the adrenal gland itself, blood cells, brain, heart, kidney, liver, nerve trunk, placenta, pituitary, sarcomas, skeletal muscles, smooth muscle, skin, thyroid, tumors (50). Also, mere separation into a predominantly visceral or peripheral locus has been attempted by use of hepatectomized or eviscerate preparations or of single limbs (14, 53). Griffith, in surveying the situation in 1949 (51), says,

> "...of the variable reports, some 52 may be interpreted as indicating general stimulation, 24

> describing inhibition, 29 indicating no effect or at least none in the absence of the liver, and 11 suggesting that the effect depends upon the concentration of epinephrine, or of the availability of oxygen or presence or absence of functioning sympathetic nerves."

It will be helpful, I believe, to mention briefly here again the Cori cycle, which was discussed in the Introduction. Epinephrine acts on muscle glycogen to cause glycogenolysis which will produce a subsequent formation of pyruvic acid and lactic acid. In the presence of O_2, the Pasteur effect will occur, and pyruvic acid will enter the Krebs' oxidative cycle, and will form CO_2 and H_2O, utilizing O_2 in the process. That this process may be involved in the calorigenic activity of epinephrine is indicated by the fact that very often in isolated tissue preparations where adequate O_2 is present, the R.Q. (CO_2/O_2) is equal to 1. In the face of excess glycogenolysis and insufficient oxygen, lactic acid will be formed. In the mammal 3/4 of this lactic acid is carried to the liver, and converted to liver glycogen, while 1/4 remains behind to be oxidized to give energy for the reformation of creatine phosphate. The energy required for the conversion of lactic acid to liver glycogen, probably comes from fat combustion, again an oxidative process. Thus in long term experiments with intact animals, the R.Q. was that of fat combustion (25). The liver glycogen is split by epinephrine to form blood glucose which is transported to the muscle to

be converted into muscle glycogen and the process starts all over again. The cycle may be represented diagramatically as follows.



Two factors appear to be rather well settled as will be shown below. First, that hyperglycemia produced by epinephrine is not sufficient to cause an increase in caloric output. Second, that muscular activity contributes only a very small fraction of the metabolic response, and then only under conditions of acute severe stress.

I should like to begin the discussion by giving a very brief resumé of the early work in the field. The studies on the effect of epinephrine on the metabolism as evidenced by alteration in the quantity and character of the gaseous or respiratory exchange were initiated by Barcroft and Dixon in 1906 (6). By means of perfusion of the coronary system of an excised heart, and determination of the CO_2 and O_2 tension of blood, they demonstrated that after addition of epinephrine, the oxygen consumption was increased 3 or 4 times. Bernstein and Falta found in 1912 (9) that in man, 0.1 mg. of epinephrine injected subcutaneously

resulted in a rapid increase in oxygen consumption which reached its height 1 hour after the injection. Anderes and Cloetta, in 1916 (5), demonstrated an increase in the volume of the blood through the lungs which accompanied the increase in oxygen absorption. Sandiford in 1919 (103) was the first to demonstrate that epinephrine did not exert its effect through the thyroid gland. This was confirmed by Marine and Lenhart in 1920 (85) in thyroidectomized rabbits. Jumping for a moment to 1946, De Visscher (119) showed that thyroidectomy in the rabbit did not completely abolish the action of epinephrine on gaseous exchange until 20 days after operation. Subsequent injection of thyroid hormone returned the response to normal, providing large doses of thyroxin were used. Small doses did not return the response to normal. He concluded that the action of the thyroid hormone was to sensitize the tissues to epinephrine calorigenic activity.

The first attempt to show that the calorigenic action of epinephrine was something over and above the mere increase in blood glucose was by Boothby and Sandiford in 1922 (12). They had 44 experiments on human subjects in which the increase in heat production, R.Q. and blood sugar from the injection of 100 gm. of glucose was compared with the results obtained in 22 experiments following subcutaneous injection of 0.5 mg. of epinephrine. It was

noted that the injection of glucose caused a 9% increase in heat production for an increase of blood sugar of 105%, while from epinephrine there was a 20% increase in heat production with only a 37% increase in blood sugar, and less of an increase in R.Q.

It was not until 1923 that Boothby and Sandiford introduced the term "calorigenic action" (12). It was this paper which was classical for its well controlled observations in which the effect of muscular activity was ruled out, a variable which complicated most of the preceeding reports. They used unanesthetized dogs which were trained to lie perfectly still throughout the experiment. They watched the dogs carefully making note of any movement at all. To be sure of a permanent record of this there were 2 pneumographs around the dog. The first passed over the upper part of the chest just behind the forelegs, in such a manner that the slightest movements of the forelegs or head were indicated on the Kymograph tracing. The second passed over the lower part of the trunk just in front of the hind legs so that movements of the hind legs as well as those of the tail were recorded. It was found that the involuntary twitching of the leg which occasionally occurred had no effect on the metabolic rate. Many of the observations may have been on sleeping dogs, since they had no way of telling

whether the dog was awake or asleep.

Epinephrine was infused intravenously, at the rate of 0.6 to 2.5 gamma/kg./minute for 6 to 13 minutes. The rise in pulse rate, and respiratory rate, and respiratory volume paralleled the increase in metabolic rate. They postulated at this time that the effect of epinephrine was general on all types of cells, and since they had shown that the effect could not be attributed entirely to an increase in blood sugar they suggested that the metabolites of the utilized blood sugar may be involved.

There are two other outstanding papers which should be mentioned, showing that the calorigenic action of epinephrine is something besides the effect of a hyperglycemia. Corkill, Dale and Marks showed in 1930 (33) that epinephrine injected into eviscerate cats caused an increased oxygen consumption. This contradicted the results of Soskin (108) of 3 years earlier who obtained the opposite result. The difference may possibly be explained by the work of Cori (27, 28) on frogs, which showed that as the hepatectomized animals began to die, epinephrine lost its calorigenic action. This indicates that failure to obtain a calorigenic response in the absence of the liver, at least in the frog, might actually be due to a failure of the animal, rather than the absence of the liver. Corkill's injection of epinephrine was made

much sooner than Soskin's injection and the metabolic rate fell faster than in Soskin's experiments. Hunt and Bright had shown earlier in 1926 (68) that tying off the liver of cats did not remove the calorigenic response to epinephrine, although it was significantly lower in the controls. Since in the fasted animals the only source of hyperglycemia is the liver, epinephrine produced its action without a hyperglycemia. The fact that the liver was not present in these cats, indicates that the source of the calorigenic response was probably from the Krebs' cycle, or from the oxidation of lactic acid. Glycolysis is also an exothermic reaction, and thus muscle glycolysis could have contributed to the calorigenic response.

The possibility that epinephrine produced its calorigenic response by some other means besides the blood glucose increase, first suggested by Boothby and Sandiford in 1923 (12) was given due consideration by Cori and Cori in 1928 (29) in their work on fasted rats. They gave epinephrine subcutaneously in a dose of 0.02 mg./100 gm. of body weight. They found that 3 hours after the epinephrine was injected, the total body glycogen, with the exception of the liver, fell 57 mg. They calculated that if the glycolyzed muscle glycogen was completely oxidized, 57 mg. should produce an R.Q. of 0.74, while the R.Q. actually observed was 0.715. Colwell and Bright (25) also

demonstrated that if experiments on cats lasted for 3 hours epinephrine infusion produced a fat R.Q. They therefore concluded that the increase in oxygen utilization was not entirely due to the oxidation of glycogen, and suggested that it might be the conversion of lactic acid to liver glycogen. In a later work in 1931, Cori and Buchwald (28) expounded upon the mechanism. They explained that Meyerhof had shown that for each O_2 equivalent of 1 mol of lactic acid used above the basal oxygen consumption, 2-5 mols of lactic acid may be reconverted to glycogen. Since epinephrine caused an increase in muscle lactic acid as well as an increase in caloric output, it was natural to think of the two as having some connection. If there was some connection, then epinephrine injection in frogs should cause less lactic acid to appear under aerobic than anaerobic conditions. A reference to previous studies showed this to be true. Whereas anaerobic conditions caused an excess of 306 mg. of lactic acid per kg. of frog, aerobic caused an excess of 169 mg./kg; thus creating a difference of 137 mg. He calculated mathematically that 25.3 cc of oxygen were needed to oxidize the part of the excess lactic acid that was slated to become oxidized (4:1). In other words, the frog should have consumed an extra amount of 25.3 cc of oxygen/kg. to oxidize the lactic acid. They put this to the test in immobilized frogs giving 0.05 to 0.1 mg. of

epinephrine subcutaneously. They computed that the total extra oxygen utilized during the 3 hour period was 28 cc/kg. of frog. This, as can be seen, compared favorably with the theoretical 25.3 cc, which he had previously calculated. Thus they concluded that the effects of epinephrine in the frog inducing its calorigenic response was mediated through the increased muscle glycolysis causing an increased lactic acid production and the subsequent oxidation of lactic acid and the reconversion to muscle glycogen.

However, the lactic acid oxidation provided energy for the reconversion of muscle glycogen only in the frog. In the mammal, almost the only place the lactic acid can be converted to glycogen is in the liver, and it is highly probable that in the mammal the source of energy is from the conversion of lactic acid to liver glycogen. Cori has consequently shown that the oxidative energy required to convert the lactic acid is derived from fat oxidation in rats. He has shown that in the 24 hour fasted rat (29) the increase in heat production is at the expense of the fat combustion following epinephrine. He showed this by determining the urea nitrogen, and finding it unchanged from the control, which indicated that the protein was not being utilized. The R.Q. of 0.715, when only fat and carbohydrate are being used, can be shown mathematically to be due 99% to the combustion of fat and 1% to the

combustion of carbohydrate. In the following paper (30) he described the effect of epinephrine injection in the rat in the postabsorptive state, and showed that of the total increase of heat production, the breakdown of fat contributed most of the calories; the increase in fat calories was greater than the increase in carbohydrate calories.

In the last paper of this series (31), Cori showed that in rats which were absorbing glucose from the alimentary canal, the extra calories following epinephrine injection were furnished entirely by fat, despite the increase in blood sugar level. There has been tendered no explanation of this phenomenon, and it is not known if the epinephrine affects fat metabolism directly or indirectly.

This theory would serve to explain some of the reports of failure to elicit a large calorigenic response in the hepatectomized animals. Since the reconversion of lactic acid takes place only in the liver, in the same area as fat oxidation, a major source of the calorigenic response would be removed with liver ablation.

From 1927 to 1939 Griffith and his colleagues published a series of papers dealing with the effect of epinephrine injected arterially to the hind limb of an anesthetized cat (52, 53, 54, 56). He assumed that

practically all of the epinephrine would be used by the leg. He tried a variety of dosages ranging from 0.002 gamma to 2 gamma/kg./minute for 5 minutes and found that in all cases there resulted a decrease in blood flow and a decrease in oxygen consumption of the hind limb. He took this together with his earlier negative studies in the isolated limb and muscle as indicating that epinephrine did not produce its increased oxygen utilization by acting on peripheral tissue. In 1947 Griffith and his co-workers (57) shifted the injection to the intravenous route. They injected doses varying from 0.5-8 gamma/kg./minute for 5 minutes. They found that the rate of blood flow through the leg was either increased or decreased, but on the average, was increased; maximally by the smallest dose and least by the largest. Glucose retention by the tissues of the leg was invariably increased as was the lactic acid level in the blood, whether flow rate was increased or decreased. The oxygen utilization of the blood was found to vary directly with the rate of blood flow. They concluded that neither a carbohydrate plethora nor an increased lactic acid output appeared to have an effect on the respiratory metabolism of the leg tissues, at least under the conditions of their experiment.

In 1949 (51), the same workers repeated their experiment, this time including a measurement of arterial

blood pressure, leg volume, and other changes resulting from epinephrine injection and glucose injected in amounts sufficient to produce an equivalent hyperglycemia.

Epinephrine was injected at the rate of 4 gamma/kg./minute for 5 minutes and glucose at the rate of 50 mg./kg./minute for 5 minutes. They produced a hyperglycemia of 77 and 82 mg.% respectively. Glucose transfer in the leg was increased by 120% by epinephrine and by 88% by glucose indicating that epinephrine influences blood sugar uptake. The average effect of epinephrine on blood flow was to increase it about 3% while glucose increased it 28%. Again the effect was variable. The leg volume decreased markedly when the blood flow increased. The arterial pressure rose with the blood flow. The oxygen utilization again varied with the blood flow and not with the rate of glucose uptake. Thus the injection of glucose increased the oxygen consumption 19% while epinephrine increased it 5%. At this time Griffith attempted to explain the diverging results.

He suggested that an increased influx of carbohydrate into the tissues of the leg stimulated the respiratory metabolism by the specific dynamic action of carbohydrate. Increased CO_2 formation caused vasodilation resulting in an increased blood flow and in an increased O_2 supply. This was shown by the glucose infusion.

In the epinephrine infusion, vasoconstriction of varying degrees occurs depending on the tissue conditions in the anesthetized animal. Arteriolar constriction could be sufficient to block passage of the red cells, but still permit a flow of plasma of high glucose content. Glucose could then enter the tissues, but no O_2 would be available for utilization. Variations in the degrees of vasoconstriction could then account for the variable amounts. However, it should be borne in mind that this situation holds for the experimental conditions that Griffith used and might not apply to the unanesthetized animal.

The outstanding objection to this theory is that one would expect an increased O_2 utilization in the muscle after the epinephrine had worn off. However, Griffith's measurements extended to 30 minutes after the injection of epinephrine and there was no indication of a subsequent increase in O_2 utilization. However, if Griffith's postulate is true the decreased O_2 presentation to the tissues would prevent the oxidation of lactic acid and pyruvic acid in the leg and all oxidation would occur in the liver; thus, giving an increase in O_2 utilization in the body as a whole without an apparent effect in the limb. Thus, the results of Griffith are not in conflict with the theory of Cori.

The literature dealing with the effect of epinephrine on O_2 consumption in vitro presents a confusing picture, indeed. As mentioned in the beginning of this discussion, almost all organs have been tested. A complete bibliography is given by Griffith (50) and indicates that in almost every tissue epinephrine may cause O_2 consumption to go up, down, or stay the same. For example, the following reports present evidence for increase in O_2 utilization following epinephrine in the liver: Gottschalk (47, 48), Masing (86), Nukariya (96), Reinwein and Singer (99), Smirnova and Sverev (105); and in muscle: Ahlgren (3, 4), and Macht and Bryan (83). DeMeio (87) has claimed that the O_2 consumption of liver remains unchanged after epinephrine administration and Paasch and Reinwein (98), Nakao (90), Schattenstein and Zyukova (104), DeMeio (87), and Walaas and Walaas (121) have made similar claims for the effect on muscle. On the other hand, there have been the reports of De Cloedt and Van Canneyt (21), Euler (38, 39), Neuschlosz (91), and Abderhalden (1) that epinephrine causes a rise in O_2 consumption in isolated liver preparations. Similar claims to an increase have been made for muscle by De Cloedt and Van Canneyt (21), Klein (76), Goor (46) and Euler (38, 39, 40). Almost all of the techniques were very similar and little if any explanation can be offered for the amazing variety of

results. In most cases the only variable between the various experiments was the dosage of epinephrine used, but the literature dealing with the effect of the dosage of epinephrine on O_2 consumption is as confusing as the literature cited above. (See Griffith (50)). Griffith, himself, (50), stated that he is,

> "...unable to evaluate such evidence except from a quantitative point of view; from this standpoint it may be pointed out again that if those results indicating no effect or a decrease in O_2 consumption are set off against those which indicate an increase the first two will be found to outnumber the third ... we have seen no acceptable or accepted explanation for so many failures to show a stimulatory effect."

In view of the fact that Griffith, a renowned investigator in the field, and probably many others like him, can only use the force of numbers to give weight to the answer to this problem, it is hoped that the data set forth in this section will serve to add weight to one side. Also, some of the observations made in the experiments with epinephrine alone, may give some clues as to the conflicting results.

INTRODUCTION TO EXPERIMENTAL WORK

The foregoing review of the literature has indicated that although some order can be placed in the rather confusing results obtained on the effect of epinephrine on carbohydrate metabolism *in vivo*, the conglomeration of results *in vitro* are so chaotic that any experiments which lend weight to one or another side of the issue are believed to be valuable in a final classification of the mechanism of action of the response. That epinephrine produces a rise in blood sugar is indisputable, as is the fact that this rise is due in a large measure to the conversion of liver glycogen to blood glucose. One of the most important questions is whether the rise is due, in part, to a failure of the tissues, particularly muscle tissue, to remove glucose from the blood, and/or to utilize the glucose once picked up.

The previous sections of this thesis have uncovered some interesting observations. First, that the adrenergic blocking drugs decrease the hyperglycemia in animals, but fail to show any effect on the lacticacidemia resulting from epinephrine injections, at least in the dog. This may be interpreted in several ways:

1. The blocking agent exerts its effect on liver glycogenolysis and not muscle glycolysis.

2. If the hypothesis offered above (that epinephrine causes part of the hyperglycemia by decreasing the rate of glucose uptake and/or glucose utilization) is true, then the blocking agents might act by removing that effect of epinephrine.

3. The blocking agent could reduce the effect of epinephrine on both liver and muscle glycogenolysis. In this case, the failure to block the lacticacidemia might be due to a circulatory accumulation of the lactic acid which is still being produced.

The other observation is that antihistamines which had formerly been thought of as fairly specific have now been shown to block epinephrine-hyperglycemia in the rabbit, although this effect was not demonstrated by most compounds in the dog. One wonders if the effect of these antihistamines might be species specific.

It was thought that one of the best methods to shed some light on the answers to some of these problems was the Warburg technique for the study of isolated tissues. The rat was chosen as the best experimental animal, since as Gemmill (44) first advocated, in rats weighing less than 180 grams, the diaphragm is rarely thicker than 0.5 mm. allowing for easy transfer of oxygen in the reaction flask. The rat diaphragm has been used

almost exclusively by other workers for the study of muscle metabolism with preparations other than mince.

The experiment was designed to run in the presence of a high glucose environment. For this reason the tissue was bathed in a medium containing 200 mg.% glucose. The experiment was further designed so that in the course of a single experiment on muscle it was possible to measure the glucose uptake, glycogen synthesis and R.Q. (hence glucose burned). The summation of all these measurements make it possible to get some insight as to changes in carbohydrate balance under the influence of epinephrine and under the influence of blocking drugs. Experiments measuring the rate of liver glycogenolysis and oxygen consumption were also included, in order to help explain some of the effects of the blocking agents.

The adrenergic blocking drugs, DHO-180 and SY-21, were chosen for study. The former was chosen because it was effective in such low dose in rabbits and was without apparent effect in dogs. The latter was chosen because it is known to be a potent blocking drug when tested on the other effects of epinephrine, yet only succeeded in producing a partial blockade of the hyperglycemia in both rabbits and dogs. In addition, it seemed desirable to select two chemical types of adrenergic blocking drugs; DHO-180 belonging to the ergot alkaloid class, while

SY-21 is representative of the 2-haloalkylamine type. Neoantergan was chosen as a representative of the antihistamines since it was so effective in rabbits, and has been considered to be one of the most specific antihistamine drugs.

METHODS

A. The rat diaphragm.

For these experiments male albino rats of the Sprague-Dawley strain were used. They were supplied by the Charles River Breeding Laboratory. Only rats weighing 85 to 110 gms. were utilized. All animals were allowed to rest as quietly as possible for at least one hour prior to an experiment. After all the apparatus was prepared and the solutions added to the flask, the rat was quickly removed from its cage and killed by decapitation. The blood was allowed to drain for 30 seconds, after which a mid-ventral incision was made in the skin and the muscle from the urinary aperture to the clavicle. The diaphragm was freed from the abdominal viscera by cutting the hepatic ligament and the other omenta close to the diaphragmatic musculature. The sternum was clipped with a forceps, and the lateral walls of the diaphragm were freed from the rib cage by cutting close to the inner wall of the ribs. The sternum was then cut, and the diaphragm was freed from the lungs by cutting the pleural attachments close to the diaphragm. The dorsal wall was freed by cutting close to the dorsal body wall. The diaphragm was thus removed and placed in a petri dish filled with cold phosphate-buffered

saline (described below). In order to keep the saline cool, the petri dish was rested on an ice bath. The central ligamentous portion was carefully dissected out together with all other visible tissue other than muscle, and then the organ was finally bisected by cutting the short connecting strip that attached it to the remaining portion of the sternum. To this time, the only place the tissue was grasped was by the attached portion of the sternum. This method of dissection allowed a minimum of manipulation and cutting of the diaphragm itself.

B. The rat liver slice.

Rats used for the experiments on liver slices were killed by the same method as described above. The abdominal viscera were exposed as detailed above and the ventral lobe of the liver was quickly excised and immediately placed in a chilled Stadie-Riggs microtome. Sections of 0.5 mm. were immediately cut. The first section was discarded, and the remaining sections were immediately placed in a petri dish filled with ice-cold phosphate-buffered saline and set in an ice bath until the succeeding portions were cut.

The medium used was a phosphate-buffered saline recommended by Tuerkischer and Wertheimer (117) as a convenient simple medium which readily supports glycogen

synthesis. It consisted of:

0.74% saline

0.018% Na_2HPO_4

0.003% NaH_2PO_4

Glucose was added to this medium just before it was added to the flasks in amounts such as to have a final concentration of 0.2% glucose. This mixture gave a pH of 7.1 which was altered to 6.95 when epinephrine was added.

The direct method of Warburg (118) was used to determine the rate of oxygen uptake in an atmosphere of oxygen, $Q_{O_2}^{O_2}$, and the rate of carbon dioxide production in an atmosphere of oxygen, $Q_{CO_2}^{O_2}$. To this end, 0.2 ml. of 20% KOH was added to the center well of those flasks which were to be used for the measurement of $Q_{O_2}^{O_2}$. The lip of the well was lined with grease to prevent leakage. At this point, the appropriate amount of blocking agent to be tested was added and followed by an amount of medium sufficient to make a final fluid volume of 2.0 ml. This volume included the 0.2 ml. of KOH added to those flasks measuring $Q_{O_2}^{O_2}$. The flasks, of course, were all prepared before sacrifice of the animals. As soon as the hemidiaphragms or liver slices were added to the flasks, 0.2 ml. of epinephrine (see below for concentrations) freshly removed from the refrigerator, was added to the side arms.

The side arms of the control received phosphate-buffered saline prepared with exactly the amount of acid as was used to make up the epinephrine solution, so that both the control and experimental tubes had ostensibly the same acid solution added to them.

Following the completion of these preparations, the flasks were attached to the manometers, and the entire fitting was placed in the Warburg water bath which was maintained at 37°C. The total time from the first decapitation to the last placing of seven manometers and a thermobar into the bath was about 20 minutes for the experiments on the diaphragm, and about 15 minutes for the liver experiments.

When all the flasks were in the bath, rubber tubes leading from an oxygen supply tank were attached to the side arms, and the flasks were flushed with 100% oxygen for 10 minutes. During this time, the shaker apparatus was set in motion at 90 strokes/minute. After 10 minutes the oxygen inlet tubes were removed, all cocks were closed, and an additional 5 minutes was allowed for further equilibration. At the end of this period, the cocks were opened and epinephrine was tipped in. The manometers were then zeroed off and readings were taken every 10 minutes for 2 hours in the earlier experiments and every 30 minutes for 1 hour in the later experiments.

On a given day, the manometer setup consisted of a thermobar (for measuring changes due to atmospheric conditions, containing everything but the diaphragmatic tissue) two or three pairs of manometers, and two additional manometers which were removed immediately after the equilibration. It was from these last two manometers that initial values for muscle glycogen, glucose concentration, and liver glycogen were taken. It was believed that this method for obtaining initial values would control the effects of handling the tissue. In order to further this control, the manometers were not always set up in the same order, i.e., the earliest excised tissue was not always placed in the first flask.

The epinephrine used was pure l-epinephrine base (supplied by Parke-Davis, Co.). It was first made in a concentration of 1:1000 using distilled water and dilute HCl. Further dilutions were prepared from this stock solution by diluting with phosphate-buffered saline. In order to determine the best dose of epinephrine to be used, the following concentrations were employed with the diaphragmatic tissue.

0.M.

1.6×10^{-5} M.

1.6×10^{-6} M.

1.6×10^{-7} M.

These concentrations refer to the final concentration i.e., after the epinephrine was tipped into the flask.

N-9-Fluorenyl-N-ethyl-2-chloroethylamine (SY-21), dihydroergocornine (DHO-180), and Neoantergan were the blocking drugs selected for this study. The final concentration decided on was that which was calculated as equal to or slightly below the blood concentration effective in blocking or reducing epinephrine-induced hyperglycemia in the rabbit, if all the dose injected were equally distributed throughout the blood volume. These concentrations were:

SY-21 -- 10^{-5} M.

DHO-180 -- 10^{-5} M.

Neoantergan -- 10^{-4} M.

Again, these concentrations refer to the final concentration after mixing with the medium. The solutions of blocking drug were prepared by starting with the dry salt in the case of SY-21 and Neoantergan, and a stock solution of DHO-180, containing 0.5 mg./ml. They were prepared fresh and diluted with phosphate-buffered saline on the morning of a given experiment and placed in the refrigerator until ready for use.

At the end of one or two hours, the manometers and flasks were removed from the bath and the flasks were

quickly detached. The tissue was removed, blotted dry on filter paper, quickly rinsed in iced phosphate-buffered saline, blotted dry again and weighed in a Roller-Smith balance. The tissue was placed in 1 ml. of 30% KOH and then the glycogen content determined by the method of Good, Kramer and Somogyi (45). The glycogen is expressed in terms of wet tissue weights. A 0.2 ml. aliquot of the medium was analyzed by the micromethod of Somogyi (45) for the glucose concentration and an additional aliquot was reserved for the determination of pH. In early experiments, 0.1 ml. aliquot was used for the determination of lactic acid by the micromethod of Barker and Summerson (8), but under these aerobic conditions, there was insufficient lactic acid produced to be detected by this method in my hands.

Effect of Various Blocking Agents on Epinephrine-Induced Effects on Carbohydrate Metabolism in the Isolated Rat Hemidiaphragm

Drug Used	# of Expts	R.Q.	Glucose uptake mg/gm/hr[2]	P[1]	% Recovered	P[1]
Control	12	1.0	5.39 ± 0.30		93.4 ± 2.1	
Epinephrine 1.6×10^{-6}M	12	1.0	5.99 ± 0.70	<0.02 >0.01	67.6 ± 2.0	<0.01
SY-21 10^{-5}M	9	1.0	4.97 ± 0.41		90.7 ± 3.0	
SY-21 + Epinephrine	9	1.0	5.04 ± 0.45	>0.5	91.3 ± 1.4	>0.5
DHO-180 10^{-5}M	9	1.0	4.99 ± 0.39		93.3 ± 1.4	
DHO-180 + Epinephrine	9	1.0	5.77 ± 0.42	<0.01	67.2 ± 1.6	<0.01
Neoantergan 10^{-4}M	15	1.0	4.77 ± 0.41		93.5 ± 1.8	
Neoantergan + Epinephrine	15	1.0	5.09 ± 0.65	0.1	67.6 ± 2.1	<0.01

1. "P" determined by group analysis by comparing drug & epinephrine with drug alone.

2. Expressed as per gm. of wet tissue weight.

Effect of Various Blocking Agents on Epinephrine-Induced Effects on Carbohydrate Metabolism in the Isolated Rat Hemidiaphragm

(continued)

Drug Used	Glucose burned mg/gm/hr[2]	P[1]	Glycogen[3] increase mg/gm/hr[2]	P[1]	Glucose utilized mg/gm/hr[2]	P[1]
Control	1.61 ± 0.23		3.43 ± 0.18		5.04 ± 0.31	
Epinephrine 1.6×10^{-6}M	1.88 ± 0.29	< 0.05 > 0.02	2.16 ± 0.31	< 0.01	4.04 ± 0.39	< 0.01
SY-21 10^{-5}M	1.49 ± 0.24		3.02 ± 0.44		4.51 ± 0.41	
SY-21 + Epinephrine	1.60 ± 0.29	0.4	3.01 ± 0.46	> 0.5	4.61 ± 0.49	> 0.5
DHO-180 10^{-5}M	1.69 ± 0.14		2.99 ± 0.28		4.68 ± 0.32	
DHO-180 + Epinephrine	1.91 ± 0.11	< 0.01	1.98 ± 0.24	< 0.01	3.89 ± 0.26	< 0.01
Neoantergan 10^{-4}M	1.57 ± 0.31		2.91 ± 0.20		4.48 ± 0.40	
Neoantergan + Epinephrine	1.79 ± 0.32	0.05	1.68 ± 0.27	< 0.01	3.47 ± 0.52	< 0.01

1. "P" determined by group analysis by comparing drug & epinephrine with drug alone.

2. Expressed as per gm. of wet tissue weight.

3. Expressed as glucose.

Effect of Various Blocking Agents on Epinephrine-Induced Effects on Carbohydrate Metabolism in the Isolated Rat Diaphragm.

1) Effect of Epinephrine

a) R.Q. - 1.00

b) Glucose uptake - increases

c) % accounted for - decreases

d) Glucose burned - increases

e) Glycogen increases - decreases

f) Glucose utilized - decreases

Interpretation - The finding that under the influence of epinephrine the diaphragm formed less glycogen can be interpreted as either a decreased glycogenesis or an increased glycolysis. The work of Tuerkischer and Wertheimer (117), which has been discussed above, suggests that the latter possibility is more nearly correct. Thus, at the same time that epinephrine caused an increase in glucose uptake it decreased the total amount of the glucose uptake which was actually utilized. This indicates that there was an accumulation of some intermediate product. The evidence presented by other workers suggests that this product was hexosemonophosphate (see discussion).

2) Effect of SY-21 plus Epinephrine

a) R.Q. - 1.00

b) Glucose uptake - Unchanged

c) % accounted for - Unchanged

d) Glucose burned - "

e) Glycogen increase - "

f) Glucose utilized - "

Interpretation - All the effects of epinephrine on the aspects of carbohydrate metabolism which have been measured were blocked by SY-21.

3) Effect of DHO-180 plus Epinephrine

a) R.Q. - 1.00

b) Glucose uptake - increased

c) % accounted for - decreased

d) Glucose burned - increased

e) Glycogen increase - decreased

f) Glucose utilized - decreased

Interpretation - All of the effects of epinephrine on the aspects of carbohydrate metabolism which have been measured remained after treatment with DHO-180.

4) Effect of Neoantergan plus Epinephrine

a) R.Q. - 1.00

b) Glucose uptake - Unchanged

c) % accounted for - decreased

d) Glucose burned - increased

e) Glycogen increase - increased

f) Glucose utilized - decreased

Interpretation - The only explanation that can be offered for the failure of the glucose uptake to rise following epinephrine is the wide variability of the data. It is believed that a larger series of experiments would show this glucose uptake to rise. With the exception of this phase all of the aspects of carbohydrate metabolism which have been measured persisted after treatment with Neoantergan. If the increase in glucose uptake is found to be truly blocked by Neoantergan, it may offer an explanation for the potentiation of epinephrine-induced hyperglycemia seen in dogs. If the elevated blood glucose is not removed at an accelerated rate such as is apparent in these experiments, the blood glucose will rise still higher.

Effect of Graded Doses of Epinephrine on Oxygen Utilization of the Isolated Rat Hemidiaphragm During the First and Second Hours.

Interpretation of Results

Epinephrine in a concentration of 1.6×10^{-7} M did not alter the oxygen consumption during either the first or second hour. However, a ten-fold increase in concentration significantly increased the oxygen consumption during both the first and second hours. A hundred-fold increase in concentration, i.e. 1.6×10^{-5} M also increased the rate of oxygen consumption during both the first and second hours, but did not increase the rate above that with 1.6×10^{-6} M concentration. It was therefore decided that a concentration of 1.6×10^{-6} M of epinephrine would be used. This concentration has been shown by other workers to produce maximum glycolysis in the isolated liver (107).

Effect of Graded Doses of Epinephrine on O_2 Utilization of Isolated Rat Hemidiaphragms During the First Hour

Treatment	# of Expts	O_2 Consumed ml/gm/hr[2]	P[1] vs. control	P[1] vs. Epi. 1.6 X 10^{-7}M	P[1] vs. Epi. 1.6 X 10^{-6}M
Control	21	1.107 ± 0.200			
Epinephrine 1.6 X 10^{-7}M	5	1.063 ± 0.202	> 0.5		
Epinephrine 1.6 X 10^{-6}M	22	1.383 ± 0.194	< 0.01	< 0.01	
Epinephrine 1.6 X 10^{-5}M	5	1.339 ± 0.250	< 0.05 > 0.02	< 0.1 > 0.05	> 0.5

1 - "P" determined by group analysis

2 - Wet weight of tissue

Effect of Graded Doses of Epinephrine on O_2 Utilization of Isolated Rat Hemidiaphragms During the Second Hour

Treatment	# of Expts	O_2 Consumed ml/gm/hr[2]	P[1] vs. control	P[1] vs. Epi. 1.6 X 10^{-7}M	P[1] vs. Epi. 1.6 X 10^{-6}M
Control	9	0.949 $\pm$ 0.162			
Epinephrine 1.6 X 10^{-7}M	5	0.999 $\pm$ 0.212	>0.5		
Epinephrine 1.6 X 10^{-6}M	10	1.251 $\pm$ 0.226	<0.01	<0.1 >0.05	
Epinephrine 1.6 X 10^{-5}M	5	1.168 $\pm$ 0.221	0.05	>0.5	>0.5

1 - "P" determined by group analysis

2 - Wet weight of tissue

The Effect of Epinephrine (1.6×10^{-6}M) on O_2 Consumption of the Isolated Rat Hemidiaphragm During the 2nd Hour

Treatment	# of Expts	R.Q.	O_2 Consumed ml/gm/hr[2]	P[1] vs. control 2nd hr.	P[1] vs. control 1st hr.	P[1] vs. Epi. 1st hr.
Control	9	1.07	0.949 $\pm$ 0.162		<0.05 >0.02	<0.01
Epinephrine 1.6×10^{-6} M	10	1.04	1.251 $\pm$ 0.226	<0.01	<0.1 >0.05	0.1

1 - "P" determined by group analysis
2 - Wet weight of tissue

The Effect of Epinephrine (1.6×10^{-6} M) on Oxygen Consumption of the Isolated Rat Hemidiaphragm During the Second Hour.

Interpretation of Results

During the second hour, the rate of oxygen consumption of the control diaphragms fell slightly but significantly as compared to the control rate of the first hour. When epinephrine was added to the medium, the mean rate during the second hour was also less than the mean rate for the first hour with epinephrine. However, the variability in the response was so large, that this decrease was not statistically significant. This second hour oxygen consumption rate with epinephrine was still significantly higher than the controls during this period, but had fallen to the point where it was not significantly different from the controls of the first hour. Hence, statistically, one cannot demonstrate any difference between the control rate for the first hour and the rate with epinephrine for the second hour.

It is suggested that this is an indication of a beginning failure of the action of epinephrine so that the rate begins to approach normal. One clue to explain the drop in oxygen utilization is suggested by the R.Q. It will be noted that the R.Q. of the controls rose during

the second hour to 1.07 from 1.00. This suggests a shift during the second hour from glucose oxidation exclusively to an oxidation of glucose with simultaneous fat and/or protein storage. It is believed that, in this case, it is probably fat. Chernick, Masoro, and Chaikoff (20a), using C^{14}-labeled glucose, have demonstrated that the isolated rat diaphragm is capable of converting glucose to fatty acids. The storage of fat during the second hour by converting the glucose to fat would release some oxygen from internal metabolism which could be utilized in the place of external, or supplied oxygen. In such an instance, the rate of oxygen utilization would tend to decrease from external measurements. Similarly, in those diaphragms treated with epinephrine, the R.Q. rose to 1.04. This can again be explained as above, by the storage of fat. It will be noted that in those diaphragms incubated with epinephrine, the R.Q. did not rise to the 1.07 of the controls. It is likely that under the influence of epinephrine, there was an accumulation of hexosemonophosphate during the first hour. During the second hour, as has been hypothesized, the effect of epinephrine begins to decrease, and this hexosemonophosphate is then oxidized. Thus, the epinephrine-treated diaphragms use more oxygen during the second hour than the controls, and the R.Q. is kept lower. Further evidence in

substantiation of the failure of epinephrine activity during the second hour is offered in the section dealing with "Effect of epinephrine on glycogen synthesis in the isolated diaphragm."

Effect of Various Blocking Agents on the Epinephrine-Induced Calorigenic Effect on the Isolated Rat Hemidiaphragm

Drug Used	# of Expts	O_2 Utilization ml/gm/hr[2]	P[1] vs. control ± Epi.	P[1] vs. control ± Epi.	P[1] vs. drug alone
Control	21	1.107 ± 0.200			
Epinephrine 1.6×10^{-6}M	22	1.383 ± 0.194	< 0.01		
SY-21 10^{-5}M	9	1.104 ± 0.179	> 0.5	< 0.01	
SY-21 / Epinephrine	9	1.200 ± 0.210	0.3	< 0.05 > 0.02	0.3
DHO-180 10^{-5}M	9	1.254 ± 0.084	< 0.05 > 0.02	< 0.1 > 0.05	
DHO-180 / Epinephrine	9	1.421 ± 0.080	< 0.01	> 0.5	< 0.01
Neoantergan 10^{-4}M	15	1.161 ± 0.230	0.4	< 0.01	
Neoantergan / Epinephrine	15	1.331 ± 0.226	< 0.01	0.4	< 0.05 > 0.02

1. "P" determined by group analysis

2. Expressed as wet weight

Effect of Blocking Agents on the Calorigenic Action of Epinephrine in the Isolated Rat Diaphragm.

Interpretation of Results

1. The Effect of Epinephrine

Epinephrine in a concentration of 1.6×10^{-6} mols caused a rise in the oxygen consumption of 0.27 ml./gm./hr. This was equivalent to a rise of 24.3% over the control value. This experiment was designed, for the most part, as a paired data experiment. However, it was noted that although there was a variability in the responses between the halves of the same diaphragm, those diaphragms treated with epinephrine consistently used more oxygen than those without epinephrine. The possibility that this effect was due to the slight acidity of the epinephrine, was ruled out by preparing a solution of diluting fluid alone, with as much acid added to it as was added to the epinephrine. The experiment was also controlled so that those diaphragms receiving epinephrine were not always prepared in the same position, i.e., control and experimental tissues were prepared alternately. As many factors as could be thought of were controlled so that this increase in the oxygen consumption due to epinephrine is believed to be a specific effect. A series of

experiments was performed to determine the R.Q. of the reaction, and since the R.Q. was almost 1.0 in every case, the increase in oxygen consumption is considered to be a reflection of the burning of glucose.

2. The Effect of SY-21

It was noted that SY-21 in a concentration of 10^{-5} M did not alter the normal rate of oxygen consumption. When epinephrine was added to the medium, the mean oxygen consumption rose 0.10 ml./gm./hr. This represents a mean increase of 9.0% over the rate with SY-21 alone. This difference was not significant. The tissue treated with SY-21 and epinephrine used only 1/3 as much oxygen over the controls as did the tissue treated with epinephrine alone. This difference was significant. Since no difference could be shown between the oxygen consumption of the diaphragm treated with SY-21 and epinephrine and the untreated diaphragm, it may be concluded that SY-21 reduces the calorigenic effect of epinephrine in the isolated rat diaphragm to the point of complete blockade.

3. The Effect of DHO-180

This dihydrogenated ergocornine in a concentration of 10^{-5} M exerted a significant calorigenic action on the isolated muscle. It raised the mean oxygen consumption 13.8% above the control values. This increase

in oxygen consumption brought the value to almost that in response to epinephrine in the otherwise untreated tissue. When epinephrine was added to a DHO-180 treated diaphragm, the mean oxygen consumption was raised an additional 13.6% to produce a significant increase over the rate with the DHO-180 alone. The rate, however, did not significantly exceed that of the epinephrine alone despite the calorigenic activity of DHO-180 alone. This means that with respect to the total increase in oxygen consumption resulting from epinephrine, there was a partial reduction produced, i.e., from a mean 24.3% increase to a mean 13.6% increase. However, a complete inhibition, obviously, did not occur.

4. The Effect of Neoantergan

This antihistamine, in a concentration of 10^{-4} M did not significantly alter the rate of oxygen consumption of the isolated muscle. When epinephrine was added to the Neoantergan treated diaphragm, the oxygen consumption increased 14.7%. Here again there is the suggestion of a partial reduction in the calorigenic effect, ie., from 24.3% to 14.7%, but no suggestion of a complete blockade.

Summary - SY-21 (10^{-5} M) completely blocked the epinephrine-induced calorigenic effect in the isolated muscle. There is a suggestion that DHO-180 (10^{-5} M), itself slightly

calorigenic, produced a partial blockade, but the blocking influence of this compound on the oxygen consumption of the diaphragm is not believed to contribute considerably to the reduction of oxygen consumption in the intact animal. This opinion is offered in view of the observation that the oxygen consumption of the diaphragm treated with DHO-180 and epinephrine was not different from that of the tissue treated with epinephrine alone. Similarly, the results with Neoantergan (10^{-4}M) suggested that this drug produced a slight reduction in oxygen consumption in epinephrine-treated diaphragms.

Effect of Graded Doses of Epinephrine on Glycogen Synthesis of the Isolated Rat Diaphragm During Two Hours of Incubation

Treatment	# of Expts	[3]Glycogen value after 2 hrs. mg/gm^2	pl vs. control	pl vs. Epi. 1.6 X $10^{-7}M$	pl vs. Epi. 1.6 X $10^{-6}M$
Control	10	3.47 ± 0.30			
Epinephrine 1.6 X $10^{-7}M$	5	3.40 ± 0.24	>0.05		
Epinephrine 1.6 X $10^{-6}M$	9	4.24 ± 0.27	<0.01	<0.01	
Epinephrine 1.6 X $10^{-5}M$	5	4.24 ± 0.25	<0.01	<0.01	>0.5

1 - "p" - group analysis
2 - Wet weight of tissue
3 - Expressed as glucose

Effect of Graded Doses of Epinephrine on Glycogen Synthesis of the Isolated Rat Diaphragm During Two Hours of Incubation.

Interpretation of Results

The influence of graded doses of epinephrine on the alteration in glycogen after two hours incubation paralleled the influence of graded doses of epinephrine on oxygen utilization. That is, 1.6×10^{-7} M was without effect and a ten-fold increase showed a significant effect. A hundred-fold increase caused no greater effect than a ten-fold increase.

The apparent discrepancy, i.e., more glycogen with epinephrine than without it is believed to be a time factor and is discussed under the section dealing with "Effect of epinephrine on glycogen synthesis in the rat diaphragm".

The data concerning the effect on glycogen together with the effect on oxygen utilization were responsible for the final decision to use epinephrine in a concentration of 1.6×10^{-6} M.

Effect of Various Blocking Agents on Epinephrine-Induced Effect on Glycogen Synthesis in the Isolated Rat Hemidiaphragm

Drug Used	# of Expts	0 Time Glycogen[3] mg/gm/hr[2]	Total Glycogen[3] after 1 hr mg/gm/hr[2]	P[1] vs. control ± Epi.	P[1] vs. control ± Epi.	P[1] vs. drug alone
Control	12	1.62 ± 0.18	5.13 ± 0.17			
Epinephrine 1.6×10^{-6}M	12		3.86 ± 0.31	< 0.01		
SY-21 10^{-5}M	9	1.72 ± 0.15	4.72 ± 0.44	< 0.01	< 0.01	
SY-21 + Epinephrine	9		4.71 ± 0.46	< 0.01	< 0.01	> 0.5
DHO-180 10^{-5}M	9	1.72 ± 0.12	4.69 ± 0.28	< 0.01	< 0.01	
DHO-180 + Epinephrine	9		3.67 ± 0.24	< 0.01	0.2	< 0.01
Neoantergan 10^{-4}M	15	1.68 ± 0.09	4.61 ± 0.20	< 0.01	< 0.01	
Neoantergan + Epinephrine	15		3.38 ± 0.27	< 0.01	< 0.01	< 0.01

1. "P" determined by group analysis
2. Expressed as wet weight
3. Expressed as glucose

The Effect of Blocking Agents of Epinephrine-Induced Effects on Glycogen Synthesis in the Isolated Rat Diaphragm.

Interpretation of Results

1) Effect of Epinephrine

A concentration of epinephrine of 1.6×10^{-6} M caused a mean reduction in the total glycogen value of 24.8%. This was a true reduction. It is interesting to note that there was a mean increase in oxygen consumption of 24.3% which, since the R.Q. was 1.0, presumably was used to burn glucose. This leads to the speculation that the glucose which would have gone to glycogen was oxidized instead, under the influence of epinephrine. It is not possible to tell whether this reduction in total glycogen after one hour, represents a decrease in glycogen synthesis or a splitting of glycogen already formed.

It was noted that if incubation were allowed to continue for an additional hour, i.e. for two hours, that there was a peculiar shift in the glycogen synthesis values. The control value for glycogen changed from a value after one hour of 5.13 ± 0.17 mg./gm., to one of 3.47 ± 0.30 after two hours of incubation. On the other hand, the glycogen value of diaphragms treated with 1.6×10^{-6} M of epinephrine shifted from a glycogen value

of 3.86 ± 0.31 mg./gm. after one hour, to 4.24 ± 0.27 after two hours of incubation. Thus, after two hours of incubation, there is apparently a reversal of the effect of epinephrine. It is possible to explain this discrepancy by the hypothesis previously offered that the effect of epinephrine starts to wear off after one hour of incubation. The mass of hexosemonophosphate which had been accumulated during the first hour (1/3 of the glucose taken up may have been in this form) is now available for oxidation. Therefore, it becomes the precursor for glycolysis instead of muscle glycogen. The glucose which is taken up during this second hour of incubation, is now probably converted to muscle glycogen. The control diaphragms, however, continue to call upon their glycogen reserves for glycolysis. A similar situation was observed by Cohen (22) who noted that the muscle glycogen of the epinephrine-treated diaphragms did not decrease as did the glycogen of the untreated diaphragms during incubation.

2) Effect of SY-21

A concentration of 10^{-5} M of SY-21 itself reduced the total mean glycogen value of the isolated diaphragm after incubation 8.0%. This reduction did not reach the level of the reduction produced by epinephrine. When epinephrine was added to a diaphragm pretreated with SY-21, there was again a mean reduction of 8.2% of the untreated

diaphragm bringing the total glycogen level almost exactly to that with SY-21 alone, which left it still higher than that with epinephrine alone. It may be concluded that although SY-21 itself caused a decrease in glycogen synthesis, it completely blocked the effect of epinephrine in this response.

3) Effect of DHO-180

DHO-180 in a concentration of 10^{-5} M produced a mean reduction in the total glycogen value of 8.6% of the control value. It should be recalled that a mean increase of 12.6% in the oxygen consumption was also produced. This decrease in glycogen synthesis was not as low as that produced by epinephrine. When epinephrine was added to the DHO-180 treated diaphragm, an additional reduction of 21.8% in the mean glycogen value occurred. This approaches the reduction of 24.8% seen with epinephrine alone. Although the final mean total value for the glycogen following DHO-180 and epinephrine is 4.9% less than that with epinephrine alone, this decrease is not statistically significant. It may be concluded that the epinephrine factor in glycogen synthesis was not influenced by DHO-180 in the isolated muscle.

4) Effect of Neoantergan

Neoantergan in a concentration of 10^{-4} M reduced

the mean total glycogen value 10.2% of the control value which was not as low as that produced by epinephrine. The addition of epinephrine to a diaphragm pretreated with the antihistaminic caused an additional mean reduction of 26.7%. This should be compared with the reduction of 24.8% due to epinephrine in an otherwise untreated diaphragm. The combined effect of a 10.2% reduction due to Neoantergan and the 26.7% reduction due to the addition of epinephrine, brought the final glycogen value below that found with epinephrine in an otherwise untreated diaphragm. Thus, it may be concluded that Neoantergan does not reduce the epinephrine factor in glycogen synthesis, and may actually potentiate it.

Summary

Epinephrine caused a reduction in glycogen synthesis. SY-21 (10^{-5} M) blocked the epinephrine induced effect on glycogen synthesis, although it itself reduced the glycogen synthesis. DHO-180 and Neoantergan themselves reduced the rate of synthesis and did not influence the action of epinephrine.

Effect of Various Blocking Drugs on Epinephrine-Induced Calorigenic Effect on the Isolated Rat Liver Slice

Drug Used	# of Expts	O_2 Utilization ml/gm/hr[2]	p[1] vs. control & Epi.	p[1] vs. control & Epi.	p[1] vs. drug alone
Control	9	1.109 ± 0.115			
Epinephrine 1.6×10^{-6}M	9	1.430 ± 0.138	<0.01		
DHO-180 10^{-5}M	9	1.116 ± 0.148	>0.5	<0.01	
DHO-180 + Epinephrine	9	1.127 ± 0.139	>0.5	<0.01	>0.5
SY-21 10^{-5}M	9	1.169 ± 0.137	0.3	< 0.01	
SY-21 + Epinephrine	9	1.250 ± 0.108	<0.02 >0.01	<0.01	0.2
SY-73 10^{-5} M	9	1.160 ± 0.065	0.1	<0.01	
SY-73 + Epinephrine	9	1.409 ± 0.083	<0.01	>0.5	<0.01
Neoantergan I 10^{-4}M	9	1.115 ± 0.131	>0.5	<0.01	
Neoantergan I + Epinephrine	9	1.367 ± 0.088	<0.01	0.3	<0.01
Neoantergan II 2×10^{-4}M	9	1.150 ± 0.081	0.4	< 0.01	
Neoantergan II + Epinephrine	9	1.394 ± 0.058	<0.01	0.5	<0.01

1. "p" determined by group analysis

2. Expressed as wet weight

The Effect of Various Blocking Agents on Epinephrine-Induced Calorigenic Effect on the Isolated Rat Liver Slice.

1) The Effect of Epinephrine

Like the effect in the isolated muscle preparation, epinephrine (1.6×10^{-6} M) significantly increased the oxygen consumption of the liver slice. This increase was 22.4% over the control value.

2) The Effect of DHO-180

This derivative of ergocornine did not significantly alter the resting oxygen consumption of the liver slice. It was found to completely block the epinephrine-induced increase in oxygen utilization.

3) The Effect of SY-21

This 2-halogenated ethylamine, used in the same concentration as was used in the isolated muscle preparation, showed a significant blockade of the epinephrine-induced calorigenic effect on the liver slice. Actually, however, this should be considered a reduction in the response rather than a blockade. It will be noticed from the statistics that the drug alone did not significantly increase the resting oxygen consumption. When epinephrine was added to the SY-21 treated diaphragm, the oxygen consumption rose 12.7% above that of the control rate. Although this was not

significantly different from the rate with SY-21 alone, it is significantly greater than the rate without any drug added.

In order to test if this effect might be due to the halogenated ethylamine grouping, SY-73, an hydroxylated ethylamine was tested. This agent is without adrenergic blocking activity in the intact animal and did not block the epinephrine-calorigenic effect on isolated liver.

4) Neoantergan

Neoantergan was tested in a concentration of 10^{-4} M, the same concentration as was used in isolated muscle studies. It was without any appreciable effect on the calorigenic action of epinephrine. This concentration was the same as would exist in the animal if 3 mg./kg. were injected and all of it were evenly distributed throughout the blood. In order to see the effect of a larger concentration, 2×10^{-4} M was added to the preparation, and again, there was no effect on the increase in oxygen consumption following epinephrine.

Summary

Epinephrine (1.6×10^{-6} M) caused an increase in the oxygen utilization of isolated rat liver slices. DHO-180 completely blocked the effect of epinephrine. SY-21

significantly reduced the response to epinephrine but did not completely block it. SY-73, not an adrenergic blocking drug, but an agent with the ethylamine grouping was without effect. Neoantergan was without effect even in doses larger than that which would usually be injected into the intact animal.

Effect of Various Blocking Drugs on Epinephrine-Induced Glycogenolysis in Isolated Liver Slices of the Rat

Drug Used	# of Expts	0 Time Glycogen[3] mg/gm[2]	Total Glycogen[3] after 1 hr. mg/gm/hr[2]	P[1] vs. control & Epi.	P[1] vs. control & Epi.	P[1] vs. drug alone
Control	9	17.55 ± 0.21	13.64 ± 0.36			
Epinephrine 1.6×10^{-6}M	9		7.51 ± 0.28	<0.01		
DHO-180 10^{-5}M	9	17.34 ± 0.29	13.72 ± 0.43	>0.5	<0.01	
DHO-180 + Epinephrine	9		13.53 ± 0.30	0.5	<0.01	0.3
SY-21 10^{-5}M	9	17.38 ± 0.28	13.24 ± 0.25	<0.02 >0.01	<0.01	
SY-21 + Epinephrine	9		12.94 ± 0.35	<0.01	<0.01	<0.05 >0.02
SY-73 10^{-5}M	9	17.61 ± 0.31	13.61 ± 0.29	>0.5	<0.01	
SY-73 + Epinephrine	9		7.64 ± 0.31	<0.01	0.4	<0.01
Neoantergan I 10^{-4}M	9	17.52 ± 0.20	13.46 ± 0.41	0.3	<0.01	
Neoantergan I + Epinephrine	9		7.72 ± 0.82	<0.01	0.5	<0.01
Neoantergan II 2×10^{-4}M	9	17.60 ± 0.23	13.57 ± 0.27	>0.5	<0.01	
Neoantergan II + Epinephrine	9		7.53 ± 0.24	<0.01	>0.5	<0.01

1. "P" determined by group analysis
2. Expressed as wet weight
3. Expressed as glucose

The Effect of Various Blocking Agents on Epinephrine-Induced Glycogenolysis in the Isolated Liver Slice of the Rat.

1) Effect of Epinephrine

Epinephrine (1.6×10^{-6} M) reduced the liver glycogen by 44.9% of the control value after one hour.

2) Effect of DHO-180

DHO-180 completely blocked the glycogenolytic effect of epinephrine on the isolated rat liver. It should be noted that this compound was without effect on the isolated muscle preparation. These data suggest that DHO-180 is selective in its action on the liver. From this, one may infer that the blockade of epinephrine-induced hyperglycemia in the intact rabbit, with this agent, is due to its effect of preventing liver glycogenolysis due to epinephrine. This logically would result in a complete blockade of the hyperglycemia such as was seen in rabbits. Even though the effect of epinephrine in muscle was present, the only way it could contribute to the hyperglycemia was to send lactic acid to the liver for glycogenesis. However, at the liver the glycogenolysis of this new glycogen, even if it were formed would be blocked by DHO-180.

3) Effect of SY-21

SY-21 in a concentration of 10^{-5} M caused a

significant reduction in the glycogenolysis due to epinephrine. SY-73, an ethylamine compound without adrenergic blocking activity in the intact animal, was without effect. In view of the combined results of the effects of SY-21 in liver and muscle, it is suggested that the primary action of SY-21 is on the muscle. Some glycogenolysis is still present following epinephrine in its action on the liver. This may explain why SY-21 succeeded in only reducing the epinephrine-induced hyperglycemia in rabbits and dogs and not completely blocking the response as did DHO-180 in rabbits. The primary cause of the epinephrine-induced hyperglycemia is probably its effect on the liver. The effect on muscle probably only serves to sustain the effect. If a given agent blocks the effect of epinephrine on the muscle but only reduces the effect on the liver, it is logical that it will only reduce the hyperglycemia and not completely block it.

4) Effect of Neoantergan

This antihistamine, in a dose of 10^{-4} M and 2×10^{-4} M, the latter being a very high concentration, was without effect on the liver glycogenolysis due to epinephrine.

Summary

Epinephrine causes a glycogenolysis of the

isolated liver slice. This effect is blocked by DHO-180, indicating that there is a selective action for DHO-180 on the liver in this response. It is suggested that it is through its action on the liver that this agent causes complete blockade of epinephrine-induced hyperglycemia in the intact rabbit. SY-21 succeeded in reducing the response of epinephrine. This suggests that the complete blockade in muscle in addition to the reduction of the effect in liver is reponsible for the reduction rather than the complete blockade of epinephrine-induced hyperglycemia in the intact rabbit and dog. SY-73, an agent without adrenergic blocking action was without effect in this response. Neoantergan, an antihistaminic effective in reducing the hyperglycemia of intact rabbits, was without effect on the isolated rat tissues even in large doses.

DISCUSSION

In the isolated diaphragm, the experiments dealing with the effect of graded doses of epinephrine indicate that a maximum effect is achieved with a dose of epinephrine corresponding to a concentration of 1.6×10^{-6} M. This concentration is the same as that found to produce maximum glycolysis of the isolated liver slices by other workers (116).

Under the conditions of this experiment, where 200 mg.% of glucose was supplied to the diaphragm of an unfasted rat at the beginning of the experiment there was an increase in the rate of oxygen utilization during the first hour of incubation with epinephrine. This increased rate was apparently due to an accelerated burning of the carbohydrate, since the R.Q. was 1.0. During the second hour of incubation, the rate of oxygen utilization was still elevated in the epinephrine-treated diaphragms, but the elevation was less than during the first hour. A clue to the mechanism of this decreased rate was supplied by the R.Q. which rose to a figure above 1.0 during the second hour period, indicating storage of fat and/or protein. In such an instance, oxygen would become available from the metabolic conversion of glucose to fat or protein, and would be

utilized for further oxidations. External measurements would, therefore, indicate a decreased rate of oxygen consumption, although the tissue may be respiring at the same rate. Although the conversion of carbohydrate to fat is usually thought of as occurring in the liver, Chernick et al. (20a) have shown that the isolated rat diaphragm is also capable of converting C^{14}-labeled glucose to fatty acids.

Epinephrine also caused a decrease in glycogen synthesis during the first hour of incubation. In the presence of glucose in the medium, such as there was in these experiments, this might be due to a decreased conversion of glucose to glycogen, or an increased glycogenolysis. The studies of Tuerkischer and Wertheimer (117) demonstrated that the increase in glycogenolysis with epinephrine, in the absence of glucose in the medium, was almost the same as the decrease in glycogen synthesis with glucose in the medium. Therefore, it is likely that at least part of the apparent decrease in glycogen synthesis was a reflection of an increase in glycogenolysis.

Sutherland (115), in a recent review of his studies on the influence of epinephrine in phosphorylase systems in liver and muscle, has directed attention to evidence that epinephrine exerts its prime effect on the

phosphorylase of the tissues. The amount of active phosphorylase found in tissues represents a balance between inactivation and resynthesis of phosphorylase. Sutherland and Cori (116), Walaas and Walaas (121), and Ellis, Anderson and Turtle (37), have demonstrated that the amount of active phosphorylase in isolated liver and muscle tissue treated with epinephrine is markedly increased. It was suggested, in view of the fact that epinephrine does not inhibit enzymatic inactivation of phosphorylase as does fluoride that its mechanism of action was to actually promote the synthesis of this enzyme.

The further interesting fact is that the direction of the phosphorylase activity is apparently reversed, i.e., the reaction proceeds mainly in the direction of glycogen breakdown. That the addition of epinephrine to isolated muscle and liver results in a glycogen depletion is too well known to warrant documentation. Hegnauer and Cori (64) clearly demonstrated that the increased glycogen breakdown in frog muscle incubated anaerobically with epinephrine was accompanied by an accumulation of hexosephosphate and a corresponding decrease in inorganic phosphate. Riesser (100) and the Walaases (121) also showed that there was this apparent reversal in direction of phosphorylase effect in isolated

rat diaphragms. The experiments reported herein do not conclusively demonstrate an increased glycogenolysis in the presence of glucose in the medium, but they do show that under these conditions, epinephrine caused a decrease in glycogen synthesis. This, coupled with a decrease in the amount of glucose accounted for, suggests that epinephrine caused an accumulation of intermediates other than glycogen in these experiments. The postulate that the direction of phosphorylase activity is reversed when the tissue is under the influence of epinephrine, together with the results of Tuerkischer and Wertheimer (117) would indicate that glycogenolysis contributed to part of these intermediates.

Thus, evidence has been presented by other workers that there is an accumulation of hexosemonophosphate following epinephrine administration to an isolated muscle. If one postulates that the action of epinephrine begins to wear off during the second hour, the diaphragm, during this period, would have a substantial amount of hexosemonophosphate available for oxidation, while the untreated diaphragm would still need to call upon its reserve of glycogen. Thus, during the second hour period, the control diaphragms would be using glycogen at a faster rate than the epinephrine-treated tissues and would account for reversal in the glycogen pattern. Further evidence

pointing to this postulate of a lessening of the action of epinephrine during the second hour is afforded by the R.Q. during this period. Whereas the R.Q. of the control tissues rose to 1.07, that of the epinephrine treated diaphragm rose to only 1.04. A release of the stored hexose-phosphate would require additional amounts of oxygen for oxidation, and hence would still be using larger amounts of oxygen than the control tissue even though fat storage were occurring, resulting in a lower R.Q. than the controls.

We have succeeded in this experiment in accounting for only 2/3 of the glucose removed from the medium by the diaphragm treated with epinephrine in terms of glycogen and oxidized glucose. These findings indicate that the remainder of the glucose is in the form of one or more intermediate products.

Epinephrine was found to induce a slight but significant rise in glucose uptake by the isolated diaphragm. It is not known how important this increase is to the entire animal organism. At first glance it seems surprising that the epinephrine should increase the uptake of glucose at the same time as it decreases the total amount of glucose utilization. However, further examination reveals that although the total amount of glucose utilized is decreased, the amount of glucose oxidized is increased,

and the decreased utilization is at the expense of glycogen formation. This effect is, actually, a process which makes more energy available to the tissue. Teleologically, we may consider this action of epinephrine on the muscle during conditions of stress as one which not only increases the total energy output, but also increases carbohydrate reserve of the tissue in case the supply of carbohydrate should for some reason be decreased.

Walaas and Walaas (121), performed an experiment very similar to the one reported here with epinephrine and rat diaphragms. For an unknown reason the results with oxygen consumption and glucose uptake conflict with our own results. It was noted in our experiments that the oxygen consumption and the glucose uptake increased in every case when the diaphragms were incubated with epinephrine. However, in the Walaases' experiment there was noted a decrease in the glucose uptake and no change in oxygen consumption. However, these workers also found that there was less glucose accounted for in the epinephrine-treated diaphragms than in the controls. One wonders if the difference in results could not be attributed to the time spent before incubation amounting to 1/2 to 3/4 of an hour and the difference in measurement of initial values. Their initial values were taken

before equilibration started. In our own experiments the initial values were taken just after equilibration and before incubation.

In the liver, it was noted that epinephrine also exerted this calorigenic action of about the same magnitude as was found in muscle. The classic glycogenolytic action of epinephrine on the liver was also quite evident.

In addition to the report of Von Euler (38) who found that ergotamine blocked the calorigenic effect of epinephrine on minced frog muscle, there have been some reports on the effect of adrenergic blocking drugs in reducing the increase in oxygen consumption when epinephrine was administered in vivo. Cappo (16) and Orestano (97) obtained negative or doubtful results with ergotamine in the intact man and rat. On the other hand, Harangezo-Oroszy (59) demonstrated positive results in the anesthetized rat. Lundholm, in 1949 (82), gave epinephrine in the dose of 20 gamma/kg. subcutaneously to cats and blocked the calorigenic effect with ergotamine, in a dose of 20 gamma/kg. intramuscularly, which did not itself lower the oxygen utilization. In a later work (81) he found that 200 gamma/kg. of ergotamine intramuscularly was necessary to block the effect of 20 gamma/kg. of norepinephrine. That dose of ergotamine, however, produced a decrease in oxygen consumption. He concluded from his

experiment that since the dose of ergotamine which blocked epinephrine did not lower oxygen consumption in the absence of epinephrine, and doses of ergotamine which blocked nor-epinephrine did, that nor-epinephrine is actually the sympathetic mediator. His conclusion is rather queer since he tested only 2 doses of ergotamine, 20 gamma and 200 gamma/kg.

De Vleeschhouwer (120) showed that Dibenamine, SY-28 and SY-30 will all block the calorigenic action of epinephrine and nor-epinephrine in dogs which are anesthetized. No doses of blocking drugs were reported.

Issekutz, a German (70), has shown that DHO-180 and DHE-45 are without effect in reducing the calorigenic action of epinephrine in anesthetized rats.

The only report found dealing with the effect of a haloalkylamine in vitro was that of Harvey, Wang and Nickerson (61) who studied the effect of a 2-haloalkylamine, SKF 688A, on mouse liver homogenates treated with epinephrine. The glycogenolysis and glucose production were measured and it was found that the increase in these effects produced by epinephrine was blocked by graded doses of the blocking drug ranging from 10-40 gamma/ml. The degree of blockade was proportional to the dose used.

In the previous section of this thesis, it has been suggested, in view of the data obtained, that the

influence of adrenergic blocking and antihistamine drugs on epinephrine-induced effects on carbohydrate metabolism may vary from species to species. Therefore, the results of experiments with rat tissues *in vitro* should be considered as possibly being specific for the rat. It is also necessary to emphasize that conditions *in vitro* only simulate, and by no means exactly reproduce, the conditions *in vivo*. The writer is aware of the dangers in explaining results *in vivo* by observations made *in vitro*. Even more dangerous is an explanation of the results with one species obtained *in vivo* with the observations made with another species *in vitro*. However, the results obtained with the isolated tissues of the rat make it possible to speculate on the various factors producing the results found in the intact rabbit and dog. Therefore, the following discussion of mechanisms should be regarded only as speculative, and not as factual explanations.

The influence of SY-21 on these responses to epinephrine suggests an explanation for the effect of this agent on epinephrine-induced hyperglycemia in rabbits and dogs. It will be remembered that SY-21, although a potent adrenergic blocking agent, even in a dose of 2mg./kg. succeeded only in reducing the hyperglycemia of both rabbits and dogs. In the isolated rat tissue, it was

noted that, whereas SY-21 completely blocked the response in muscle, it only partially blocked the effect in liver tissue. Since the hyperglycemia produced by epinephrine is probably due primarily to the liver glycogenolysis, (the muscle effects serving to sustain the action by restoring liver glycogen from lactic acid) a partial blockade of the liver processes would, logically, only result in a partial blockade of the hyperglycemia. Since SY-21 blocked the effects of epinephrine in isolated rat muscle, the failure for this agent to reduce epinephrine-induced lacticacidemia in dogs is still an open question. Although, species specificity should be considered, a circulatory factor should also be considered. With a dose as large as 2 mg./kg. of SY-21, the injection of epinephrine would result in a decrease in blood pressure, and probably in some resultant pooling of blood. This would allow an accumulation of lactic acid so that when measured, it would appear higher than normal. This latter explanation would give some clue to the failure of the agents used in the dog to block the lacticacidemia produced by epinephrine injection.

The first suppression of the glycogenolytic effect of epinephrine <u>in vitro</u> was accomplished by Frolich and Pollack (43) and Morita (89). These workers succeeded in blocking the glycogenolytic effect of epinephrine in

ergotoxin perfused livers. Houssay and Gerschman (67) successfully produced the blockade in a similar experiment using ergotamine and dihydroergotamine. Tuerkischer and Wertheimer (117) using incubated rat diaphragms produced a blockade of the glycogenolysis with ergotamine. However, Hegnauer and Cori (64) were unable to block the increase in lactic acid and hexosemonophosphate production in isolated frog muscle with ergotamine. In their experiments, the muscles were incubated in a nitrogen atmosphere.

Ellis, Anderson and Turtle (37) studied the effect of epinephrine on liver slices from rats and rabbits. It was found that there was a marked increase in glucose liberation and phosphorylase activity when the slices were incubated with epinephrine. Rabbit liver slices gave a larger increase in these reactions than the rat liver slices. It was found that dihydroergotamine blocked the effects of epinephrine in the liver.

The action of dihydroergocornine, which gave a suggestive reduction of the increase in oxygen utilization induced by epinephrine in the diaphragm, and a complete blockade of all the effects measured in the liver, gives a clue to its mechanism of action in the intact rabbit and rat (36). If, again, the primary cause for hyperglycemia in the intact animal as a result of epinephrine injection was the outpouring of glucose from the liver, then

blockade of the lysis of liver glycogen should result in a complete blockade of the hyperglycemia. Since the effect on muscle only sustains the hyperglycemia by replenishing glycogen from lactic acid, then this new glycogen is also blocked by DHO-180 and hence the blood sugar would still not become elevated.

Neoantergan was found to be an effective blocking agent of epinephrine-induced hyperglycemia in the rabbit, but in these experiments in the rat it was found to be without blocking activity, even in high concentrations. Indeed, its action on isolated muscle tissue indicated that it might potentiate the effects of epinephrine by increasing the rate of glycogenolysis and decreasing the rate of glucose uptake. These latter effects suggest some explanation for the action of some antihistamines to potentiate the hyperglycemia in dogs. One is forced to conclude that the blocking action of this antihistaminic agent in the rabbit is due to some non-specific action or to some effect on carbohydrate metabolism which is rabbit specific. The action of Pitocin, Pitressin, and ephedrine to inhibit epinephrine-induced hyperglycemia in the rabbit, suggests that the rabbit may be sensitive to agents which produce circulatory changes. The ability of several antihistamines to potentiate the pressor effects of epinephrine, suggests that

in the presence of epinephrine these agents also induce circulatory changes, which might have some effect on carbohydrate metabolism in the rabbit. Other such non-specific mechanisms in which carbohydrate metabolism is only indirectly affected can be postulated, but the proof for any such mechanism is still lacking.

GENERAL SUMMARY AND CONCLUSIONS

When epinephrine is injected into an intact animal, one of the characteristic changes that can be observed is an hyperglycemia. There are two possible mechanisms by which the blood sugar could be increased in the fasted animal. The first is an increase in the rate of liver glycogenolysis which releases glucose into the blood stream. The second is a decreased rate of glucose uptake by the tissues which results in an accumulation of glucose in the blood stream. The action of epinephrine has been postulated to be on both of these mechanisms.

It has been suggested that the process referred to in the literature as glucose utilization should be considered as two separate processes. The first has been termed "glucose uptake", and was defined as the process of removal of glucose from the blood stream by the tissues and the phosphorylation of this glucose. The second process has been termed "glucose utilization", and was defined as the conversion of phosphorylated glucose to glycogen, carbon dioxide, water, and lactic acid. The basis for this suggested separation was the finding that these two processes, glucose uptake and glucose utilization, were capable of varying independently

of each other *in vitro*.

In the intact organism, it is difficult to measure these two processes independently, and the method used has been to measure the A-V differences in blood glucose. By the standards set in the above definitions this is a measure of glucose uptake. It may be called glucose utilization only by assuming that the glucose utilization, as defined above, varies directly with the glucose uptake. This assumption is felt to be false in view of the observation reported herein that, at least in the presence of epinephrine, the glucose utilization of the isolated rat diaphragm may vary independently from the glucose uptake. In addition, it has been pointed out in the literature (32) that the methods for determining the blood glucose concentration are of such a low order of accuracy, that the error in determination alone may be sufficient to show the A-V differences to go up, down or stay the same. It is believed that this factor may explain the divergent views expressed in the literature regarding the action of epinephrine on glucose uptake *in vivo*. Furthermore, part of the effect seen *in vivo* may be due to the action of insulin and adrenocortical hormones.

The observations reported herein, on the influence of epinephrine on the isolated rat diaphragm

demonstrated that the rate of glucose uptake increased slightly but significantly. In addition, the rate of glucose utilization decreased. It has been shown that this decrease in glucose utilization by the isolated muscle was at the expense of glycogen synthesis. Therefore, despite the decrease in overall glucose utilization, there was still an increase in the amount of glucose oxidized. The isolated rat diaphragm exhibited an R.Q. of 1.0 during the first hour of incubation with epinephrine. During the second hour, the R.Q. was found to rise above 1.0 and was taken to indicate the conversion of carbohydrate to fat. The isolated rat diaphragm has previously been observed to convert carbohydrate to fat (20a).

If the author can be permitted to speculate on an explanation for the responses in intact animals by the observations made in isolated tissue preparations, it may be said that the primary or early effect of injected epinephrine is an induced hyperglycemia by means of an increased liver glycogenolysis. This increased blood glucose is picked up by the muscles at an increased rate. Once the glucose is phosphorylated by the muscles, less is converted to glycogen then would be if the hyperglycemia was due to other reasons than epinephrine injection. On the other hand, there is an increase in

glycolysis as a result of epinephrine injection causing a lacticacidemia. This increase in glycolysis is not sufficient to balance the decrease in glycogen synthesis and the result is a storage of intermediate; probably hexosemonophosphate. Emphasis should be placed on the statement that these suggested mechanisms in the intact animal are the primary or early effects of epinephrine. In the intact animal, the presence of a hyperglycemia would result in a secretion of insulin. Therefore, the later responses following epinephrine injection would be the result of the combined actions of epinephrine, insulin, and adrenocortical hormone.

A number of adrenergic blocking drugs belonging to a series of ethly homologues of Aryl-2-haloalkylamines have been synthesized recently which are capable of inhibiting most of the excitatory effects of epinephrine. The data reported herein, demonstrated that most of these drugs were effective in inhibiting the hyperglycemic action of epinephrine in the rabbit. Furthermore, it has been suggested that the effectiveness of these agents, at least in the rabbit, roughly parallels their potency as inhibitors of other excitatory effects of epinephrine. If the structures of some of the more potent adrenergic blocking drugs are changed, by replacing the halogen with an hydroxyl group, or by

converting the ethyl homologue to a methyl homologue, the resulting compounds lose or have a reduced adrenergic blocking activity. Such compounds were also unable to block epinephrine-induced hyperglycemia.

When these agents were injected into dogs, the hyperglycemia induced by an infusion of epinephrine was reduced. However, they failed to reduce the lactic-acidemia induced by epinephrine. In view of the finding that one of these agents, SY-21, reduced the glycolysis induced by epinephrine in the isolated rat diaphragm, at the risk of transferring from in vitro experiments with one species to in vivo experiments of another, it is suggested that the failure to reduce lacticacidemia in the dog was not due to a failure to inhibit muscle glycolysis. It has been suggested that the lacticacidemia following SY-21 and epinephrine is due to a circulatory accumulation of lactic acid. In the presence of an adrenergic blocking drug in the dose used (2 mg. of SY-21 per kg.) epinephrine causes a decrease in blood pressure, and presumably a pooling of blood. Under such conditions, although the actual amount of lactic acid entering the blood stream might be decreased, it would not be carried to the liver as fast as it would if the blocking drug were not present. Therefore, measurement of a given aliquot of blood would show a lacticacidemia that was not

due to muscle glycolysis.

SY-21 was found to completely inhibit all of the effects of epinephrine on carbohydrate metabolism which were measured in the isolated rat diaphragm. On the other hand it only partially inhibited the glycogenolysis induced in the isolated rat liver slice by epinephrine. Hence, since the primary action of epinephrine in producing an hyperglycemia is to induce liver glycogenolysis, one would expect to find only a partial reduction of epinephrine-induced hyperglycemia with SY-21. This was found to be the case in both the rabbit and the dog.

Dihydroergocornine was found to block epinephrine-induced hyperglycemia in the rabbit, but was without effect in the dog. The reason for this failure in the dog is believed to be insufficient dosage. Dogs were unable to tolerate doses higher than 0.05 mg./kg. without retching and vomiting. In the studies on the surviving tissues of the rat, it was found that DHO-180 produced a complete inhibition of liver glycogenolysis induced by epinephrine, but only a suggestion of inhibition of the effects of epinephrine on carbohydrate metabolism in the diaphragm. Furthermore, DHO-180, itself, reduced the glycogen synthesis and increased the oxygen uptake of the diaphragm, so that its effect

summed with the reduced effect of epinephrine to give final values similar to that produced by epinephrine in an otherwise untreated diaphragm. Because of the complete inhibition seen in the isolated liver studies, it is not surprising that DHO-180 produced a complete suppression of hyperglycemia in the intact rabbit.

Since several antihistamines can influence the pressor effects of epinephrine (76, 77), several of these agents were tested for their ability to influence epinephrine-induced hyperglycemia in the rabbit. The ability of these agents to inhibit the rise in blood sugar due to epinephrine in the rabbit paralleled their ability to inhibit the action of histamine on the bronchioles and intestine of the guinea pig. Since large doses of aminophylline did not reduce the hyperglycemia, it is suggested that the effectiveness of some of these agents was not related to their ethylenediamine structure. The effectivensss of some of these agents does not appear to be related to their atropine-like or local anesthetic properties since atropine, cocaine, and procaine were unable to inhibit epinephrine-induced hyperglycemia. On the other hand, Pitressin, Pitocin, and ephedrine did reduce the rise in blood sugar induced by epinephrine. In view of this, it is suggested that the antihistamines, in the presence of epinephrine, perhaps act through some circulatory

changes which influence carbohydrate metabolism only indirectly.

In dogs, of three antihistamines tested only Chlorothen reduced epinephrine-induced hyperglycemia, but did not influence the lacticacidemia. On the other hand, Histadyl and Chlortrimeton, which blocked the blood sugar rise in the rabbit, potentiated the hyperglycemia in the dog. Furthermore, Neoantergan, one of the most potent inhibitors of epinephrine-induced hyperglycemia in the rabbit, did not influence the effects of epinephrine on carbohydrate metabolism in the isolated rat liver and diaphragm. These observations together with those reported in the literature suggest that the ability of the antihistamines to inhibit epinephrine-induced hyperglycemia in the rabbit is species specific.

It is concluded that it is a general property of the more potent adrenergic blocking drugs to inhibit epinephrine-induced hyperglycemia in several mammalian species. This property exists both *in vivo* and *in vitro*. Since these agents by themselves do not cause hypoglycemia *in vivo* or reduce glycogenolysis or glycolysis *in vitro*, it is suggested that their mechanism of action is to block the effect of epinephrine rather than influencing carbohydrate metabolism directly. Therefore,

these agents make convenient research tools for the study of carbohydrate metabolism. Through their use, it is possible to study the influence of a variety of substances on carbohydrate metabolism without the complicating presence of epinephrine influences.

Bibliography

1. Abderhalden, E., and Gellhorn, E.
Vergleichende Versuche über die Wirkung von l-und d-adrenalin auf den Gaswechsel von Organen in verschiedenem zustande.
Pflüger's Arch. f. d. ges. Physiol. 212: 523, 1926

2. Adler, L., and Lipschitz, W.
Die Wirkung von Hormonen auf die Zelloxydationen und den Wärmehaushalt des Organismus.
Arch. f. Exp. Path. und Pharmak. 95: 181, 1922

3. Ahlgren, G.
Respiration of tissues.
Klin. Wchnschr., 3: 667, 1924

4. Ahlgren, G.
Mikrorespirometrische Untersuchungen über die Hormonwirkungen. II. Adrenalin.
Skan. Arch. Physiol., 47: 275, 1926

5. Anderes, E., and Cloetta, M.
Der beweis für die Kontraktilität der Lungengefässe und die Beziehung zwishen Lungendurchblutung and O_2 - Resorption.
Arch. f. Exp. Path. und Pharmak., 79: 301, 1916

6. Barcroft, J., and Dixon, W.E.
The gaseous metabolism of the mammalian heart. Part I.
J. Physiol. 35: 182, 1906

7. Barger, G.
The alkaloids of ergot.
In Handbuch der Experimentellen Pharmakologie.
6: 84, 1938

8. Barker, S.B. and Summerson, W.H.
The colorimetric determination of lactic acid in biological material.
J. Biol. Chem., 138: 535, 1941

9. Bernstein and Falta, W.
Über die Einwirkung von Adrenalin, Pituitrinum infundibulare und Pit. glandulare auf den respiratorischen Stoffwechsel.
Verhandl I. deutch Kong. f. inn. Med. 29: 536, 1912

10. Blancher, M.
Action, sur l'hyperglycémie adrénalinique du diéthylamino-méthyl-3-benzodioxane (833F) et de quelques éthers-oxydes phénoliques voisins.
Compt. rend. Soc. de Biol. 115: 1185, 1934

11. Blum, E.
Cited by Boothby and Sandiford (12)
Arch. f. Klin. Med., 7: 146, 1901

12. Boothby, W.M. and Sandiford, I.
The calorigenic action of adrenalin chlorid.
Amer. J. Physiol. 66: 93, 1923

13. Bovet, D. and Maderni, P.
Action sympathicolytique de quelques amines à fonction éther-oxyde phénolique.
Compt. rend. Soc. de Biol., 114: 980, 1933

14. Cammer, L. and Griffith, F.R., Jr.
Adrenalin and the metabolism of peripheral tissues.
Amer. J. Physiol., 125: 699, 1939

15. Cannon, W.B. and Rapport, D.
Studies on the conditions of activity in endocrine glands.
Amer. J. Physiol., 58: 308, 1921

16. Capo, R.,
Antagonismo fra adrenalina e ergotamina e ricambio respiratorio.
Riforma Med., 46: 1347, 1930

17. Chen, G. and Clarke, I.G.
The influence of diphenhydramine hydrochloride and epinephrine on glucose metabolism in the rabbit.
J. Pharmacol. & Exp. Therap., 93: 175, 1948

18. Chen, G. and Clarke, I.G.
The effect of N-(2-chloroethyl)-N-Ethyl-1-naphthalene-methylamine HCl on blood glucose and liver glycogen.
J. Pharmacol. & Exp. Therap., 101: 181, 1951

19. Chen, G. and Russel, D.
Influence of diphenhydramine on blood pressure response to epinephrine in dog under adrenergic blockade.
Proc. Soc. Exper. Biol. & Med., 74: 298, 1950

20. Chen, K.K.
Personal communication.

20a. Chernick, S.S., Masoro, E.J., and Chaikoff, I.L.
The in vitro conversion of C^{14}-labeled glucose to fatty acids.
Proc. Soc. Exper. Biol. & Med., 73: 348, 1950

21. de Cloedt, J. and Van Canneyt, J.
Influence of insulin on respiration of tissues.
Compt. rend. Soc. de Biol., 91: 92, 1924

22. Cohen, J.A.
Effect of adrenaline on the utilization of glucose.
Biochem. et. Biophys. Acta., 3: 231, 1949

23. Cohen, J.A.

Influence of various hormones on utilization of glucose.

Biochem. et. Biophys. Acta., 4: 535, 1950

24. Cohen, J.A. and Needham, D.M.

Effect of adrenalin injection in utilization of glucose in muscle extracts.

Biochem. et. Biophys. Acta., 6: 141, 1950

25. Colwell, A.R. and Bright, E.M.

The use of constant glucose injections for the study of induced variations in carbohydrate metabolism.

Amer. J. Physiol., 92: 555, 1930

26. Cori, C.F.

Mammalian carbohydrate metabolism.

Physiol. Reviews, 11: 143, 1931

27. Cori, C.F. and Buchwald, K.W.

Effect of epinephrine on the oxygen consumption of frogs before and after hepatectomy.

Proc. Soc. Exper. Biol. & Med., 28: 104, 1930

28. Cori, C.F. and Buchwald, K.W.

The calorigenic action of epinephrine in frogs before and after hepatectomy.

J. Biol. Chem., 92: 367, 1931

29. Cori, C.F. and Cori, G.T.
The mechanism of epinephrine action. I. The influence of epinephrine on the carbohydrate metabolism of fasting rats, with a note on new formation of carbohydrates.
J. Biol. Chem., 79: 309, 1928

30. Cori, C.F. and Cori, G.T.
The mechanism of epinephrine action. II. The influence of epinephrine and insulin on the carbohydrate metabolism of rats in the post-absorptive state.
J. Biol. Chem., 79: 321, 1928

31. Cori, C.F. and Cori, G.T.
The mechanism of epinephrine action. III. The influence of epinephrine on the utilization of absorbed glucose.
J. Biol. Chem., 79: 343, 1928

32. Cori, C.F., Fischer, R.E., and Cori, G.T.
The effect of epinephrine on arterial and venous plasma sugar and blood flow in dogs and cats.
Amer. J. Physiol., 114: 53, 1935

33. Corkill, A.B., Dale, H.H., and Marks, H.P.
The respiratory quotient of the eviscerated spinal cat.
J. Physiol., 70: 86, 1930

34. Eadie, G.S.

A comparison of the glycogenolytic responses to epinephrine administered by the subcutaneous and intravenous routes.

Amer. J. Physiol., 90: 711, 1929

35. Ellis, S.

The effect of amines on the blood sugar of the rat.

J. Pharmacol. & Exp. Therap., 101: 92, 1951

36. Ellis, S. & Anderson, H.L.

Effect of sympathomimetic amines and sympatholytics on blood sugar and lactate of the rat.

Fed. Proc., 9: 269, 1950

37. Ellis, S., Anderson, H.L., Jr., and Turtle, M.A.

A study of the mechanism of action of epinephrine on liver slices.

J. Pharmacol. & Exp. Therap., 106: 383, 1952

38. Euler, U.V.

Zur Kenntnis des Antagonismus zwischen Adrenalin und Ergotamin.

Arch. f. Exper. Path. und Pharmak., 139: 373, 1929

39. Euler, U.V.

"Über die Abhängigkeit der oxydationssteigernden Wirkung des Adrenalins von der Unversehrtheit der Innervation.

Arch. f. Exper. Path. und Pharmak., 143: 209, 1929

40. Euler, U.V.

Studies on the influence of adrenaline and insulin on tissue oxidation with special reference to the importance of innervation.

Skand. Arch. Physiol., 59: 123, 1930

41. Folin, O. and Malmros, H.

An improved form of Folin's micro-method for blood sugar determinations.

J. Biol. Chem., 83: 115, 1929

42. Freis, E.R., Stanton, J.R., and Wilkins, R.W.

Effects of certain dihydrogenated alkaloids of ergot in hypertensive patients.

Amer. J. M. Sc., 216: 163, 1948

43. Fröhlich, A. and Pollak, L.

Über Zuckermobilisierung in der überlebenden Kaltblüterleber.

Arch. f. Exper. Path. und Pharmak., 77: 265, 1914

43a. Fulton, J.F.

A textbook of physiology.

16th Ed., W.B. Saunders Co., Phila., 1949

44. Gemmill, C.L.

Effect of insulin on glycogen content of isolated muscles.

Bull. Johns Hopkins Hosp., 66: 232, 1940

45. Good, C.A., Kramer, H., and Somogyi, M.
The determination of glycogen.
J. Biol. Chem., 100: 485, 1933

46. Goor, H.v.
Increase insulin content of the blood after reflex stimulation of the vagus.
Arch. nēeland, physiol., 14: 535, 1929

47. Gottschalk, A.
The influence of hormones on oxidative carbohydrate catabolism.
Klin. Wchnschr., 3: 1356, 1924

48. Gottschalk, A. and Pohle, S.
Untersuchungen über den Mechanismus der Adrenalin hyperglycämie.
Klin. Wchnschr., 1: 1310, 1922

49. Grant, R.L.
Effect of N, N-dibenzl-2-chloroethylamine (Dibenamine) on blood sugar response to insulin and epinephrine.
Fed. Proc., 8: 202, 1949

50. Griffith, F.R., Jr.
Fact and theory regarding the calorigenic action of adrenaline.
Physiol. Reviews, 31: 151, 1951

51. Griffith, F.R., Jr., Cole, C.D., and Thomas, D.B.
Comparative effect of intravenous glucose and adrenalin on blood flow, oxygen utilization and glucose retention by hind leg tissues of anesthetized cats.
Amer. J. Physiol., 157: 205, 1949

52. Griffith, F.R., Jr., and Emery, F.E.
Some metabolic effects of clamping visceral arteries, splanchnic vasoconstriction and adrenal and hepatic stimulation; with special reference to the calorigenic action of adrenin and sympathin.
Amer. J. Physiol., 111: 369, 1935

53. Griffith, F.R., Jr., and Hummel, L.E.
Action of adrenalin on metabolism of peripheral tissues.
Proc. Soc. Exper. Biol. & Med., 27: 1033, 1930

54. Griffith, F.R., Jr., Lockwood, J.E., and Emery, F.E.
Adrenalin and blood lactic acid: Effect of evisceration.
Amer. J. Physiol., 123: 432, 1938

55. Griffith, F.R., Jr., Lockwood, J.E., and Emery, F.E.
Adrenaline hyperglycemia: Proportionality with dose.
Amer. J. Physiol., 126: 299, 1939

56. Griffith, F.R., Jr., Lockwood, J.E., and Emery, F.E.
Adrenalin lactacidemia: Proportionality with dose.
Amer. J. Physiol., 127: 415, 1939

57. Griffith, F.R., Jr., Omachi, A., Lockwood, J.E., and Loomis, T.A.
The effect of intravenous adrenalin on blood flow, sugar retention, lactate output and respiratory metabolism of peripheral (leg) tissues in the anesthetized cat.
Amer. J. Physiol., 149: 64, 1947

58. Hanson S.
Action de la yohimbine sur les effets de l'adrénaline à l'égard du sucre du sang.
Compt. rend. Soc. de biol., 101: 603, 1929

59. Harangozo-Oroszy, M.V., and Issekutz, Jr., B.V.
Über den Vergleich des Sauerstoffverbrauchs des lebenden und überlebenden Muskels.
Arch. f. Exp. Path. und Pharmak., 200: 146, 1942

60. Harvey, S.C., Nickerson, M. and Stover, K.
Blockade of epinephrine-induced hyperglycemia.
Fed. Proc., 9: 283, 1950

61. Harvey, S.C., Wang, C.Y., and Nickerson, M.
Blockade of epinephrine-induced hyperglycemia.
J. Pharmacol. & Exp. Therap., 104: 363, 1952

61a. Hawk, P.B., Oser, B.L., and Summerson, W.H.
Practical physiological chemistry
12th Ed., Blakiston Co., Phila., 1947

62. Hecht, H.H. and Anderson, R.B.
Influence of Dibenamine (N, N-dibenzyl-2-chloro-ethylamine) on certain functions of the sympathetic nervous system in man.
Amer. J. Med., 3: 3, 1947

63. Hecht, H.H., Crandall, R. and Samuels, A.J.
Adrenergic blockade in man by a new imidazole derivative C-7337.
Fed. Proc., 9: 283, 1950

64. Hegnauer, A.H., and Cori, G.T.
Influence of epinephrine on chemical changes in isolated frog muscle.
J. Biol. Chem., 105: 691, 1934

65. Henderson, F.G., and Chen, K.K.
A new adrenergic blocking agent.
Fed. Proc., 8: 301, 1949

66. Hermann, H. and Vial, J.
Sur quelques actions pharmacodynamiques du 2-benzl-imadazoline (C-3259).
Compt. rend. Soc. de Biol., 136: 803, 1942

67. Houssay, B.A., and Gerschman, R.
Accion del aloxano sobre la glucogenolisis hepatica por adrenalina.
Rev. Soc. argent. de biol., 23: 28, 1947

68. Hunt, H.B., and Bright, E.M.
Locus of the calorigenic action of adrenalin with observations on tissue metabolism.
Amer. J. Physiol., 77: 353, 1926

69. Ingle, D.J.
The physiological action of the adrenal hormones.
In Chemistry and Physiology of Hormones. pg. 83
Publication of the Am. Assoc. Advancement Sci.
Science Press Printing Co., Lancaster, 1944

70. Issekutz, B., sen., and Gyermek, L.
Die Wirkung von Dihydroergotamin und Dihydroergocornin auf den gäswechsel.
Arch. Int. Pharma. et de Thérap., 78: 174, 1949

71. Kerwin, J.F., Herdegen, T.F., Heisler, R.Y., and Ullyot, G.E.
Adrenergic blocking agents: I. N-(9-fluorenyl)-2-chloroethylamine series.
J. Am. Chem. Soc., 72: 940, 1950.

72. Kerwin, J., Ullyot, G.E., Fellows, E.J. and Macko, E.
Adrenolytic activity of a series of N-(9-fluorenyl)-beta-chlorethylamines.
Fed. Proc., 8: 308, 1949

73. Klein, O.

Hormones and water metabolism in diabetes after insulin and pituitary extract.

Ztschr. f. Klin. Med., 100: 485, 1924

73a. Komrad, E.L., and Loew, E.R.

Inhibition of epinephrine-induced hyperglycemia with adrenergic blocking drugs.

Amer. J. Physiol. 165: 66, 1951

74. Laurin, E.

Ergotoxin und Adrenalinhyperglykämie.

Biochem. Ztschr., 82: 87, 1917

75. Litchfield, J.T., Adams, M.R., Goddard, L., Jaeger, M.S., and Alonse, L.

5-Halo-2-thenyl derivatives of N, N-dimethyl-N'-2-pyridyl-ethylenediamine as antihistaminics.

Bull. Johns Hopkins Hosp., 81: 55, 1947

76. Loew, E.R.

Pharmacology of antihistamine compounds.

Physiol. Reviews, 27: 542, 1947

77. Loew, E.R.

Antihistamine agents in allergy; Pharmacology of Benadryl and specificity of antihistamine drugs.

Ann. N.Y. Acad. Sc., 50: 1142, 1950

78. Loew, E.R., Achenback, P.A., and Micetich, A.
Adrenergic blocking drugs. V. Blocking of excitatory responses to epinephrine and adrenergic nerve stimulation with N-alkyl-N-(2-chloroethyl)-benzhydrylamines.
J. Pharmacol. & Exp. Therap., 97: 441, 1949

79. Loew, E.R., and Micetich, A.
Adrenergic blocking drugs. II. Antagonism of histamine and epinephrine with N-(haloalkyl)-1-naphthalene-methylamine derivatives.
J. Pharmacol. & Exp. Therap., 94: 339, 1948

80. Loew, E.R., and Micetich, A.
Adrenergic blocking drugs. IV. Antagonism of epinephrine and histamine with 2-(2-biphenyloxy)-2'-chlorodiethylamine derivatives.
J. Pharmacol. & Exper. Therap., 95: 448, 1949

81. Lundholm, L.
The effect of l-nor-adrenalin and ergotamine on the oxygen consumption of guinea pigs.
Acta. Physiol. Scand., 18: 341, 1949

82. Lundholm, L., and Mohme, E.
Effects of adrenaline and ergotamine on the oxygen consumption.
Acta. Physiol. Scand., 16: 367, 1949

83. Macht, D.I., and Bryan, H.F.
Effect of epinephrine and ephedrine on muscle oxydase.
Arch. Internat. de Pharmacodyn. et de Thérap. 52: 148, 1936

84. Mann, F.C., and Magath, T.B.
Studies on physiology of liver; Effect of administration of glucose in condition following total extirpation of liver.
Arch. Int. Med., 30: 171, 1922

85. Marine, D., and Lenhart, C.H.
The influence of glands with internal secretions on the respiratory exchange. I. Effect of the subcutaneous injection of adrenalin on normal and thyroidectomized rabbits.
Amer. J. Physiol., 54: 248, 1920

86. Masing, E.
Über Zuckermobilisierung in der überlebenden Leber nebst Bemerkungen über die Sauerstoffatmung der Leber.
Arch. f. Exper. Path. und Pharmakol., 69: 431, 1912

87. De Meio, R.H.
Action of adrenaline on tissue respiration *in vitro*.
Rev. Soc. Argent. de biol., 17: 102, 1941

88. Miculicich, M.
Über den Einfluss von Ergotoxin auf die Adrenalin und Diuretinglykosurie.
Arch. f. Exp. Path. und Pharmakol., 69: 133, 1912

89. Morita, S.
Untersuchungen über die zuckertreibende Wirkung adrenalinähnlicher (sympathomimetischer) Substanzen.
Arch. f. Exp. Path. und Pharmakol., 78: 245, 1915

90. Nakao, T.
Über den Messungsapporat des respiratorischen Gaswechsels nach Zunta-Geppert, sowie über die Einflusse der vegetativen Nervengifte auf diesen.
Jap. J. M. Sc., IV, Pharmacol., 10: 37, 1937

91. Neuschlosz, S.M.
Respiration of cancer cells.
Klin. Wchnschr., 3: 57, 1924

92. Nickerson, M.
The pharmacology of adrenergic blockade.
J. Pharmacol. & Exp. Therap. Part II., 95: 27, 1949

93. Nickerson, M., and Goodman, L.S.
Pharmacological properties of new adrenergic blocking agent; N-N-dibenzyl-2-chloroethylamine (Dibenamine).
J. Pharmacol. & Exp. Therap., 89: 167, 1947

94. Nickerson, M., and Gump, W.S.
The chemical basis for adrenergic blocking activity in compounds related to Dibenamine.
J. Pharmacol. & Exp. Therap., 97: 25, 1949

95. Nitzescu, I.I.
L'ergotamine, la yohimbine et le réaction glycémique des hemorragies. Contribution à l'étude du mécanisme de l'hyperglycémie post-hémorragique.
Compt. rend. Soc. de Biol., 100: 386, 1928

96. Nukariya, S.
Untersuchungen über den Sauerstoffverbrauch der uber lebenden Säuger tier leber, unter dem Einfluss von sympathischen und parasympathischen Giften und Aminosauren.
Sei-I-Kwai M.J., 46: 1, 1927

97. Orestano, G.
Action of sympathetic and parasympathetic drugs on respiratory exchange. I. The action of adrenaline.
Bull. Soc. ital. biol. spec., 8: 379, 1933

98. Paasch, G., and Reinwein, H.
Studien über Gewebsatmung; der Einfluss von Thyroxin, Adrenalin und Insulin auf den Sauerstoffverbrauch von uberlebendem Rattenzwerchfall.
Biochem. Ztschr., 211: 468, 1929

99. Reinwein, H., and Singer, W.
Studien über gewebsatmung. IV. Mitteilung Der Einfluss von Thyroxin, Adrenalin und Insulin auf den Sauerstoffverbrauch überlebender Leberzellen.
Biochemische Zeitschrift, 197: 152, 1928

100. Riesser, O.
Glycogen synthesis in surviving rat diaphragm and the in vitro effect of hormones and vitamins.
Biochem. et Biophys. Acta, 1, 208, 1947

101. Rothlin, E.
Zur Pharmakologic des Sympathicolyticums Dihydroergotamin DHE 45.
Schweiz. med. Wchnschr., 76: 1254, 1946

102. Rothlin, E.
Pharmacology of natural and dihydrogenated alkaloids of ergot.
Bull. Schweiz. Akad. d. med. Wissensch, 2: 249, 1947

103. Sandiford, I.

Clinical metabolism. Papers from the Mayo Foundation and the Medical School of the University of Minnesota.

I: 527-529, Philadelphia, 1919

103a. Sayers, G.

The adrenal cortex and homeostasis

Physiol. Reviews, 30: 241, 1950

104. Schattenstein, D.I., and Zyukova, M.A.

The nervous regulation of tissue respiration. II. The effect of atropine and adrenaline on tissue respiration.

Arch. Sci. biol. (U.S.S.R.), 40: 72, 1936

105. Smirnova, L.G., and Sverev, V.V.

The adrenaline syndrome in the analysis of shock condition. II. The influence of prolonged intravenous injections of adrenaline upon the gas exchange of tissues.

Bull. biol. med. exper. U.S.S.R., 2: 459, 1936

106. Soga, T.

Die Wirkung des Adrenalins auf den Blutzucker und das Muskelglykogen und über seine Beziehung zur Leber.

Jap. J.M. Sc. IV, Pharmacol., 12: 21, 1940

107. Somogyi, M.

Studies of arteriovenous differences in blood sugar. V. Effect of epinephrine on the rate of glucose assimilation.

J. Biol. Chem., 186: 513, 1950

108. Soskin, S.

On the "Calorigenic Action" of epinephrine.

Amer. J. Physiol., 83: 162, 1927

109. Soskin, S., Essex, H.E., Herrick, J.F., and Mann, F.C.

Comparative influence of epinephrine and of dextrose on the utilization of sugar by the muscles, determined with the aid of thermostromuhr measurements of blood flow.

Amer. J. Physiol., 118: 328, 1937

109a. Soskin, S., and Levine, R.

Carbohydrate metabolism.

Univ. of Chicago Press, Chicago, 1946

110. Soskin, S., Levine, R., and Taubenhaus, M.

Effect of added glucose on rate of appearance of free sugar in liver brei.

Proc. Soc. Exp. Biol. & Med., 42: 689, 1939

111. Soskin, S., Priest, W.S., and Schutz, W.J.

The influence of epinephrine upon the exchange of sugar between blood and muscle.

Amer. J. Physiol., 108: 107, 1934

112. Spühler, O.

Die experimentelle Untersuchung eines neuen Sympathicolyticum, des Dihydroergotamins (DHE-45).

Schweiz. med. Wchnschr., 77: 28, 1947

113. Stone, C.A., and Loew, E.R.

Adrenergic blocking drugs: III. Effects of 2 halogenated ethylamines on pressor responses to epinephrine, nicotine and adrenergic nervous reflexes.

J. Pharmacol. & Exp. Therap., 94: 350, 1948

114. Susina, S.V., and Unna, K.R.

Effects of antihistamine agents on epinephrine-induced hyperglycemia in the dog.

J. Pharmacol. & Exp. Therap., 101: 34, 1951

115. Sutherland, E.W.

The effect of the hyperglycemic factor and epinephrine on enzyme systems of liver and muscle.

Ann. N.Y. Acad. Sci., 54: 693, 1951

116. Sutherland, E.W., and Cori, C.F.

Effect of hyperglycemic-glycogenolytic factor and epinephrine on liver phosphorylase.

J. Biol. Chem., 188: 531, 1951

117. Tuerkischer, E., and Wertheimer, E.
In vitro synthesis of glycogen in diaphragms of normal and alloxan-diabetic rats.
Biochem. J., 42: 603, 1948

118. Umbreit, W.W., Burns, R.H., and Stauffer, J.R.
Manometric techniques and tissue metabolism.
Burgess Publishing Co., Minn., 1949

119. DeVisscher, M.
Rôle de la sécrétion thyroidienne dans l'action métabolique de l'adrénaline.
Compt. rend. Soc. de biol., 140: 1205, 1946

120. DeVleeschhouwer, G.R., Delaunois, A.L., and Verbeke, R.
Influence de l'adrénaline, de la L-noradrénaline et de quelques adrénolytiques de synthèse sur les échanges respiratoires.
Archiv. Int. de Pharm. et de Thérap., 81: 400, 1950

121. Walaas, O. and Walaas, E.
Effect of epinephrine on rat diaphragm.
J. Biol. Chem., 187: 769, 1950

122. Winter, C.A.
Study of comparative antihistaminic activity of 6 compounds.
J. Pharmacol. & Exp. Therap., 90: 224, 1947

THE INFLUENCE OF BLOCKING AGENTS ON EPINEPHRINE-INDUCED EFFECTS ON CARBOHYDRATE METABOLISM IN VIVO AND IN VITRO

Abstract of a Dissertation

Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

BOSTON UNIVERSITY GRADUATE SCHOOL

by

Eugene Leslie Komrad
S.B., Long Island University, 1948
A.M., Boston University, 1949

Department: Medical Sciences

Field of Specialization: Physiology

Major Instructor: Professor Earl R. Loew

Abstract

Under conditions of general body stress, the adrenal medulla is called upon to secrete its hormone, epinephrine. As a result of the influences of this hormone, a variety of changes in the resting physiology of the organism are incurred, which usually aid the individual in meeting the stressful challenge. One of the first changes which is known to occur as a result of the action of endogenous or exogenous epinephrine is a generalized hyperglycemia. Although this fundamental observation has been known for half a century, a complete understanding of the mechanism of action is still lacking. In the fasting animal, it could logically result from an increased liver glycogenolysis and/or a decreased glucose uptake by the tissues. A review of the literature has shown that whereas all workers are agreed on an increased liver glycogenolysis as a result of epinephrine activity, they are far from agreement on the latter phase, the glucose uptake. The results of this study indicate that much of the confusion is due to a misuse of terms, for it has been demonstrated that, at least *in vitro*, the glucose uptake of muscle tissue increases, while the amount of glucose utilized decreases following epinephrine. The extra glucose is stored in

the form of hexosemonophosphate.

There have recently been made available a number of compounds belonging to the aryl-2-haloalkylamine series, of which Dibenamine is a type compound, which have the ability to inhibit many of the actions of exogenous or endogenous epinephrine. For this reason, they are known as adrenergic blocking drugs. It was of interest to study a number of these compounds to determine their ability to inhibit epinephrine-induced hyperglycemia. A standard intravenous dose of 4 gamma/kg. was used as the challenging dose of epinephrine on the basis of a dose-response curve and the previous report that it was within physiologic range. The following compounds were tested. They are listed by the aryl portion of the molecules: 2-orthobenzyl phenoxyethyl; 1-phenyl-2-N-methylbenzyl; 9-fluorenyl; 1-naphthylmethyl; methylthionaphthalene; 2-biphenylyloxy; benzhydryl; and one dihydrogenated derivative of ergot, dihydroergocornine. It is suggested that the effectiveness of 2-haloalkylamines in diminishing or blocking epinephrine-induced hyperglycemia in rabbits is roughly related to their potency as measured by antagonism of the other effects of epinephrine. The basis of this suggestion is that the most potent drugs block or diminish the blood sugar rise in a dose of 2 mg./kg., while the less potent homologues require a

dose of 5 mg./kg. to obtain the same effect. The least potent of these agents did not reduce the hyperglycemia even in a dose of 10 mg./kg. In addition, the methyl homologue of one of the adrenergic blocking drugs is known to have a markedly reduced epinephrine antagonism, and did not have any effect on the hyperglycemia. Likewise, when the halogen is replaced by an hydroxyl radical, and the resulting agent loses its epinephrine reversal action, it also loses its ability to block epinephrine-induced hyperglycemia. A review of the literature, together with the data obtained, seems to indicate that it is a general property of the adrenergic blocking drugs to inhibit the hyperglycemic effect of epinephrine in the rabbit. Also, it is to be expected that larger doses of blocking drug are required to inhibit epinephrine-induced hyperglycemia than epinephrine-induced hypertension. The hypertensive action of epinephrine is due to an algebraic summation of a pressor and depressor phase. Hence, a slight inhibition of the pressor response becomes magnified by the depressor action. However, in the glycemic effect of epinephrine, only a hyperglycemic phase is present, and would require larger doses of blocking drug to induce an equivalent diminution to that found in the blood pressure response.

Certain of the available synthetic antihistaminic drugs are known to increase or decrease some of the effects of epinephrine. Nevertheless, they have been generally regarded as fairly specific for their blocking action of histamine. A number of these agents were studied to determine their effect on epinephrine-induced hyperglycemia in rabbits. The same challenging dose of 4 gamma/kg. injected intravenously was used. When the antihistamines were injected intravenously and slowly, they did not produce any rise in the blood sugar level of fasting rabbits. The following compounds have been described as being among the most potent antihistamines when the effects of histamine on bronchioles and intestines of guinea pigs were used as test objects: Thenylpyramine (Histadyl); Chlorothen; Pyranisamine (Neoantergan); Tripelenamine (Pyribenzamine); Phenergan; Chlorprophenpyridamine (Chlortrimeton); and 1-(10-acridyl)-2-dimethylaminoethane. HCl. These were also found to be most effective in diminishing epinephrine-induced hyperglycemia in rabbits. It is believed that this activity is not due to the ethylenediamine structure which is present in most of these compounds, since two active compounds tested did not have this structure. In addition, aminophylline was ineffective even when injected in amounts representing at least twice as much ethylenediamine

as contained in an effective dose of antihistamines of the ethylenediamine type. Furthermore, Methapheneline (Diatrin) and Thonzylamine (Neohetramine) which do contain the ethylenediamine structure, did not markedly inhibit the hyperglycemia. A number of other compounds were tested which have been reported to have only weak antihistaminic activity, and these agents had only a very weak inhibiting action. It is unlikely that the activity of these compounds is related to their local anesthetic properties, since cocaine and procaine did not influence epinephrine-induced hyperglycemia. In addition, the finding that atropine was ineffective, makes it unlikely that the activity is related to the atropine-like action of some of these compounds. However it is noteworthy that Pitressin, Pitocin, and ephedrine were all effective in reducing the hyperglycemia. Since these three agents all produce vasoconstriction and other circulatory changes, it suggests that an interference in the distribution of hyperglycemia due to a redistribution of blood, or some other such circulatory change influencing carbohydrate metabolism indirectly, may be the mechanism of action of some of the antihistamines.

Epinephrine was infused intravenously into trained, unanesthetized dogs and the effect of Histadyl

and Chlortrimeton on the resulting hyperglycemia and lacticacidemia was examined. Interestingly, these agents, most potent in rabbits, were without effect in dogs; indeed, they potentiated the maximum rise in blood sugar. Chlorothen, however, did significantly reduce the hyperglycemia, although none of these agents reduced the lacticacidemia. The adrenergic blocking drugs N-9-fluorenyl and 1-phenyl-2-N-methyl of the 2-haloalkylamine series were also tested in dogs and found to reduce the hyperglycemia, although they did not influence the lacticacidemia. Dihydroergocornine was without effect in dogs, but this is believed to be a dosage effect.

Finally, the effect of a derivative of ergot, dihydroergocornine (DHO-180), a member of the aryl-2-haloalkylamine series, N-9-fluorenyl (SY-21), and an antihistamine, pyranisamine (Neoantergan) were tested for their ability to influence epinephrine-induced effects on carbohydrate metabolism <u>in vitro</u>. A standard concentration of 1.6×10^{-6} M of l-epinephrine was selected as the challenging dose for use with the isolated diaphragm and liver slice of the rat. In the muscle tissue, epinephrine was found to increase the oxygen utilization and the amount of glucose uptake; however, the amount of glycogen synthesized and glucose utilized was decreased. In the liver slice, epinephrine caused

an increase in glycogenolysis and oxygen utilization. SY-21 was found to completely block the influence of epinephrine on muscle, whereas it only partially inhibited the effect in liver tissue. This suggests an explanation for the partial inhibition of the hyperglycemia in rabbits, since only a partial reduction in liver glycogenolysis would only result in a partial reduction of the hyperglycemia.

Since SY-21 completely inhibited the glycolysis induced by epinephrine in the isolated rat diaphragm, it is suggested that the failure of this drug to reduce the lacticacidemia in the dog was not due to a failure to inhibit glycolysis. SY-21, in the presence of epinephrine causes a drop in blood pressure and presumably blood flow. Therefore, it is possible that lactic acid would be accumulated in the blood stream, and measurements of a given aliquot of blood would reveal a high lactic acid concentration although the rate of muscle glycolysis was not elevated.

Contrary to the effects of SY-21, were the effects of DHO-180 which exhibited only a partial inhibition of the effects in muscle, but a complete inhibition of the effects in liver. Hence, it is to be expected, as was found, that <u>in vivo</u> this agent would produce a complete inhibition of epinephrine-induced

hyperglycemia.

Neoantergan was without activity when added to the surviving rat diaphragm and liver even in high concentration. This finding, together with those in the intact dog, suggest that the ability of the antihistamines to inhibit epinephrine-induced hyperglycemia in the rabbit is species specific.

It is concluded that epinephrine induces its hyperglycemic action primarily through an increased liver glycogenolysis. A general property of the more potent adrenergic blocking drugs is to inhibit epinephrine-induced hyperglycemia in several mammalian species. This property exists both in vitro and in vivo. Their mechanism of action is believed to be a blockade of the effect of epinephrine rather than any direct effect on carbohydrate metabolism.

Autobiography

I was born in New York City, New York, on the 20th of July, 1928; the first child of my parents, Isadore and Helen Komrad. I received my elementary school education in four different schools in New York City, graduating from Public School #80, in the Bronx, New York in 1942. By passing a competitive examination, I was admitted to Townsend Harris High School, an experimental three year preparatory school. This school was closed a year after my entrance, and I then entered the De Witt Clinton High School and graduated from the Honor School Section in June, 1945. Having been awarded a scholarship to Long Island University, I attended the College of Arts and Sciences until June, 1948, at which time I received a Bachelor of Science degree cum laude.

Having decided to continue my studies in the field of the physiology of the endocrine glands, I entered Boston University Graduate School in September, 1948 to work under Dr. Leland C. Wyman in adrenal physiology. I received the Master of Arts degree in Biology in June, 1949. I then transferred to the Division of Medical Sciences to work under Dr. Earl R. Loew of the Department of Physiology. From July of 1949 until July of 1952 I held the position of Research Fellow in Physiology in the Department of Physiology in the Boston University School of Medicine; and at the same time, pursued courses leading to the doctorate. In August, 1952 I moved to my present position in New Orleans, Louisiana. I now hold the title of Instructor in Physiology at the School

of Medicine at the Tulane University of Louisiana, and Research Associate at the Alton Ochsner Medical Foundation where I am pursuing the study of endocrinology under Dr. Albert Segaloff.