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Title A COMPARISON OF SOME OF THE ADRENERGIC EFFECTS OF GUANETHIDINE, PHENOXYBENZAMINE, AND DICHLOROISOPROTERENOL

Abstract approved (Major Professor)

Guanethidine was studied for its ability to block the blood sugar and lactic acid effects of norepinephrine and isoproterenol using dogs for the test animals. A comparison was made with phenoxybenzamine, a known alpha blocking agent, and dichloroisoproterenol, a known beta blocking agent. Guanethidine appeared to block the blood sugar and lactic acid effects of norepinephrine as well as phenoxybenzamine. Guanethidine appeared to be not as effective as dichloroisoproterenol in blocking the blood sugar and lactic acid effects of isoproterenol. Normal saline was included as a stimulator in the test to disclose the effect of guanethidine on the blood sugar and

lactic acid levels. The effect of guanethidine on the blood sugar and lactic acid levels was not significantly different from the blood sugar and lactic acid effects of the other blocking agents. It appeared that guanethidine could be classified as an alpha blocking agent.

A COMPARISON OF SOME OF THE ADRENERGIC EFFECTS OF GUANETHIDINE, PHENOXYBENZAMINE, AND DICHLOROISOPROTERENOL

by

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INTRODUCTION

Historical Development of Adrenergic Concepts

History of Receptors

At the turn of the century it was discovered that an extract of the adrenal gland, when injected into a vein, would cause a marked increase in blood pressure (25),(39). It was also determined that the changes in the animal following the injection resembled very closely the changes which occurred following sympathetic stimulation (22). Shortly after the action of the crude extract was discovered, the active principle was isolated and identified (2). Administration of the purified derivative, epinephrine, did not give results that matched the effect of sympathetic stimulation too well. Instead, the activity of norepinephrine, a substance not then known to be an adrenergic mediator, more closely resembled the effect of sympathetic stimulation (12).

In attempting to affix an activity site to the extract, it was first thought that the effect produced was

due to an action on the sympathetic nerve. The possibility was rejected when it was learned that the activity could still be seen when the extract was injected into a denervated animal. It was concluded that the activity of the extract was exerted upon the effector organ (22). Because both excitatory and inhibitory actions were produced, it was thought that an explanation of the phenomena was beyond reach (22). T. R. Elliott offered an explanation to the effect that there was possibly a site on the muscle that would respond to the epinephrine released at the adjoining nerve ending (14), but was discouraged from continuing his line of thought by Langley, Elliott's professor (13). It was not until Loewi's experiment on frogs' hearts, nearly twenty years later, that the concept of a specific chemical receptor was proposed (29). Another twenty-five years passed by before Ahlquist proposed his two-receptor theory of adrenergic action (1). Since Ahlquist offered his theory, others have attempted to modify it somewhat, but without complete success. One theory proposed three receptors (21), another suggested four (17), later modified by the author to agree more closely with Ahlquist's theory (18).

One of the reasons for the relucatance to immediately accept the two-receptor theory was that all of the chemical mediators of sympathetic stimulation had not been isolated (27), although the substances were known. Another reason was that unknowlingly many indirect acting agents were studied along with the physiological mediators, and it was difficult to believe that only two receptors were present to respond to such a diverse group of stimulatory drugs (21). A possible mechanism of action of the indirect acting agents has been shown by the use of reserpine. Reserpine has been shown to deplete the catechol amine supply, mainly norepinepherine, of adrenergic tissue (7), (9), (10). Following such depletion by reserpine, the indirect acting agents do not produce any effect. If the reserpinized animal is next perfused with norepinephrine and again treated with an indirect acting agent, the indirect acting of an agent will again show activity (8). It has been suggested that the activity of the indirect acting agents is to release the adrenergic mediator and the mediator produces the adrenergic response (8), (28). Ahlquist's two-receptor theory was aided by the discovery of the

action of reserpine in that it was possible to account for the activity of the indirect acting agents.

History of Adrenergic Agents

The slow progress in the search for the chemical mediators of the adrenergic system parallels, to a degree, the development of the receptor theory. Indeed, the development of the receptor theory, the discovery of the adrenergic agents, and the development of adrenergic blocking agents are inseparable. Long gaps have appeared between discoveries. After the effect of the crude adrenal extract was discovered an active principle was sought. The first adrenergic mediator was discovered and isolated about six years later, in 1901 (2). The formula was fairly well understood, and by another four years' time the mediator, epinephrine, had been produced synthetically (11). The isolation and identification of a second mediator did not follow as soon as might be expected, especially since the substance was known. In 1946 von Euler reported finding noradrenaline in adrenergic nerve fiber (15). Goodall later found norepinephrine in mammalian heart and adrenal glands (19). Fortunately, it did not take so long for the isolation of what is possibly the

third sympathetic mediator, although it did take another ten years. In 1956 Lockett reported the isolation of isoproterenol from the lung tissue of the cat (27). It had been known for some time that the responses to the mediators differed from organ to organ, and from mediator to mediator. The difference in action was the basis for Ahlquist's two-receptor theory (1). The receptors giving generally an excitatory response when stimulated Ahlquist called, for convenience, alpha receptors. The receptors giving generally inhibitory responses were named beta (1). In keeping with Ahlquist's system of naming, the stimulatory drugs were classified as to the receptor stimulated. Norepinephrine stimulated the alpha receptors, isoproterenol, the beta, and epinephrine stimulated both (1).

History of Adrenergic Blocking Agents

When it was found that certain of the ergot alkaloids would reverse the rise in blood pressure produced by sympathetic stimulation (12), agents were sought which would control the blood pressure in hypertensive individuals, particularly those agents which did not produce a profound drop in the blood pressure or other undesirable effects. To make the search for hypotensive agents

productive, a knowledge was necessary of what physiological agent or agents produced hypertension, as well as where and how the activity of these physiological agents could be blocked in the body. It was discovered that some agents could block sympathetic activity at the adrenergic ganglia (6), but the same agents also blocked the parasympathetic ganglia (35), (20, p. 169). The next advance in combating hypertension was made by Fourneau and Bovet in 1933 when they introduced the dioxane derivatives (16). In the late 1940's and early 1950's, the beta-haloalkylamines, including phenoxybenzamine (34), were reported (30), (38), (19). The beta-haloalkylamines primarily block the alpha receptors (20, p. 212). The alpha blocking agents do not control the positive chronotropic and inotropic effects on the heart (37), which leaves the alpha blocking agents with something to be desired in the control of hypertension. In 1958 Powell and Slater reported on the beta blocking effects of dichloroisoproterenol (40). Although dichloroisoproterenol is not used therapeutically, it is a useful research tool (20, p. 213).

It had been known for sometime that the beta receptors could be incompletely blocked by some agents, such as

ergot, that also blocked the alpha receptors. Some of the beta receptors could also be blocked by such drugs as ephedrine, a substance usually considered to be an adrenergic stimulator (37). Because of other more predominate pharmacological actions of the drugs possessing beta blocking properties, the drugs were nearly useless for research purposes. Following the disclosure of dichloroisoproterenol's effect on the beta receptors, it has been possible to explore research areas not previously available. As an example, the use of dichloroisoproterenol gives some insight into the possible areas in the body from which blood sugar is released following adrenergic stimulation (33).

Receptor Sites and Blockade

While a large volume of information on the various stimulators and blocking agents of the receptor site has been amassed, very little has been disclosed about the receptor site itself. It has been determined that the excitatory activity is usually associated with structures carrying a small cationic head, as does norepinephrine, a primary amine (4). The secondary and tertiary amines have less excitatory effect due to the increase in the size of

the cationic head. The positive charge is not allowed to come as close to the receptor site. When the substituent on the amine is large enough, as is the isopropyl group in isoproterenol, an inhibitory (beta) activity is seen (4). It would appear that the inhibitory action is a function of the hydroxyl-containing ring and that the excitatory function is a property of the side chain (4). When halo-substituents are made in the ring, the inhibitory action is blocked as occurs with dichloroisoproterenol (40), and other fluoro- and chloro-substituted compounds (24). Particularly strong evidence for the alpha activity being associated with the side chain was presented by Levy and Ahlquist, who observed that when the alcoholic -OH of dichloroisoproterenol is replaced by a chlorine, the property of blocking the beta receptor is lost, and the molecule becomes an alpha blocker instead (24).

The binding properties of the adrenergic agents allow some insight into the nature of the adrenergic receptors. It has been shown that norepinephrine appears not to be metabolized during the process of initiating a contraction, and that a pairing of opposite charges accounts best for the development of a rapid response. It has been

suggested that the beta-haloalkylamines produce their block by first forming an ethyleniminium ion which could effect an esterification with an anionic portion of the receptor. A phosphate anion has been offered as the possible point of esterification. Two possibilities have been suggested as the biochemical carriers of the phosphate group: (a) a specific protein phosphorylated on a functional group, and (b) an enzyme-bound cofactor containing one or more phosphate groups. It has been hypothesized that an enzyme-bound adenosinetriphosphate may be cyclized to 3',5'-adenosinemonophosphate (4). The complete make-up of the receptor site has not been elucidated, but at least a portion of the receptor has been postulated. It was also suggested that the cyclization of ATP to 3',5'-AMP would account for the effect of catechol amines on glycogenolysis (4).

Synopsis of Recent Studies on Guanethidine

Maxwell, et al. have reported that a dose of 15 mg. per kilogram of guanethidine produced a transient fall in the blood pressure followed by a moderate hypertension lasting about 45 minutes, and then a gradual decline in the blood pressure. In the anesthetized normotensive

dog the decline averaged 14 mm. of mercury. In the non-anesthetized dog the same dose lowered the blood pressure ten to twenty-five millimeters of mercury with the effect lasting four to ten days (31). It was also shown that, in both the renal hypertensive and neurogenic hypertensive dog, guanethidine produced a lowering of the blood pressure which lasted from nine to twelve days (31). In a second study by Maxwell et al. (32), it was reported that the pressor response to norepinephrine, epinephrine, Cobefrine® and Epinine® were significantly augmented, and the responses to dopamine, synephrine, and Neo-synephrine® were not significantly effected when pretreated with guanethidine, 15 mg. per kilogram, 48 hours prior to the stimulatory drugs. The pressor responses to Propadrine® and ephedrine were antagonized by guanethidine. When the dose of the two amines was increased by five to ten times a pressor response was produced. The pressor response of amphetamine, tyramine, methamphetamine, Paredrinol®, phenylethylamine, and Vonedrine® following guanethidine was antagonized, and an increase of five to ten times in the dose of the amine produced only slight

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pressor effects. The site of action for guanethidine was placed at the receptor site in smooth muscle. It was postulated that guanethidine could cause a reduction in the transmitter stores in vascular tissue similar to the reduction caused by reserpine. Norepinephrine was thought to be diminished in the intraneuronal sites in arterial tissue (32). Bien (5) supported the theory of reserpine-like action of guanethidine in his report given at the Ciba Foundation Symposium on Adrenergic Mechanisms. Green (20) suggested that the action of guanethidine, although resembling reserpine in the depletion of tissue catechol amines, may be fundamentally different from that of reserpine. Green also suggested that a possible reason for the release of catechol amines *in situ* by guanethidine may be a secondary effect of an undefined excitatory action on adrenergic nerve tissue (20).

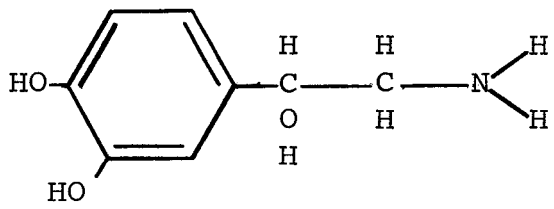
STATEMENT OF PROBLEM

A review of the literature on guanethidine did not disclose the effect of guanethidine on the blood sugar and lactic acid levels, neither guanethidine's own effect on the blood sugar and lactic acid, nor the effect on the rise in blood sugar and lactic acid caused by adrenergic stimulation. In addition there was no statement as to whether guanethidine was primarily an alpha blocking agent, a beta blocking agent, or both. It appeared of interest to further the study of guanethidine to determine whether guanethidine had an effect on the blood sugar and lactic acid either by itself or with an infusion of adrenergic agent, and to determine whether guanethidine produced alpha or beta block, or both.

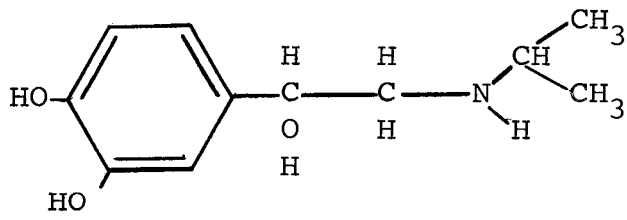
To compare the effect of guanethidine, one predominately alpha stimulator and one predominately beta stimulator was chosen. Normal saline containing the same preservatives as the two stimulators was used as a control. Norepinephrine (levarterenol, l-a-(aminomethyl)-3,4-dihydroxybenzyl alcohol bitartrate), was chosen as the alpha stimulator (1), and isoproterenol (isopropyl-levarterenol, a-(isopropylaminomethyl)-3,4-dihydroxybenzyl

alcohol hydrochloride), was chosen as the beta stimulator for the experiment (1).

A known alpha blocking agent and a known beta blocking agent were chosen to compare with guanethidine. The alpha blocking agent was phenoxybenzamine (N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)-benzylamine hydrochloride) (30), (34) and the beta blocking agent was dichloroisoproterenol (1-(3,4-dichlorophenyl)-2-isopropylaminoethanol hydrochloride) (40). Guanethidine, ([2-(octahydro-1-azocinyl)-ethyl] -guanidine sulfate), was compared with the other two blocking agents for its effect on changes in blood sugar and lactic acid normally produced by infusion of an adrenergic agent.

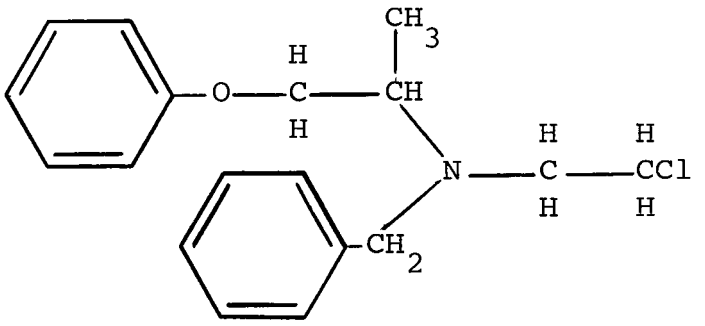


NOREPINEPHRINE

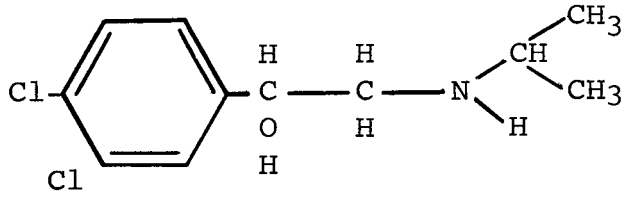


ISOPROTERENOL

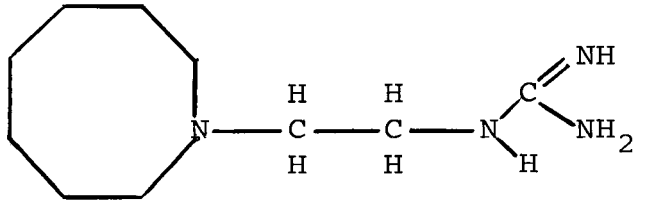
Figure 1. Structural Formulas of Stimulators



PHENOXYBENZAMINE



DICHLOROISOPROTERENOL



GUANETHIDINE

Figure 2. Structural Formulas of Blockers

EXPERIMENTAL PROCEDURE

Experimental Design

Statistically, the experiment was a three by three factorial design with four replications. The adrenergic blockers and guanethidine constituted one factor; the adrenergic stimulators and normal saline constituted the other factor. The three by three design gave nine treatments, and with the four replications made 36 individual tests. The 36 tests were completely randomized from a table of random numbers (26, p. 507). Four mongrel dogs, two of each sex, weighing between ten and twenty kilograms, were the test animals. The random order was followed in all cases except when randomization assigned an animal previously treated with guanethidine to be used in less than ten days. A ten-day period was allowed for the animal to recover from the dose of guanethidine (31),(32). When randomization assigned an animal that had been treated with guanethidine in a previous test to be used again in less than ten days, the next animal and next treatment was chosen, and the treatment assigned the

guanethidinized animal was done after the expiration of ten days.

Experimental Methods

The general procedure was the same for all treatments except with guanethidine. The longer time of onset of action for guanethidine required a slightly different procedure.

General Procedure

The animal to be used was fasted for 24 hours prior to the test and then anesthetized using 35 mg. per kilogram of sodium pentobarbital intraperitoneally. Upon loss of the palpebral reflex, a sample of blood (about one and one-half milliliters) was withdrawn from the cephalic vein. A one-milliliter portion of the sample was pipetted into nine milliliters of the deproteinizing solution (1 ml. of 10% sodium tungstate to 9 ml. of N/12 sulfuric acid) (24), and the mixture was immediately shaken. The stimulator drug was then administered by constant infusion into the saphenous vein using a Harvard Infusion Apparatus (Harvard Apparatus Co., Dover, Mass.). The rate of infusion was 0.73 ml. per minute, and the dose of the

adrenergic agent calculated to be contained in that volume of normal saline. Three more one-milliliter samples were taken at 20-minute intervals starting five minutes after the onset of infusion. The blood samples were deproteinized in the same manner as the first sample. After the last sample was taken, the adrenergic blocking agent was administered by disconnecting the tube from the syringe in the infusion apparatus and injecting the blocking agent through the infusion tube. The dose of the blocking agent was diluted with enough normal saline to make ten milliliters and the dilution administered over a period of five to seven minutes. The infusion needle was then removed, and one hour and fifteen minutes was allowed for onset of action of the blocking agent. Following the elapse of the onset time, the infusion was restarted and three more samples were taken in the same manner at the same time intervals as the three samples taken prior to the administration of the blocking agent. The deproteinized blood samples were then analyzed for blood sugar (36) and blood lactic acid (3).

The doses used were: norepinephrine, 3 mcg./Kg./min.; and isoproterenol, 1.5 mcg./Kg./min. The normal saline was infused at the same rate as the two catecholamines,

0.73 ml. per minute. The catecholamines and saline solution contained 0.1% each sodium bisulfite and chlorobutanol as a preservative. The doses for the blocking agents were: phenoxybenzamine, 5 mg./Kg.; dichloroisoproterenol, 8 mg./Kg.; guanethidine, 10 mg./Kg. Each blocking agent was tested with each stimulating drug and normal saline in all animals in the test.

Procedure for Guanethidine

The slower onset of guanethidine required the omiting of the three samples immediately prior to the administration of the blocking agent. Twenty-four hours prior to the test the animal was lightly anesthetized with sodium pentobarbital (20 mg./Kg. IP) and the dose of guanethidine injected into the cephalic vein. The animal was not fasted prior to the administration of guanethidine, but was fasted for the following 24 hours. On the day of the test, the animal was anesthetized with 35 mg. per kilogram of sodium pentobarbital intraperitoneally. Upon loss of the palpebral reflex, a control sample of blood was withdrawn from the cephalic vein and deproteinized, the stimulating agent was administered by constant infusion, and three samples taken in the same

manner described in the general procedure. The samples were then analyzed for blood sugar and lactic acid.

RESULTS AND STATISTICAL ANALYSIS

Results for Blood Sugar

The average values obtained for the blood sugar are shown in Table I and figures 3-6. The values obtained for all animals with each stimulator prior to block were averaged together to show the amount of increase in the blood sugar produced by the stimulator. Table I shows that there was an average increase in blood sugar of 3.6 mg.% between the control value and the last of the three samples. The average increase is somewhat lower than that reported by van der Pol in his study of norepinephrine and isoproterenol (41). The method employed by van der Pol was different in that a single, larger dose was given, and the time interval was longer. At the end of one and one-half hours van der Pol showed an average increase of 18 mg.% in the rat. The average increase in blood sugar produced by isoproterenol prior to block was 59.7 mg.%. The increase obtained by van der Pol averaged 24 mg.%. The differences between the results of the two tests may be due to the different method employed as well as the different animals.

TABLE I

AVERAGE VALUES FOR BLOOD SUGAR

Norepinephrine

	Control	Before Block			After Block		
	1	2	3	4	5	6	7
Alpha	79.8	71.5	78.7	87.2	78.5	94.1	101.1
Beta	71.8	68.4	79.5	71.5	54.7	70.0	68.1
Guan-ethidine	76.6				79.4	82.4	79.9
Alpha and Beta	75.8	70.0	79.1	79.4			

Isoproterenol

Alpha	74.0	101.0	137.5	156.6	83.2	148.2	163.5
Beta	79.4	96.3	124.3	116.1	61.6	68.3	60.9
Guan-ethidine	70.2				95.9	139.1	146.9
Alpha and Beta	76.7	98.7	130.9	136.4			

Normal Saline

Alpha	81.4	74.9	72.6	75.3	70.5	65.7	69.3
Beta	78.7	78.3	78.1	72.7	56.0	54.8	60.1
Guan-ethidine	78.8				81.9	83.2	80.9
Alpha and Beta	80.1	76.6	75.4	74.0			

Normal saline produced an average decrease of 6.1 mg.% prior to block.

Following administration of the blocking agents, there were some marked changes in the blood sugar levels. By determining the difference between the control blood sugar level and the value obtained for the last sample, it is seen that there was an increase in blood sugar when either phenoxybenzamine or guanithidine was used to block the effect of norepinephrine. The increase in blood sugar with phenoxybenzamine apparently was not due to the blocker, as phenoxybenzamine showed a decrease in blood sugar of 12.1 mg.% when used with normal saline. McCutcheon has reported that phenoxybenzamine does not always block the hyperglycemic effect of adrenergic stimulation (33). There was a rise of 3.3 mg.% in the blood sugar when norepinephrine was blocked by guanethidine, but guanethidine itself showed a rise in the blood sugar of 2.9 mg.% when used with normal saline solution. Dichloroisoproterenol, a beta blocker, gave a decrease in the blood sugar of 3.7 mg.% when used to block norepinephrine. It appears that dichloroisoproterenol is a better alpha blocker than either phenoxybenzamine or guanethidine. The results obtained do not follow those

reported by Levy and Ahlquist (24) who stated that dichloroisoproterenol had little effect as a blocker of alpha receptors.

The results obtained when the blocking agents were used with the beta stimulator, isoproterenol, were more typical (Table I). Neither phenoxybenzamine nor guanethidine produced a block of the hyperglycemic effect of isoproterenol. The rise in blood sugar with phenoxybenzamine was 89.5 mg.%, and for guanethidine, 76.9 mg.%. Dichloroisoproterenol produced a decrease of 18.5 mg.% in the blood sugar when used to block the effects of isoproterenol.

Statistical Analysis for Blood Sugar

The difference between the control blood sugar level and the final blood sugar level was taken as the statistic to be analyzed. Analysis of variance was made, and the least significant difference (LSD) between the means computed was determined. For convenience in calculation, a constant of 33.5 was added to all values obtained in the chemical analysis of blood sugar. The 5% level of significance was chosen for both the analysis of variance

TABLE II

STATISTICAL LAYOUT FOR BLOOD SUGAR

Blockers / Stimulators	Phenoxy-benzamine (Alpha)	Dichloroiso-proterenol (Beta)	Guanethidine (Guan)
Norepinephrine	77.5	33.5	26.2
	33.5	14.0	17.7
	56.8	44.6	37.6
	51.6	28.0	65.5
Isoproterenol	99.5	15.0	111.2
	114.5	0.0	103.8
	206.1	22.1	114.9
	71.8	23.0	111.1
Normal Saline	18.7	15.0	41.5
	24.0	20.7	40.4
	28.8	13.4	28.4
	14.2	10.4	32.0

Numbers represent the difference between the control level and the final blood sugar level plus a constant of 33.5.

TABLE III

TOTALS AND MEANS FOR BLOOD SUGAR

(B) Blockers / (A) Stimulators	Alpha	Beta	Guan	Totals (B)
Norepinephrine	219.4 54.85	120.1 30.03	147.0 36.75	486.5 40.54
Isoproterenol	491.9 122.98	60.1 15.03	441.0 110.25	993.0 82.75
Normal Saline	85.7 21.43	59.5 14.88	142.3 35.58	287.5 23.96
Totals (A)	797.0 66.42	239.7 19.98	730.3 60.86	1767.0 49.08
Totals for Dogs	438.1 Dog #1 48.7	368.6 Dog #2 40.9	552.7 Dog #3 61.4	407.6 Dog #4 45.3

To constitute a significant difference, the individual means must differ by at least 31.99.

TABLE IV

ANALYSIS OF VARIANCE CALCULATIONS
FOR BLOOD SUGAR

Preliminary Calculations

Type of Total	Total of Squares	No. of Items Squared	Observations per Squared Item	Total of Squares per Observation
Correction	3,122,289.00	1	36	86,730.25
Observations	152,465.86	36	1	152,465.86
Blockers	1,226,003.18	3	12	102,166.93
Stimulators	1,305,387.50	3	12	108,782.29
Treatment	555,362.02	9	4	138,840.51
Dogs	799,412.62	4	9	88,823.62

Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Dogs	2,093.37	3	697.79	1.4522
Blockers	15,436.82	2	7,718.41	3.9406*
Stimulators	22,052.04	2	11,026.02	22.9470*
Interaction	14,621.53	4	3,655.38	7.6074*
Error	11,531.98	24	480.50	
Total	65,735.61	35		

Significance level, 5% F with 3 and 24 d.f. = 3.0088
2 and 24 d.f. = 3.4028
4 and 24 d.f. = 2.7763

*Denotes that F is in the critical region, and the results are significant.

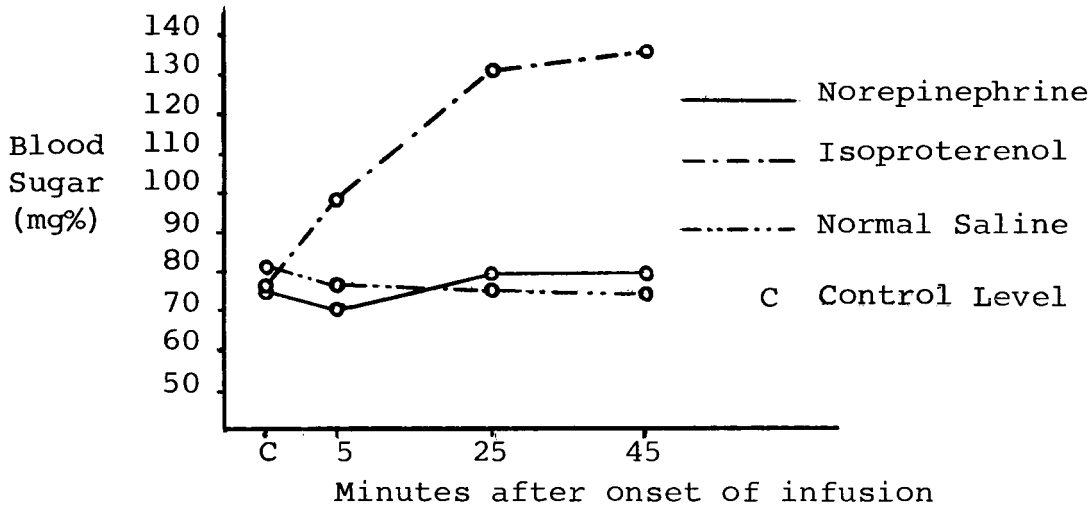


Figure 3. Average Blood Sugar Levels Before Block

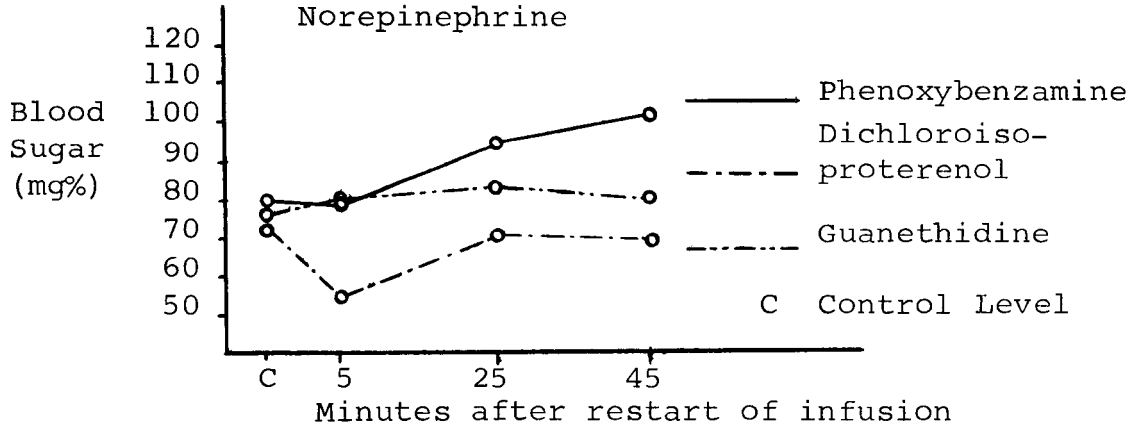


Figure 4. Average Blood Sugar Levels After Block

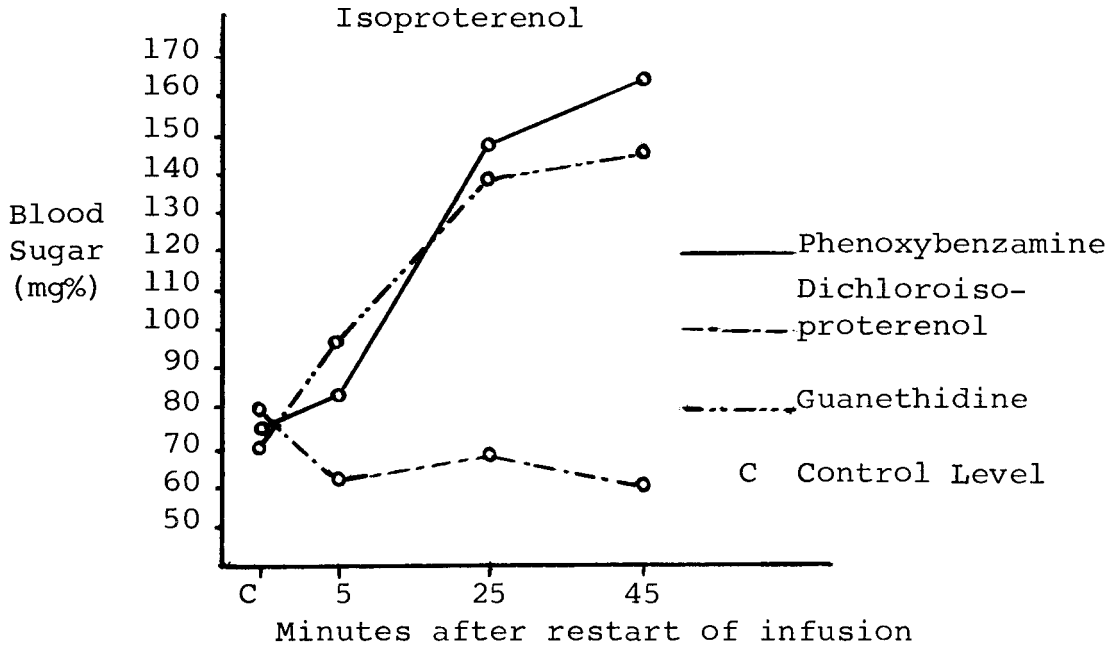


Figure 5. Average Blood Sugar Levels After Block

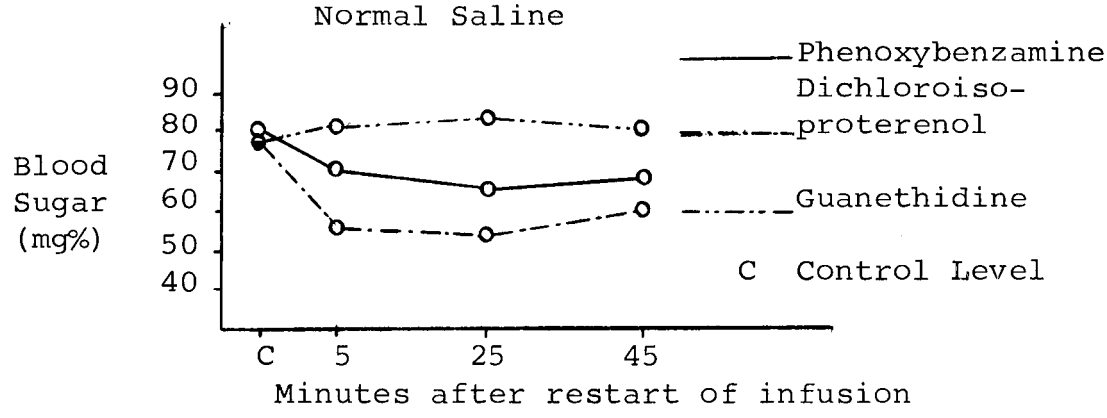


Figure 6. Average Blood Sugar Levels After Block

and the LSD. F-values at the 5% level were as follows: for 4 and 24 d.f., 2.7763; for 3 and 24 d.f., 3.0088; and for 2 and 24 d.f., 3.4028 (26, p. 521).

The analysis of variance (Table IV) shows that the computed F-values are in the critical region at the 5% level, and the results are significant for the blockers, the stimulators, and the interaction. The computed F-value for the effect of the animals is outside the critical region at the 5% level, and is not significant.

In calculating the least significant difference, the following formula was used (26, p. 326):

$$LSD_{.05} = t_{.025_{24}} \sqrt{\frac{2(\text{Error M.S.})}{4}}$$

The formula is for a two-tailed t-test at the 5% significance level with 24 degrees of freedom. The value derived by the formula was 31.99. To show a significant difference the computed means must differ by more than the derived value, 31.99.

The test of the means of the observations for least significant difference was as follows:

a. Norepinephrine: no significant difference between the means of any of the three blockers.

b. Isoproterenol: no significant difference between the blocking effect of phenoxybenzamine and guanethidine; a significant difference between the blocking effect of dichloroisoproterenol and phenoxybenzamine, and between dichloroisoproterenol and guanethidine.

c. Normal saline: no significant difference between the means of any of the three blockers.

d. Phenoxybenzamine: no significant difference between the blocking effect of phenoxybenzamine when used with norepinephrine or normal saline; a significant difference between blocking the effect of norepinephrine and blocking the effect of isoproterenol; a significant difference between blocking the effect of normal saline and blocking the effect of isoproterenol.

e. Dichloroisoproterenol: no significant difference in the blocking effect when used with any of the three stimulatory drugs.

f. Guanethidine: no significant difference between the blocking effect when used with norepinephrine or normal saline, a significant difference in blocking the effect of norepinephrine and blocking the effect of

isoproterenol; a significant difference between blocking the effect of normal saline, and blocking the effect of isoproterenol.

Results for Lactic Acid

The average values obtained for lactic acid are shown in Table V and figures 7-10. The same general results were obtained from the chemical analysis although the results are different when the blockers were used with normal saline. From the graphs for blood sugar and lactic acid, it can be seen that the order of the blocking action is: for blood sugar, guanethidine, phenoxybenzamine, dichloroisoproterenol; and for lactic acid, dichloroisoproterenol, guanethidine, phenoxybenzamine.

Statistical Analysis of Lactic Acid Effects

The difference between the control blood lactic acid level and the final lactic acid level was taken as the statistic to be analyzed. Analysis was made in the same manner as for blood sugar. For convenience in calculation, a constant of 4.0 was added to all values obtained in the chemical analysis of lactic acid. The 5% level of

significance was chosen for both the analysis of variance and LSD. The same F-values apply as for the blood sugar analysis. The results of the analysis of variance (Table VIII) were the same as the results of the analysis of variance for blood sugar except that the F-value obtained for the blockers was outside the critical region, and was not significant.

The same formula was used in calculating the LSD for lactic acid as was used for blood sugar. The value calculated as the least significant difference between the means was 16.12. The same series of comparisons was made for lactic acid as for blood sugar, and the results were the same in each case as they were for blood sugar.

a. Norepinephrine: no significant difference between the means of any of the three blockers.

b. Isoproterenol: no significant difference between the blocking effect of phenoxybenzamine and guanethidine; a significant difference between the blocking effect of dichloroisoproterenol and phenoxybenzamine, and between dichloroisoproterenol and guanethidine.

c. Normal saline: no significant difference between the means of any of the three blockers.

d. Phenoxybenzamine: no significant difference between the blocking effect of phenoxybenzamine when used with norephinephrine or normal saline; a significant difference between blocking the effect of norepinephrine and blocking the effect of isoproterenol; a significant difference between blocking the effect of normal saline and blocking the effect of isoproterenol.

e. Dichloroisoproterenol: no significant difference in the blocking effect when used with any of the three stimulatory drugs.

f. Guanethidine: no significant difference between the blocking effect when used with norepinephrine or normal saline, a significant difference in blocking the effect of norepinephrine and blocking the effect of isoproterenol; a significant difference between blocking the effect of normal saline, and blocking the effect of isoproterenol.

TABLE V

AVERAGE VALUES FOR LACTIC ACID

Norepinephrine

	Control	Before Block			After Block		
	1	2	3	4	5	6	7
Alpha	5.9	5.7	7.2	8.1	10.1	11.9	12.2
Beta	7.4	6.8	6.0	7.2	9.4	9.6	9.5
Guan-ethidine	6.0				5.2	6.1	10.6
Alpha and Beta	6.6	6.3	6.6	7.7			

Isoproterenol

Alpha	7.1	6.9	18.1	16.3	22.0	24.8	30.4
Beta	5.1	11.3	20.7	21.8	8.0	8.8	8.7
Guan-ethidine	6.6				8.6	19.0	29.0
Alpha and Beta	6.1	9.1	19.4	19.1			

Normal Saline

Alpha	6.5	4.6	4.8	5.0	5.2	4.6	4.1
Beta	5.6	5.2	5.6	4.4	7.2	6.6	7.4
Guan-ethidine	6.3				5.4	5.1	5.3
Alpha and Beta	6.1	4.9	5.2	4.7			

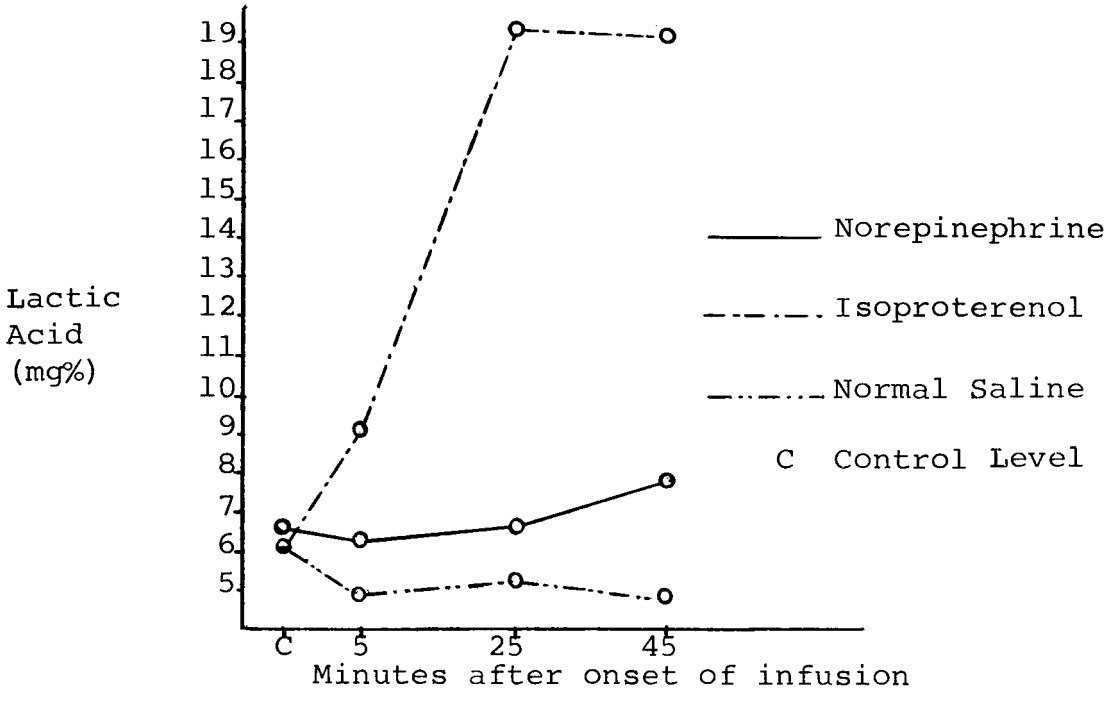


Figure 7. Average Lactic Acid Levels Before Block

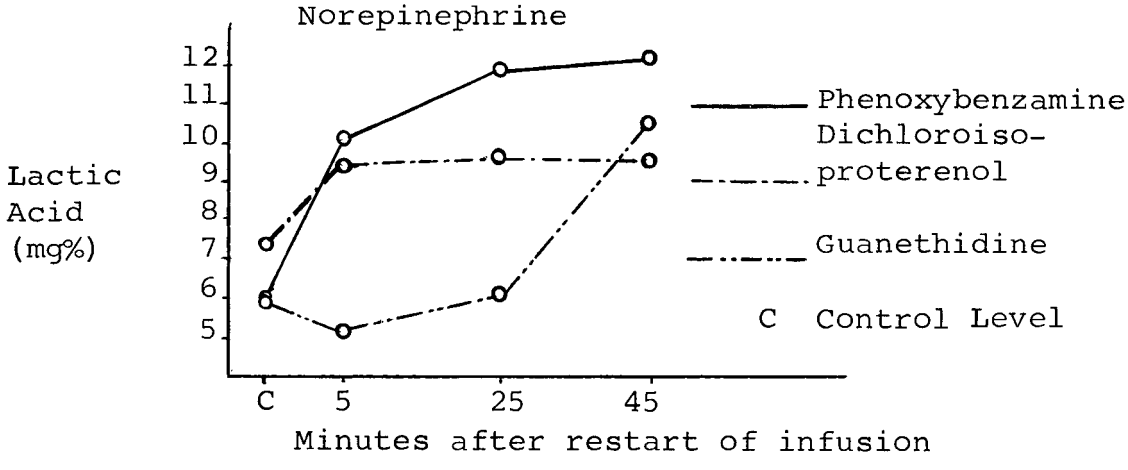


Figure 8. Average Lactic Acid Levels After Block

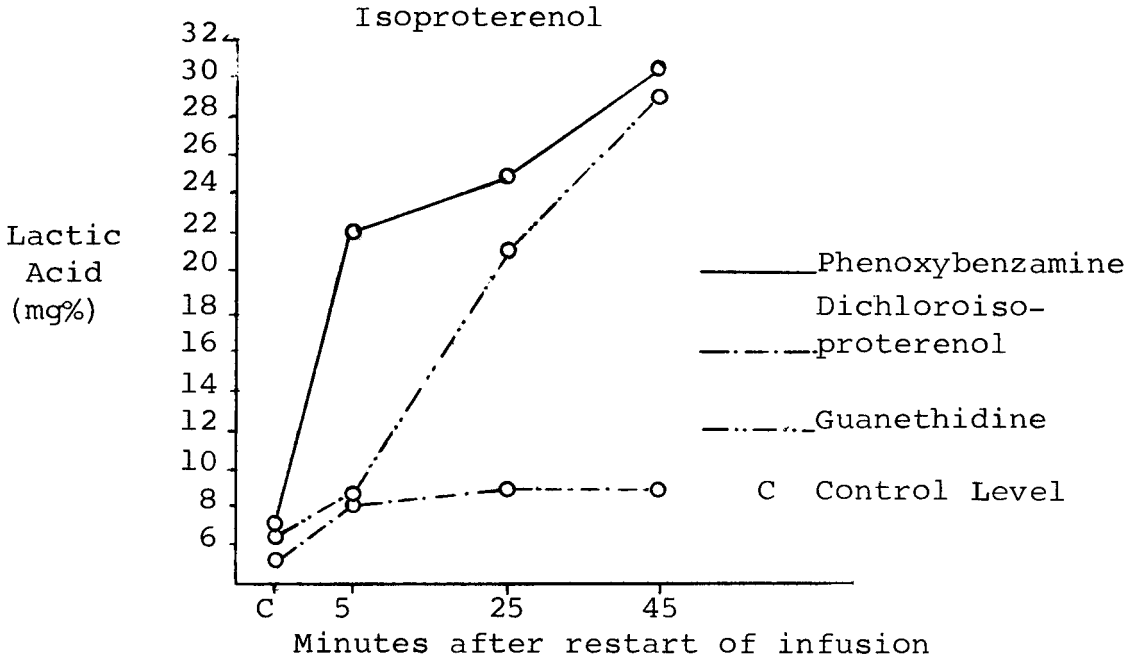


Figure 9. Average Lactic Acid Levels After Block

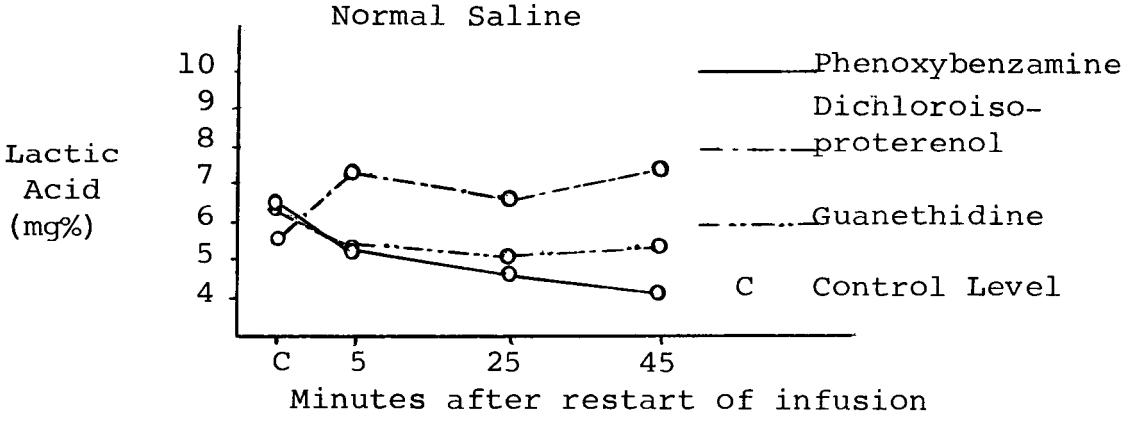


Figure 10. Average Lactic Acid Levels After Block

TABLE VI

STATISTICAL LAYOUT FOR LACTIC ACID

Blockers / Stimulators	Phenoxybenzamine (Alpha)	Dichloroisoproterenol (Beta)	Guanethidine (Guan)
Norepinephrine	4.2	2.5	7.6
	17.0	15.7	4.8
	3.9	2.7	18.1
	16.1	3.5	3.9
Isoproterenol	0.0	11.2	27.6
	38.2	4.9	26.9
	33.1	4.4	20.0
	37.7	9.7	31.1
Normal Saline	0.1	3.5	2.4
	1.8	5.3	4.0
	3.9	8.2	2.0
	0.7	6.0	3.3

Numbers represent the difference between the control level and the final lactic acid level plus a constant of 4.0.

TABLE VII

TOTALS AND MEANS FOR LACTIC ACID

(B) Blockers / (A) Stimulators	Alpha	Beta	Guan	Totals (B)
Norepinephrine	41.2	24.4	34.4	100.0
	10.3	6.1	8.6	8.5
Isoproterenol	109.0	30.2	105.6	244.8
	27.3	7.6	26.4	20.4
Normal Saline	6.5	23.0	11.7	41.2
	1.6	5.8	2.4	3.4
Totals (A)	156.7	77.6	151.7	386.0
	13.1	6.5	12.6	10.7
Totals for Dogs	59.1	118.6	96.3	112.0
	Dog #1 6.6	Dog #2 13.2	Dog #3 10.7	Dog #4 12.4

To constitute a significant difference, the individual means must differ by at least 16.12.

TABLE VII

ANALYSIS OF VARIANCE CALCULATIONS
FOR LACTIC ACID

Preliminary Calculations

Type of Total	Total of Squares	No. of Items Squared	Observations per Squared Item	Total of Squares per Observation
Correction	148,996.00	1	36	4,138.78
Observations	8,567.76	36	1	8,567.76
Blockers	53,589.54	3	12	4,465.80
Stimulators	71,624.48	3	12	5,968.71
Treatment	28,128.70	9	4	7,032.18
Dogs	39,376.46	4	9	4,375.16

Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Dogs	236.38	3	78.79	1.4555
Blockers	327.02	2	163.51	3.0205
Stimulators	1,829.93	2	914.96	16.9020*
Interaction	736.45	4	184.11	3.4019*
Error	1,299.20	24	54.13	
Total	4,428.98	35		

Significance level, 5% F with 3 and 24 d.f. = 3.0088
2 and 24 d.f. = 3.4028
4 and 24 d.f. = 2.7763

*Denotes that F is in the critical region, and the results are significant.

DISCUSSION

Blood Sugar Effect

In the analysis of variance, the item marked "Blockers" tests whether or not the blockers had any effect on the outcome of the experiment. The calculated F-value was larger than the F-value from the table which indicates that the blocking agents collectively did have an effect, but analysis of variance does not disclose how the blood sugar was affected by the individual blockers. The item marked "Stimulators" tests whether or not the stimulatory drugs had an effect. The calculated F-value exceeded the F-value from the table indicating that the blood sugar was affected by the stimulatory drugs, but, again, the effect of a particular stimulator is not disclosed. The "Interaction" tests whether or not the blockers and stimulators together had an effect on the outcome of the experiment. The calculated F-value was higher than the F-value from the table which shows that the blood sugar was affected by the interaction of the blockers and stimulators, but nothing can be determined for a specific blocker or stimulator interaction.

The calculated F-value for the item marked "Dogs" in Tables IV and VIII discloses whether or not the differences in the animals used had a significant effect on the outcome of the experiment. The calculated F-value was less than the F-value from the table, therefore the differences in the animals did not significantly affect the outcome of the experiment. The information derived from the analysis of variance concerning the stimulators and blocking agents was too general to be useful, and the test of least significant difference between the means was used to show the difference between the blocking agents in relation to the stimulatory drugs.

The LSD allows the determination of the following:

a. The lack of significant difference in the blood sugar levels when the blocking agents were used with norepinephrine means that each blocking agent was equally effective in blocking the blood sugar effects of norepinephrine.

b. In blocking the blood sugar effects of isoproterenol, the lack of significant difference between guanethidine and phenoxybenzamine means that the two were equally effective as blocking agents. The significant difference between dichloroisoproterenol and both guanethidine and

phenoxybenzamine shows that, because the blood sugar was higher using either guanethidine or phenoxybenzamine than it was using dichloroisoproterenol, dichloroisoproterenol is a significantly better blocker of the blood sugar effects of isoproterenol.

c. The lack of significant difference between the three blockers when used with normal saline means that none of the blockers themselves had a significantly different effect on the blood sugar. The slight rise of 2.1 mg.% in the blood sugar seen with guanethidine and the lowered blood sugar seen with the other two drugs were within the limits of experimental error.

d. Phenoxybenzamine is a significantly better blocker of the blood sugar effect of norepinephrine than it (phenoxybenzamine) is of isoproterenol. The differences seen between normal saline and both norepinephrine and isoproterenol are not pertinent to the study other than to show that normal saline did not cause an increase in blood sugar.

e. Dichloroisoproterenol served as an equally effective blocker of the blood sugar effect of both norepinephrine and isoproterenol. The slightly better block

with isoproterenol was within the limits of experimental error, and was not significant.

f. Guanethidine shows a significantly better block of the blood sugar effect of norepinephrine than of isoproterenol.

Lactic Acid Effect

The statistical analysis of the values obtained in the chemical analysis of lactic acid followed the statisical analysis of blood sugar except that the F-value for "Blockers" was outside the critical region. The interpretation is that collectively the blockers did not have an effect on the lactic acid levels caused by the stimulators. Individually, however, the blockers had an effect on the lactic acid levels as disclosed by the test for the least significant difference.

The differences between the means for lactic acid support those for blood sugar and aid in establishing the type of block produced by guanethidine.

General Discussion

In their original report on dichloroisoproterenol, Powell and Slater indicated that only a slight block, if any, was produced against the excitatory effects of epinephrine and norepinephrine (40). Levy and Ahlquist (23) report that there is no block of the blood sugar effect of isoproterenol by dichloroisoproterenol. The result of this experiment shows that dichloroisoproterenol was equally as effective as phenoxybenzamine in blocking the blood sugar effect of norepinephrine, and inspection of the mean values obtained (figure 2) discloses that there is a possibility that dichloroisoproterenol may be a better blocker of the blood sugar effect of norepinephrine than phenoxybenzamine. The experiment is in agreement with McCutcheon who reported an effective block of both blood sugar and lactic acid by dichloroisoproterenol when used with norepinephrine (33).

An examination of the structural formula of guanethidine (figure 2) should lead to the prediction that blockade of the alpha receptors would be expected rather than blockade of the beta receptors. Levy and Ahlquist

have shown that beta block is associated with halo-substitution of the ring (24), and Belleau (4) suggests that alpha activity is associated with alteration of the side chain. From the structure of guanethidine, it can be seen that there is no halo-substituted ring and that the side chain is altered away from that configuration producing the most effective alpha activity (4). The statistical analysis of the data obtained in the experiment discloses that guanethidine is an effective alpha blocker, and that guanethidine is not an effective beta blocking agent which agrees with what might be supposed from an inspection of the structural formula of guanethidine. The enhanced sensitivity to norepinephrine shown by some muscular tissue, and reported by Maxwell and others (20, (31), (32) was not evident for blood sugar and lactic acid levels in this experiment. The higher blood sugar levels obtained by guanethidine with normal saline, while not significant, make further investigation of guanethidine an interesting possibility.

CONCLUSIONS

Some of the conclusions that may be drawn from the experiment concerning guanethidine are:

1. Guanethidine appears to be as effective as phenoxybenzamine, a known alpha blocking agent, in blocking the alpha receptors.

2. Guanethidine appears to be not as effective as dichloroisoproterenol, a known beta blocking agent, in blocking the beta receptors. Guanethidine appears to block the beta receptors only poorly, if at all.

3. Guanethidine appears to be a significantly better blocker of the alpha receptors than of the beta receptors.

4. Guanethidine appears to have only a very slight (2.1 mg.%) positive effect on the blood sugar, twenty-four hours after administration.

5. Guanethidine appears to have a slight (1.4 mg.%) negative effect on the lactic acid level, twenty-four hours after administration.

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