THE METABOLIC EFFECTS OF ANALOGUES OF ARTERENOL IN CONJUNCTION WITH VARIOUS MONO-AMINE OXIDASE INHIBITORS

by

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INTRODUCTION

Within the past few years there has been an increased interest in the metabolism of epinephrine and some related analogues. Those studied here are isoproterenol, ethyl norepinephrine and N-isopropyl ethyl arterenol[1]. This interest has ranged from studies of the possible different metabolic steps in the early metabolism of these analogues to their final degradation (26,p. 379-380) (3,p. 1-26) (54,p. 25P) (42,p. 593).

Throughout the literature very little has been said as to what causes the breakdown or oxidation of epinephrine and other sympathomimetics. Richter, in 1940, listed five possible enzyme systems responsible for the inactivation of epinephrine in vivo (56,p. 361). These five possible enzyme systems are:

1. Catechol oxidase
2. Cytochrome oxidase
3. Amine oxidase
4. Peroxidase
5. Psuedo phenylase

These five enzymes may oxidize epinephrine as follows:

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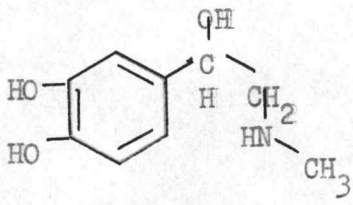
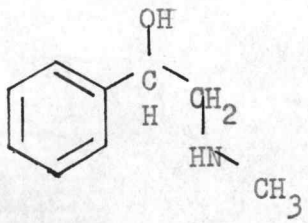
1. Isuprel (isoproterenol), Butanephrine (ethyl norephrine) and Win-3046 or Isoetharine (N-isopropyl ethyl arterenol) are trade names of Winthrop-Stearns Company.

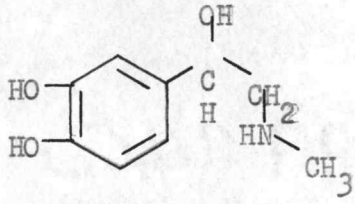


catechol oxidase

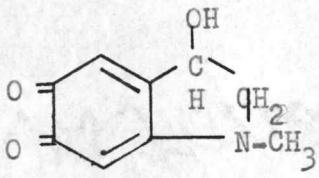


EPINEPHRINE

N-METHYL PHENYL ETHANOLAMINE

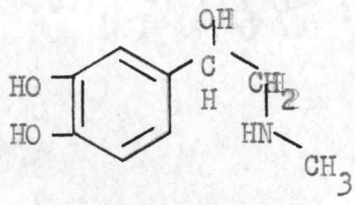


cytochrome oxidase

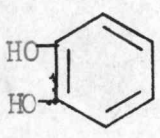


EPINEPHRINE

ADRENOCHROME



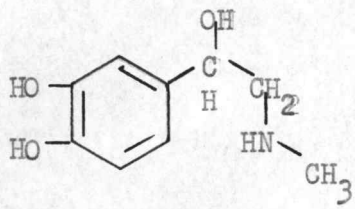
psuedo phenylase



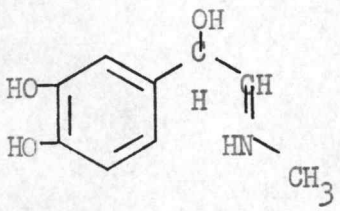
+CH_3-CHOH-CH_2-$NHCH_3$

EPINEPHRINE

CATECHOL

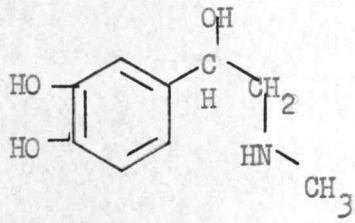


peroxidase phenylase

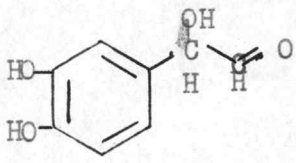


EPINEPHRINE

1(3-4 DIHYDROXYPHENYL) -2-METHYLIMINO ETHANOL



mono-amine oxidase



EPINEPHRINE

α-HYDROXY (3-4 DI-HYDROXYPHENYL ACET-ALDEHYDE

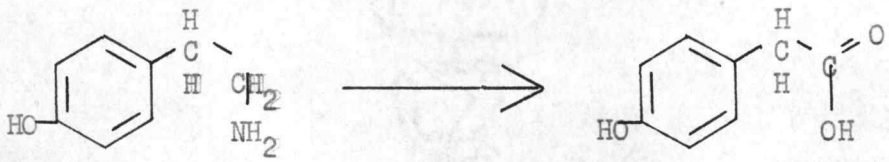
A study of past and current literature indicates there is still a great deal of controversy as to which of these enzyme systems or combination of systems is the most important for the inactivation of epinephrine and its analogues. Most theories have accumulated during the past decade; each theory explaining a possible true mechanism whereby epinephrine and its analogues may be degradated.

Bacq favors catechol oxidase (4,p. 343) while Gaddum and Kwiatowski favor amine oxidase (30,p. 98). Other investigators (11,p. 2187-2190) favor cytochrome oxidase and finally Richter (55,p. 25P) has stated that epinephrine is not oxidized by any enzyme but is esterified *in vivo* resulting in a sulfate or a glutamate.

As early as 1937, Blaschko, Richter and Schlossmann (11,p. 2187) came out with the idea as has Alles, *et al* in 1943 (1,p. 487) that epinephrine oxidase (Richter's catechol oxidase), tyramine oxidase (amine oxidase) and a third enzyme, aliphatic amine oxidase were one and the same enzyme, amine oxidase. Following this reasoning, vonEuler recently (1955) suggested that peroxidase may be the enzyme in lieu of amine oxidase. He believes his theory to be supported by the fact that peroxidase has the power to transfer catechol amines into inactive compounds (72,p. 27). All these theories are attempts to explain the means by which epinephrine is actually degradated.

Interesting studies made by Schayer, Smiley and Kapla in 1952 (58,p. 550-551) and Schayer in 1955 (60,p. 286) indicate that at least 50 percent of epinephrine administered to rats was inactivated by the loss of the $-NHCH_3$ group, presumably through the action of amine oxidase. This work was done with radioactive C^{14} on the alpha carbon of epinephrine.

Schayer, this time using radioactive tyramine (57,p. 60-63) was able to obtain p-hydroxyphenylaceticacid after amine oxidase had oxidized tyramine. Thus, he was able to show that an enzyme, amine oxidase, had produced the following reaction:



TYRAMINE

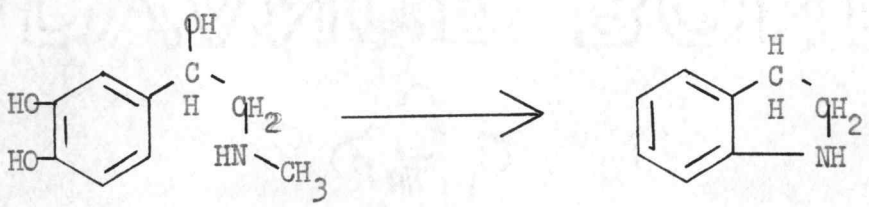
p-HYDROXYPHENYLACETIC ACID

In this reaction there is the intermediate formation of the corresponding aldehyde, p-hydroxyphenylacetaldehyde. The possibility also exists that an intermediate substance is formed midway between tyramine and p-hydroxyphenylacetaldehyde. This being p-hydroxyphenylacetimine, $HO-C_6H_4-CH_2CH=NH$. Interestingly enough this would be the product of Richter's peroxidase (56,p. 361). Other work has been done to support the concept that amine oxidase is

the main effective inactivating enzyme for catechol amines (16,p. 317) (19,p. 784-787).

Other supporters of the amine oxidase mechanism of epinephrine degradation are Friendenwald and Herrmann (28, p. 413-414). Their method was to test the oxygen uptake in tissues subjected to cyanide, which suppresses the cytochrome oxidase system, and amphetamine, which suppresses the amine oxidase system. They found that cyanide had no effect on oxygen uptake, but that amphetamine completely suppressed the oxygen uptake in tissues. Therefore, they attribute the oxidation of epinephrine to amine oxidase.

Also, there are many studies which point out the possibility that amine oxidase has nothing to do with epinephrine oxidation. Bacq, (3,p. 1-26) as mentioned above, favors the theory that catechol oxidase is the important enzyme in the degradation of epinephrine and its relatives. His reasoning for this is based on the oxidation of epinephrine to indole, as shown



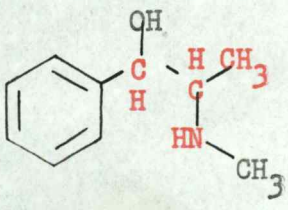
EPINEPHRINE INDOLE

However, he does not completely commit himself to the catechol oxidase system. He also mentioned that Richter's

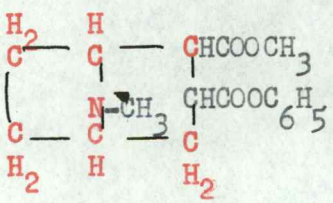
sulfo-conjugation is a good possibility.

vonEuler (71,p. 25) (72,p. 27) could find no significant effect upon testing liver and spleen for epinephrine and norepinephrine after amine oxidase inhibitors. Griesemer and Wells (32,p. 284) found that inactivation of the enzyme mono-amine oxidase does not alter the action of epinephrine in pharmacological systems. It can readily be seen that there is still much controversy concerning the action of amine oxidase and the degradation of epinephrine and its analogues.

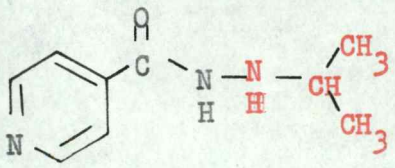
Bacq (3,p. 1-26) and Blaschko (7,p. 415-458) have pointed out that the necessary chemical structure for the inhibition of amine oxidase is an isopropyl amine grouping in the molecule. The following, containing such groupings, are the inhibitors of amine oxidase used in this study:



EPHEDRINE



COCAINE



IPRONIAZID

Areas in red are the molecular groupings (isopropyl amines) which Bacq and Blaschko say might possibly cause the inhibition of amine oxidase.

Blaschko and Duthie (12,p. 348-349) in 1945, made a study of the inhibition of amine oxidase by using amidine,

-C=NH , type structures. They found that pentamidine was
NH_2

a powerful <u>in vitro</u> inhibitor of amine oxidase. Their method was to use rabbit liver (which contains the enzyme) and test it for tyramine oxidation before and after treatment with pentamidine.

Another theory in the structural requirements for the inhibition of amine oxidase has been put forth by Zeller, <u>et al</u>. (79,p. v). They state that phenylhydrazine is a good inhibitor not only of mono-amine oxidase but also of di-amine oxidase. They follow the line that iproniazid is a strong mono-amine oxidase inhibitor. It can be seen that iproniazid is a hydrazide structure as is phenylhydrazine. Zeller states that the main requisite for mono-amine oxidase inhibitors is the hydrazone structure, $=N-NH_2$. It can be seen however, that ephedrine and cocaine lack any type of nitrogen-nitrogen bonding. Even so, these two compounds have been found by various investigators to have an inhibiting type action on mono-amine oxidase (2,p. 596) (30,p. 98) (47,p. 407-408) (49,p. 301) (64,p. 593).

In 1940, Philpot (49,p. 301) found that by using guinea pig liver as a source of mono-amine oxidase it could be shown that the oxidation of tyramine and epinephrine could be inhibited from 60% to 100% by ephedrine, cocaine and some relatives of cocaine. Philpot states that the possible mechanism of action in epinephrine potentiation by cocaine

and ephedrine is that they compete with the epinephrine for absorption on the enzyme surface. This is similar to Gaddum and Kwiatowski's theory that ephedrine inhibits the enzyme, mono-amine oxidase, therefore allowing epinephrine to accumulate and produce its sympathomimetic effect (30, p. 98). It must be kept in mind that Philpot's work was done completely in vitro.

There has been other work completed that agrees with the above in showing that cocaine has an inhibitory action on mono-amine oxidase and thus allows epinephrine to concentrate and produce its typical action (45,p. 224) (66, p. 334).

It can also be seen that numerous authors have directly controverted the above findings. For example, Brown and Boxill (14,p. 654) could demonstrate no abnormal hypertensive effects with epinephrine after pretreatment with cocaine. They administered epinephrine four different ways. Single doses of the drug before and after cocaine and progressively increasing doses of epinephrine before and after cocaine. They state that there was no significant change in blood pressure.

The work of another group, Polonovski, Connard and Schmitt (51,p. 1980) parallels the research of Brown and Boxill. Their views are that cocaine, in high concentrations, does not potentiate sympathomimetic action on blood

pressure. A few studies of the action of cocaine *in vitro* also seem to indicate that it does not potentiate sympathomimetic amines (51,p. 1980).

There is also conflicting evidence regarding the action of ephedrine in combination with epinephrine and some analogues. Blaschko in 1938 (8,p. 7P) made the statement that ephedrine is not oxidized by mono-amine oxidase, whereas epinephrine is oxidized by the enzyme. This was the basic development of his theory that isopropyl amine derivatives act as inhibitors and that ethyl amine derivatives act as substrates for mono-amine oxidase. Again, in 1938 Gaddum, *et al* (30,p. 98) postulated that the mechanism of epinephrine potentiation by ephedrine is analagous to acetylcholine potentiation by physostigmine. Their experiments were done by perfusing the compounds through the ear veins of rabbits.

On the negative side, Furchgott (29,p. 183-265) could show in only a few experiments any demonstrable potentiation of epinephrine with ephedrine in vasodilation of rabbit aorta. Burns (18,p. 237) also rarely observed any potentiation with small arteries of rats.

Up to this point it is clear that there are volumes of conflicting evidence on the subject of mono-amine oxidase inhibition by ephedrine and cocaine. There also appears to be conflicting evidence with regard to

1-isonicotinyl-2-isopropyl hydrazine (iproniazid, Marsilid[2]).

Although iproniazid is a recent drug (1951) much clinical work and experimentation has been done with it. The drug was first produced in 1951 by Dr. H. H. Fox of the Hoffmann-LaRoche Laboratories for its possible action in tuberculosis. The reasoning behind this was that the parent compound of iproniazid is isoniazid, a compound used successfully in tuberculosis. However, iproniazid was found to be of less value in tuberculosis than isoniazid and, as a consequence, was nearly lost from the medical profession (24,p. 1-3).

During its application as a tuberculostatic agent it was found that a few severely depressed patients exhibited a stimulation of the central nervous system. This stimulation was said to change the mood of the individual. Thus the terms "mood elevator" and "psychic energizer" were coined (24,p. 3). Since that time it has been shown that iproniazid had a remarkable effect on the personalities of certain depressed patients (13,p. 11).

E.A. Zeller, from Northwestern University Medical School, deserves much credit for giving the impetus to the investigation into the inhibition of mono-amine oxidase by iproniazid. Among the numerous investigations completed

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2. Marsilid is the trade name of Hoffmann-LaRoche, Inc. for iproniazid.

by Zeller, important ones have been his work with iproniazid on the inhibition of the enzyme in bacteria (76,p. 350), in smooth muscle preparations (33,p. 182), in the structural requirements for inhibition (79,p. v) and more recently in in vitro mitochrondrial isolation methods (77,p. 273). The results of Corne (22) agree with Zeller in the fact that these mitochrondrial tests showed an inhibition of mono-amine oxidase within two hours. However, this is not in agreement with Delay and Buisson (25,p. 51-55) who found that by treating depressed psychiatric patients with iproniazid it takes from a few days to as many as three weeks for any observable change to be noted.

In 1953, Zeller and Griesemer studied the possible potentiating effect with iproniazid on sympathomimetic amines. With a dose of 0.18 millimoles of iproniazid per kilogram of cat they found a strong potentiation in the contraction of the nictitating membrane. However, they found no potentiation in blood pressure (31,p. 701).

Other work done by Zeller and associates indicate that iproniazid is a very effective mono-amine oxidase inhibitor in mitochrondrial isolation tests (77,p. 273) (78,p. 460).

Kamijo, et al. in 1956 (39,p. 218) found that iproniazid first depresses then potentiates the action on the nictitating membrane of the eye of the cat from both

cervical sympathetic trunk stimulation and intra-arterial injections of epinephrine, norepinephrine and tyramine. This group also found that mono-amine oxidase was definitely inhibited in the liver, brain and kidney. They state that the depression obtained first in the nictitating membrane was due to adrenergic blockage. The reasoning behind this potentiating property is not that enzymatic inhibition occurs but rather an interference with the penetration of the amine to the intracellular site and, as a consequence, a prolonged action at the receptor site.

Other workers who have found possible potentiation of sympathomimetics by iproniazid are:

1. Corne, et al, who found that doses of 0.4 millimole per kilogram of iproniazid in dogs (sub-cutaneously) completely inhibits mono-amine oxidase in the liver (22,p. 339).
2. Griesemer, while working with iproniazid pretreated aortic strips found 60% less contraction with epinephrine. He also stated, like Kamijo, that there is the possibility of some adrenergic blocking action (33,p. 182).
3. Schayer and Wu, who found that iproniazid produces a significant increase in the total tryptamine level of the urine (58, p. 63).
4. Tickner, while studying amine oxidase inhibition by antihistaminics postulated that the sympathomimetic action exhibited by some antihistamines is due to their inhibition of mono-amine oxidase (65,p. 609).

To support the argument that iproniazid does not potentiate sympathomimetic amines, the following references are cited:

1. Benson, et al, while studying the toxicology of iproniazid found only minor pharmacological effects (5,p. 385-386).
2. Friend, while giving infusions of norepinephrine before and after iproniazid in man found no significant differences in blood pressure (27,p. 63).
3. Griesemer, et al, this time studying blood pressures in cats found no increase in the pressure with iproniazid and epinephrine (33,p. 182).
4. Schmitt, while doing the same type of work as Kamijo (39,p. 218) found quite the opposite effects. That is, Schmitt found that a pre-injection of iproniazid not only did not increase the nictitating contractions in cats but actually decreased the contractions when treated with epinephrine (63,p. 2574).
5. Udenfriend, Weissback and Boydanski found that iproniazid is a poor in vitro inhibitor of serotonin (5-hydroxytryptamine) in peripheral tissues. They state that amphetamine and procain amide are not effective in vivo (66,p. 259).

In the majority of experiments liver, brain, spleen and other organs were used either to test oxygen uptake or ammonia release (28,p. 665) (32,p. 284) (77,p. 273) (78,p. 460). However, it is felt that removing an organ from its natural habitat might effect its oxidative abilities. In an attempt to determine whether one enzyme or another, or a combination of enzyme systems, are acting, the test may better be done in vivo. The action of the different systems may be accounted for if they are eliminated by a pharmacological inhibition. That is, to produce a pharmacological inactivation of one or several enzyme systems and test the subsequent results.

It was felt that if one system could be eliminated and this system was responsible for epinephrine oxidation, then a potentiation of epinephrine would occur due to its decreased oxidation.

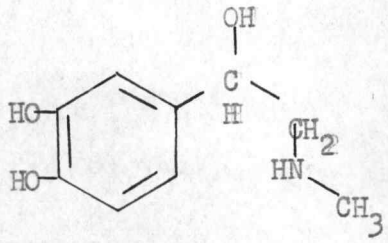
Amine oxidase was the system selected for research for the following reasons:

1. There is a large variety of drugs that inhibit amine oxidase *in vitro*. A few that have been shown to act in this manner are amphetamine (28,p. 413-414), butyn (68,p. 298), cocaine (49,p. 307) (66,p. 331), ephedrine (30,p. 98), iproniazid (35,p. 282-283), methylene blue (75,p. 8P), sulfhydral groups (7,p. 415-458), and antihistamines (7,p. 415-458).
2. Results of the research done in this field are extremely varied.
3. The majority of work done has been carried out by *in vitro* methods. It was felt that more work must be done *in vivo* to contribute to the knowledge of the enzymatic oxidation of epinephrine.

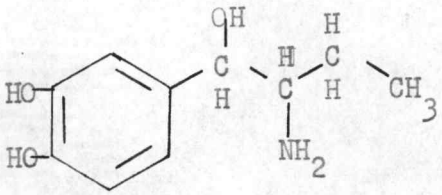
The *in vitro* studies that have been done may be summed up by quoting Richter (56,p. 361):

> "It is therefore impossible to draw any conclusions as yet from these *in vitro* experiments as to the way which adrenalin is inactivated in the tissues *in vivo*".

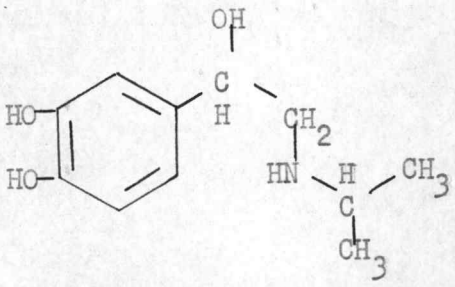
In this study the amine oxidase inhibitors iproniazid, cocaine and ephedrine were used with the following sympathomimetic compounds:



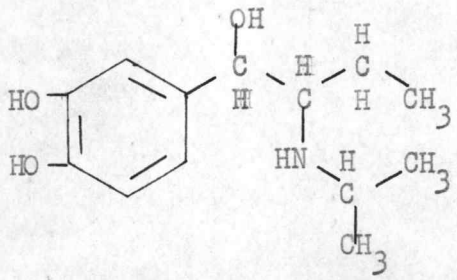
EPINEPHRINE



ETHYL-NOREPINEPHRINE
(BUTANEPHRINE)



N-ISOPROPYL ARTERENOL
(ISUPREL)



ETHYL N-ISOPROPYL ARTERENOL
(WIN-3046)

These four sympathomimetic substances were chosen because each is an adrenergic compound, they are related chemically (sterically), their relation to mono-amine oxidase has not been previously studied and they elicit both similar and varied effects (70,p. 526) (17,p. 21P-22P) (43,p. 45) (73,p. 552). Some effects produced by the compounds have been summarized by Lands, et al.(43,p. 45). For example, Win-3046, Isuprel and Butanephrine all produce depressor responses. According to Lands their depressor order is:

Isuprel - 1
Win-3046 - 10 times less effective
Butanephrine - 100 times less effective

From the responses obtained from these sympathomimetic substances it was postulated that if mono-amine oxidase was responsible for their oxidation, and if the inhibitors of mono-amine oxidase cited above were used, then a potentiation of sympathomimetic effects could be expected.

EXPERIMENTAL

METHOD I

Twenty mongrel dogs of either sex weighing between seven and fifteen kilograms were used in this study. Each was anesthetized with 35mg/Kg pentobarbital intraperitonealy. This was supplemented, if needed, with small doses of pentobarbital intravenously. The animals were fasted for approximately 18 hours prior to the experiment and between experiments the animals were allowed to exercise freely and to take food and water ad libidum.

A total of four experiments were done per animal. These four experiments constituted one complete cycle. A total of four cycles represents one completed series as shown in Table I.

TABLE I

	Cycle I	Cycle II	Cycle III	Cycle IV
Exp. I	EPI control	BUT control	ISU control	WIN control
Exp.II	EPI→COC	BUT→COC	ISU→COC	WIN→COC
Exp.III	EPI→EPH	BUT→EPH	ISU→EPH	WIN→EPH
Exp.IV	EPI→IPR	BUT→IPR	ISU→IPR	WIN→IPR

EPI:Epinephrine
BUT:Butanephrine
ISU:Isuprel
WIN:Win-3046

COC:Cocaine
EPH:Ephedrine
IPR:Iproniazid

Controls shown in Table I form the basis of standard curves to be correlated with the three subsequent experiments which were run after the inhibitors had been given.

All the inhibitors were given intramuscularly except doses immediately preceding the experiment which were administered intravenously. The following regime was used:

1. Cocaine
   50 mg (IM) was given the day before the experiment and 50 mg (IV) was given immediately preceding the experiment.

2. Ephedrine
   50 mg (IM) was given the day before the experiment and 50 mg (IV) was given immediately preceding the experiment.

3. Iproniazid
   50 mg (IM) was given every day for three days before the experiment and 50 mg (IV) was given immediately preceding the experiment.

Ephedrine and cocaine have been known to act within a relatively short period of time (64,p. 571) (40,p. 665) whereas iproniazid takes a considerably longer period of time to act (52,p. 179) (37,p. 190-193). Therefore, ephedrine and cocaine were given one day prior to the experiment and iproniazid was given three days prior to experimentation.

Experiments in conjunction with iproniazid were always conducted last because of the extended time necessary for the drug to act (52,p. 179) (37,p. 190-193).

The inhibitors were dissolved in distilled water and kept in a refrigerator at 8° Centigrade. The amines were dissolved in a preservative of 0.1 per cent chlorobutanol and 0.1 per cent sodium bisulfite. The amines were all

CONSTANT VOLUME INFUSION PUMP

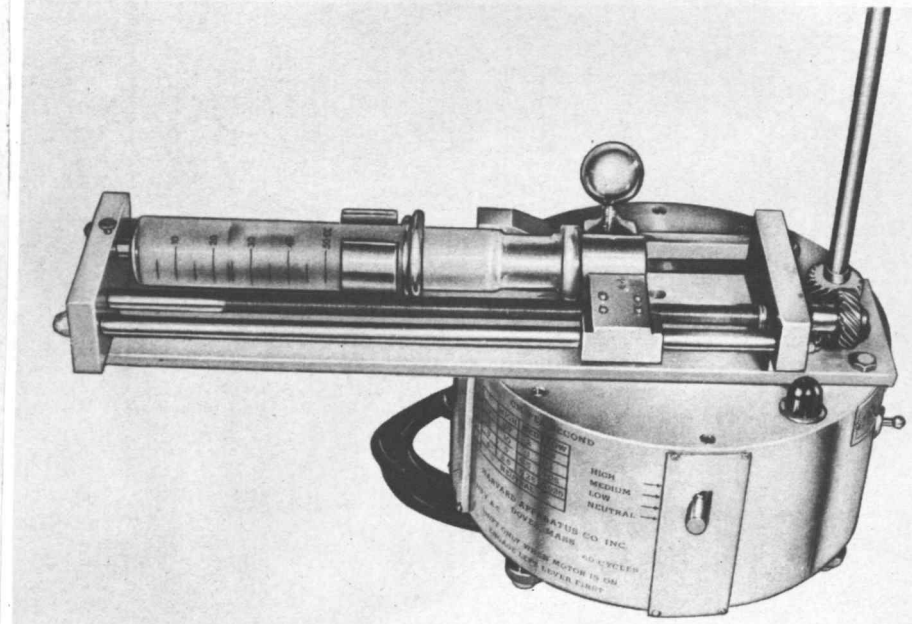


FIGURE I

infused intravenously.

Constant infusions of the drugs were made by the use of a Harvard Constant Volume Infusion Pump (Figure I). The speeds of administration were either 1 ml/2.5 minutes or 1 ml/5 minutes. The solutions of amines were diluted with physiological saline and administered at either

of the two speeds to achieve the correct dosage per minute.

The amine dosage may be listed as follows:

TABLE II

SYMPATHOMIMETIC AMINE DOSAGE PER MINUTE

EPI	BUT	ISU	WIN
1.5 r	25.0 r	1.06 r	29.5 r
3.0 r	50.0 r	5.40 r	59.0 r
6.0 r	67.6 r	10.80 r	118.0 r
11.5 r	101.4 r	20.00 r	236.0 r
15.0 r	250.0 r	40.00 r	472.0 r
30.0 r	500.0 r	107.60 r	958.0 r
74.0 r	888.0 r	-	-

Each dose level indicated in Table II was infused for fifteen minutes. The total length of the infusion time was between 90 minutes and 105 minutes depending on the drug used.

At the end of each fifteen minute infusion period a two milliliter blood sample was taken. This was used for the determination of blood sugar and lactic acid. Both blood sugar and lactic acid were quantitatively determined from venous blood samples by the Nelson test for reduced sugars (48,p. 375-380) and the Baker and Summerson test for blood lactic acid (36,p. 569).

Blood pressure, respiration, pulse and temperature were recorded as follows:

1. Blood pressure
   Blood pressure was obtained with a mercury manometer using sodium citrate (4%) in the system. Recordings were made on a smoked drum of an electric kymograph throughout the entire experiment. The carotid or femoral artery was exposed through an incision and cannulated with a glass arterial cannula.

2. Respiration
   A nasal cannula connected to a tambour was used to record respiration during the first three experiments of a series. For the fourth experiment in each series a tracheotomy was performed. This was done to facilitate the carotid cannulation.

3. Pulse
   The pulse was obtained by placing a stethoscope on the left chest of the animal and timing the contractions of the heart with a stop watch.

4. Temperature
   Temperature was recorded rectally in degrees centigrade.

Heparin was used as an anticoagulant throughout the experiments in a dosage of 3 mg/Kg. Preliminary experiments were done to test the possibility that heparin may have an effect on blood sugar and blood lactic acid. The results of this preliminary study proved negative (see Table III).

RESULTS AND DISCUSSION

TABLE III

THE MEAN EFFECT OF HEPARIN ON BLOOD SUGAR AND LACTIC ACID*

	CONTROL	POST-HEPARIN
BLOOD SUGAR	71.8 mg%	67.00 mg%
LACTIC ACID	9.12 mg%	10.13 mg%

*Average of seven trials.

It can be seen from the following that the differences between the controls and the findings after heparin are not significant.

BLOOD SUGARS (mg%)

CON	POST-HEP
72	66
69	69
76	76
84	83
60	54
84	66
58	55

ANALYSIS OF VARIANCE OF BLOOD SUGARS

With 6 and 6 degrees of freedom and 5% significance level $F > 0.1718$ and $F < 5.8197$.

	CON	POST-HEP
Σy	503	468
$(\Sigma y)^2$	253009	219024
$\frac{(\Sigma y)^2}{n}$	36144	31289
Σy^2	36797	32079
SS	653	792
s^2	108.8	132

$F = 0.8242$ with 6 and 6 degrees of freedom. NOT SIGNIF.

LACTIC ACID (mg%)

CON	POST-HEP
8.78	7.79
15.64	10.99
9.52	20.10
7.13	7.19
9.14	10.11
8.14	6.04
5.48	8.79

ANALYSIS OF VARIANCE OF LACTIC ACIDS

With 6 and 6 degrees of freedom and 5% significance level $F > 0.1718$ and $F < 5.8197$.

	CON	POST-HEP
Σy	63.83	70.96
$(\Sigma y)^2$	4074.27	5035.32
$\frac{(\Sigma y)^2}{n}$	582.03	719.33
Σy^2	653.00	852.24
SS	70.96	132.91
s^2	11.82	26.15

$P = 0.4520$ with 6 and 6 degrees of freedom. NOT SIGNIFICANT

These findings support J. Erick Jorpes (38,p. 93) who states that heparinized blood appears to be normal and may be used for the determination of blood sugar. Since the results of the trials were not significant heparin was used throughout the work.

BLOOD PRESSURE

The action of epinephrine on blood pressure shows the typical pressor response. According to Ahlquist (2,p. 596) and later by Vander Pol (70,p. 526) this hypertension occurs largely from alpha receptor stimulation. The greatest pressor response occurred when the last and greatest dose was infused. At this point the dose was 74 r/minute and the blood pressure reached 160 mm Hg. This represents a rise of 37 mm Hg above the initial pressure. When comparing the rise in blood pressure of epinephrine to that of epinephrine preceded by the mono-amine oxidase inhibitors, the following may be seen.

TABLE IV

DIFFERENCES BETWEEN INITIAL AND FINAL BLOOD PRESSURES OF EPINEPHRINE CONTROL AND EPINEPHRINE PLUS INHIBITORS*

DRUG	INITIAL PRESSURE	FINAL PRESSURE	DIFFERENCE	INCREMENT FROM CONTROL
EPI control	123	160	+37	--
EPI+EPH	131	188	+57	+20
EPI+COC	107	173	+66	+29
EPI+IPR	120	137	+17	-20

*Averages of three experiments for each drug.

Ephedrine when used with epinephrine potentiates blood pressure. This may be seen in Table IV. There is an increase in blood pressure that is 20 mm greater than the rise seen in the epinephrine control. Since ephedrine has

been established as a mono-amine oxidase inhibitor (30,p. 98) (8,p. 7P) it may be postulated that this effect on blood pressure, when used with epinephrine, is due to inhibition of the enzyme. Kunz, Bobb and Green (41,p. 453) also obtained a marked degree of potentiation in the vasoconstriction of arteries of rats with epinephrine-ephedrine combination.

TABLE V

DIFFERENCES BETWEEN INITIAL AND FINAL BLOOD PRESSURES OF BUTANEPHRINE, ISUPREL AND WIN-3046 CONTROLS AND IN CONJUNCTION WITH INHIBITORS*

DRUG	INITIAL PRESSURE	FINAL PRESSURE	DIFFERENCES	INCREMENT FROM CONTROL
BUT control	111	81	-30	--
BUT+EPH	128	93	-35	+ 5
BUT+COC	123	79	-44	+ 14
BUT+IPR	137	91	-46	+ 16
ISU control	113	39	-74	--
ISU+EPH	133	55	-78	+ 4
ISU+COC	105	41	-64	- 10
ISU+IPR	135	41	-94	+ 20
WIN control	110	61	-49	--
WIN+EPH	114	64	-50	+ 1
WIN+COC	117	62	-55	+ 6
WIN+IPR	106	44	-62	+ 13

*Averages of three experiments for each drug.

No significant potentiation was seen with ephedrine as an inhibitor before Butanephrine, Isuprel or Win-3046. In the doses that were used in this study these three sympathomimetic amines cause a depressor action. The descending

FIGURE 2A

EFFECTS ON BLOOD PRESSURE FROM EPINEPHRINE & EPINEPHRINE PLUS INHIBITORS
(AVERAGES OF THREE DOGS)

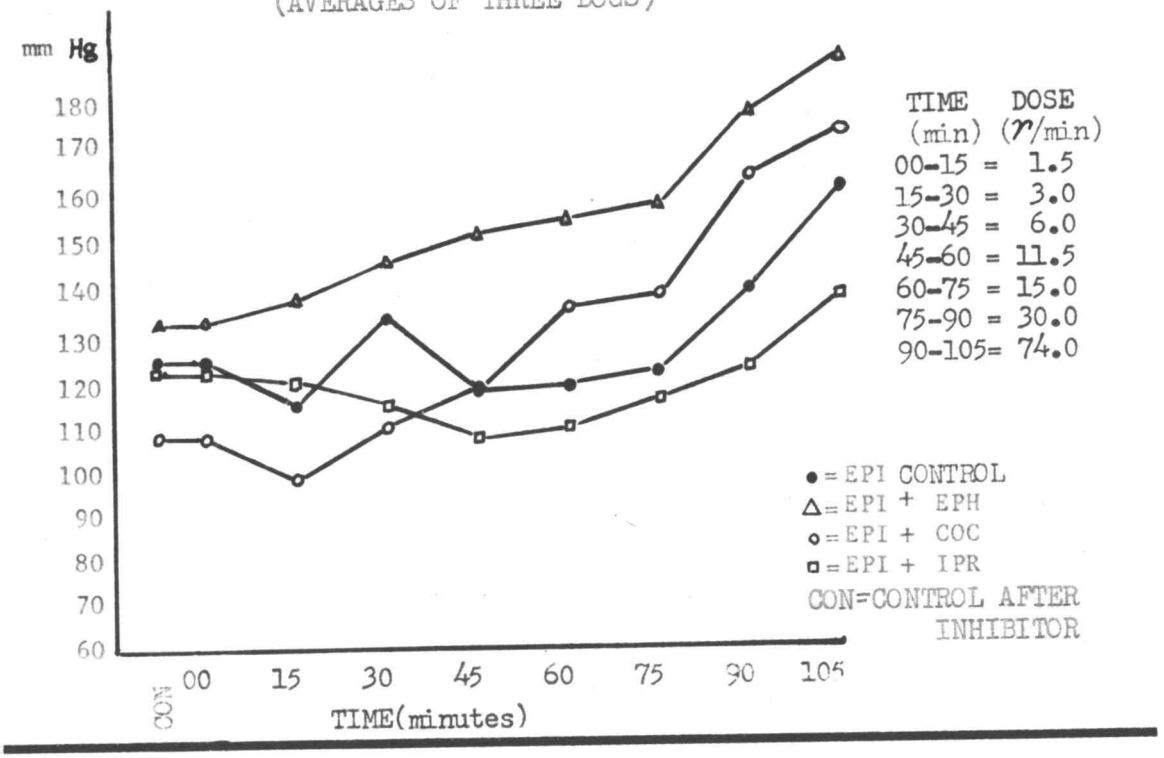
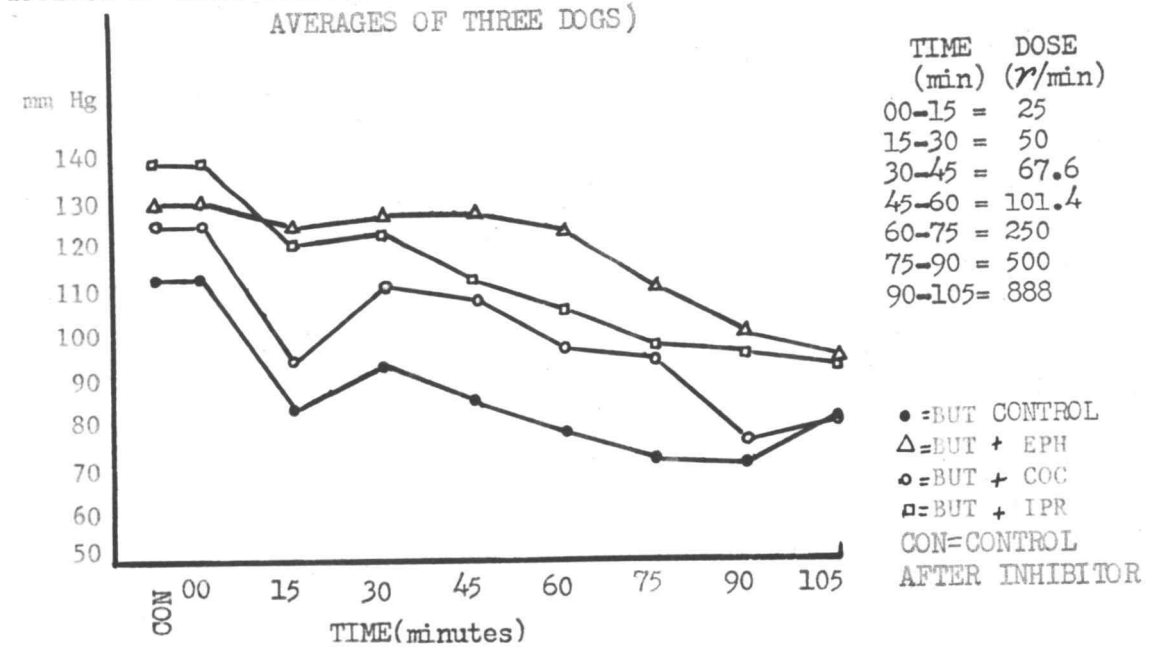


FIGURE 2B

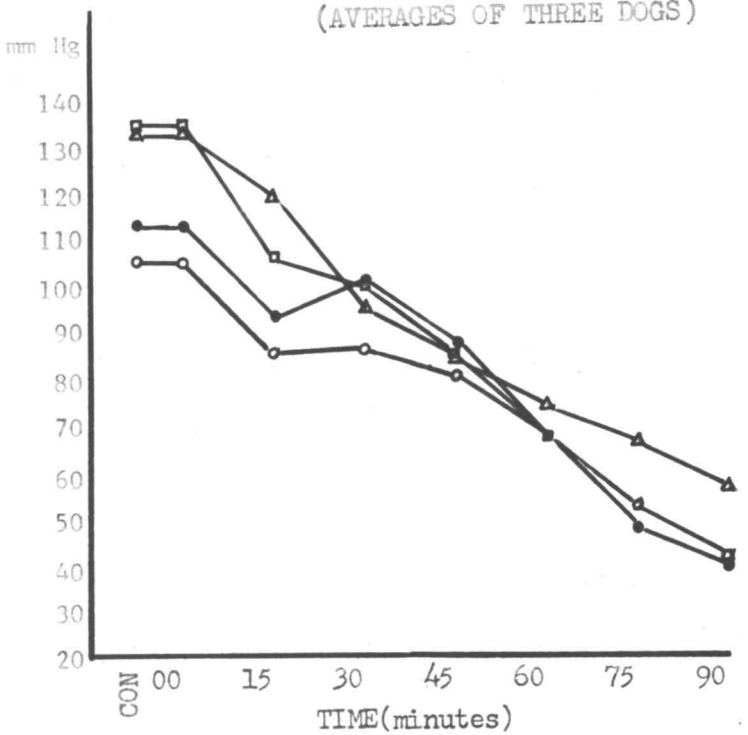
EFFECTS ON BLOOD PRESSURE FROM BUTANEPHRINE & BUTANEPHRINE PLUS INHIBITORS
AVERAGES OF THREE DOGS)



order of this depressor action is Isuprel > Win > Butanephrine (see Table V). With ephedrine this order of depressor action is unchanged; however, there is no increased depressor action. That is, an injection of ephedrine previous to infusion of Isuprel, Win-3046 or Butanephrine is ineffective in altering the blood pressure response seen without it (Figures 2B, 3A and 3B). This would tend to indicate that either ephedrine does not act as a mono-amine oxidase inhibitor or this is not the mechanism by which these three drugs are oxidized. But it must be remembered that epinephrine produces a stronger alpha effect than Isuprel, Win-3046 or Butanephrine which are predominantly beta stimulators (44,p. 45) in the order given. This, in effect, may possibly be the reason for the potentiation of epinephrine and not that of the beta stimulators. An interesting item in regard to Butanephrine is that in very large doses it produces an alpha action (19). It can be seen from Table V that the greatest decrease in blood pressure of the three depressor substances in combination with ephedrine is Butanephrine. This is 5 mm Hg whereas Isuprel and Win-3046 shows 4 and 1 mm, respectively. This increased depressor response with Butanephrine is perhaps negligible; however, it is greater than either Isuprel or Win-3046 and may be attributed to the fact that Butanephrine possesses stronger

FIGURE 3A

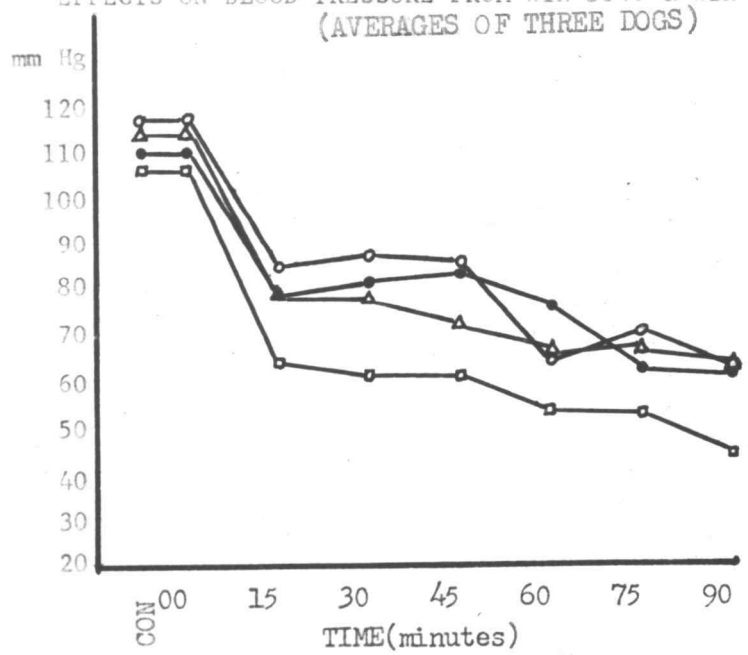
EFFECTS ON BLOOD PRESSURE FROM ISUPREL & ISUPREL PLUS INHIBITORS
(AVERAGES OF THREE DOGS)



TIME (min)	DOSE (γ/min)
00-15 =	1.06
15-30 =	5.4
30-45 =	10.8
45-60 =	20.0
60-75 =	40.0
75-90 =	107.6

FIGURE 3B

EFFECTS ON BLOOD PRESSURE FROM WIN 3046 & WIN 3046 PLUS INHIBITORS
(AVERAGES OF THREE DOGS)



TIME (min)	DOSE (γ/min)
00-15 =	29.5
15-30 =	59
30-45 =	118
45-60 =	236
60-75 =	472
75-105 =	958

alpha receptor stimulation.

With Isuprel and Win-3046, both strong beta stimulators, the pressure differences between the control and those with ephedrine are not significant.

The action of ephedrine on sympathomimetic amines seems to depend upon the amine that is used. Those compounds that are predominantly alpha receptor stimulators appear to be potentiated while the beta receptor stimulators are not affected. Following this reasoning then, it may be postulated that the enzyme, mono-amine oxidase, is inhibited by ephedrine in respect to the action of an alpha type adrenergic drug.

Cocaine, as an inhibitor before epinephrine, exhibited the greatest degree of potentiation in blood pressure. Here a rise of 66 mm Hg was seen which is 29 mm Hg above the control increment. Other authors have stated that cocaine definitely potentiates the pressor action of epinephrine (15,p. 192) (45,p. 224) (49,p. 301) (64,p. 571) (66,p. 331). In a very recent study (1959) Trendelenberg (67,p. 64) found an increase in response to blood pressure when using cocaine in conjunction with norepinephrine. The mechanism of the potentiation of a rise in blood pressure by epinephrine-cocaine combination may be summed up as either or both of the following (47,p. 407) (66,p. 331) (21,p. 143) (49,p. 301):

1. Cocaine prevents the destruction of epinephrine by competing with the enzyme responsible for epinephrine destruction, i.e., mono-amine oxidase inhibition.

2. Cocaine may cause an increased sensitivity or permeability in the receptor cell to epinephrine.

Philpot (49,p. 301) was the first to indicate that cocaine inhibits mono-amine oxidase. As stated above, a potentiated blood pressure was seen with epinephrine-cocaine combination. This potentiation may be said to be due to the action of cocaine claimed by Philpot.

Although it appears in Table V that Butanephrine plus cocaine exhibits a potentiated blood pressure, Figure 2B shows no such potentiation. This graph reveals a control Butanephrine-cocaine 12 mm Hg above the Butanephrine control and 2 mm Hg lower at the final pressure. This may be considered negligible. Therefore, it may be stated that there is only a slight indication of potentiation of blood pressure with Butanephrine-cocaine combination.

The same is also true for Isuprel and Win-3046. In fact, with Isuprel the control drop in blood pressure exceeds the Isuprel-cocaine drop by 10 mm Hg. It can be seen from the graph, Figure 3A, that the difference is negligible and, therefore, cocaine has no effect on the depressor action of Isuprel. This is in agreement with

Lands (44,p. 14) who found that cocaine increases the pressor response of epinephrine but has no effect on the depressor response of Isuprel.

The action on blood pressure of Win-3046 preceded by cocaine is definitely negligible. The increment of the control when compared to the Win-cocaine combination increment is but 6 mm Hg. Figure 3B shows that this combination closely parallels the control Win-3046.

Therefore cocaine appears to act very similarly to ephedrine on blood pressure. That is, it potentiates alpha type stimulating sympathomimetics and appears to have no effect on the beta type amines.

When comparing Table V with Figure 2A it can be seen that iproniazid does not potentiate the blood pressure rise of epinephrine but, on the contrary, appears to depress or block the pressor action. This agrees with Griesemer, et al. (33,p. 182) who found that aortic strips pretreated with iproniazid produced a 50 per cent less contraction with 10^{-7} M epinephrine. They state that there appears to be some adrenergic blocking action from iproniazid. In another study by Griesemer (30) in which cats were used, no increased blood pressure was found with epinephrine plus iproniazid. Rebhum, Feinberg and Zeller (53,p. 218) however, found a potentiation with toxic doses of epinephrine-iproniazid combination over epinephrine alone.

Friend (27,p. 63) could find no significant difference in blood pressure when using arterenol with and without iproniazid.

It may then seem reasonable at this point to agree with La Brosse (42,p. 593) who states that the reason epinephrine is not potentiated by iproniazid in vivo is that mono-amine oxidase is not the principal enzyme responsible for epinephrine metabolism.

When the animal was pretreated with iproniazid and then infused with Butanephrine the affect on blood pressure exhibited no potentiation, whereas with Win-3046, there is a slightly potentiated depressor action. An interesting item is that the depressor relation between these three drugs still exists, i.e., ISU > WIN > BUT.

The blood pressure effect with Isuprel exhibits an increased depression of 90 mm Hg which is 20 mm lower than the Isuprel control (Table V). This may be considered a potentiation since the initial blood pressure of the Isuprel-iproniazid combination is much greater than the corresponding Isuprel control pressure and the final pressures are very close. It can be seen from Figure 3A that as the third infusion was started (10.8 r/minute) the curve follows the control very closely.

However, from Table V and Figure 3B, it can be seen that Win-3046 exhibits only a slight potentiation in

blood pressure fall when the animal was pretreated with iproniazid.

These animals that have been infused with the sympathomimetic amines after pretreatment with iproniazid appear to be falling into a definite pattern. Isuprel, the most powerful beta drug used, exhibited the greatest degree of potentiated blood pressure fall; Win-3046, less depressant than Isuprel, showed a fair potentiation in pressure fall after iproniazid; Butanephrine, a still milder beta type drug, showed no change over the control; and, epinephrine, an alpha type drug, exhibited an inhibition of blood pressure rise when used with iproniazid.

In summary, the blood pressure effects may be stated as follows:

1.	EPI+EPH	: + +	1.	BUT+EPH	: -
2.	EPI+COC	: + + +	2.	BUT+COC	: -*
3.	EPI+IPR	: - -	3.	BUT+IPR	: -
1.	WIN+EPH	: 0	1.	ISU+EPH	: 0
2.	WIN+COC	: 0	2.	ISU+COC	: 0
3.	WIN+IPR	: +	3.	ISU+IPR	: + +

\+ : degree of potentiation beyond control
0 : no effect beyond control.
\- : degree of adrenergic blockade
* : slight action

Again it can be seen that if one follows Ahlquist's theory, alpha type drugs (EPI) show an increased rise in blood pressure with ephedrine and cocaine but a blockade in blood pressure with iproniazid. On the other hand,

beta type drugs (WIN, ISU) show no effect beyond the control with ephedrine and cocaine but a potentiation with iproniazid.

It can be seen that the more powerful the beta stimulator the greater is the potentiation by iproniazid. As one moves in the direction of more alpha type drugs less potentiation and greater blockage occurs.

PULSE

Since pulse may be considered as being due to a change in pressure within a system of elastic tubes, set up by the blood being pumped by the heart, a change in pulse may result from a reflex response to pressure changes due to vasoconstriction or vasodilation. When considering drug action another possibility of changes in pulse rate exists. Drugs may have a direct action on the chronotropic receptors in cardiac tissues thus effecting pulse rate.

Under normal conditions a rise in arterial blood pressure stimulates the vagus nerve causing cardiac inhibition (Marey's Law). However, under the action of drugs this may or may not take place.

In the case of the epinephrine infusion this phenomenon appears to hold until the last dose was administered (Figure 4A). At this point a vagal escape must have occurred since the blood pressure was still climbing throughout the last infusion.

Figure 4A shows the pulse rates of epinephrine and epinephrine preceded by the enzyme inhibitors. It can be seen that the control, the epinephrine plus ephedrine and the epinephrine plus iproniazid follow Marey's Law relatively closely up to the point of the 15 r/minute infusion. The increment of the epinephrine control showed a total

FIGURE 4A

EFFECTS ON PULSE FROM EPINEPHRINE & EPINEPHRINE PLUS INHIBITORS

(AVERAGES OF THREE DOGS)

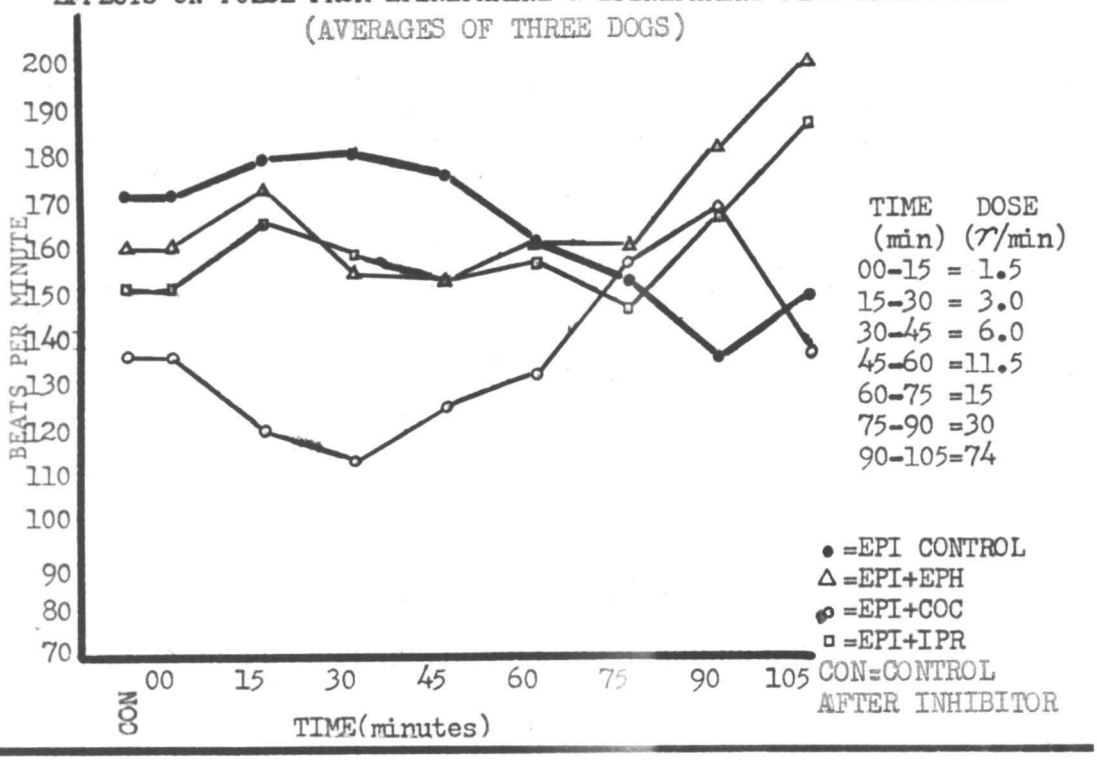
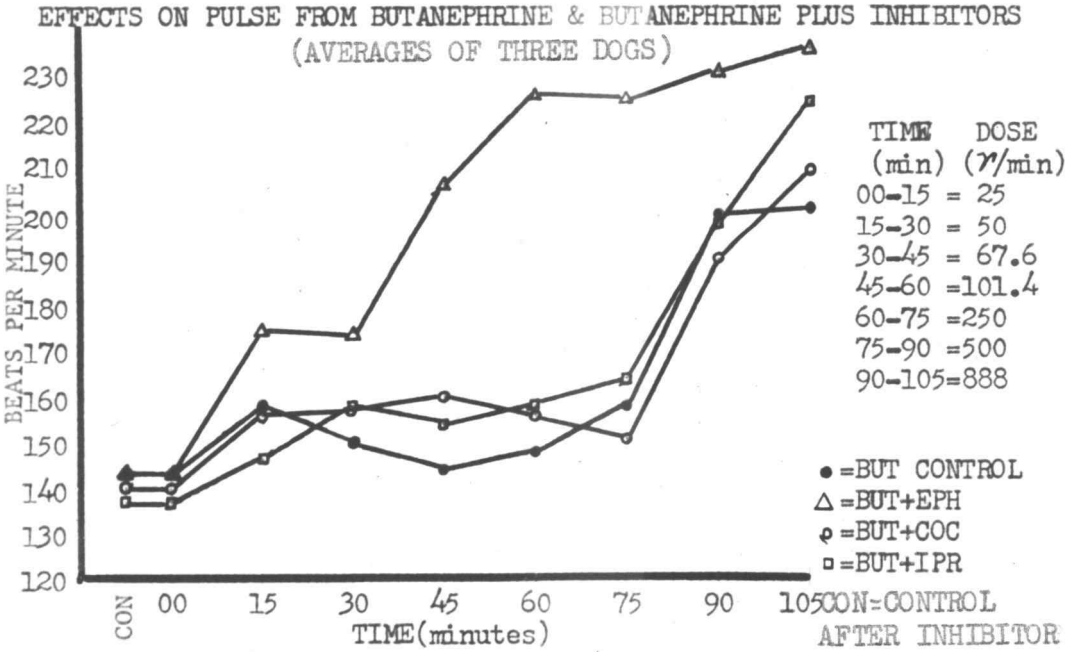


FIGURE 4B

EFFECTS ON PULSE FROM BUTANEPHRINE & BUTANEPHRINE PLUS INHIBITORS

(AVERAGES OF THREE DOGS)



decrease of 22 beats per minute. Both epinephrine preceded with ephedrine and epinephrine preceded by iproniazid show an increase of 40 and 35 beats per minute, respectively. Also by studying the graphs in Figure 4A it appears that this potentiation is mediated by a vagal escape. A potentiated vagal escape may result from:

1. A decreased parasympathetic effect.
2. A decreased blood pressure.
3. An increased sympathetic effect.

The first and second possibilities may be ruled out for the following reasons:

1. A decreased parasympathetic effect and an increased sympathetic effect produce similar results and since a sympathetic substance was being infused a decreased parasympathetic effect seems unlikely and may be ruled out.
2. A decreased blood pressure is not occurring.

Ruling out these two possibilities, the third may be considered which is an increased sympathetic effect or potentiation of pulse rate under epinephrine in conjunction with ephedrine and iproniazid.

One other possibility does exist, however, and that is that the mono-amine oxidase inhibitors ephedrine and iproniazid may cause a stimulation of the chronotropic receptors of the heart. The evidence for this may be that the epinephrine control curves of blood pressure and pulse exhibit Marey's Law. With the inhibitors both the

blood pressure and pulse rate climb which nullifies Marey's Law, indicating a possible stimulation of chronotropic receptors.

The effect of cocaine on pulse, when used as a pre-treatment with epinephrine, are varied and difficult to interpret. One of the first effects noted is the early escape of the vagus occurring at the end of the second fifteen minute infusion (3 r/minute). The pulse then climbs rapidly and steadily to a rate of 166 beats per minute, or a rate of 31 beats greater than the curve of the epinephrine-cocaine control shows. Up to this point a potentiated pulse rate may be assued. But as can be seen from Figure 4A during the last infusion a decrease in pulse rate occurred. This decrease extends down to the control level of 135 beats/minute. Since the blood pressure was still rising under the sympathomimetic effect of the amine, and a vagal escape was present, the vagus probably would not come back into play. Without the vagus the possibility exists that with this particular dose of epinephrine preceded by cocaine an inhibitory action of the chronotropic receptors of the heart occurs. This, then, could account for the bradycardia taking place, whereas with the other combinations of drugs an apparent stimulation of these receptors occurred.

TABLE VI

DIFFERENCES BETWEEN INITIAL AND FINAL PULSE RATES OF EPINEPHRINE, BUTANEPHRINE, ISUPREL AND WIN-3046 CONTROLS AND IN CONJUNCTION WITH INHIBITORS*

DRUG	INITIAL RATE	FINAL RATE	DIFFERENCES	INCREMENT FROM CONTROL
EPI control	170	148	-22	--
EPI+EPH	159	199	+40	+66
EPI+COC	135	135	0	0
EPI+IPR	150	185	+35	+57
BUT control	142	200	+58	--
BUT+EPH	141	235	+94	+36
BUT+COC	139	208	+69	+11
BUT+IPR	136	223	+87	+29
ISU control	156	246	+ 90	--
ISU+EPH	142	266	+124	+34
ISU+COC	130	235	+105	+15
ISU+IPR	148	211	+ 63	-27
WIN control	148	176	+28	--
WIN+EPH	152	236	+84	+56
WIN+COC	147	215	+68	+40
WIN+IPR	145	209	+64	+36

*Average of three experiments for each drug

The effect of mono-amine oxidase inhibitors in conjunction with epinephrine on pulse showed a potentiation in two of the drugs used and a possible potentiation in a third (cocaine). This potentiation was caused by an increased effect of the sympathomimetic substance presumably through the inhibition of mono-amine oxidase.

Pulse rate under Butanephrine showed two interesting items. The first is the apparent ineffectiveness of cocaine and iproniazid when used prior to the Butanephrine infusion. As seen in Figure 4B these curves lie close to the control curve. The other item of interest is the potentiation occurring from the Butanephrine-ephedrine combination. It can be seen in the curve that there is a definite and progressive rise in the pulse well above the increase associated with the control. Since the blood pressure decreases while the pulse rate increases under Butanephrine, it can be assumed that no vagal effect occurs and a vasodilation is responsible for the hypotension. The heart then, must increase in rate to compensate for this hypotension. However, this decrease in blood pressure under Butanephrine plus ephedrine is not great. Therefore, the potentiated pulse could be due to a direct action on the heart. That is, an increased action of the amine through the inhibition of the enzyme mono-amine oxidase by ephedrine.

The pulse under Win-3046 (Figure 5B) shows a resemblance to the pulse under Butanephrine. Ephedrine can be seen to definitely potentiate the pulse of Win-3046. Table IV indicates an increase of 84 beats/minute which is 56 over the control increase. This tachycardia may be a reflex action occurring from vasodilation.

In reviewing the blood pressures of Figure 3B, it can be seen that Win-3046, Win-3046 plus ephedrine and Win-3046 plus cocaine are exceptionally close to one another in the hypotension produced. Considering this a normal response, one would assume that the corresponding pulse rates would increase proportionately. Obviously, this is not the case as can be seen in Figure 5B. With ephedrine pretreatment the pulse of Win-3046 is definitely potentiated. With cocaine pretreatment, there is a potentiation but to a smaller degree than with ephedrine. No greater vasodilation occurs with Win-3046 in combination with cocaine as the blood pressures indicate; and there is no greater vagal response elicited as seen by the pulse rates. Therefore, it seems highly possible that the potentiation that is occurring is a result of an excessive action Win-3046 on the cardiac muscle. This could be brought about by an inhibition of mono-amine oxidase.

Iproniazid fails to elicit any potentiation on pulse rate with Win-3046 until the last infusion. At this point

FIGURE 5A

EFFECTS ON PULSE FROM ISUPREL & ISUPREL PLUS INHIBITORS

(AVERAGES OF THREE DOGS)

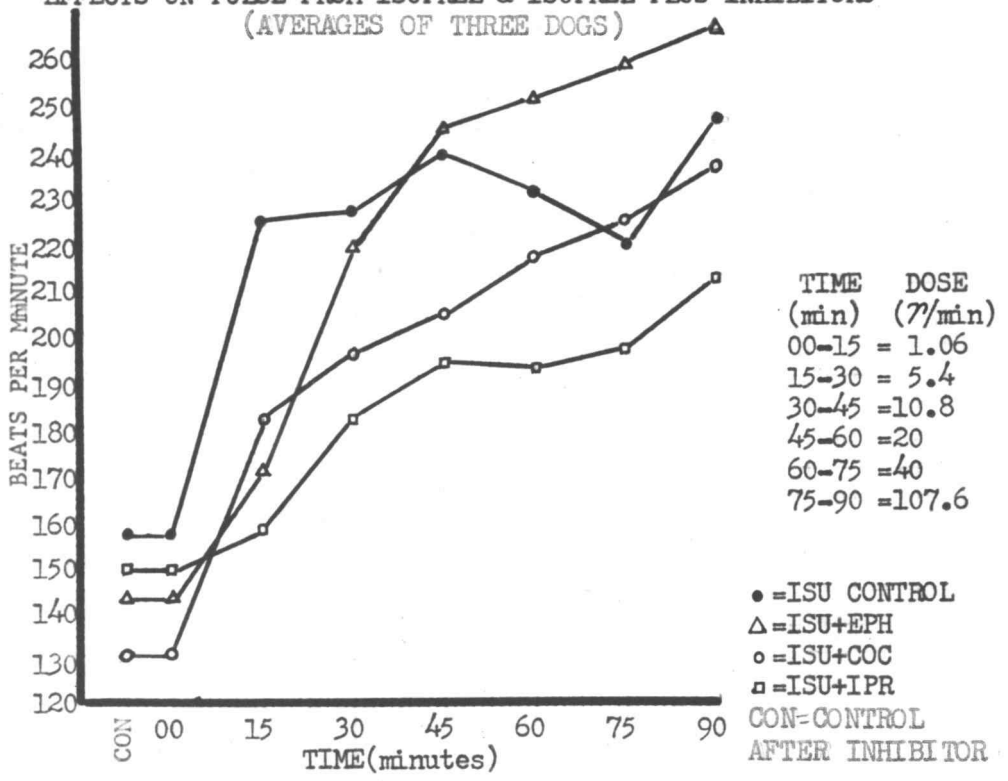
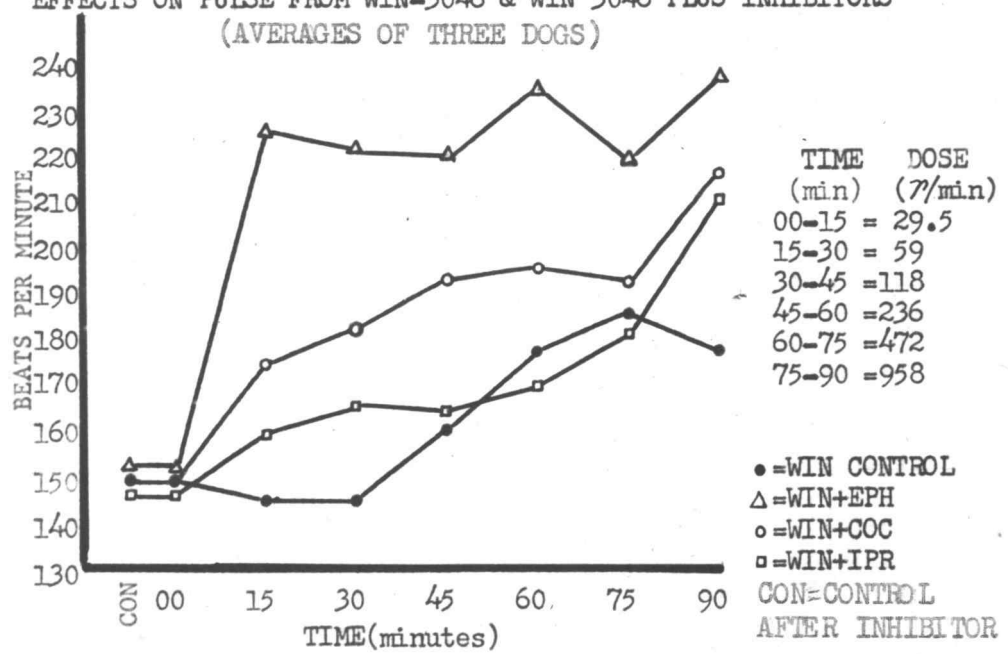


FIGURE 5B

EFFECTS ON PULSE FROM WIN-3046 & WIN 3046 PLUS INHIBITORS

(AVERAGES OF THREE DOGS)



there is a surge in pulse which corresponds to a slightly potentiated hypotension. The action of iproniazid when used as a pretreatment before the amine infusions shows no spectacular action on blood pressure or pulse.

When measuring the pulse rate under Isuprel (Figure 5A) ephedrine pretreatment appears to induce a slight potentiation. Cocaine pretreatment has no effect while iproniazid before Isuprel exhibits a slight inhibitory action on pulse. Here again, the question arises as to the action of iproniazid. In some areas it appears to have definite potentiating qualities; in other areas the effect seems to block the adrenergic action of the amine. This blocking action of iproniazid appears to be the case when considering pulse. The increase in pulse rate of Isuprel-iproniazid combination was somewhat less than the increase seen in the control, being 27 beats/minute below the control level. Here again we have a situation that nullifies Marey's Law, and appears to be directly opposite to iproniazid in combination with epinephrine. That is, when iproniazid is used as a pretreatment to epinephrine an increased blood pressure and an increased pulse were seen. When iproniazid is used with Isuprel a decreased blood pressure and a decreased pulse rate is seen. This may be interpreted by saying that strong alpha type drugs when in combination with iproniazid tend to stimulate the

chronotropic receptors of the heart, while strong beta type drugs in combination with iproniazid tend to inhibit or depress these receptors.

Mono-amine oxidase inhibitors in combination with sympathomimetic amines exhibit varied pulse rate responses. These responses are as follows:

1. EPI+EPH : ++
2. EPI+COC : +
3. EPI+IPR : ++

1. ISU+EPH : +
2. ISU+COC : 0
3. ISU+IPR : -

1. BUT+EPH : +++
2. BUT+COC : 0
3. BUT+IPR : 0

1. WIN+EPH : +++
2. WIN+COC : ++
3. WIN+IPR : 0

\+ : degree of potentiation beyond control
0 : no effect beyond control
\- : degree of adrenergic blockade

BLOOD SUGAR

In dogs, normal blood sugars are in the vicinity of 75 to 80 mg%. Walker, Boyd and Asimov (74,p. 487) and Griffith (34,p. 151-187) state that epinephrine causes liver and muscle glycogenolysis with resulting hyperglycemia. In reviewing the two receptor theory of Ahlquist (2,p. 586-600) it is seen that he places glycogenolysis as purely an alpha type phenomenon. That is, hyperglycemia is brought about through stimulation of the alpha receptors by the sympathomimetic amines. If this is the case, it is seen that all of the amines tested exhibited an alpha type response, i.e., hyperglycemia. In other words, the liver appears to be predominantly composed of alpha type receptors. These sympathomimetic amines cause hyperglycemia by increasing the breakdown of glycogen to glucose in the liver. Walker, Boyd and Asimov (74,p. 487) feel that epinephrine exhibits a type of catalytic action in glycogenolysis. This helps to explain, on a biochemical level, the mechanism of hyperglycemia produced by epinephrine.

Table VII reveals only two cases of any type of blood sugar potentiation; one with cocaine and the other with iproniazid. Cocaine showed a greater potentiation of blood sugar when used with epinephrine (Figure 6A) and a milder

potentiated hyperglycemia with Win-3046 (Figure 7B). Iproniazid, when used with the two beta drugs Win-3046 and Isuprel, appears to cause a slight potentiation (Figures 7A and 7B).

Here again, as with blood pressure, the main potentiation occurs with an alpha drug. It can also be seen from Figure 6A that the epinephrine-ephedrine combination appears to produce a potentiation of blood sugar. However, the increment between the initial and final level is exactly the same as the increment of the control, which is 160 mg%.

The effect of iproniazid in combination with beta type sumpathomimetic amines on blood sugar exhibits a slight degree of potentiation, indicating some enzymatic inhibition. The slightly decreased hyperglycemia during the infusion of the third, fourth and fifth doses of epinephrine can be seen to rise rapidly to attain a final increment of but 1 mg% less than the control which is negligible.

The effect of Butanephrine after pretreatment with the mono-amine oxidase inhibitors on blood sugars presents a different and interesting response. As can be seen from the graphs in Figure 6B none of the inhibitors potentiated the effect. In fact, all appeared to cause a depressed hyperglycemia when comparing them to the control.

FIGURE 6B

EFFECTS ON BLOOD SUGAR FROM BUTANEPHRINE & BUTANEPHRINE PLUS INHIBITORS

(AVERAGES OF THREE DOGS)

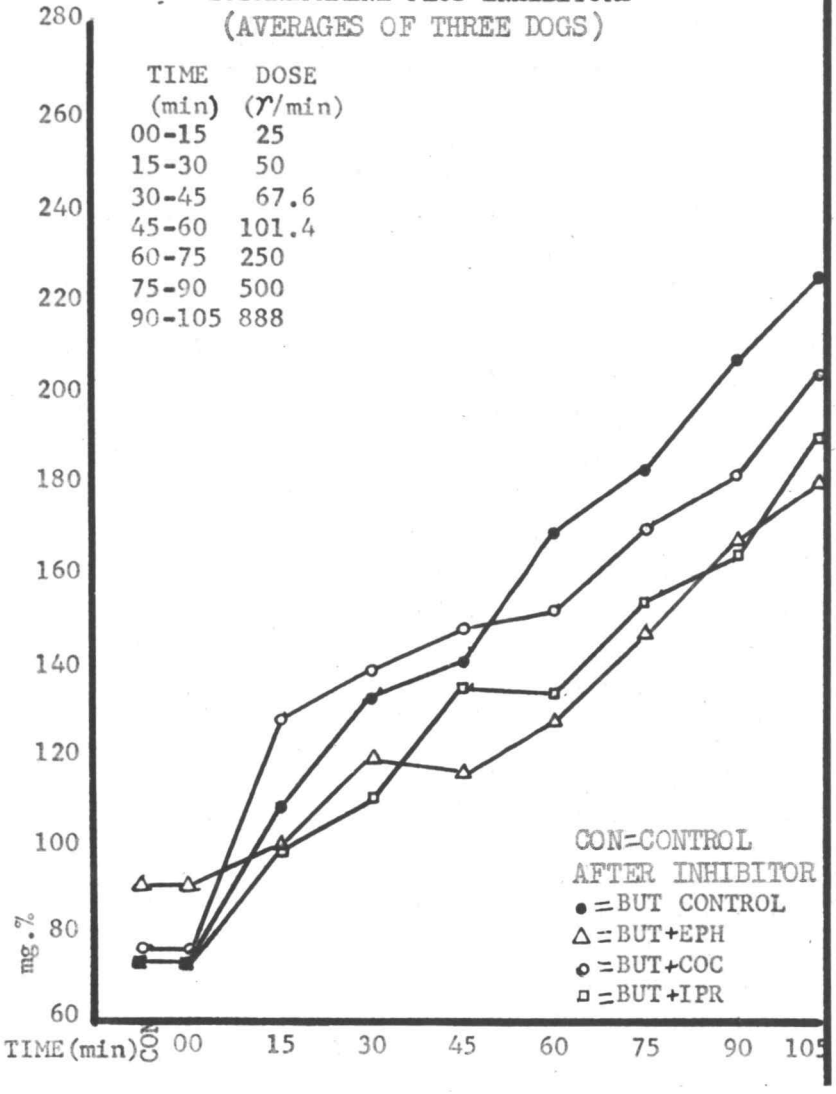
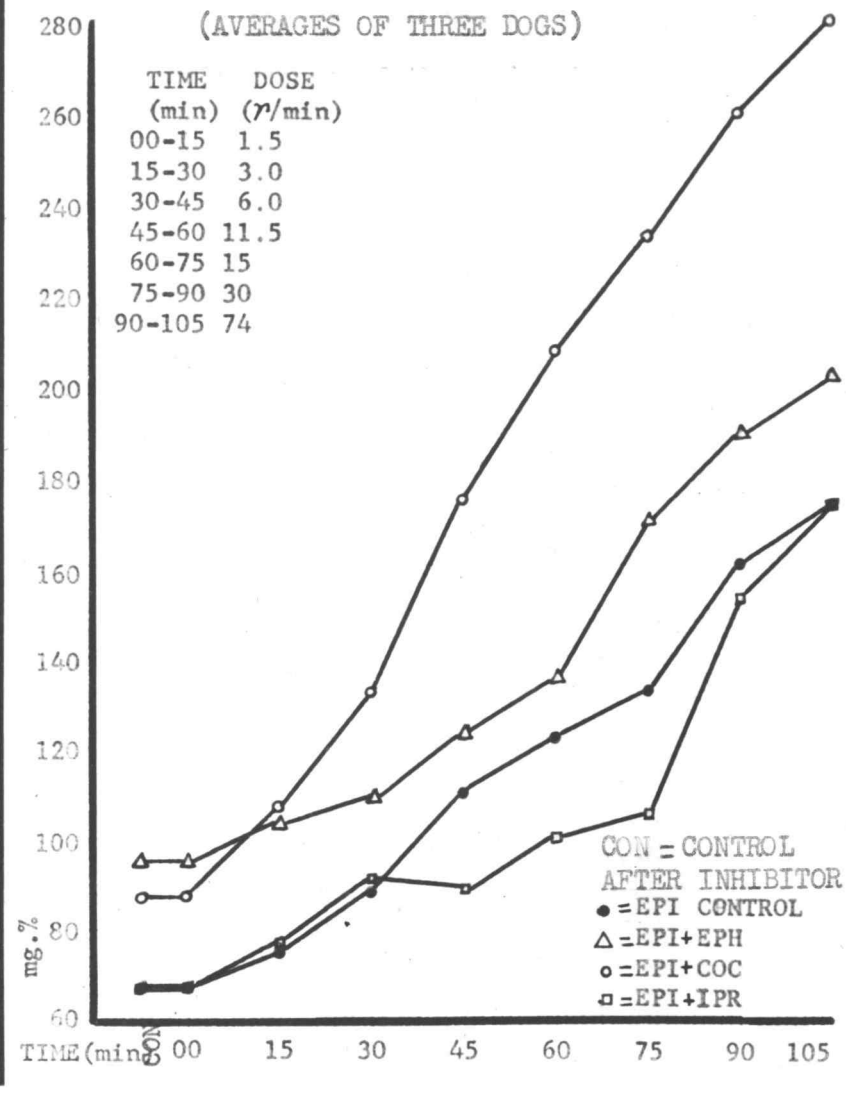


FIGURE 6A

EFFECTS ON BLOOD SUGAR FROM EPINEPHRINE & EPINEPHRINE PLUS INHIBITORS

(AVERAGES OF THREE DOGS)



This effect on blood sugar may be brought about by a decreased output of glucose from the liver or an increased utilization of glucose by the muscles. Under conditions of anaerobic metabolism any increased utilization of glucose by the muscles will cause a concomitant increase in blood lactic acid. In reviewing the blood lactic acid graphs of Butanephrine (Figure 8B) it can be seen that no increased lactacidemia occurs beyond the control. Therefore, it may be assumed that the hyperglycemia produced by Butanephrine in conjunction with the inhibitors is lessened because of a decreased glucose output from the liver.

This seems to indicate a type of adrenergic blockade.

The above interpretation is presuming that blood sugar is the direct cause of blood lactic acid. It is conceivable that the opposite may be true. However, at this point, the receptors of the liver are known to be mainly alpha (26,p. 380) while those of the muscles remain unclear.

Under Isuprel it can be seen from Table VII and Figure 7A that cocaine has no effect on blood sugar above that of the control, while iproniazid had a tendency to cause a slight potentiation. However, with the monoamine oxidase inhibitor ephedrine an effect is seen that is similar to Butanephrine plus ephedrine. And that is, a blockade of the liver receptors. This same effect is

TABLE VII

DIFFERENCES BETWEEN INITIAL AND FINAL BLOOD SUGARS OF EPINEPHRINE, BUTANEPHRINE, ISUPREL AND WIN-3046 CONTROLS AND IN CONJUNCTION WITH INHIBITORS*

DRUG	INITIAL SUGAR	FINAL SUGAR	DIFFERENCE	INCREMENT FROM CONTROL
EPI control	67	173	+106	--
EPI+EPH	95	201	+106	0
EPI+COC	87	279	+192	+86
EPI+IPR	68	173	+105	- 1
BUT control	73	222	+149	--
BUT+EPH	90	178	+ 88	-61
BUT+COC	76	202	+126	-23
BUT+IPR	72	188	+116	-33
ISU control	84	183	+ 99	--
ISU+EPH	93	162	+ 69	-30
ISU+COC	84	181	+ 97	- 2
ISU+IPR	76	193	+117	+18
WIN control	90	232	+142	--
WIN+EPH	98	223	+125	-17
WIN+COC	88	264	+176	+34
WIN+IPR	76	247	+171	+29

*Average of three experiments for each drug.

FIGURE 7B

EFFECTS ON BLOOD SUGAR FROM WIN-3046 & WIN-3046 PLUS INHIBITORS

(AVERAGES OF THREE DOGS)

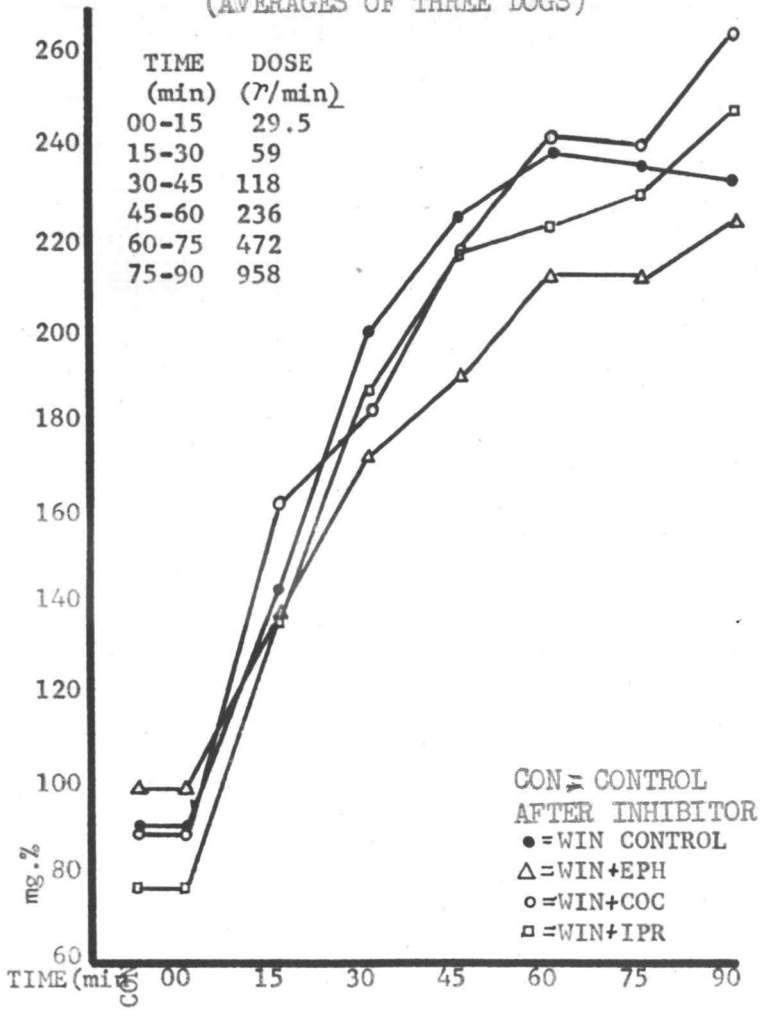
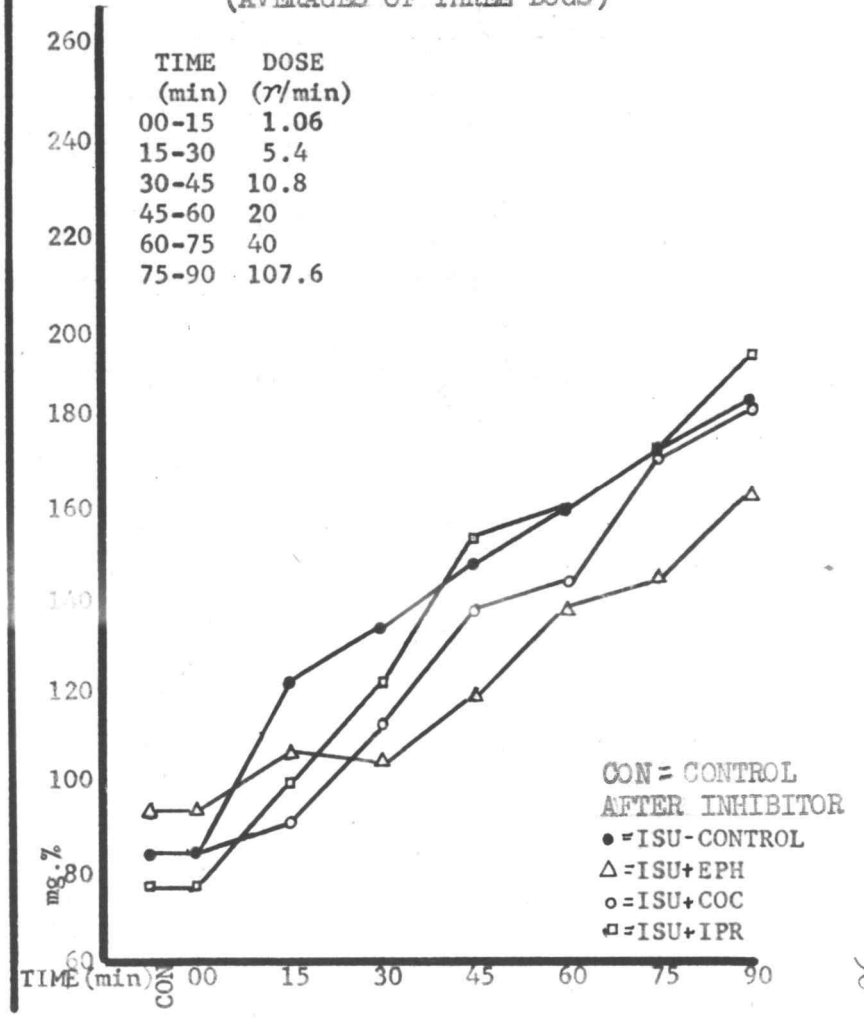


FIGURE 7A

EFFECTS ON BLOOD SUGARS FROM ISUPREL & ISUPREL PLUS INHIBITORS

(AVERAGES OF THREE DOGS)



also seen with Win-3046 in conjunction with ephedrine (Figure 7B). These results seem to indicate that ephedrine possesses an adrenergic blocking effect on blood sugars when used with beta type drugs.

Blood sugar was slightly potentiated by the combination of iproniazid plus Win-3046 and cocaine plus Win-3046.

A summary of the effects on blood sugar may be tabulated as follows:

1. EPI+EPH : X
2. EPI+COC : +++
3. EPI+IPR : 0

1. ISU+EPH : -
2. ISU+COC : 0
3. ISU+IPR : +

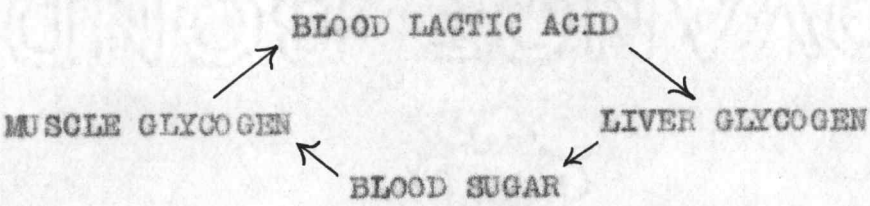
1. BUT+EPH : -
2. BUT+COC : -
3. BUT+IPR : -

1. WIN+EPH : -
2. WIN+COC : +
3. WIN+IPR : +

X : additive effect.
+ : degree of potentiation beyond control.
0 : no effect beyond control.
- : degree of adrenergic blockage.

LACTIC ACID

The metabolism of blood sugar and lactic acid is evidently closely related. The formation and removal of lactic acid from the circulation is shown by the Cori Cycle:



It can be seen that any increase in lactic acid is due to an increase in muscle glycogenolysis or an increase in the formation of blood glucose from the liver.

Under epinephrine, Figures 8A and 6A show that both an increased lactacidemia and a hyperglycemia occur. This may be interpreted as a definite increase in metabolism of the liver and the muscles.

Along with this increased metabolism of the liver and muscles the body is attempting to compensate for the increased acidity by increasing the amount of available oxygen by an increase in respiration. It is known that if oxygen is available to the organism no great increase in lactic acid will ordinarily occur (26,p. 726). Under epinephrine, then, an increased blood sugar and lactic acid occurs while the increased respiration is probably a reflex mechanism due to the increased acidity of the blood.

Epinephrine preceded by ephedrine seems to produce a lesser increase in metabolism than the control. It has been shown that this combination exhibits no great potentiation on blood sugar and, as can be seen from Figure 8A, the degree of increased lactacidemia is 7.24 mg% less than the control. Since glycogenolysis, according to Ahlquist, is an alpha type response to adrenergic stimulation, ephedrine may be considered to have partially blocked this action.

In iproniazid pretreated animals epinephrine produced a similar effect. However, the amount of adrenergic blockade is not as great as that shown with the epinephrine-ephedrine combination. Under the former, an increased lactacidemia occurs which is above that produced by the ephedrine combination but still below the control (Figure 8A) with no effect on blood sugar (Figure 6A) indicating a lesser degree of blockade.

It can be seen from Figure 8A that epinephrine plus cocaine has no greater effect on lactic acid formation than epinephrine alone. The fact that cocaine with epinephrine produces a tremendous hyperglycemia (Figure 6A) may be explained by the fact that cocaine may prevent the muscles from utilizing the available sugar. The sugar then accumulates in the blood. Concomitantly, the muscles are producing lactic acid at a normally increasing rate under the effect of the drug.

TABLE VIII

DIFFERENCES BETWEEN INITIAL AND FINAL BLOOD LACTIC ACID OF EPINEPHRINE, BUTANEPHRINE, ISUPREL AND WIN-3046 CONTROLS AND IN CONJUNCTION WITH INHIBITORS*

DRUG	INITIAL ACID	FINAL ACID	DIFFERENCE	INCREMENT FROM CONTROL
EPI control	6.32	34.63	28.31	-
EPI+EPH	7.93	29.00	21.07	- 7.24
EPI+COC	7.91	34.95	27.04	- 1.27
EPI+IPR	10.91	33.20	22.29	- 6.02
BUT control	8.23	41.00	32.77	-
BUT+EPH	8.35	33.79	25.44	- 7.33
BUT+COC	7.02	37.50	30.08	- 2.69
BUT+IPR	7.18	39.10	31.92	- 0.85
ISU control	9.67	46.25	36.28	-
ISU+EPH	7.16	37.81	30.65	- 5.63
ISU+COC	7.72	37.46	29.74	- 6.54
ISU+IPR	10.71	49.96	39.25	+ 2.97
WIN control	10.65	47.04	36.39	-
WIN+EPH	12.73	43.79	31.06	- 5.33
WIN+COC	7.06	40.43	33.37	- 3.02
WIN+IPR	11.66	36.30	24.64	-11.75

*Average of three experiments for each drug.

When considering Butanephrine it can be seen that the same type of pattern is followed but to a smaller degree. Ephedrine in conjunction with Butanephrine again causes a large change in increment as seen in Table VIII. This effect may be attributed to the same metabolic processes as were shown under the epinephrine plus ephedrine combination. Also, as under the epinephrine-cocaine combination, Butanephrine preceded by cocaine produces a negligible change on lactic acid production. The mechanism, however,

EFFECTS ON LACTIC ACID FROM EPINEPHRINE & EPINEPHRINE PLUS INHIBITORS
(AVERAGES OF THREE DOGS)

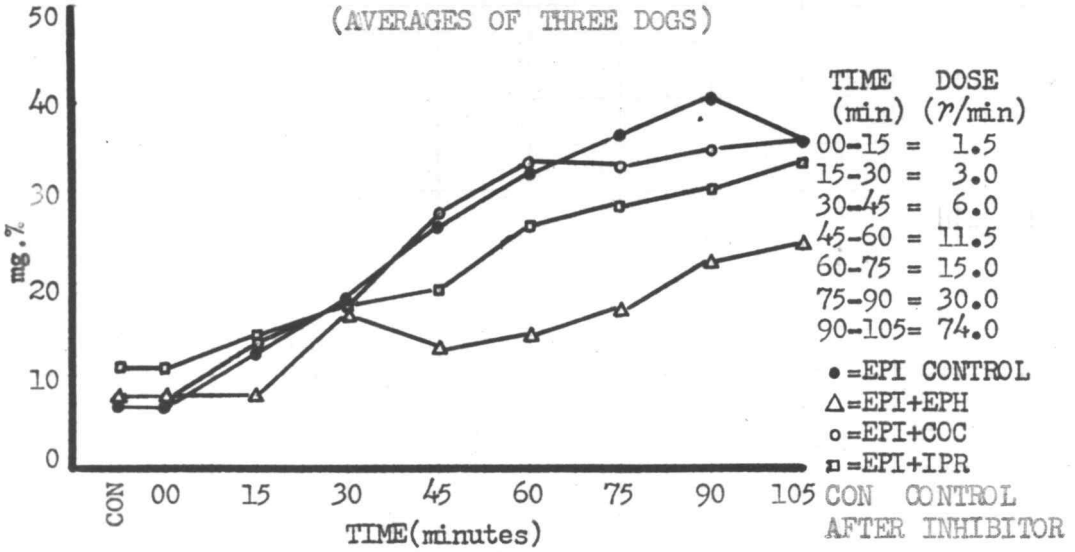


FIGURE 8A

EFFECTS ON LACTIC ACID FROM BUTANEPHRINE & BUTANEPHRINE PLUS INHIBITORS
(AVERAGES OF THREE DOGS)

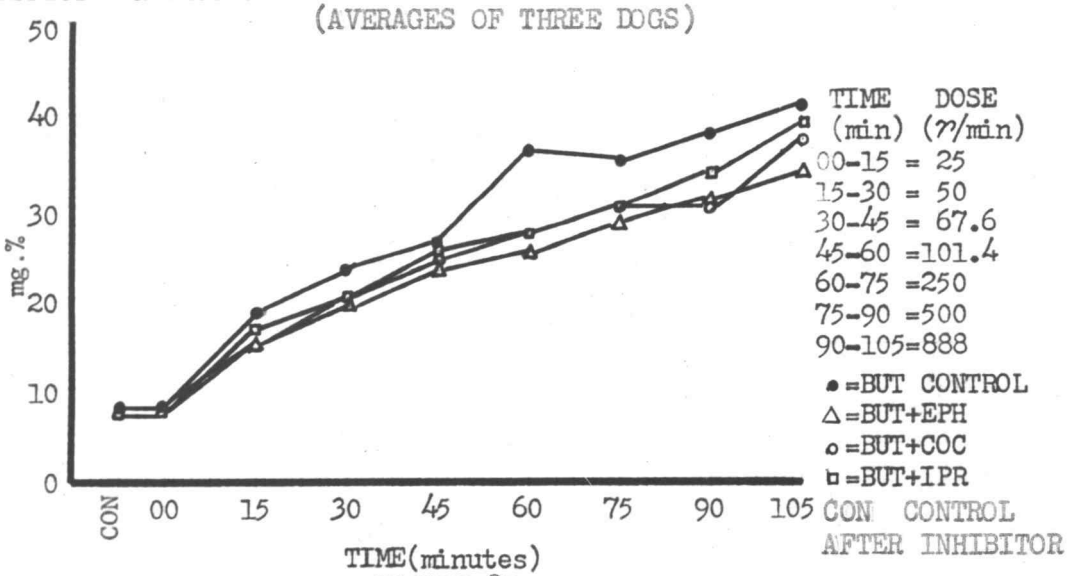


FIGURE 8B

appears to be different from that which occurred under the epinephrine-cocaine combination. With Butanephrine and cocaine it appears that the muscles are able to utilize the available sugar, whereas under epinephrine and cocaine it was stated that the muscles were unable to utilize the sugar. This can be seen from the blood sugar graphs, Figures 6A and 6B, respectively.

The lactic acid production, however, was not augmented in either case by the use of cocaine. Thus, it may be stated that cocaine when used with an alpha type sympathomimetic amine, such as epinephrine, tends to inhibit the muscles from utilizing excess sugar; whereas, under a mild beta type drug, Butanephrine, utilization of blood sugar is not impeded.

It can be seen from Figure 8B that in iproniazid pretreated animals with Butanephrine infusion there is very little effect on lactic acid production beyond the control. It is a steadily increasing state similar to the Butanephrine control. As a consequence, the decreased hyperglycemia seen under Butanephrine plus iproniazid can be attributed to an inhibition of the sugar output from the liver, i.e., a partial adrenergic blockade of the liver receptors.

Isuprel and Win-3046, as stated previously, as considered to be predominantly beta type sympathomimetics. In Table VIII it can be seen that with these two sympathomimetics

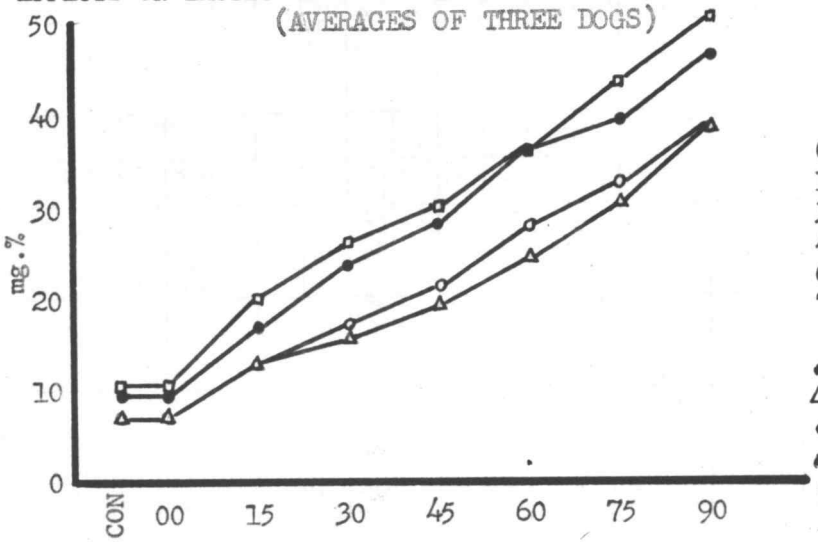
all increments but the Isuprel plus iproniazid are less than the control levels. Isuprel in conjunction with iproniazid is considered to be parallel to the control.

These decreased increments indicate a decreased glycogenolysis in the muscles. When considering the blood sugar graphs, Figures 7A and 7B, ephedrine decreases the hyperglycemia produced by both Isuprel and Win-3046. This is interpreted as a decrease in liver glycogenolysis. This decreased blood sugar by two beta type amines appears to be another case of adrenergic blockade.

When cocaine is used before infusions of Isuprel or Win-3046 a picture is presented that is slightly different than when ephedrine is used. In all four cases the lactic acids are similar in that they are slightly blocked, but the blood sugars are different. The amines in conjunction with cocaine exhibit no change in blood sugar beyond the control. However, when used with ephedrine both Isuprel and Win-3046 show a decreased hyperglycemia. Cocaine then, seems to produce a decrease in the mobilization of muscle glycogen with very little effect on liver glycogenolysis.

As can be seen from the following table the combination of drugs fall into a definite pattern. It should be noted that under all conditions both the lactic acids and blood sugars showed a rise under the action of the amines. When used with the inhibitors a rise in lactic

EFFECTS ON LACTIC ACID FROM ISUPREL & ISUPREL PLUS INHIBITORS
(AVERAGES OF THREE DOGS)

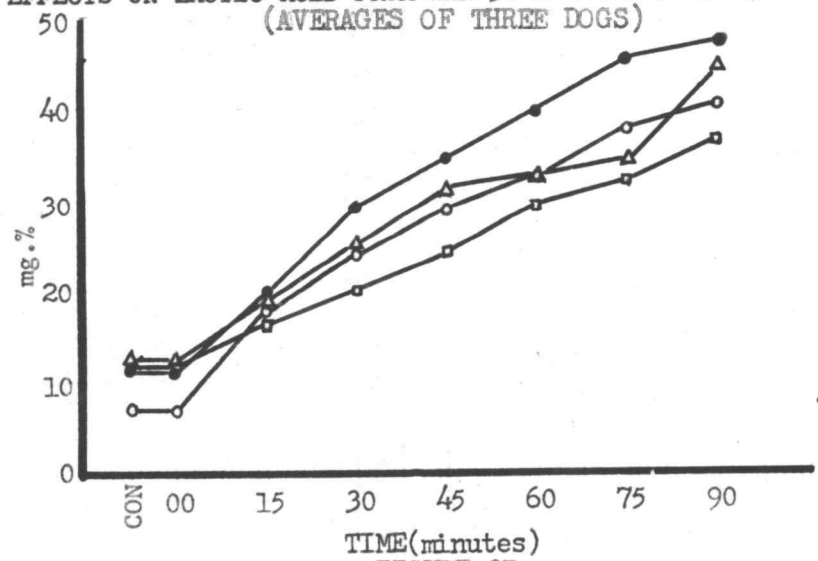


TIME (min)	DOSE (γ/min)
00-15 =	1.06
15-30 =	5.4
30-45 =	10.4
45-60 =	20.0
60-75 =	40.0
75-90 =	107.6

●=ISU CONTROL
△=ISU+EPH
○=ISU+COC
□=ISU+IPR
CON= CONTROL
AFTER INHIBITOR

FIGURE 9A

EFFECTS ON LACTIC ACID FROM WIN-3046 & WIN-3046 PLUS INHIBITORS
(AVERAGES OF THREE DOGS)



TIME (min)	DOSE (γ/min)
00-15 =	29.5
15-30 =	59
30-45 =	118
45-60 =	236
60-75 =	472
75-90 =	958

●=WIN CONTROL
△=WIN+EPH
○=WIN+COC
□=WIN+IPR
CON = CONTROL
AFTER INHIBITOR

FIGURE 9B

acids and blood sugars still took place but to varying degrees from the controls.

TABLE IX

A COMBINED SUMMARY OF INHIBITORS ON THE RISE OF BLOOD SUGAR AND LACTIC ACID

DRUG COMBINATION	LACTIC ACID	BLOOD SUGAR
EPI control	0	0
BUT control	0	0
WIN control	0	0
ISU control	0	0
EPI+EPH	- -	X**
BUT+EPH	- -	-
WIN+EPH	-	-
ISU+EPH	-	-
EPI+COC	0	+++
BUT+COC	-*	-
WIN+COC	-*	+*
ISU+COC	-	0
EPI IPR	-	0
BUT IPR	0	-
WIN IPR	- -	+
ISU IPR	+*	+

+ : degree of potentiation beyond control.
0 : no effect beyond control.
- : degree of adrenergic blockade.
* : slight action.
**: additive effect.

As can be seen from Table IX the action of ephedrine on beta type drugs decreases both muscle glycogenolysis and liver glycogenolysis. The inhibitory type action on lactic acids by beta type drugs pretreated with ephedrine is also seen with alpha type sympathomimetics, but to a larger extent. That is, as one moves in a direction away from

the more beta type drugs toward the more alpha type the lactic acid production moves from a slight inhibition to a greater inhibition. The blood sugars move in the opposite direction, only at a slower rate.

When cocaine pretreatment is used, beta type drugs act similarly to the alpha type under ephedrine. Thus, there is a decreased muscle glycogenolysis with little change in liver glycogenolysis beyond the control. As the drugs become more alpha in action, when used with cocaine, the muscle glycogen breakdown becomes more like the control curve while the liver glycogen breakdown passes through a stage of slightly decreased glycogenolysis (Butanephrine) to a stage of marked hyperglycemia (epinephrine).

When iproniazid is used as a mono-amine oxidase inhibitor with sympathomimetic amines it can be seen that strong beta type drugs show a potentiated blood sugar. The more alpha type drugs have either no effect beyond the control or a slight inhibitory action. With the exception of a slight increase in lactic acids shown with the Isuprel after iproniazid, the over all effect is a depression of lactic acid formation.

RESPIRATION

The effect on respiration of sympathomimetic amines is that of stimulation. Throughout all the experiments an increased respiration was noted. This increase in respiration is probably brought about through the decreased pH of the blood which is a direct action of increased lactic acid. It has been shown that all the sympathomimetics used in this work increase lactic acid content of the blood.

With all the experiments no greater increase in respiration was noted when the animals were pretreated with a mono-amine oxidase inhibitor than under the control conditions. This indicates that as the enzymes were being inhibited, allowing the sympathomimetic substances to accumulate, there was little change in effect on respiration.

TEMPERATURE

The effects of these drug combinations on temperature is not great. Figures 10A, 10B, 11A and 11B indicate that there is no great variance from the control when the animals were pretreated with the amine oxidase inhibitors.

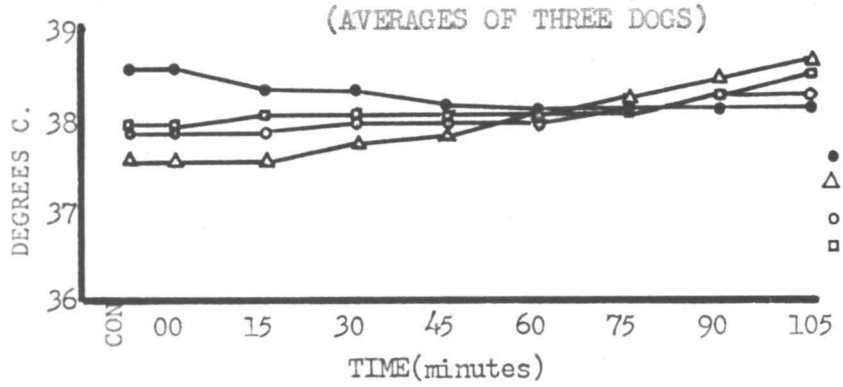
With epinephrine there appears to be a steady state in and around 38° Centigrade. Isuprel, however, tends to cause the temperature to increase with or without the inhibitors. This would indicate that Isuprel caused either an increased metabolism or a vasoconstriction. However, vasoconstriction does not occur with Isuprel and there is no apparent increase in metabolism with or without the inhibitors. The cause of the rise of temperature by Isuprel is obscure and further research is needed to elucidate the cause.

The Butanephrine control, after one hour of infusion, appears to cause a hyperthermia whereas when the inhibitors are used before the infusion the increased temperature did not occur. Since Cameron, et al. (20,p. 326-331) found that Butanephrine was an extremely powerful vasodilator, the hyperthermia that occurs is probably due to an increased metabolism. When the inhibitors were used before the Butanephrine infusion a decrease in both blood sugar and lactic acid production occurred. This decrease in

metabolism may explain the reason why Butanephrine in conjunction with the inhibitors was ineffective in causing a temperature response like the control.

Under Win-3046, it may be considered that the control, the Win-3046 plus cocaine and the Win-3046 plus iproniazid did not effect temperature. However, when ephedrine is used before the infusion of Win-3046 an increased temperature occurs. This hyperthermia is probably due to a combination of vasoconstriction and an increased metabolism caused by the drug combination.

EFFECTS ON TEMPERATURE FROM EPINEPHRINE & EPINEPHRINE PLUS INHIBITORS
(AVERAGES OF THREE DOGS)

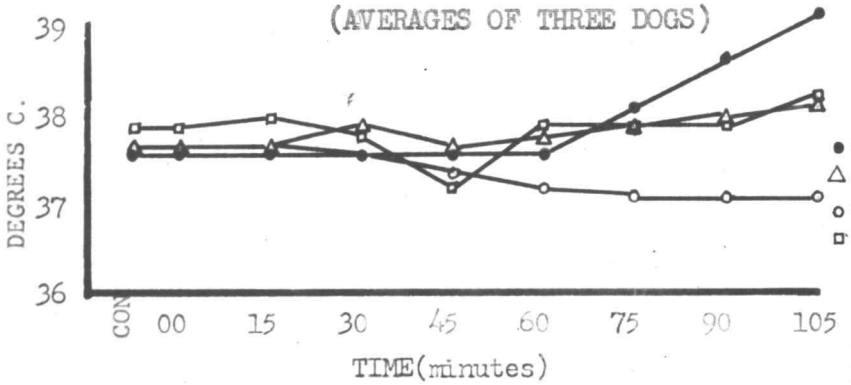


● =EPI CONTROL
△ =EPI+EPH
o =EPI+COC
□ =EPI+IPR

TIME (min)	DOSE (r/min)
00-15	1.5
15-30	3.0
30-45	6.0
45-60	11.5
60-75	15.0
75-90	30.0
90-105	74.0

FIGURE 10A

EFFECTS ON TEMPERATURE FROM BUTANEPHRINE & BUTANEPHRINE PLUS INHIBITORS
(AVERAGES OF THREE DOGS)

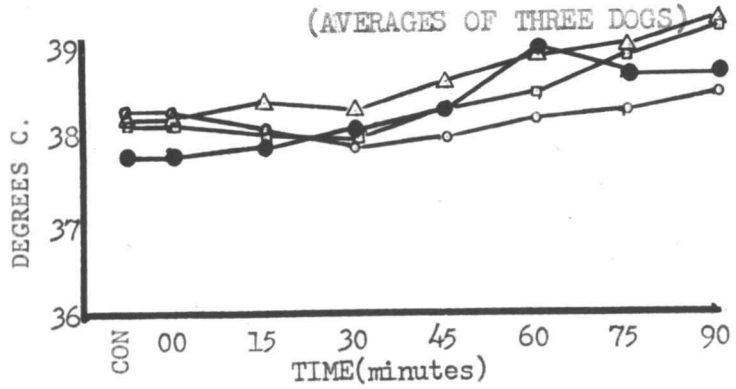


● =BUT CONTROL
△ =BUT+EPH
o =BUT+COC
□ =BUT+IPR

TIME (min)	DOSE (r/min)
00-15	25
15-30	50
30-45	67.6
45-60	101.4
60-75	250
75-90	500
90-105	888

FIGURE 10B

EFFECTS ON TEMPERATURE FROM ISUPREL & ISUPREL PLUS INHIBITORS
(AVERAGES OF THREE DOGS)

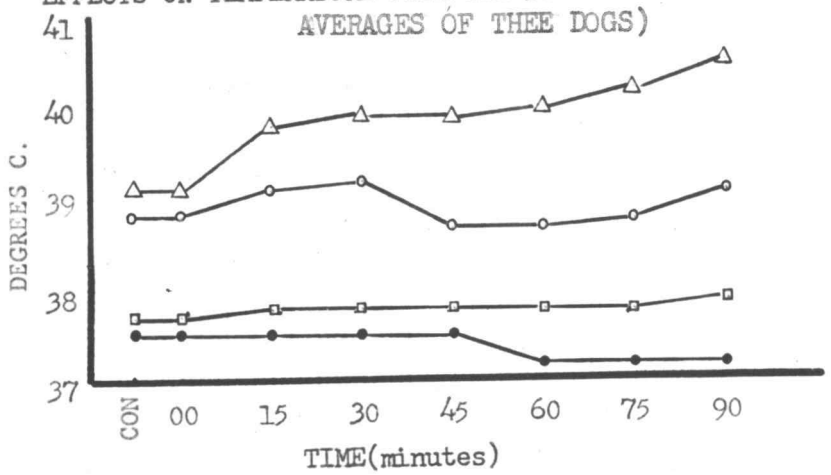


●=ISU CONTROL
△=ISU+EPH
○=ISU+COC
□=ISU+IPR

TIME (min)	DOSE (γ/min)
00-15	1.06
15-30	5.4
30-45	10.8
45-60	20.0
60-75	40.0
75-90	107.6

FIGURE 11A

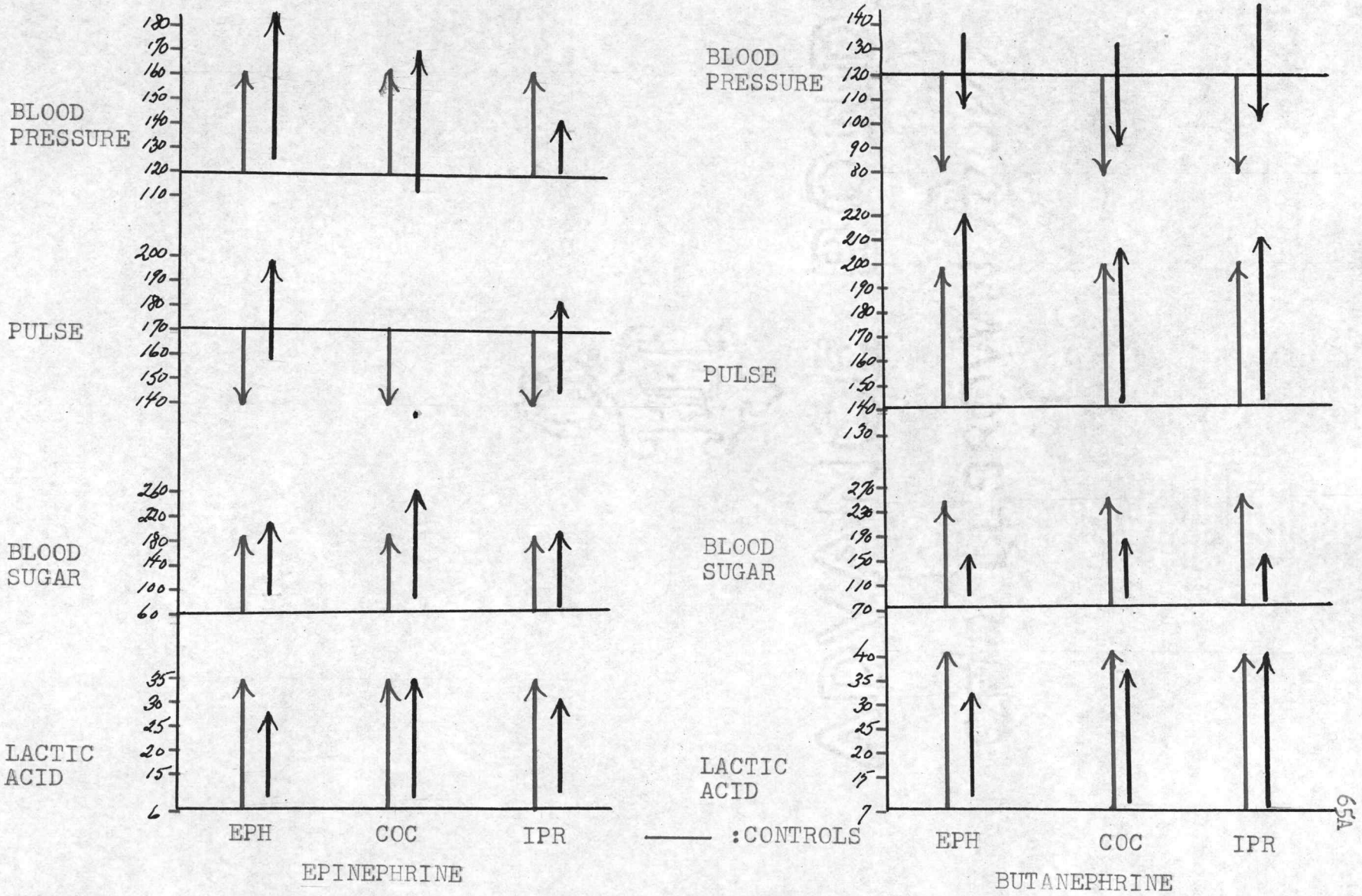
EFFECTS ON TEMPERATURE FROM WIN-3046 & WIN-3046 PLUS INHIBITORS
AVERAGES OF THEE DOGS)

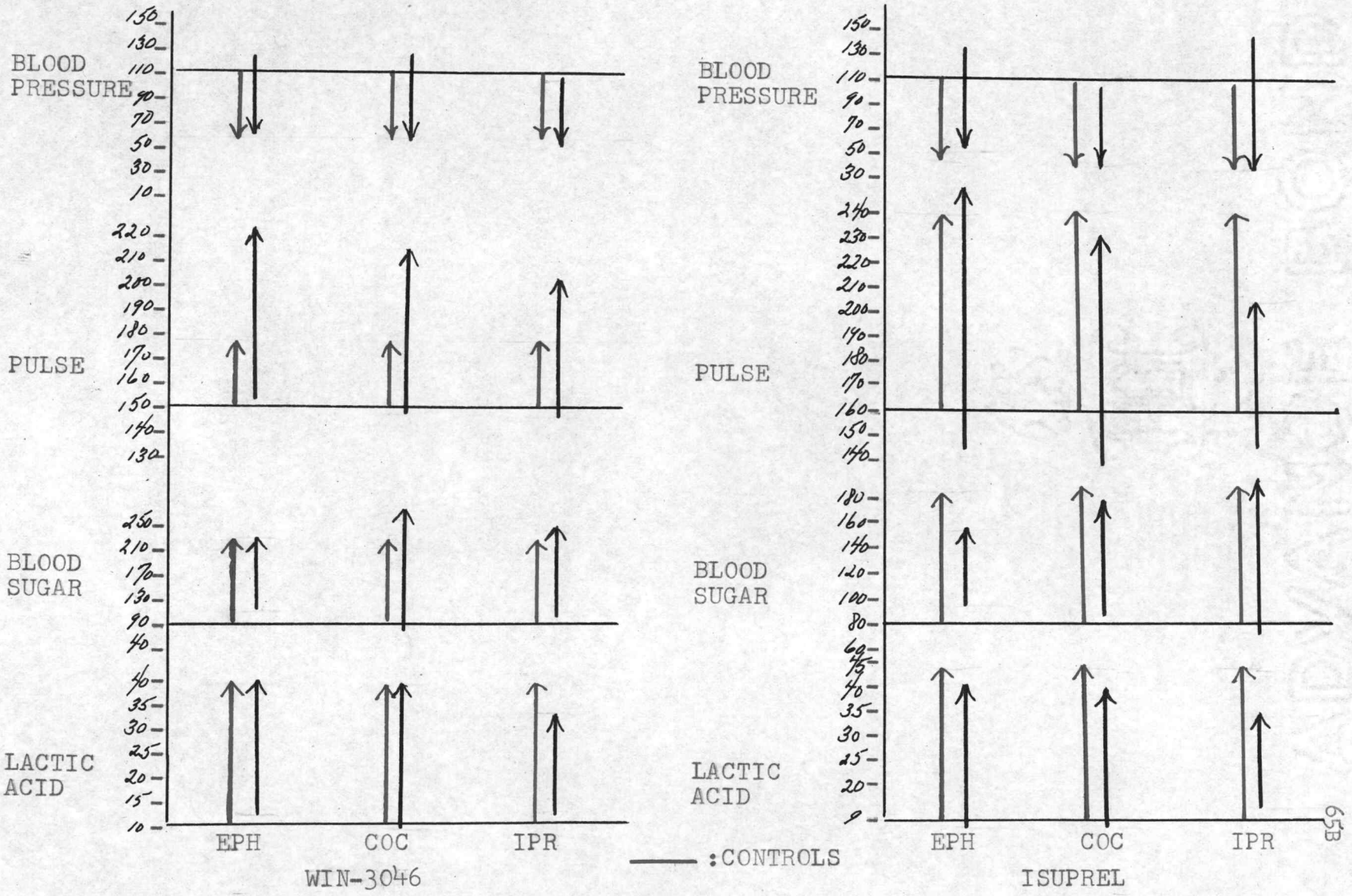


●=WIN CONTROL
△=WIN+EPH
○=WIN+COC
□=WIN+IPR

TIME (min)	DOSE (γ/min)
00-15	29.5
15-30	59
30-45	118
45-60	236
60-75	472
75-90	958

FIGURE 11B


BLOOD PRESSURE
180
170
160
150
140
130
120
110
PULSE
200
190
180
170
160
150
140
BLOOD SUGAR
260
220
180
140
100
60
LACTIC ACID
35
30
25
20
15
6
EPH
COC
IPR
EPINEPHRINE
BLOOD PRESSURE
140
130
120
110
100
90
80
PULSE
220
210
200
190
180
170
160
150
140
130
BLOOD SUGAR
270
230
190
150
110
70
LACTIC ACID
40
35
30
25
20
15
7
EPH
COC
IPR
BUTANEPHRINE
——— :CONTROLS


BLOOD PRESSURE
150
130
110
90
70
50
30
10
PULSE
220
210
200
190
180
170
160
150
140
130
BLOOD SUGAR
250
210
170
130
90
LACTIC ACID
40
45
40
35
30
25
20
15
10
EPH
COC
IPR
WIN-3046
BLOOD PRESSURE
150
130
110
90
70
50
30
PULSE
240
230
220
210
200
140
180
170
160
150
140
BLOOD SUGAR
180
160
140
120
100
80
60
LACTIC ACID
45
40
35
30
25
20
0
EPH
COC
IPR
ISUPREL
——:CONTROLS

SUMMARY AND CONCLUSION

The preceding graphs on pages 64 and 65 summarize the relationship between the experimental values and those of the controls for all of the experiments. The control values represent the control curves which are the results of infusions of the sympathomimetic amines without the inhibitors. Each is the average of three experiments.

1. When epinephrine is infused after ephedrine it is concluded that no inhibition of mono-amine oxidase takes place. This follows from the fact that even though there is a potentiation of blood pressure and pulse under these conditions, there is no increase in blood sugar and lactic acid which would probably occur if the enzyme were inhibited.

It is concluded that some inhibition of mono-amine oxidase may occur when epinephrine is infused into an animal pretreated with cocaine. This is reasoned from the fact that not only are the blood pressure and pulse augmented but also there is a rise in blood glucose while the muscles remain at the control level of acid output.

It is also concluded that when epinephrine is infused after iproniazid some type of adrenergic blockade occurs. This is apparent from the lessened blood pressure rise and concomitant pulse increase and

a decreased lactic acid output in the face of a blood sugar equal to the control level.

2. When ephedrine is used prior to the infusion of Butanephrine two mechanism occur. 1) An adrenergic blockade causes a decreased hypotension and a decreased rise of blood sugar and lactic acid. 2) Stimulation of the chronotropic receptors of the heart stimulates pulse rate.

It is concluded that the adrenergic responses of Butanephrine infusion after cocaine or iproniazid are blocked. Even though Butanephrine alone produced a slight hypotension, when used with the inhibitors the blood pressure does not decrease to the extent of the control even though the pulse remains unchanged. Also, the fact that the blood sugar and lactic acid curves rise to a lesser extent than the control, points to the conclusion that when Butanephrine is infused after treatment with cocaine or iproniazid an adrenergic blockade occurs.

3. After ephedrine, Win-3046 acts similarly to Butanephrine after ephedrine. That is, there appears to be a stimulation of the chronotropic receptors of the heart. It can also be concluded that the decreased lactic acid indicates a reduced ability of the muscles to make use of the slight excess of the glucose in the

circulation. This may be interpreted as a mild degree of mono-amine oxidase inhibition.

Very slight enzymatic inhibition is seen when the combination of Win-3046 and iproniazid are used. It resulted in a slight increase in both blood pressure and blood sugar above the control. Lactic acid production dropped below the control curve indicating the muscles were effected to a lesser extent than the liver.

4. Isuprel infusion after ephedrine pretreatment shows an action very similar to Win-3046 or Butanephrine after ephedrine, only to a milder degree. This action may be due to a stimulation of the chronotropic receptors of the heart and a mild adrenergic blockade.

When cocaine was used before Isuprel no appreciable response occurred beyond the control.

A mono-amine oxidase inhibition appeared to be present in the Isuprel-iproniazid combination. Since the blood pressure decrease was potentiated and the increased pulse rate was partially blocked, it seems reasonable to assume that this is due to an increase in the effect of Isuprel. Potentiation of blood sugar also points toward an enzymatic inhibition by iproniazid.

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