A STUDY OF ADRENALINE VASODILATATION IN SKELETAL MUSCLE

A Thesis

Presented to

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by

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ABSTRACT

Lundholm has proposed that relaxation of smooth muscle by adrenaline is due to an increase of lactic acid resulting from a stimulation of glycogenolysis by adrenaline. However, some evidence against Lundholm's theory has been obtained by other workers.

The present work has been undertaken to further investigate the mechanism involved in the relaxation of smooth muscle of the blood vessels. It was made possible by the discovery of a new blocking agent of adrenergic inhibitory actions, 1-(3,4-Dichlorophenyl)-isopropylaminoethanol (Lilly 20522 or DCI).

It was found that this drug blocks effectively the vasodilator response to adrenaline and other sympathomimetic amines at a dose considerably lower than that necessary to block vasodilatation by sodium lactate, but does not affect glycogenolysis due to adrenaline. On the basis of these studies, it appears that adrenaline vasodilatation is due to a direct action of adrenaline and that it is unlikely that lactic acid production is the direct cause of relaxation.

ACKNOWLEDGMENTS

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Appreciation is also expressed to the staff of the Department of Pharmacology, whose help and co-operation made this work possible, and especially to Mrs. Anne Swarthager for the typing of the manuscript.

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SECTION I

LITERATURE REVIEW

Since Oliver and Schafer (54) reported on the action of extracts of adrenal medulla on the cardiovascular system in 1895 much attention has been given to the potent vasopressor effect of the catechol hormones. Less work has been done on the vasodepressor effect of adrenaline which was first described by Moore and Purington (48) in 1900. Although this depressor effect can be easily demonstrated in cats and dogs and under certain conditions in man, it has been considered as a phenomenon of lesser importance than the cardiac and vasopressor effects.

Vasodilatation may be considered in the same category of sympathomimetic actions as the relaxant effects on certain other smooth muscles. The musculatures of the gastrointestinal tract and the bronchi are relaxed by adrenaline while the sphincter muscles of the stomach and intestine are contracted as a rule. Its effect on the urogenital system is most variable and complicated, depending on different factors namely, species, dosage and methods used. The relaxing effect on the intestine was under investigation by Ott as early as 1899 (53). It has been known for a long time that low concentration of adrenaline may dilate blood vessels supplying skeletal muscles. The first report of vasodilatation due to adrenaline was that of Bardier and Frenkel (7) in 1899 who observed that suprarenal extracts produced a vasodilatory action on the kidney and spleen. This report has never been confirmed, all other investigators observing only pure constriction. Many other observations have been made in animals as evidence for hypotensive and vasodilatory effects of adrenaline. After the report by Moore and Purington (48), Cannon

and Lyman in 1913 (13), and in 1916 Hoskin and Gunning (36), showed that an increase in blood flow accompanied the decrease in blood pressure. It has been shown since, that adrenaline skeletal muscle dilatation occurs quite consistently and much more markedly in cats and dogs than in rabbits.

In humans, the first observation on dilatation of the muscle bed was made by Grant and Pearson in 1938 (28). By means of plethysmography they found that a single intravenous injection of adrenaline caused a great increase of calf blood flow. In 1946, Allen, Barcroft and Mihoin (2) found a marked transient increase in blood flow in the forearm during the intravenous infusion of adrenaline (10 µg/min). This was followed by a sustained and less marked vasodilatation. The blood pressure, heart rate and vasomotor nervous control were shown not to be factors in the observed increases in flow. They showed that intra-arterial infusion (1-3 µg/min) of adrenaline caused only the transient dilatation. These workers considered this phenomenon to be the result of the direct action of adrenaline on the blood vessels of the limb. Their work was confirmed by Duff and Swan in 1951 (22). The second phase of dilatation during intravenous infusion of adrenaline was at first thought to be mediated by sympathetic nerves (2), but in Whelan's series of experiments (63) intravenous adrenaline caused a sustained increase in blood flow even in the acutely sympathectomized or nerve-blocked forearm as well as in normal forearm. This result indicated that the sustained vasodilatation was independent of the nervous reflex effect. In 1956 Barcroft and Cobbold (5) found a parallelism of

lactic acid formation and the sustained increase in blood flow during intravenous infusion of adrenaline in the human subjects. They thought that lactic acid might be partly responsible for the second phase of vasodilatation, but they gave no definitive evidence for such a causal relationship. As for the marked transient vasodilatation Allen et al. (2) produced evidence to show that it was due to a local action of adrenaline on the muscle vessels, but the basic cause and the precise statement on the causation are still not available (6). Anatomically, vasodilatation was found to be localized in the skeletal muscle, because the skin of the forearm tested was pale and the dilatation is more conspicuous in the forearm than in the hand (6).

Secretion of catechols from the adrenal medulla can also provoke skeletal muscle vasodilatation (14). The secretion of these hormones is induced reflexly under many conditions such as asphyxia — for review, see Celander (14). Measuring adrenal vein blood, he estimated the output of adrenaline to be 2-3 μg/kg/min as compared to the resting output of 0.1 μg/kg/min. There is controversy on the major changes in the relative proportions of adrenaline and noradrenaline secreted from the medulla. The latter possesses negligible vasodilator properties. In 1949, Holtz and Schumann suggested the selective release of noradrenaline from adrenal medulla as a major importance in circulatory homeostasis during a decreased baroreceptor discharge (9). Their results are not in accordance with those of Kaindl and Euler (37) and Euler and Follow (23), who found no selectivity in the hormone secretions. Whether or not

there is some selectivity in the nature of the hormones released, it is important to consider that maximal dilatation of muscle bed vessels is obtainable even when adrenaline represents only 25% of the total catechol amines released (Largy et al. (40)).

Despite the work of Uvnäs and his coworkers (61, 62) which indicates that most if not all the sympathetic vasodilator fibers in the cat are cholinergic there remains the possibility of the existence of adrenergic vasodilators in this outflow which was shown by Barcroft et al. (6) to become activated during the fainting reaction in man.

The functional significance of adrenaline vasodilatation is certainly an important one, but the mechanisms of its effect has not been understood. Previous workers have attempted to explain it by the following mechanisms:

(1) Release of histamine from the liver if adrenaline is given intravenously.

(2) Action of an oxidation product of adrenaline.

(3) Release of certain dilator substances from muscles.

(4) Direct effect of adrenaline on effector cells.

As early as 1926 Burn and Dale (11) had observed that the vasodilator effect following intravenous injection of adrenaline was regularly greater and quicker in onset by some six seconds than that following the intra-arterial injection. They interpreted this difference as an indication that the vasodilator effect was not due to direct effect of adrenaline but to a histamine-like principle liberated from the liver as adrenaline passed through its vessels.

Staub in 1946 (59) also found an increase in the level of plasma histamine during intravenous infusion of 20 µg/min of adrenaline and suggested that this was responsible for muscle dilatation. However, Monger and Whelan (47) were unable to confirm Staub's observations using either intra-arterial or intravenous adrenaline.

Bacq and Heirman (4) have stated that vasodilation may be induced by adrenoxine, a possible oxidation product of adrenaline. They stated that previous oxidation might account for the vasodilating action of adrenaline. However, there is no evidence that adrenaline is oxidised to adrenoxine in vivo, and Furchgott has shown that this mechanism is probably not responsible since adrenaline is effective in decreasing the tone of Dibenamine-treated aortic strips under anaerobic conditions (26, 27).

It has long been known that adrenaline causes the release of lactic acid from skeletal muscle (see e.g., Cori (17)). An extensive series of studies by Lundholm and Holmo-Lundholm (42, 43, 45, 46) led them to conclude that relaxation of smooth muscle by adrenaline is secondary to its glycogenolytic effect. They supported their hypothesis by the following evidence obtained from isolated tracheal muscle, gut, coronary vessels and uterus as well as measurements of skeletal muscle blood flow in the intact animal:

(1) Lactic acid and the relaxant effect are simultaneously produced when adrenaline is added to isolated rabbit gut, guinea pig uterus and bovine tracheal muscle.

(2) Metabolic inhibitors such as copper and fluoride and few others abolish both the relaxing effect and production of

lactic acid.

(3) Subsequent addition of $NaHCO_3$, in other words, neutralization of the formed lactic acid by alkalinisation, weakens the dilator response, while acidity *per se* does not have as much effect as does adrenaline.

(4) Lactic acid itself, when added to the preparation, causes the isolated organ to relax; intra-arterial infusion of lactic acid in equivalent amounts to those released by adrenaline can duplicate the vasodilatation effect produced by adrenaline.

(5) The threshold of adrenaline for lactic acid production, like that for vasodilation, is lower than that for vasoconstriction.

This evidence appears to be very impressive. Their hypothesis is very attractive insofar as it removes a great difficulty in our thinking on this problem. This difficulty is succinctly stated by Celander (14) "It is hard to conceive that the direct effect of l-adrenaline on the smooth muscle cells of muscular blood vessels at a low dosage should be a relaxation while the same substance on the same substrate at a higher dosage would bring about a constriction".

However, none of the evidence presented is direct and the hypothesis is thus based mainly on correlation and on results using a wide variety of factors such as inhibitors which do not have specific actions.

While there is no doubt that adrenaline causes the release of lactic acid from smooth and skeletal muscle (for review see Ellis (24)

), and while no one doubts the importance of lactate as a readily oxidizable substrate during the "alarm reaction" (see e.g., Drury (19)), we felt like many other workers that Lundholm's hypothesis was far from proving a causal relationship between glycogenolysis and smooth muscle relaxation.

There is some body of evidence which tends to disagree with their hypothesis.

(1) Bentley (10) was unable to confirm the parallelism of increased intestinal lactate content and adrenaline relaxation.

(2) If $NaHCO_3$ is used to replace NaCl in the medium instead of being added as an additional salt, no effect on adrenaline relaxation could be noted by Bentley.

(3) Ramos (57) found $NaHCO_3$ to be a stimulant of intestinal contractions when used in the same manner as it was by Lundholm.

(4) The use of copper and fluoride has been criticized by Bentley who showed that these inhibitors have direct stimulant as well as non-specific blocking effects. Copper, for example, inhibited relaxation due to added lactate as well as to adrenaline.

(5) In contrast to Furchgott's observations on aortic strips quoted above, Ramos (57) showed that adrenaline no longer relaxes the intestine under anaerobic conditions. Glycogenolysis should not be decreased anaerobically.

(6) Furchgott showed that adrenaline relaxed rabbit intestinal strips which were depleted by endogenous substrate and then

supplied with non-glycolizable substrate as a source of energy. In rabbit aortic strip relaxation can also be induced by isoproterenol regardless of the substrate added.

In view of the above discrepancies, and especially because of Furchgott's observations, it was felt that a direct effect of adrenaline on the smooth muscle cells should not be discounted as an explanation for the observed vasodilatation.

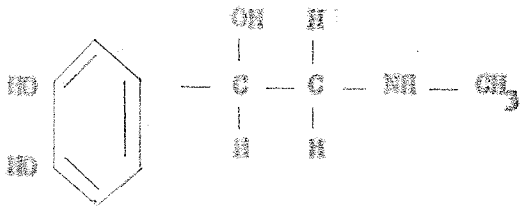
Although the physical, chemical and morphological properties of drug receptors have not been identified, they have been extremely useful as a concept. It has been thought that most drugs act by combining with a certain intra- or extracellular component of cells. Combination with receptors is the primary step and is followed by a chain of reactions resulting in a definite response.

The concept of receptors was first proposed by Langley in 1905 (39). Dale, (18) was the first to use the concept to explain the various actions of adrenaline. Recently Ahlquist classified the reactions to sympathomimetic amines into those involving α-and those involving β-receptors. Each of these receptors may mediate either "excitatory" or "inhibitory" actions depending on the organ in which it is found. β-receptors are assumed to be involved in the relaxation of most smooth muscle such as the skeletal muscle vessels and the bronchi and in cardiac stimulation. This concept of dual receptors was established by observing the sometimes overlapping ability of a series of sympathomimetic amines to cause certain effects.

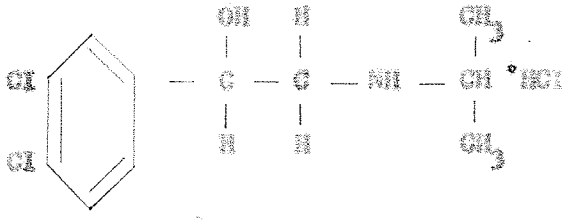
The use of adrenergic blocking agents also has served to dis-

tinguish the various receptor types. Dale (15) in his classical paper had demonstrated the inhibitory action of adrenaline by selectively blocking the excitatory receptor with ergot alkaloids. In general, the adrenergic blocking agents block only the α-receptor responses to adrenaline (see Nickerson's review (51)) and may thus unmask the presence of significant but subordinate β-receptors. Blockade of β-receptors has never been clearly demonstrated, although from time to time certain agents such as ephedrine, PI 38 and butylnorsympathol (15) have been reported to have this effect.

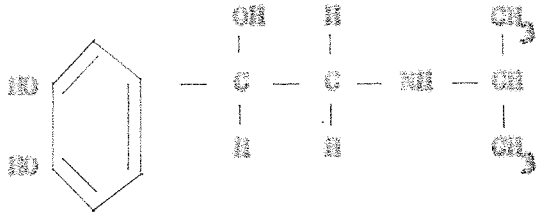
Recently Powell and Slater (56) have reported on a new compound, 1-(3,4-dichlorophenyl)-2-isopropylaminoethanol (DCI, LAlly 20522), a dichloro-analogue of isoproterenol which they showed to block responses to sympathomimetic amines involving β but not those involving α-receptors. Thus adrenaline no longer caused relaxation of bronchi or isolated tracheal chains after DCI while the vasopressor action was potentiated. It is interesting to note the close structural similarity of these compounds.



Adrenaline



Lilly 20522 (DCI)



Isopro-te-renol

The hypothesis was proposed that this compound selectively inhibits the β-receptors with which adrenaline combines to trigger inhibitory responses. The blocking action is most probably competitive.

It is apparent then, that blockade of adrenergic function with DCI closely parallels Ahlquist's original delineation of β-receptors just as blockade with Dibenamine closely paralleled his conception of α-receptors. This work was undertaken in the hope that this parallelism would allow us to test directly the hypothesis of Lundholm concerning the mechanism of adrenaline-induced vasodilatation.

SECTION II

MATERIAL AND METHOD

(1) BLOOD FLOW MEASUREMENT

(2) EXPERIMENTAL PROCEDURE

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(1) BLOOD FLOW MEASUREMENT.

We were mainly interested in the change of blood flow in the skeletal muscles. The hind limb of the cat was used chiefly because it has a large proportion of skeletal muscle, and because many other previous investigators have used the similar preparations.

Selection of an instrument for blood flow measurement became an integral part of this project. There are many methods for measuring peripheral circulation. Most of them measure blood flow indirectly, such as by measuring Na^{23} clearance or volume changes. However, we feel that none are superior to the direct measurement of blood flow by means of photoelectric drop recorders, which actually record the flow of the blood 'drop by drop'. The photoelectric method was originally devised by Clements and Nyberg, 1949 (16) primarily for the measurement of venous outflow (16, 31), and has been widely used by the Scandinavian workers for the past few years. It is useful in anesthetized and heparinized animals, and involves the cannulation of the veins in which the flow is to be recorded. The blood is directed through a plastic tubing to a photoelectric drop recorder which in turn operates on the ordinate recorder on a smoked drum (Fig. 3). Assuming that the size of each drop of blood is uniform, the rate of blood flow is proportional to the number of drops of blood passing the chamber in unit time. It has the advantage of providing a continuous picture of blood flow.

However, the original device has some defects. The important one is that it has an air volume of 3 ml. enclosed in the chamber. The compression and rarefaction of the air in the chamber due to sudden increase or decrease of flow imparts incorrect reading even for venous blood flow recording. The transient error would amount to a few drops of blood. Folkow (25) has reduced this shortcoming by means of a micro-modification, which we used. However, when such a small chamber was used in experiments of long duration, blood that had splashed on the walls of the chamber and dried completely interrupted the photoelectric recording. This constituted the most technically difficult part in our experiments. To remedy this, frequent washing of the interior of the chamber was necessary. However, occlusion of the circulation would induce reactive hyperaemia later. We therefore modified his apparatus to include an additional shunt or bypass (Fig. 1). Consequently, the flow through the chamber could be interrupted at any time without affecting the flow through the limb.

Heat from an incandescent lamp was provided at a constant distance from the body of the animal to keep it warm (about 28°C - 30°C, measured with a thermometer close to the hind limb of the cat), and to keep the blood at a fairly constant temperature during its extracorporeal circulation. In agreement with other workers, we found that vasodilatation in the hind limb of cats could be elicited only when the animals were kept warm (42).

In our measurement of blood flow we were mainly interested in

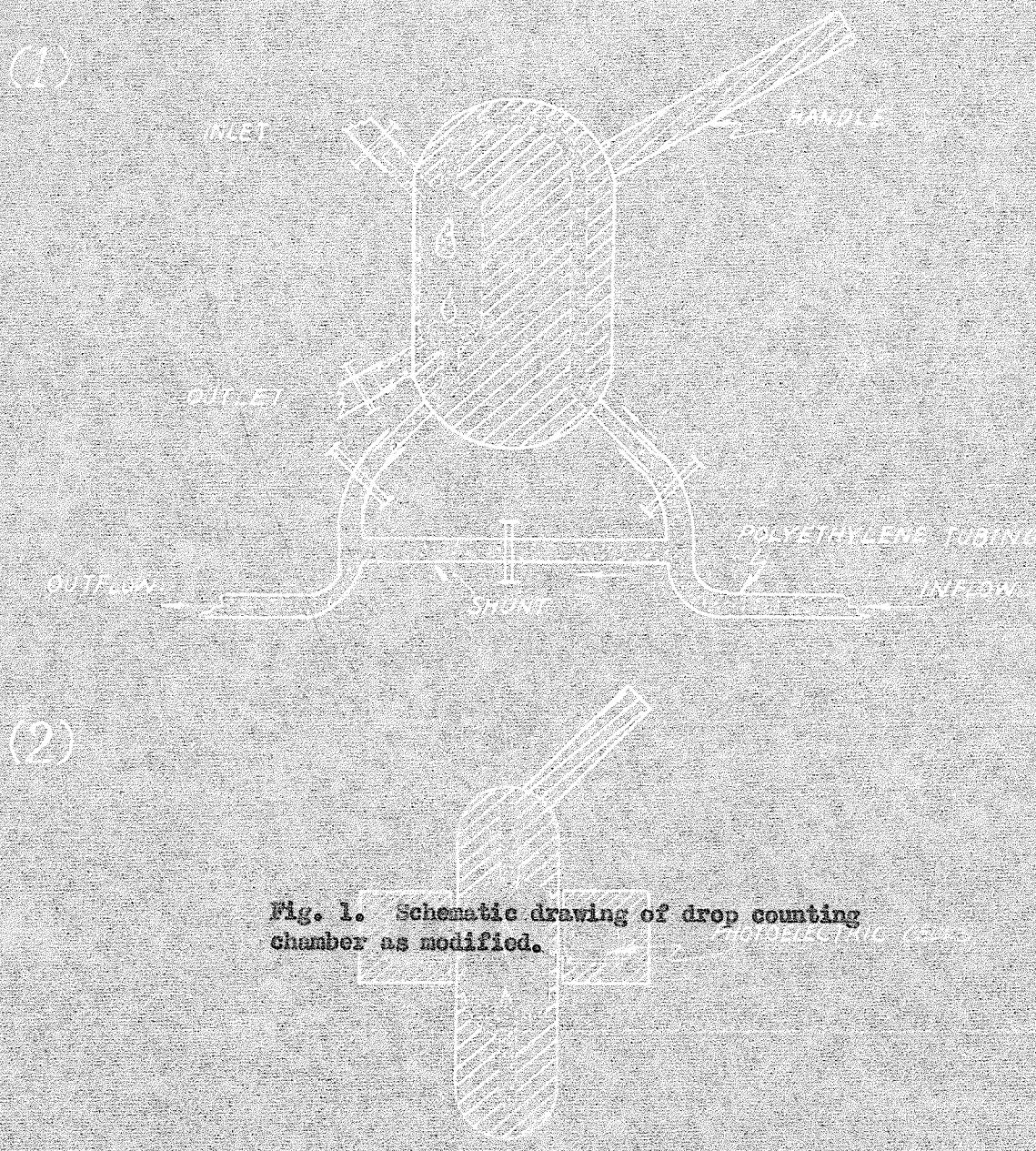
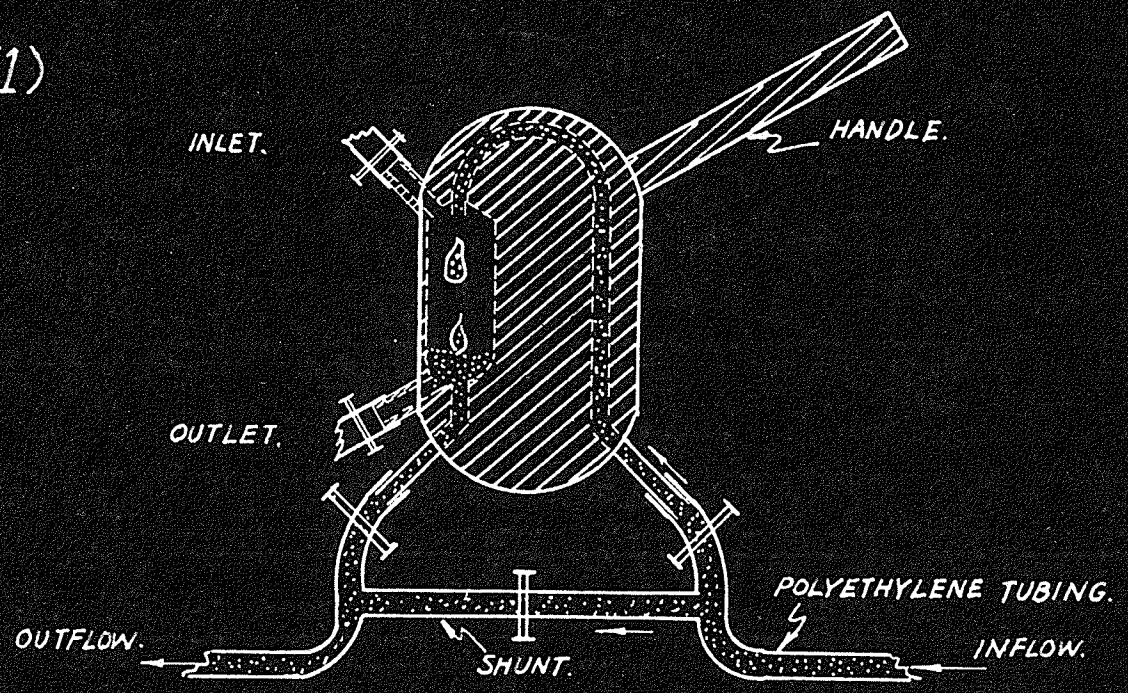
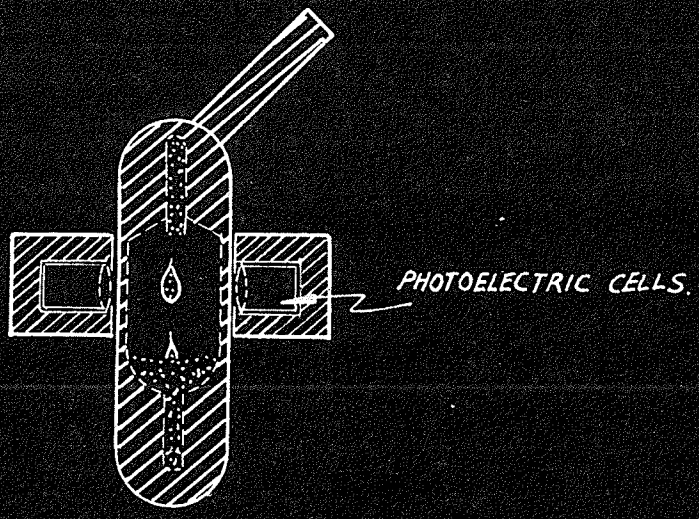


Fig. 1. Schematic drawing of drop counting chamber as modified.

(1)



(2)



SCHEMATIC DRAWING of the DROP CHAMBER.

(1) FRONT VIEW.

(2) SIDE VIEW WITH PHOTOELECTRIC CELLS.

VENOUS BLOOD.

ENCLOSED AIR.

PERSPEX.

the changes of blood flow due to each injection rather than in the actual rates of flow. Since the flowmeter recorded the rate of blood flow in drops per minute, this could serve as a unit of flow as accurately and more conveniently than the unit of ml per minute. The rate of rise of the ordinate recorder could be varied by driving it with a series of synchronous clocks, to suit the rate of femoral venous blood flow, which fell in the range of 20-300 drops per minute. Calibration of the 20 RPM and 40 RPM clocks is shown in Fig. 2. The movement of the ordinate recorder was reversed by interruption of the electrical impulse from the photoelectric cell due to the passage of each drop between it and the light source. The delay before the recorder rose again was 0.1 second and has been corrected for in the results.

The simplest and clearest way of indicating changes after each injection was to express them as percentage of the basal flow. Undoubtedly this method had some drawbacks. For example, the basal level may change from time to time. Therefore, for each injection given a new basal value was taken, in spite of the fact that the variation was not too great in most experiments. As long as this method was used for comparison purposes the author felt that the percentage deviation from basal level fairly well reflected the relative changes due to each injection, especially when the control and the experimental changes were measured from approximately equal basal flow. A plot of percent dilatation due to 0.5 µg adrenaline against the basal flow in 15 experiments showed that there was no

correlation between these parameters.

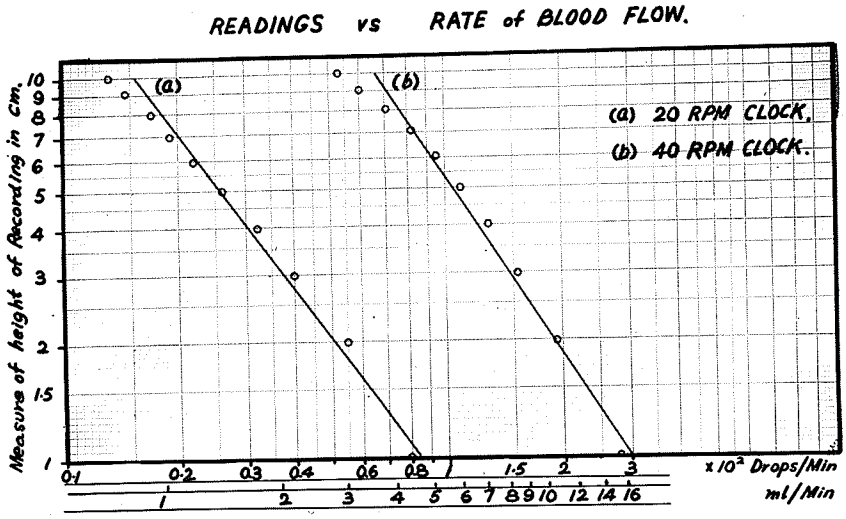


Fig. 2. Calibration curves for the two interval recorder motors.

(2) EXPERIMENTAL PROCEDURE:

Cats of either sex weighing 1.5-4 Kg were used. They were grossly healthy and were not fasting. Anaesthesia was induced with 35 mg/Kg pentobarbital intraperitoneally. This was enough to induce deep anaesthesia in the cats for 4-5 hours. Relatively deep anaesthesia was necessary because spontaneous movement of the extremities otherwise caused substantial variation in blood flow. When necessary, additional doses of 1.25 mg/Kg pentobarbital were administered intravenously during the experiments to maintain uniformity of depth of anaesthesia.

About 15 minutes after the anaesthetic was given the cat was tied by four paws to the table. Both the femoral arteries and the femoral veins were exposed (Fig. 3). The operated area was kept moist by a cotton swab soaked with 0.9% NaCl. 3 mg/Kg of heparin was used intravenously to prevent coagulation and supplemented by 1 mg/Kg every half hour. Systemic arterial blood pressure was recorded continuously from the right femoral artery using a mercury manometer. In case of more than slight bleeding during the surgical procedure, 10-20 ml of 0.9% NaCl was infused slowly intravenously.

On the left side, the femoral vein was cut open. The proximal part was ligated while the distal end was cannulated with a polyethylene tube. The outflow of venous blood was passed through the drop chamber and returned to the body via the proximal end of the cannulated femoral vein of the opposite limb. Blood was returned

through the other limb because this reduced the trauma in the leg in which blood flow was being measured.

All intravenously administered drugs were given into the venous outflow tubing by means of a 27 gauge needle. The rates of injection were such as to prevent major changes of the systemic arterial blood pressure.

For intra-arterial injection of adrenaline, isoproterenol, Na-lactate, nitroglycerine and DGI solutions during the early experiments, a fine cannula was inserted into the greater saphenous artery (Fig. 3, insert 1). However, this method produced complications upon injection of solutions. For instance, adrenaline vasodilatation could not be elicited consistently. Furthermore, in this 'saphenous artery insertion method' small amounts of saline which should not affect the flow were found to increase the blood flow. In order to eliminate this, a T-cannula about 1 cm long was inserted into the femoral artery as illustrated in Fig. 3, insert 2. A fine polyethylene tubing was attached to the side through which solution was injected by means of a 1/4 ml syringe. As a result, little or no change in flow occurred upon injection of saline. This method was adopted by Lundholm in most of his experiments (43).

However, this method still found to be unsatisfactory due to the following factors:

(1) There was a dead space in the fine tubing and the needle. It amounted to 0.2-0.3 ml. and for each injection, double the volume would have to be injected to wash in the solution.

(2) There were interruptions in each injection due to washing of solution into the blood vessel. The interruption was 2-3 seconds in duration. This affected the dilator response.

(3) Extra unnecessary saline solution was used for washing the drug into circulation.

In order to achieve better results a further 'Separate Injection Method' was devised (Fig. 3, insert 3). Three fine polyethylene tubes were attached to the side of the horizontal part of the cannula. Each drug was thus injected through a separate tubing. Usually two drugs were used in an experiment, the third tube being employed for control injection of saline or diluent. The advantages of this method are:

(1) Exact amount of diluted drug could be injected.

(2) There was no dead space.

(3) There was no interruption of injection.

(4) There was no contamination by the previous injection.

For example, saline consistently produced no effect when given immediately before or after the drug.

We found this improved device provided very satisfactory and reproducible results. The local spasm of the vessels and inconsistent recording no longer existed. The device was employed throughout after the preliminary experiments.

The circulation through the paw was interrupted by a tight ligature around the ankle. There were two reasons for doing so:

(1) To eliminate the large proportion of the skin flow of the limb.

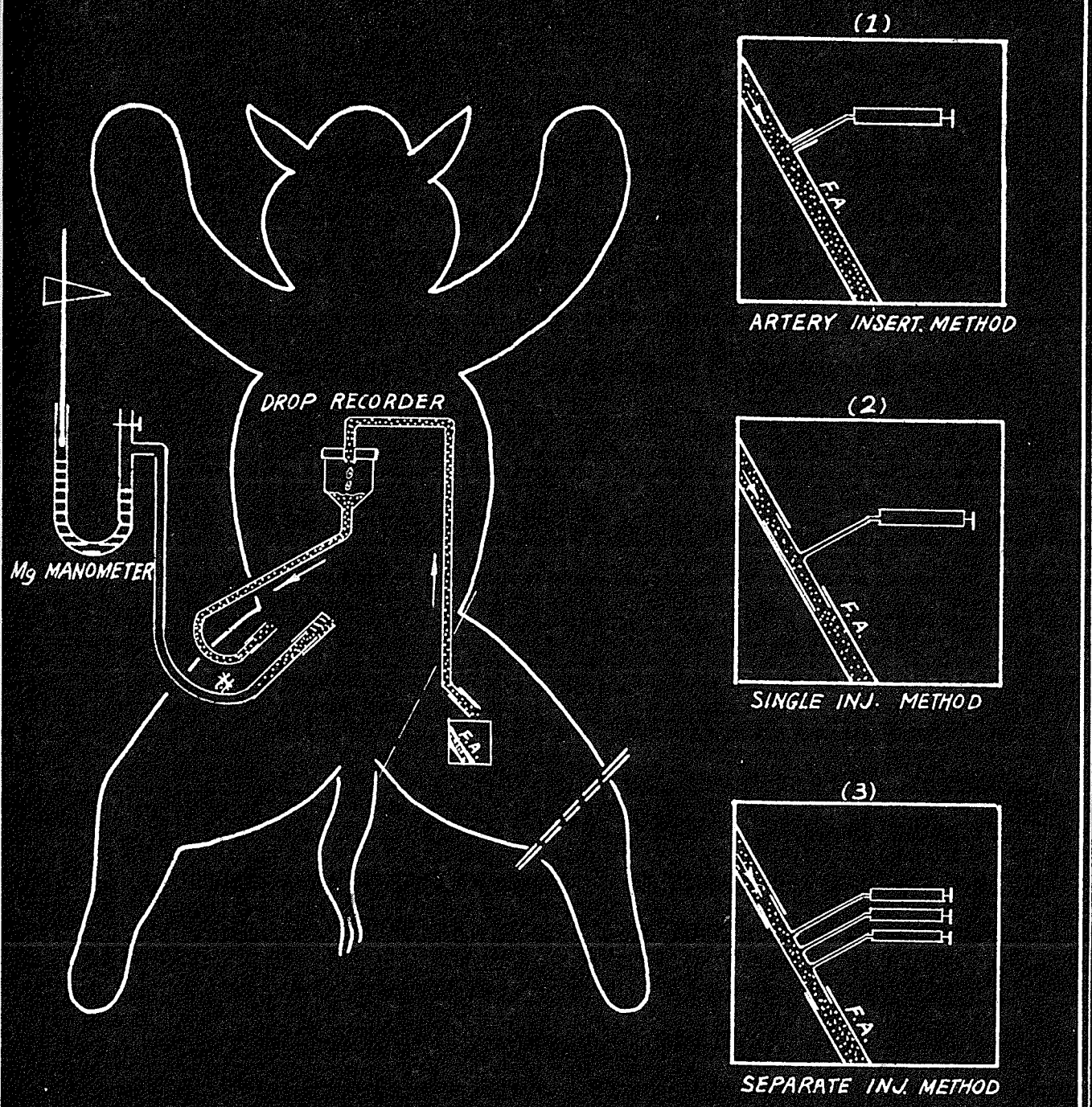


Fig. 3. Schematic drawing of the experimental setup and the methods used for the intra-arterial injection of the drugs.

(2) To avoid a great part of the A-V anastomoses found more abundantly in the paw (14).

The effectiveness of the performance of the tie was tested by insuring that the blood flow through the flow chamber was not affected by applying pressure on the paw.

Skinning of the hind limb has been proposed by some workers, most recently by Lindgren (63) to avoid the constrictor effect of adrenaline on skin blood vessels, but trauma due to skinning would conversely produce rather harmful effects on the experiments. Instead, we decided to use Dibenzyline, an adrenergic "excitatory" blocking agent, in experiments where adrenaline constriction was predominant.

Collateral circulation in the limb presented another problem. In some of the preliminary experiments the basal blood flow gradually decreased. Ligation of the observable channels to the upper part of the limb had been done but this again made for unreliable preparations due to the fact that the surgical procedure lasted too long and thus traumatized the limb excessively. This defect was remedied effectively by applying pressure around the upper thigh with a piece of heavy string. When this procedure was done the basal blood flow varied to a much lesser extent and the experiment could last from 4-6 hours.

The response of the blood vessels to intra-arterial injection was found to be quite inconsistent during the 30 to 45 minutes immediately after the surgical procedure. This instability occurs

frequently after preparation (42). Hence, the animal was always left alone on the table one half to one hour after the surgical procedure until the blood flow became stable.

Dilator drugs were given in doses sufficient to increase the flow usually by about 80-100%. In most of the experiments vasodilator drugs were injected twice for the control, then HCl was administered either intra-arterially or intravenously. The dilator drugs were repeated subsequently in the same order or at random, at approximately five minute intervals. Adrenaline solution was slightly acidified to about pH 6 so as to prevent unduly rapid oxidation. Decomposition of adrenaline could be seen by the appearance of the pink colour in the solution.

Sodium lactate was prepared fresh daily by the addition of an equimolar amount of $NaHCO_3$ solution to a stock solution of Calcium d(+) lactate (obtained from California Foundation for Biochemical Research) and subsequent filtration to remove the precipitated calcium carbonate.

Blood sampling for analysis of lactate and glucose was done as follows: A polyethylene catheter was introduced through the saphenous vein so as to sample the femoral vein blood. Drugs were given in the opposite femoral vein. Blood samples were drawn with a 1 ml. syringe and deproteinized immediately. The assay of blood glucose was done by the Method of Nelson (50) and that for lactic acid by the modified Barker and Summerson Method (8). Where necessary, solutions were freshly made up just before use, and chemical analy-

sis was done immediately after the sample was taken.

In experiments for the determination of the effect of intra-arterial adrenaline, blood samples were taken just below the outflow of the drop chamber. They were drawn at such a rate that the speed of the flow remained unaffected.

SECTION III

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

(1) PRELIMINARY EXPERIMENTS:

In a series of seven cats, the effects of DCI on the blood pressure responses to adrenaline and isoproterenol were investigated. DCI was given slowly (1-2 minutes) so as to avoid any changes in the systemic blood pressure. The effects of the sympathomimetic amines were tested before, and again 15 minutes after DCI injection. Typical experiments are illustrated in Figs. 4 & 5. As shown in Tables 1 and 2, 5 mg/Kg DCI increased the pressor response to 4 µg/Kg adrenaline by an average of 40 mm Hg or 133%, and decreased the depressor response to 0.5 µg/Kg isoproterenol by 42 mm Hg or 65%.

Considering the facts that the observed presser response to adrenaline is a summation of vasoconstriction in areas such as the skin and vasodilatation in the muscle vessels, while the depressor action of isoproterenol is due purely to the vasodilatation in these muscle vessels, it is to be expected that DCI would cause the observed potentiation of the adrenaline vasopressor effect while decreasing the isoproterenol-induced hypotension. When a smaller dose of 0.25 mg/Kg DCI was employed the increase of the pressor response to the same dose of adrenaline amounted to 20 mm Hg or 63% (Fig. 4). It appears that doses of DCI as low as 0.25 mg/Kg could be expected to block quite effectively vasodilator action of some sympathomimetic amines. The above results are in agreement with those of Powell & Slater (56), except that DCI appeared to be much more potent in our hands.

In a control experiment on blood flow responses, 0.4 µg isoproter-

enol and 0.5 ng lactate intra-arterially were given repeatedly to a cat at intervals of 15-20 minutes. Lactate produced an average of 95% increase of blood flow, isoproterenol 147%. Fig. 6 shows that the variation of isoproterenol was approximately 21 ± 8.6% and lactate 18.3 ± 7.4%. The responses fluctuated in intensity and the variations were therefore not due to the gradual reduction of the response of the blood vessels over a period of two hours.

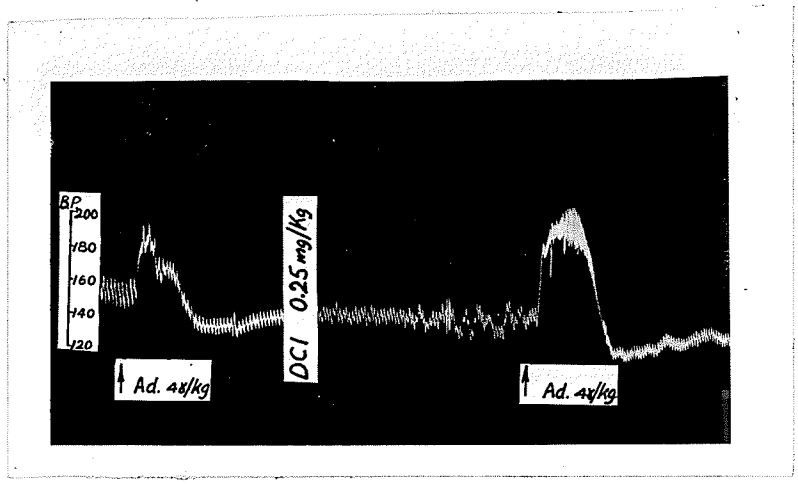


Fig. 4. The effect of DCI on the blood pressure response due to adrenaline. Cat No. 5 (below)
Blood pressure in mm Hg.
All injections were given intravenously.

TABLE 1.

Effect of DCI on blood pressure response to adrenaline.

Cats No:	B.P. Increase due to Adrenaline				Potentiation	
	Before DCI		15 mins after DCI			
	Response	Increase	Response	Increase	mm Hg	per cent
1.	B.122 A.154	32	B.106 A.163	57	25	78
2.	B.162 A.202	40	B.100 A.198	98	58	145
3.	B.162 A.185	23	B.138 A.200	62	39	170
4.	B.130 A.154	24	B.133 A.198	64	39	170
*5.	B.156 A.188	32	B.142 A.194	52	20	63
Ave. (1-4)		30		70	40	136%

Adrenaline-4γ/kg IV.
DCI- 5mg/kg IV.
*Only 0.25 mg/kg of DCI used
Blood pressure in mm Hg. B. before
A. at peak

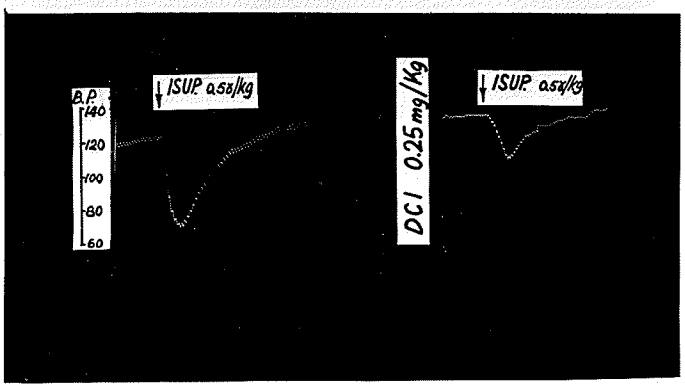


Fig. 5. The effect of DCI on the blood pressure response due to isoproterenol. Cat No. 2 (below)
Blood pressure in mm Hg.
All injections were given intravenously.

TABLE 2.

Effect of DCI on blood pressure response to Isoproterenol.

Cats No:	B.P. response due to Isoproterenol Before DCI		15 mins after DCI		Decrease in Response	
	Response	Fall	Response	Fall	mm Hg	per cent
1.	B.136 A. 72	-64	B.116 A.110	-16	38	70
2.	B.146 A. 64	-82	B. 86 A. 54	-32	50	61
Ave.		-66		-24	42	65

Isoproterenol- 0.5 µg/kg.
DCI- 5 mg/kg.
B. - before.
A. - at peak.

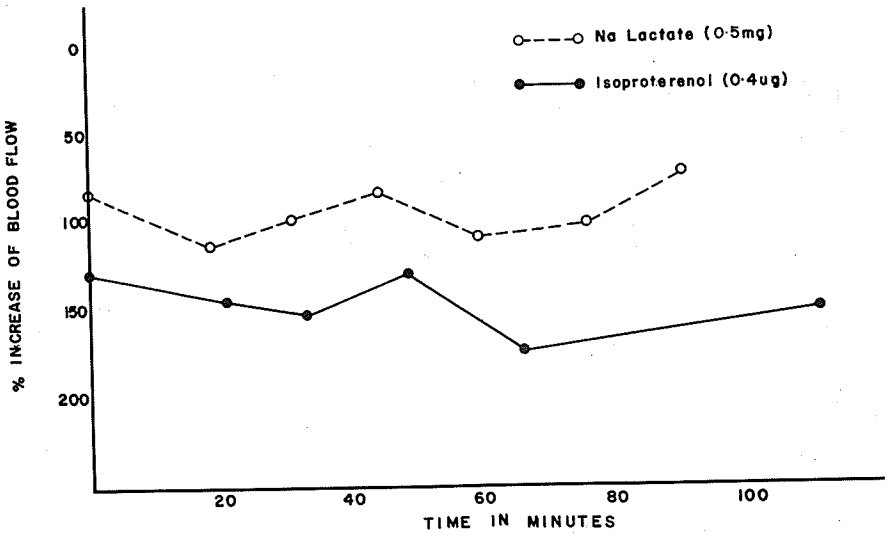


Fig. 6. Blood flow responses to repeated injections of isoproterenol and Na-lactate.

Variations for:

Na-lactate ..... 18.3 ± 7.4%

Isoproterenol ..... 22 ± 8.6%

(2) EFFECT OF INTRA-ARTERIAL INJECTION OF DCI ON BLOOD FLOW RESPONSES TO ADRENALINE, ISOPROTERENOL AND NITROGLYCERINE.

In this series DCI was given intra-arterially in doses of 0.2-1.2 mg. Tables 3 and 4 show the blockade of dilatation induced by adrenaline, isoproterenol and nitroglycerine. The dilatations due to the two sympathomimetic amines were effectively blocked by DCI ($P < .001$ for adrenaline; $P < .05$ for isoproterenol), while nitroglycerine, a general smooth muscle relaxant, was resistant to the blockade. This suggests the specific adrenergic inhibitory blocking activity of DCI. The constrictor responses to isoproterenol seen after DCI might be an artifact because they did not occur later using the improved method described above. It was probable that the local spasm of the vessels due to injection was the cause of this constriction which was quite apparent after the dilatory action was effectively blocked by DCI.

It may be noted in Table 3 that, with the exception of cat No. 10, adrenaline appeared to produce less dilatation at 0.25 µg and 1 µg than 0.5 µg. Analysis of variance shows that there is a statistical significant homogeneous variance between the dosage and vasodilator response. This was probably due to the fact that 0.25 µg was too small to give full dilator effect while the constrictor effect of higher doses of adrenaline (1 µg) overshadowed part of the vasodilatory action of adrenaline.

TABLE 3.

Preliminary experiments showing the blockades of adrenaline Vasodilatation by DCI.

No:	Adrenaline Dose µg	DCI Dose mg	Control Bl. Fl. dp/min	% Inc.	Bl. Fl. Post DCI dp/min	% Inc.
1.	1.0	2	B.29 A.39	56%	B.34 A.20	- 42%
* 2.	1.0	0.6	B.270 A.410	52%	B.230 A.230	0%
3.	0.25	0.3	B.107 A.200	87%	B.98 A.98	0%
4.	0.25	0.3	B.108 A.200	85%	B.125 A.145	16%
5.	0.5	0.2	B.125 A.200	76%	B.125 A.75	-66%
6.	0.5	0.2	B.130 A.290	123%		-31%
7.	0.5	0.3	B.105 A.210	100%	B.33 A.32	37%
8.	0.5	0.3	B.44 A.95	116%	B.44 A.23	-50%
9.	0.5	0.3	B.21 A.38	85%	B.26 A.19	-27%
10.	0.25	0.3	B.48 A.107	123%	B.50 A.64	28%

B. - before; A. - after.
* - modified Ringer's diluent used.
Constriction indicated by % increase in flow.
All injections given intra-arterially.

Statistical analysis: $n = 9$, $P = .001$, $t = 4.781$
$10.5 > 4.781$
$P < .001$

TABLE 4.

Preliminary experiments showing the effect of DCI on Isoproterenol and nitroglycerine vasodilatation.

No:	Drugs dose	DCI dose mg	Control Bl. Fl. dp/min	% Inc.	Bl. Fl. Post DCI dp/min	% Inc.
1.	Isop. 1µg	0.2	B.117 A.200	71%	B. 92 A. 62	- 31%
2.	" " 0.5µg	0.3	B. 55 A.103	88%	B. 55 A. 34	- 38%
3.	" " 0.1µg	0.3	B. 25 A. 78	212%	B. 64 A. 69	8.5%
4.	" " 1µg	0.6	B.260 A.500	93%	B.245 A.245	0%
5.	" " 0.03µg	1.2	B.100 A.189	89%	B.180 A.220	22%
6.	" " 1µg	0.2	B. 47 A.110	134%	B. 32 A. 64	100%
7.	Nitrogly. 0.1mg	0.3	B. 45 A. 67	49%	B. 55 A.100	82%
8.	" " "	0.3	B. 22 A. 59	168%	B. 22 A. 55	150%

B. = before. A. = after.
Constriction indicated by negative % increase in flow.
All injections given intra-arterially.

Statistical analysis: Difference before and after DCI for Isuprel.(Isoproterenol):

$n = 5$, $P = 0.3$, $t = 2.571$

$3.90 > 2.571$

$P < .05$

DCI itself is a vasodilator. When given intra-arterially there was a transient increase of blood flow but the blood pressure remained unaltered. It took almost a minute for the blood flow to return to normal or the basal level. Subsequent injection of either adrenaline or isoproterenol shortly after the blood flow returned to normal caused an increase in flow which appeared to be greater than that which had been observed prior to the treatment with compound DCI (Table 5). The author cannot account for this phenomenon.

TABLE 5.

Data showing the greater increase of blood flow due to injections of adrenaline or isoproterenol immediately after DCI.

Drugs*	% Increase of Bl. Fl. Before DCI	% Increase of Bl. Fl. After DCI	Extra Increase	Time Interval Of Inj. After DCI
1. Adren. 0.3	144%	150%	6%	4 Mins.
2. Isop. 0.03	60%	76%	16%	6 "
3. Adren. 0.5	88%	103%	15%	4 "
4. Isop. 0.125	91%	113%	22%	3 "

DCI - given intra-arterially.
* Dosage in µg.

(3) EFFECT OF LARGE DOSES (5-10 mg/Kg) OF DCI INTRAVENOUSLY ON BLOOD FLOW RESPONSES TO ADRENALINE, ISOPROTERENOL AND LACTATE.

Another series was done using intravenous DCI. Owing to the fact that the drug went into the whole circulation and in view of the work of others, a much higher dosage (5-10 mg/Kg) was employed. The effect on the vasodilatory actions of adrenaline, isoproterenol and lactate were tested. Twenty cats were used in this series. Dibenzyline (1 mg/Kg) was given to 6 cats (out of nine) in which the vasodilator response to small doses of adrenaline had been obscured or greatly reduced by the vasoconstrictor action. After the treatment with Dibenzyline, the constrictor action of adrenaline could not be observed either before or after DCI was given. Fig. 7 shows that the blockade of vasodilatation due to all three of the agonists occurs gradually over a period of 15-30 minutes after the injection of DCI. The duration of the block was greater than two hours. There were three cats in the adrenaline experiments in which the duration of complete blockade exceeded three hours.

Three experiments were done using 10 mg/Kg of DCI in an attempt to block the vasodilator response to nitroglycerine (0.6 mg I.A.). There was considerably more variation in the control responses to this vasodilator than to the other agents used. In one of these three experiments, there occurred great potentiation of the vasodilator response (4-5 fold increase). The other two experiments are shown in Fig. 8. It may be seen that no significant blockade could be demonstrated at this very high dose of DCI.

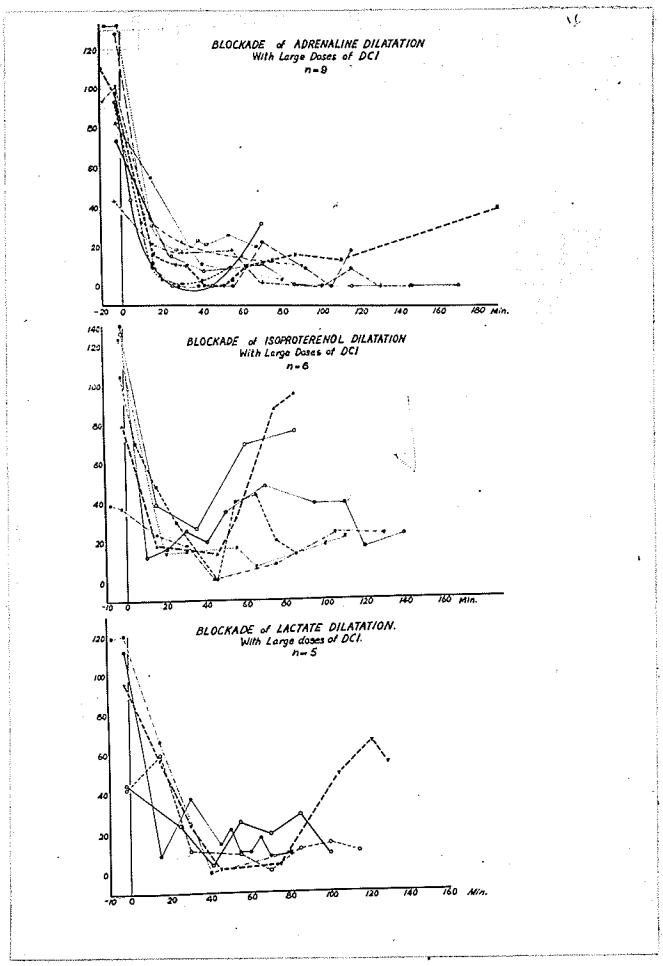


Fig. 7. The blockade of adrenaline, isoproterenol, and lactate vasodilatation by large doses (5-10 mg/kg) of DCI intravenously.

Injection of DCI at zero time.
Ordinates — percentage increase of blood flow.
abscissae — time in minutes after DCI.

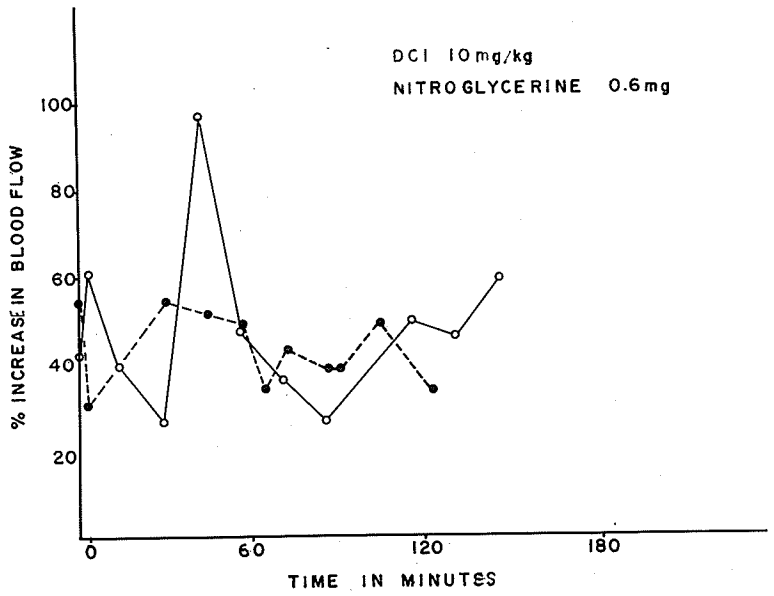


Fig. 8: The effect of intravenous DCI (10 mg/kg) on the vasodilator response to I.A. nitroglycerine in 2 experiments.

(4) EFFECT OF MINIMAL DOSES (0.25-0.5 mg/Kg) OF INTRAVENOUS DCI ON BLOOD FLOW RESPONSES TO ADRENALINE, ISOPROTERENOL AND Na-LACTATE.

DCI was found to be a more potent drug than it appeared from the work of Powell and Slater (56) who found 5 mg/Kg DCI given intravenously had no effect on the pressor response to adrenaline or noradrenaline. However, as we showed in Section 1, 0.25 mg/Kg intravenously may be expected to give some blockade on the inhibitory action of adrenaline.

Accordingly, a series of experiments was done to determine the minimal dose of DCI which would be effective in blocking the vasodilator responses to sympathomimetic amines. In agreement with the single experiment in which blood pressure responses were measured, it was found that doses of 0.25-1.0 mg/Kg of DCI would block vasodilatation induced by isoproterenol and adrenaline.

In each experiment, the responses to either adrenaline or isoproterenol, and the response to lactate were studied before and after intravenous DCI. A typical record is shown in Fig. 9. In each of four experiments, DCI had no blocking effect on lactate vasodilatation, while in the case of the sympathomimetic amines the blockade took place over a period of 10 to 20 minutes (Fig. 10). Further observations on 4 cats used in the experiments described in Section (6) show the same kind of 'differential blockade' (Table 6). In cats No. 2 and 3 (Fig. 10) the response of blood vessels to adrenaline returned to the control levels in the period of one to two hours. This gives some indication of the duration of the blocking action of DCI.

TABLE 6.

Experiments showing the "Differential Blockades" to adrenaline and lactic acid vasodilations by DCI.

Cat No:	Drugs	Dilatation			
		Before DCI		After DCI	
		dp/min	%	dp/min	%
* 1.	Adren. 0.5 µg	B.80 A.150	86%	B.37 A.37	0%
		B.40 A.80	100%	B.37 A.37	0%
	Lact. 0.5 mg	B.72 A.125	74%	B.37 A.64	126%
* 2.	Adren. 0.5 µg	B.32 A.86	173%	B.59 A.68	15%
		B.52 A.113	118%	B.62 A.74	19%
	Lact. 0.5 mg	B.58 A.115	98.5%	B.53 A.105	98.5%
3.	Adren. 0.5 µg	B.62 A.130	110%	B.36 A.38	5.5%
	Lact. 0.5 mg	B.45 A.79	75.5%	B.38 A.72	89.5%
4.	Adren. 0.5 µg	B.50 A.92	81%	B.40 A.40	0%
		B.44 A.72	63%	B.40 A.40	0%
	Lact. 0.5 mg	B.48 A.75	51%	B.41 A.75	82%
5.	Adren. 0.5 µg	B.77 A.139	80%	B.83 A.83	0%
	Lact. 0.5 mg	B.78 A.140	79.5%	B.78 A.140	79.5%
6.	Adren. 0.5 µg	B.55 A.92	68%	B.66 A.74	12%
	Lact. 0.5 mg	B.59 A.92	56%	B.66 A.100	51.6%
** 7.	Adren. 0.5 µg	B.56 A.100	88%	B.48 A.48	0%
	Lact. 0.5 mg	B.55 A.87	58%	B.40 A.80	67%
8.	Adren. 0.25 µg	B.135 A.370	175%	B.110 A.140	0%
	Lact. 0.5 mg	B.135 A.170	26%	B.106 A.154	45%
*** 9.	Isop. 0.25 µg	B.78 A.140	67%	B.78 A.78	0%
	Lact. 0.5 mg	B.78 A.125	64%	B.78 A.130	67%

Continued

TABLE 6. (Cont'd)

Cat No:	Drugs	Dilatation			
		Before DCI		After DCI	
		dp/min	%	dp/min	%
10.	Isop. 0.25 mg	B.40 A.130	230%	B.30 A.30	0%
	Lact. 0.5 mg	B.37 A.125	230%	B.30 A.92	210%
11.	Isop. 0.5 mg	B.55 A.115	111%	B.43 A.51	18.6%
	Lact. 0.5 mg	B.60 A.135	125%	B.44 A.110	137%

* For lactic acid determination see Fig. 9.
DCI = 0.25 mg/kg intravenously.
Adrenaline and Na-lactate given intra-arterially.
** 1 µg/kg DCI intravenously used.
*** Totally 0.5 µg/Kg DCI intravenously used.

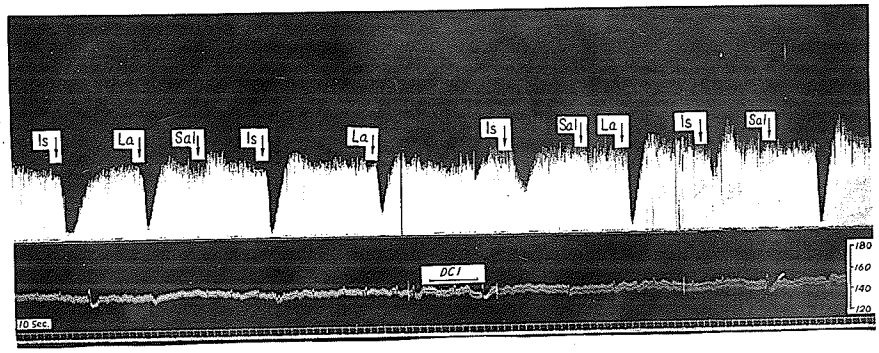


Fig. 9. Reproduction of a record obtained in an experiment described in Section 4. The upper trace is from the ordinate recorder indicating changes in flow, a decrease in the height of the record indicating vasodilatation. The record kymograph was stopped before and after the intravenous injection of DCI. The lower record is blood pressure recorded by means of a mercury manometer.

Is - Isoproterenol
La - Na Lactate
Sal - Saline

The last, unlabeled, response was to Na Lactate.

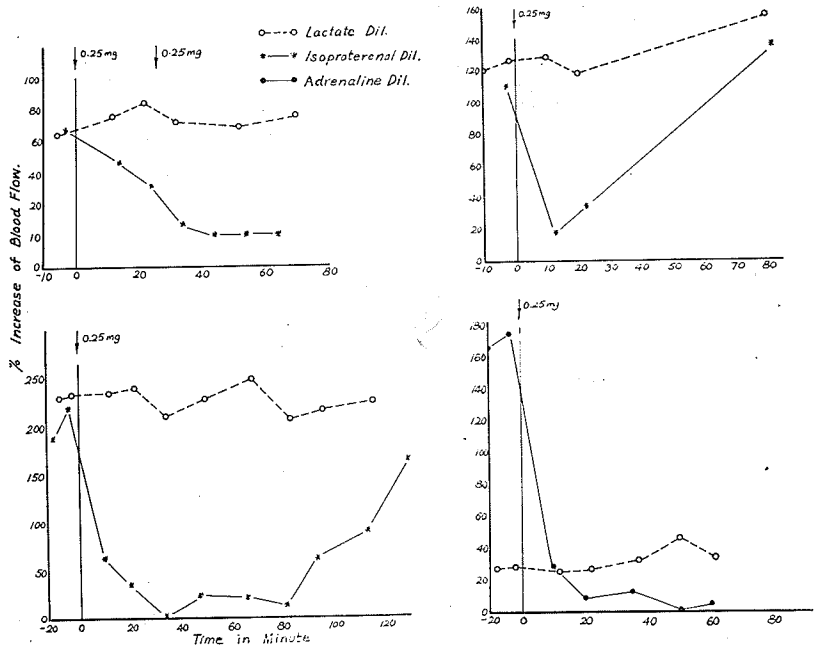


Fig. 10. The effect of minimal doses (0.25-0.5 mg/kg) intravenous HCl on adrenaline, isoproterenol and Na-lactate dilatations.

The arrows indicate the intravenous injection of HCl .
Doses indicated on the graph should be in mg/kg.
Ordinate: percent increase in flow.
Abcissa: time in minutes after the first injection of HCl.

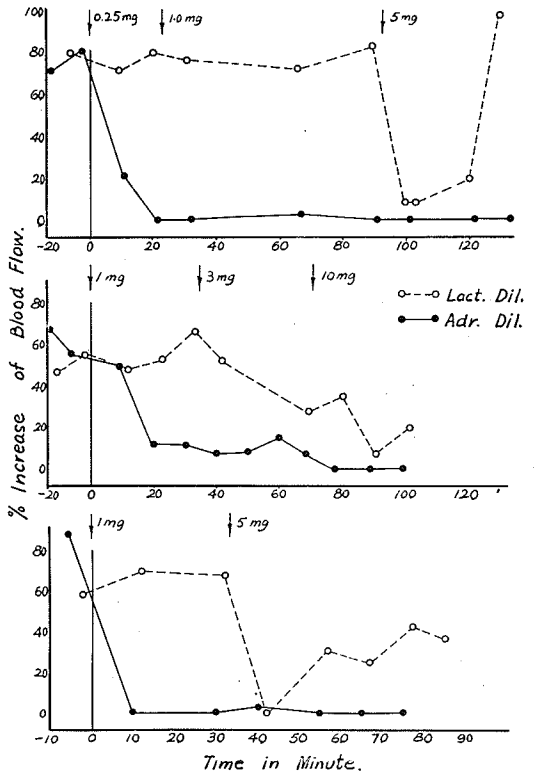


Fig. 11. The effect of stepwise increases in the dose of DCI given intravenously on adrenaline and lactate-induced vasodilatations. See Fig. 10. for other details.

In view of the apparent contrast of these results with those in Section (3), in which large intravenous doses of DCI blocked both adrenaline and lactate vasodilatation, three further experiments were done in which the dose of DCI was increased stepwise. Initially, 0.25 mg/Kg DCI induced a 'differential block' of adrenaline and lactate dilatation. Not until the dose had been increased to a total of 4-5 mg/Kg were both lactate and adrenaline vasodilatations inhibited (Fig. 11).

Since the first and last injections of DCI were separated by not more than 90 minutes (usually only 30 minutes), and the duration of action of DCI has been shown to be great, we feel that there is less error in using the total doses of DCI injected than in designating the blocking dose for lactate as the single dose injected just prior to the appearance of block.

(5) EFFECT OF DCI ON THE METABOLIC ACTIONS OF ADRENALINE.

We have shown that DCI blocked adrenaline-induced vasodilatation at a dose having no effect on lactate-induced dilatation. It is now necessary to determine whether the blockade of adrenaline was due to blockade of its glycogenolytic action.

(a) ON HYPERGLYCAEMIA DUE TO ADRENALINE.

Powell and Slater, 1958 (36) had stated that the mechanism of adrenergic blocking action of DCI was due to the combination of the drug with the β-receptors, and that the drug-receptor complex formed a fairly stable blockade. So far no investigation has been done on the metabolic effect of DCI. On the other hand, blockade of adreno-

line-induced hyperglycemia by α-receptor blocking agents, such as ergot alkaloids was reported as early as 1912 by Mieulicich (44). Later Konrad and Loew (38) echoed Rothlin's view (58) in suggesting that one of the characteristics of a potent adrenergic α-receptor blocking drugs, e.g., ergot alkaloid, was the ability to diminish adrenaline hyperglycemia in certain species. Other adrenergic blocking agents such as benzodioxanes, phenoxyethylamines, yohimbine, priscoline and dibenamine also possess this property. All these agents except ergot alkaloids require much higher doses to suppress the adrenaline-induced hyperglycemia than to block the pressor response, thus demonstrating a low coefficient of correlation between the two actions (32). Thus, the mechanism of α-receptors mediating pressor response and that of adrenaline-induced hyperglycemia cannot be considered to be identical. However, ephedrine, which was considered to be able to block certain adrenergic inhibitory action of adrenaline was also found to diminish the hyperglycemic action of adrenaline (23c). It was conceivable, therefore, that DCI might also block this reaction.

Venous blood from the femoral vein was analyzed for glucose. Figure 12 shows the changes in blood glucose level induced by 4 μg/Kg adrenaline given intravenously before and after 5 mg/Kg DCI. All three experiments indicate that adrenaline hyperglycemia was not inhibited by even high doses of DCI. DCI may be seen to exert some hyperglycemic action.

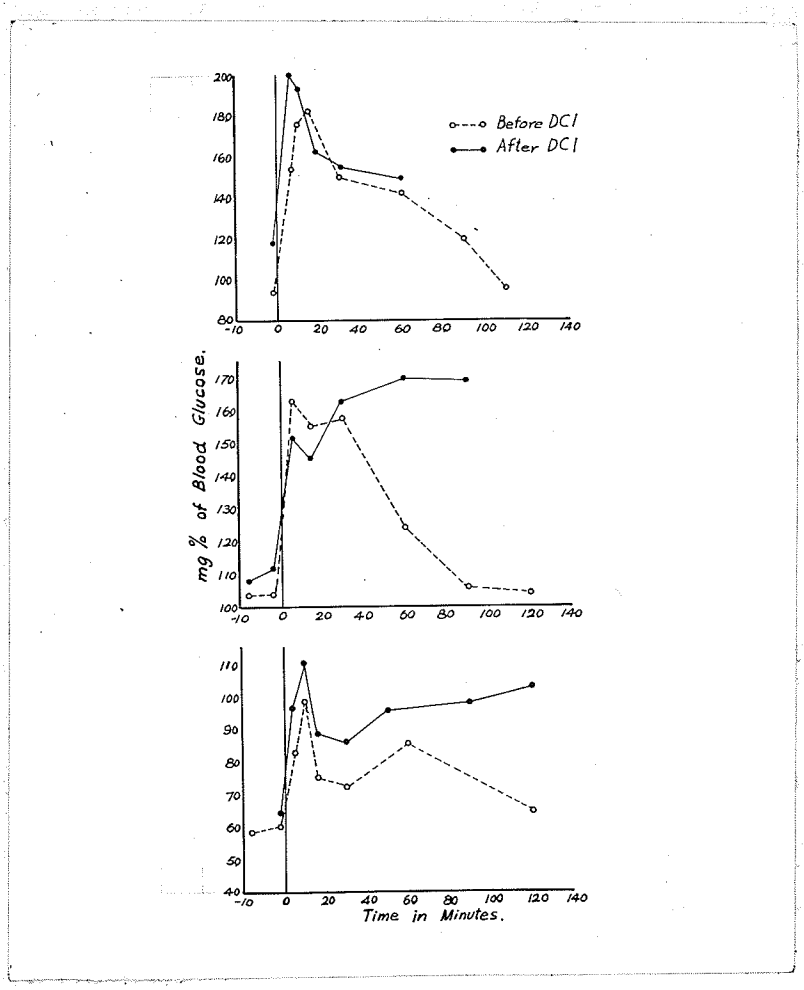


Fig. 12. The effect of 5 mg/kg DCI intravenously on adrenaline-induced hyperglycemia.

Adrenaline was given at zero time.

(b) IN LACTACIDEMIA DUE TO ADRENALINE.

The effect of DCI on adrenaline-induced lactacidemia was also studied. The blood samples for lactic acid determination were taken from the femoral vein. The validity of the method and the accuracy of blood lactate determination were first examined. One half ml containing 30, 60, and 80 mg of lactate was added separately to test tubes containing 4.5 ml of the cat venous blood. Duplicate samples were employed for comparison of results. It was found that the average percentage recovery for the first experiment was 94.5%, while that in the second experiment was 92%. (Table 7) The method, though long and tedious, provided fairly accurate results.

The basal venous blood lactate of the cats varied from 4-30 mg/100 ml (Fig. 13). After adrenaline (4-8 µg/kg) was given intravenously, the blood lactate level began to increase. In every case there was a drop at 20-30 minutes and then a secondary increase. The lactic acid level after DCI was usually higher than that observed prior to the blocking agent.

As shown in Fig. 13, DCI fails to block the lactacidemia due to adrenaline.

(6) EFFECT OF DCI ON LACTACIDEMIA DUE TO INTRA-ARTERIAL INJECTION OF ADRENALINE IN THE LIVER OF THE CAT.

It cannot be ruled out that other tissues contribute partly to the increase of lactic acid in the above experiments. Therefore, four experiments were performed in which blood flow as well as lactic acid concentrations were recorded in the venous effluent

TABLE 7.

Blood lactate recovery as determined by the Method of Parker and Summerson.

Samples	Lactic acid Conc. (mg)	Recovered (mg)	% Recovery
Control Blood	17.0) 11.0) 14.0	- - -	- - -
+30mg	40.7) 42.0) 41.3	27.3	91.0
+60mg	69.0) 78.0) 73.5	59.5	99.1
+80mg	89.0) 90.0) 89.5	75.5	94.3

Average = 94.8%

Samples	Lactic Acid Conc. (mg)	Recovered (mg)	% Recovery
Control Blood	29.0) 31.0) 30.0	- - -	- - -
+30mg	55.0) 50.8) 57.9 68.0)	27.9	93
+60mg	81.2) 87.3) 84.2	54.2	90
+80mg	101.0) 107.0) 102.7 100.0)	73.7	93

Average = 92.0%

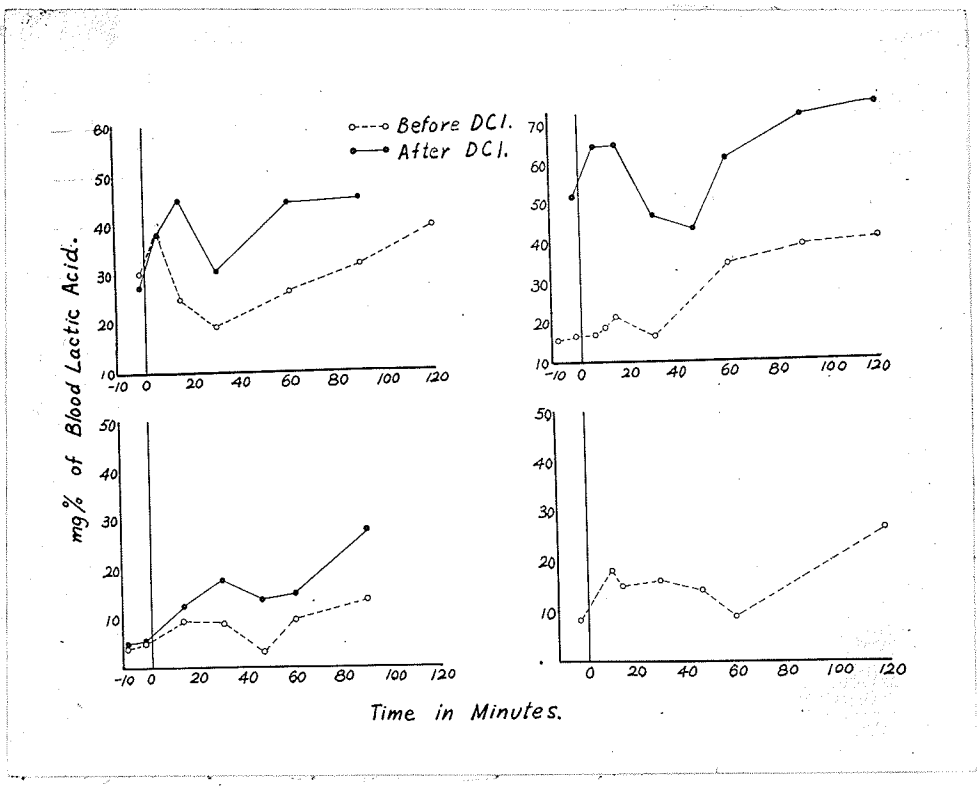


Fig. 13. The effect of 5mg/kg DCI on adrenaline-induced lactacidemia.

All injections were given intravenously.
Adrenaline (4–8µg/kg) was injected at zero time.

after intra-arterial administration of adrenaline. The experimental set up was the same as in Section 4. Blood samples were drawn at about two minute intervals from a polyethylene tubing attached to the outflow of the drop-counting chamber. Adrenaline was injected twice before and twice after DCI. DCI at a dose of 0.25 mg/Kg was employed to block the increase of blood flow due to adrenaline, while not affecting the Na-lactate-induced increase of blood flow. Results of 2 of the 4 experiments are seen in Fig. 14. In these experiments adrenaline did not seem to increase notably the venous blood lactic acid concentration in control experiments.

The increase of blood flow (80-130%) in response to control adrenaline injection may be one of the factors causing the lactic acid concentration to appear unchanged. Similar results were also observed in the three other experiments. After vasodilatation was blocked by DCI, a rise in venous effluent concentration was observed in all cases except one.

Since it is impossible to determine accurately when the blood passing through the vessels during the period of maximal dilatation would have reached the collection point, or whether any peak of lactate production could be determined accurately using this experimental design, it is also impossible to state definitively whether or not, or by how much, lactate production was inhibited by DCI. However, the fact that lactate concentration rose after blockade of vasodilation, i.e., during conditions of constant flow, indicates strongly that DCI did not block lactate production and lends strong

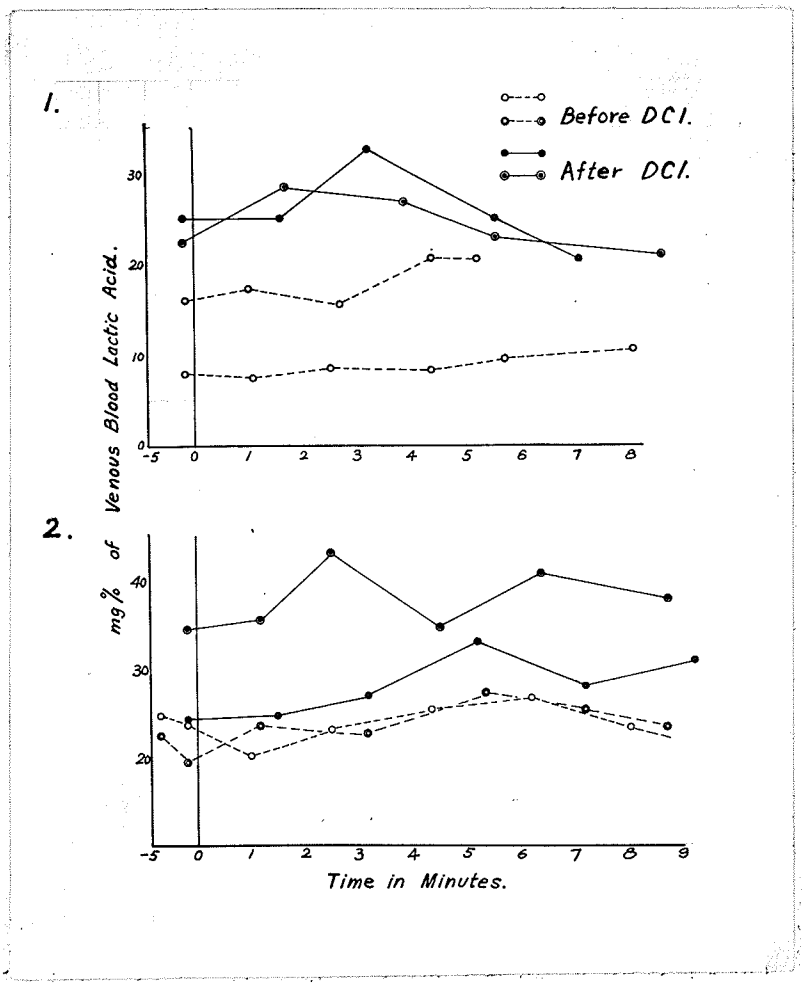


Fig. 14. The effect of DCI on lactic acid level in venous effluent blood after intra-arterial injection of adrenaline.

DCI - 0.23 mg/kg intravenously.
Adrenaline - 0.5 µg intra-arterially at zero time.

The "differential blockades" to adrenaline and lactic acid vasodilation by DCI of the same experiments are shown in Table 7.

support to the conclusion drawn from the results in Section 5 above. It is hoped that future experiments in which the total venous effluent is collected and analyzed will allow a definitive conclusion.

SECTION IV

GENERAL DISCUSSION

The results presented appear to demonstrate that the mechanism of adrenaline dilatation is distinct from that of the dilatation due to lactic acid, thus adding to the evidence against the hypothesis made by Lundholm who had attributed the vasodilator action to the metabolic effect of adrenaline. The validity of the preceeding statement is predicated upon the following analysis of the present and previously reported observations.

Two types of drugs had been utilized, namely two structurally related sympathomimetic amines, adrenaline and isoproterenol, and two general smooth muscle relaxants, nitroglycerine and lactate. Neither of the amines produces tachyphylaxis (Fig. 4), but both possess considerable dilator activity of the blood vessels.

The initial series of experiments, with regard to the effect of DCI on blood pressure response to the sympathomimetic amines in the whole circulation, concur with Powell and Slaters' results except in regard to dosage (56). After the administration of DCI the pressor response to adrenaline is enhanced, and the isoproterenol hypotension is reduced. Moreover, Powell indicates that the pressor responses to noradrenaline and carotid occlusion remain unchanged after DCI. Since noradrenaline, unlike adrenaline and isoproterenol, has negligible or no vasodilator activity, this suggests the selective blockade of the inhibitory sites.

In our regional blood flow experiments the blockade produced by DCI in appropriate doses (0.25-0.5 mg/Kg) has been shown to be specific in suppressing the dilator response of blood vessels to

both adrenaline and isoproterenol (Table 7). The lack of blockade of nitroglycerine and lactate by the same doses of DCI provides further evidence for the specificity of DCI.

Furchgott has shown (26) that adrenaline, noradrenaline and isoproterenol react with the same set of "relaxing" receptors in the smooth muscle of aortic strips, and that these are not inactivated by Dibenamine. It is known that Dibenamine blocks selectively the α-receptors. One must postulate that this smooth muscle must contain β receptors which combine with the sympathomimetic amines so as to trigger a series of reactions leading to the inhibitory response. Structural considerations show that DCI has almost the same chemical structure as isoproterenol except the phenolic hydroxyl groups have been replaced by chlorine groups. Hence, it is highly probable that the sympathomimetic amines tested and DCI all occupy the same receptor sites. Their potency differs from one another. This may be explained either by the intrinsic activity of the compound, or by its affinity to the site, or both - see Ariens (3). It was reported by Powell that DCI has very high affinity for the receptor site but very little intrinsic activity. In our experiments, however, doses of DCI as low as 0.2 mg I.A. were shown to cause vasodilatation. Also it is interesting to note that intra-arterial injections of either adrenaline or isoproterenol shortly (3-6 mins.) after the administration of DCI by the same route, an increase in flow which is greater than that which was observed prior to the treatment with DCI (Section II). However, one must consider that although this was a consistent observation, the additional

increase in blood flow did not exceed the limit of variation as shown in the control experiment (Fig. 3). Considering DCI a competitive type of antagonist, its participation in the interaction implies a relatively decreased frequency of a more effective encounter of the other compound with the receptors.

The present data have shown the specificity and high effectiveness of appropriate doses (0.25-0.5 mg/Kg) of DCI in suppressing the dilator response of blood vessels to both adrenaline and isoproterenol. On the other hand if the dose of DCI is raised by 20-40 times (5-10 mg/Kg) it is also capable of blocking Na-lactate vasodilatation but not that due to nitroglycerine. They suggest that amounts of DCI in excess to those required to "saturate" the β receptors may interrupt the mechanism of lactic acid vasodilatation, perhaps by blocking the "lactic acid inhibitory receptors". The behavior of large doses of a drug should not be mistaken as its major or predominant action. Drugs in multiple quantity of their effective dose often show unpredictable actions quite different from those shown by a minimal effective dose. One such example is the overlapping of α-blockade with β-blockade at doses of classical adrenergic (α-receptor) blocking agents 10-30 times greater than those necessary for maximal reversal of the adrenaline pressor response (29), an observation which should not be considered valid evidence against the postulate that these blocking agents block specifically the α receptors.

It is well known that adrenaline increases the concentration of active phosphorylase in liver and muscle which accelerates glycogen-

olysis and results in an increase of blood glucose and lactic acid (24). The latter comes exclusively from skeletal muscle (46). In this context it is important to note that the differential blockade of sympathomimetic dilatation is not due to blockade of the metabolic actions of these agents. This conclusion is supported mainly by the results in Section (5) showing that changes in venous blood glucose and lactic acid on injection of adrenaline are unaffected by even large doses of DCI. The attempt to determine the influence of DCI given intravenously on the production of lactic acid from a regional muscle bed in response to intra-arterial injections of adrenaline suffers from the lack of precise determination of <u>total</u> lactate output. It is possible that the minimal or nil changes in concentration of lactate in venous effluent blood observed in control experiments are due to changes in flow. The explanation is equally applicable to the slight decrease of lactic acid concentration in the initial transient dilatation due to intra-venous infusion of adrenaline in Barcroft's experiments (5). Hildes (33) had also found no increase in femoral venous lactic acid during the arterial injection of adrenaline.

After DCI, on the other hand, an increase in lactic acid concentrations was observed. While this is not proof that DCI lacks any effect on lactic acid production, it is also not consistent with blockade of such production. It appears to this writer that any possible effect must be a very minor one.

Lundholm (65) had hypothesized that adrenaline-induced lactic

acid production was the cause of dilatation. If this were so, adrenaline should still be able to increase the blood flow even though the β-receptors are blocked by DCI, provided this blockade does not interfere with metabolism of muscle glycogen induced by adrenaline. Since DCI at appropriate doses blocks selectively the β-receptors it seems likely therefore, that the "lactic acid vasodilator receptors" and the sympathomimetic "β-receptors" mediating the vasodilatory responses are quite different. In other words, completely different mechanisms or sites of action are involved in producing a similar effect with adrenaline and lactic acid.

In order to support this concept some explanation of the dual actions of adrenaline on muscle blood vessels must be made. It is known that by whatever route adrenaline injected or infused (0.1 µg I.A. injection or 0.01-0.1 µg/min. I.A. infusion or 10 µg/min. I.V. infusion) a marked but transient increase in blood flow is the first change in cats or in humans. This transient great increase of blood flow lasts for a short time, about one to one and one half minutes according to the present experiments. In higher concentrations a pure constriction results in skeletal muscle vessels as well as in most of the other blood vessels. The problem, therefore, is to explain how the same substance on the same substrate of the smooth muscle can produce relaxation or excitation by varying the dosage. The activity of a drug is a function of many factors such as its "affinity" and "intrinsic activity" (3). In 1956, Stephenson (66) and Nickerson (52)

produced evidence which shows that the maximum effect can be obtained by an agonist when occupying only a small proportion of receptors. This modification has made the Law of Mass Action, with regard to drug receptor interaction, less significant. In addition, as Furchgott had clearly stated in the review (27), "a lack of proportionality would be expected because the receptor-drug interaction is only the first step in complex process leading to the response." The modified concept may be used in the phenomenon of the dual reactions of adrenaline vasodilatation and vaso-constriction. Let us assume that in the skeletal muscle vessels the threshold of adrenaline for β-receptors, which are less numerous than α-receptors, is much lower than the threshold for these α-receptors. Thus, at appropriately low concentrations, the fraction of β-receptors combined with adrenaline is sufficiently greater than the fraction of α-receptors combined with it that vasodilatation results. However, at higher concentrations the fraction of α-receptors reacted with adrenaline increases sufficiently so that even though the threshold of the β-receptor for adrenaline is lower, vasoconstriction results. This assumption of a reaction mechanism represents a possible explanation for the behavior of adrenaline at high and low concentrations.

It is hoped that the present experimental evidence, together with the previous reported observations may serve to clarify some points about adrenaline vasodilatation.

SECTION V

SUMMARY

SUMMARY:

Studies on the mechanism of skeletal muscle vasodilatation revealed the following:

1. Contrary to the report by Lundholm who was unable to induce vasodilatation under this anesthetic, in our series of experiments increase of blood flow due to adrenaline could be produced consistently under pentobarbital anesthesia.

2. 1-(3,4-dichlorophenyl)-isopropylamino ethanol (DCI) causes vasodilatation on intra-arterial injection but blocks vasodilatation due to the subsequent injection of sympathomimetic amines if these are injected ten minutes or longer after DCI; nitroglycerine dilatation is not blocked.

3. The minimal effective dose of DCI intravenously for blockade of the vasodilator responses to intra-arterial adrenaline or isoproterenol approximates 0.25 mg/Kg, while doses of 4-10 mg/Kg are required to block vasodilatation due to Na-lactate, and no significant blockade of nitroglycerine can be shown at the highest dose.

4. DCI at a dose of 5 mg/Kg intravenously does not affect the hyperglycemia or the lactacidemia due to the intravenous injection of 4 µg/Kg of adrenaline.

5. Intravenous injection of a minimal blocking dose of DCI did not appear to block the release of lactic acid into the venous effluent blood after intra-arterial injection of adrenaline.

6. It appears to be a justifiable conclusion that vasodilatation due to adrenaline is not mediated by its glycogenolytic action.

7. A possible explanation has been offered for the fact that adrenaline in small doses dilates these vessels while large doses contract them.

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