OBSERVATIONS ON THE ACTION OF ERGOTAMINE

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HISTORY.

The search for the active principle of ergot was started soon after the introduction of the drug in medicine. Tanret (1875) was the first to crystallise ergotinine in a state of purity, and from the mother liquors of the crystalline alkaloid he obtained an active principle but failed to recognise that it was a separate substance and regarded it as amorphous ergotinine. Later, Kobert (1884) and Jacobi (1897) described the active principle as sphacelinic acid and chrysotoxine. Kobert (1884) indeed distinguished a second active principle - the amorphous alkaloid cornutine - which he claimed had pharmacological activity of a different kind and was very toxic. This preparation was not, however, chemically pure and owed its activity to a single alkaloid, a hydrate of ergotinine. Barger and Carr (1907) first isolated a principle in the form of a crystalline salt, from the caustic liquor from which ergotinine had been extracted, and named it ergotoxine. A little later, Kraft (1906) recognised that the amorphous substance left after crystallising out the ergotinine was not identical with the latter but was a second alkaloid. He showed that it could be formed from ergotinine and could be reconverted into the latter. He called it hydro-ergotinine. Barger and Carr/

Carr (1907) showed by analysis that the two alkaloids, ergotinine and ergotoxine, did differ by a mol. of water. Stoll (1920) discovered a third ergot alkaloid, ergotamine, which crystallised readily and had a physiological activity indistinguishable from that of ergotoxine. This alkaloid could be converted into an inactive isomeride ergotaminine. So four distinct alkaloids of ergot have been found out, viz., ergotinine, ergotoxine (which was isolated as hydrate of ergotinine), ergotamine and ergotaminine. Of the two alkaloids, ergotamine and ergotaminine, the latter had no physiological action. These alkaloids were evidently closely related to ergotinine and ergotoxine. Although the two less soluble ones, i.e. ergotinine and ergotaminine, had very slight physiological activity, they could, however, be changed into the active forms ergotoxine and ergotamine. The various pharmacological effects given by the alkaloids, ergotoxine and ergotamine, were of equal intensity within experimental errors and it was thought by some investigators that they were identical. But physical and chemical evidences showed that the four alkaloids were all quite different and had separate existence.

The poisonous character of ergot was recognised as a result of human suffering rather than of animal experimentation/

experimentation. In the nineteenth century extensive use of animals was made. Kobert (1884) and his pupils used the cock's comb test and also experimented upon other animals but were not very successful with the results of the action of ergot. Jacobi (1897) was equally unsuccessful. The real pharmacology of ergot starts with the work of Dale (1906) on chrysotoxine and later of Barger and Dale (1907) on pure ergotoxine. Dale's work made it clear that ergot and the various impure active preparations which had been made from it contained a principle producing a characteristic and physiologically recognisable effect and this was found to be ergotoxine. Later, when the new alkaloid ergotamine was discovered, Dale and Spiro (1922) showed that ergotoxine and ergotamine had the same pharmacological action and remarked that the isolation of ergotamine did not really change the pharmacology of ergotoxine.

Various methods of assay of ergot alkaloids were made use of, besides the cock's comb method. The rabbit's uterus proved the most satisfactory method. It was noted that ergot abolished the contracting action of adrenaline on the rabbit's uterus and this was taken advantage of by Broom and Clark (1924) and Mendez/

Mendez (1928) who used the rabbit's uterus for quantitative estimation of active principles. Other methods used were the inversion of adrenaline effect on cat's blood pressure and the frog's vessel perfusion.

The present work embodies the observations on the action of ergotamine on the bloodvessels and heart of frogs. Observations have also been made on the action of the drug on the coronary circulation of dogs, using the isolated heart-lung preparation of Starling.

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ACTION OF ERGOTAMINE ON THE BLOODVESSELS OF THE FROG.

LITERATURE.

Kobert (1884) was the first to observe the vaso-constrictor action of ergot on the peripheral arteries. Using sphacelinic acid, he observed the gangrenous phenomenon in the cock's comb and a rise of blood-pressure in the rabbit. Jacobi (1897) got the same results as Kobert on cock's comb with his sphacelotoxine (the active principle of chrysotoxine) which he thought was an important part of the alkaloid. He also observed contractions of the bloodvessels of the rabbit, cat and dog. Dale (1906) found that injection of 20 mgm. of sodium salt of chrysotoxine intravenously into an anaesthetised or pithed cat under artificial respiration with the vagi cut caused peripheral vaso-constriction and a rise in blood pressure. Later Barger and Dale (1907) found that ergotoxine, 0.5 mgm. per Kilo., injected into a pithed cat caused a rise in blood pressure due to stimulation of the plain muscular tissue of the arteries. Again Dixon (1906) observed that although ergot injected intravenously produced constriction of the splanchnic and limb vessels, per-

fusion/

perfusion of ergot through peripheral vessels tended rather to dilate than to constrict them. Dale and Spiro (1922) using ergotamine, the new alkaloid, obtained vasoconstriction following injection of 0.5 to 1 mgm. ergotamine into a decerebrate cat and they further observed no difference in the pharmacological action of ergotoxine and ergotamine. Rothlin (1923) observed an increase of the blood pressure in the rabbit by administration of 0.05 to 0.1 mgm. per Kilo. ergotamine and a decrease by 0.3 to 0.5 mgm. In the cat he observed an increase in blood pressure after 0.1 to 0.5 mgm. ergotamine per Kilo. and a further slight increase by a second dose. Later (1925) he stated that the action of ergotamine on the isolated and artificially perfused vessel had in most cases no constricting effect even in large doses. Ganter (1926) reported that when 0.5 mgm. to 1 mgm. gynergin (ergotamine) was injected intravenously into a cat, the bloodvessels dilated and with larger doses they constricted. He attributed the dilator action of small doses to sympathetic paralysis and the constrictor action of the bigger doses to direct action on the plain muscle of the bloodvessels. Burn and Dale (1926) did not obtain any vasoconstriction by ergotamine in the perfused cat's leg, on the contrary they observed slight/

slight vasodilatation. Heymans and Regniers (1927) observed intense but transient vasodilatation in the head by injecting 0.01 to 0.1 mgm. ergotamine in the perfusion tube. On perfusing the head and limbs of the rabbit with 1/75,000 to 1/85,000 of ergotamine tartrate in Ringer, they observed the same vasodilatation. In dogs and cats the vasodilatation was less constant and less intense and sometimes there was either no action or vasoconstriction instead. Hamet (1931) observed vasodilatation which passed into vasoconstriction on perfusing dogs' hind limbs with ergotamine, but after bilateral adrenalectomy he got vasoconstriction by ergotamine. Heymans, Bouckaert and Moraes (1932) more recently have shown that 0.25 mgm. ergotamine intravenously injected raised the general blood pressure and constricted the vessels of the leg and also those of the head by direct vascular action. But on perfusing the head or limbs of the dog with defibrinated blood, ergotamine tartrate, 0.5 mgm., caused intense vasodilatation. They concluded, therefore, that in the defibrinated blood there were vasotonins which would cause vasoconstriction but after ergotamine the action of the vasotonins would be reversed. They confirmed their observations on hind limbs of the frog in which they noticed that 0.1 cc. defibrinated blood in the perfusing Ringer with a pH of 7.2 caused vasoconstriction, but after 0.1 mgm. ergotamine was injected previously/

previously into the perfusion tube it caused vasodilatation. Using the Löwen-Trendelenburg preparation of the frog's bloodvessels, Pearce (1913) observed vasoconstriction by injecting 0.1 mgm. of ergotamine. Medici (1924) found slight vasoconstriction of the frog's vessels in the same preparation with 1 in 10 million to 1 in 100 million ergotamine in Ringer but practically no vascular action in Ringer free from bicarbonate. Masuda (1925) observed vasoconstriction with 0.3 cc. of 1 in 100,000 ergotoxine phosphate. Heymans et al. (1932) noticed that ergotamine tartrate gave a vasodilator action and the methane sulphonate a vasoconstrictor action of frogs' hind limbs. He believed the vasodilatation to be due probably to the acidity of the tartrate.

Working with the arteries of freshly killed animals Cow (1911) observed that ergotoxine caused constriction in all cases with but one exception, the pulmonary artery, on which no effect was produced. Cruickshank and Subba Rao (1926), working with isolated rings of systemic arteries, found that ergotamine in concentrations below 1/500,000 had no effect, 1/500,000 to 1/100,000 concentration of ergotamine caused constriction followed by slight dilatation or return to the normal/

TABLE I.

ACTION OF THE ACTIVE PRINCIPLES OF ERGOT ON THE BLOODVESSELS.

Author	Animal	Preparation	Dose	Action and Remarks
Kobert 1884	Cock	Sphacelinic Acid	-	Gangrene of Comb
[illegible]	Rabbit	"		B.P. rise
Jacobi 1897	Cock	Chrysotoxine	-	Gangrene of Comb
	Dog, cat rabbit	"		Contraction of bloodvessels
Dale 1906	Cat	Sodium salt of chryso-toxine	20 mgm.	B.P. rise and peripheral vasoconstrictinn on injection
Dixon 1906	-	Ergot	-	Contraction of splanchnic and limb vessels on injection but dilatation of peripheral vessels by direct perfusion
Barger and Dale 1907	Cat	Ergotoxine	0.5 mgm. per kilo	B.P. rise on injection
Dale and Spiro 1922	Cat	Ergotamine	0.5 to 1 mgm.	Vasoconstriction on injection
Rothlin 1923	Rabbit	"	0.05-0.1 mgm. per kilo	B.P. rise on injection
	"	"	0.3-0.5 mgm. per kilo	B.P. fall on injection
	Cat	"	0.1-0.5 mgm. per kilo	B.P. rise on injection

TABLE I contd.

ACTION OF THE ACTIVE PRINCIPLES OF ERGOT ON THE BLOODVESSELS.

Author	Animal	Preparation	Dose	Action and Remarks
Ganter 1926	Cat	Ergotamine	0.5-1 mgm.	Bloodvessels dilatation on injection but constriction with larger doses
Burn and Dale 1926	Cat	"	-	Slight vasodilatation of the legs on perfusion
Heymans and Regniers 1927	Rabbit	"	1/75000 to 1/185000	Vasodilatation on perfusion of the head and hind limbs with Ringer
	Dogs cats	"	"	Vasodilatation less constant and less intense than in rabbit and sometimes vasoconstriction instead
Hamet 1931	Dog	"	-	Vasodilatation of dog's hind limbs on perfusion which passed into vasoconstriction
Heymans, Bouckaert Moraes 1932	Dog	"	0.25 mgm.	B.P. rise and constriction of vessels on injecting the drug
	Dog	"	0.5 mgm.	Intense vasodilatation of the head and limbs on direct perfusion with defibrinated blood
Pearce 1913	Frog	"	0.1 mgm.	Vasoconstriction in a Trendelenburg preparation
Medici 1924	Frog	"	1 in 10 millions -1 in 100 millions	Slight vasoconstriction in a Trendelenburg preparation

TABLE I contd.

ACTION OF THE ACTIVE PRINCIPLES OF ERGOT ON THE BLOODVESSELS.

Author	Animal	Preparation	Dose	Action and Remarks
Masuda 1925	Frog	Ergotoxine	0.3 cc. of 1 in 10^5	Vasoconstriction in a frog Trendelenburg preparation
Heymans et al. 1932	Frog	Ergotamine methane sulphonate	0.1 mgm.	Vasoconstriction of hind limbs
	Frog	Ergotamine tartrate	-	Vasodilatation of hind limbs (believed to be due to the acidity of the tartrate)
Cow 1911	Sheep and ox mostly	Ergotoxine	-	Constriction of isolated arteries except the pulmonaries
Cruickshank Subba Rau 1926	Ox and dog	Ergotamine	Below 1/500,000	No effect on isolated systemic arteries
	"	"	1/500,000 to 1/100,000	Constriction followed by dilatation

normal, whereas higher concentrations caused pure dilatation. From the above it is perfectly clear that the results on the vascular action of ergotamine are divergent. Both vasoconstrictor and vasodilator effects have been noticed although according to the classical view ergotamine has been considered so far to be a vasoconstrictor.

The action of the active principles of ergot on the bloodvessels is embodied in Table I.

METHOD.

Observations on the bloodvessels of the frog have been made by different methods. Gaskell (1880) perfused the bloodvessels of the frog by means of a cannula in the right aorta and allowed the fluid to escape by an incision in the abdominal vein, or in the sinus venosus and observed the changes in flow; he also observed microscopically the changes in the bloodvessels of the mylohyoid muscle. Krogh (1919-20) and Fraser (1928) used the bloodvessels of the tongue, and Adler (1916) used the mesenteric vessels of frogs. The Löwen-Trendelenburg (1910) method has been used by Alday-Redonnet (1920), Schmidt (1921), Pearce (1913), Forst (1926), Medici (1924), Masuda (1925), and Mahn and Reinert (1925). Hooker (1911) perfused the whole/

whole frog by putting ~~in~~ a cannula in the bulbous aorta and allowing the perfusate to escape by a cut in the great veins. Herbst (1922) perfused through the left aorta. During the present observations. the frog vessels have either been perfused through the left aorta with perfusion of the whole, or by means of the Trendelenburg method in which only the hind limbs are perfused. Perfusion has also been carried out of the vessels of the isolated stomach and the intestines through the coeliaco-mesenteric artery.

Rana esculenta was used for the experiments and the ergotamine was the tartrate manufactured by Sandoz Chemical Works, under the name of Femergin II. Frogs kept in the laboratory for a month or more were found unsuitable for the experiments, so comparatively fresh frogs were employed. The Ringer's fluid used for perfusion contained NaCl 0.7%, KCl 0.014%, $CaCl_2$ (anhydrous) 0.012%, $NaHCO_3$ 0.05%. The pH was 8.5. The temperature of the perfusion fluid ranged between 17°C and 20°C and the perfusion pressure was between 10 and 15 cms. in different experiments. Perfusion was carried out at a constant pressure by a special device described below.

It consists of a reservoir of about 80 cc. capacity open at the top and closed at the bottom by a three-holed indiarubber bung. These holes admit three pieces/

pieces of glass tubing, one for the overflow, another for washing out the reservoir, and the third for perfusing the vessels. The overflow tube, unlike the other two, is pushed right up into the reservoir to a definite height, and thus maintains the required perfusion pressure. The tube for washing out the reservoir is closed at the bottom with a stopcock; the tube for perfusion is connected to a long straight cannula by a piece of rubber tubing. It is convenient to fit up a second reservoir and to connect this with the perfusion cannula through a Y tube. The advantage is that perfusion can be carried by the one or the other reservoir each containing a different perfusion fluid. Care is, of course, necessary at the start to be sure that the reservoirs are delivering fluid at the same hydrostatic pressure; it is advisable to check this by perfusion with each in succession, using normal Ringer; the rate of flow through the preparation should be the same in both cases. An aspiration bottle is fixed to a stand above the reservoir and from it the perfusion fluid is constantly dropping into the reservoir.

Series 1. In the first series of experiments spinal frogs were employed. The perfusion was carried out by inserting the cannula in the left aorta through the frog's ventricle. The rest of the procedure was carried/

TABLE II.

PERFUSION OF WHOLE FROG: SPINAL CORD INTACT: BRAIN DESTROYED.

Drops per min. before ergotamine	Concentration of ergotamine	Drops per min. during ergotamine perfusion at intervals of					Percentile increase in 20 mins.	
		5	10	20	25	30 mins.		
16	1/4 × 10^6	17	17	16	16	16	0.0	
14	"	14	14	17	22	24	21.4	
41	"	-	41	47	48	48	14.6	=5.8
16	"	16	17	16	16	16	0.0	
43	"	-	-	40	-	-	-7.0	
13	1/2 × 10^6	14	14	16	16	-	23.1	
22	"	-	30	37	-	40	68.2	
44	"	-	53	56	-	-	27.3	
13	"	13	18	43	42	-	223.0	=85.4
18	1/1 × 10^6	-	27	28	-	-	55.5	
11	"	-	16	19	-	22	72.7	
27	"	-	33	36	37	-	33.3	
17	"	-	25	35	41	-	105.8	
13	"	-	22	22	-	-	69.2	
13	"	-	19	26	29	-	100.0	
18	"	32	43	46	-	-	155.5	
16	"	39	56	65	-	-	308.0	=112.5
14	1/500,000	-	18	26	28	-	85.7	
15	"	18	22	27	-	-	80.0	=88.6
9	"	9	15	18	-	-	100.0	

$1/4 \times 10^6$ = 1/4,000,000, $1/2 \times 10^6$ = 1/2,000,000 $1/1 \times 10^6$ = 1/1,000,000.

carried out as described by Sharpey-Schafer (1921). The drops were allowed to flow down the legs into a funnel and thence to a drop recorder. The drops were electrically recorded on a slow kymograph and checked also by visual counting.

RESULTS.

Different concentrations of ergotamine tartrate were employed. Table II embodies the results obtained. It will be noticed that a concentration of $1/4 \times 10^6$ of ergotamine causes practically no increase in the flow, the average increases being only about 5.8 per cent. in 20 minutes. A concentration of $1/2 \times 10^6$ brings about a more marked increase, the average order being 85.4 per cent. A concentration of $1/1 \times 10^6$ gives an average increase of 112.5 per cent. and a concentration of $1/5 \times 10^5$ an increase of 88.6 per cent. From these results it is clear that a concentration of $1/2 \times 10^6$ is sufficient to increase the rate of flow through the frog's bloodvessels. A few experiments were performed to find out whether the response obtainable with a concentration of $1/1 \times 10^6$ was the maximal or whether further increase in the response was obtainable with higher concentrations. In one of them Fig.1 a vessel perfusion went on at the rate of 11 drops per minute before ergotamine was added/

added. A concentration of $1/1 \times 10^6$ of the drug increased the flow to 22 drops per minute. At this stage the perfusion fluid was changed to 1/250,000 and no further increase was found. It was, however, noted that in frogs that did not respond adequately to $1/1 \times 10^6$ concentration of ergotamine, higher concentrations did cause an increase.

The effect of washing out for varying intervals of time with normal Ringer, after perfusion with ergotamine, was next determined. In six experiments a return to perfusion with Ringer solution free from ergotamine was carried out and of these three experiments showed no change in outflow, and three resulted in a tendency to return to the rate flow found initially. Thus the ergotamine effect was only partially reversible.

INFLUENCE OF CALCIUM.

Observations on the influence of calcium alone in a frog vessel preparation have been made by a number of investigators. Hooker (1911) observed that removal of calcium from the Ringer decreased the vascular tone in frogs, whereas Alday Redonnet (1920) found that increase of calcium caused vasoconstriction in frogs. Gramentski (1927) obtained vasoconstriction by increasing $CaCl_2$ from 0.04 to 0.3% in the Ringer solution, the response increasing with concentration, and vasodilatation by perfusion with calcium-free Ringer/

Ringer. Schmidt (1921) on the other hand, observed vasodilatation in frogs when he increased the calcium chloride content of the Ringer solution from 0.024 to 0.24 per cent. Hulse (1922) obtained vasodilatation with certain concentrations of $CaCl_2$.

The action of ergotoxine in calcium-free Ringer on a frog vessel preparation was investigated by Pearce (1913) and he observed vasoconstriction. Medici (1924) did not observe any vascular action of ergotamine in calcium-free Ringer.

During the investigations on the part played by calcium on the vascular response to ergotamine, it was observed that changes in the calcium content alone of the perfusing fluid did not usually cause any variation in the rate of flow. With 0.006 per cent. $CaCl_2$ in the perfusing Ringer, out of four experiments in only one did the rate of flow increase, i.e. from 11 to 15 drops per minute after 15 minutes perfusion, but it should be stated that an increase of the same order was also noted with 0.048 per cent. $CaCl_2$ in the Ringer in one experiment out of three. From the above it is clear that changes of no great significance were brought about by a change in the calcium content of the Ringer solution. The vascular response to ergotamine was, however, modified by previous perfusion with Ringer/

TABLE III.

PERFUSION OF WHOLE FROG: SPINAL CORD INTACT: BRAIN DESTROYED.

Calcium content	Concentrations of ergotamine used	Rate of outflow after 20 mins. 100 = normal
Nil	$1/1\times10^{6}$, 1/500,000, 1/250,000	100 average for 4 exps.
0.006 per cent.	$1/1\times10^{6}$	125 " " 5 "
0.012 per cent.	$1/1\times10^{6}$	212 " " 8 "
0.048 per cent.	$1/1\times10^{6}$	200 " " 3 "

Ringer having varying percentages of calcium. From Table III it will be seen that in the absence of $CaCl_2$ ergotamine did not increase the rate of flow through the blood vessels in concentrations varying from $1/1 \times 10^6$ to 1/250,000; with 0.006 per cent. $CaCl_2$ in the Ringer the increase was slight and with 0.048 per cent. $CaCl_2$ the increase was nearly the same as that obtained with normal Ringer which contained 0.012 per cent. $CaCl_2$. Fig.2 shows the absence of vascular response with as much as 1/250,000 ergotamine in calcium free Ringer.

INFLUENCE OF pH.

During perfusion of the whole frog with salt solution, Gaskell (1880) observed vasoconstriction by 1/10,000 NaOH and vasodilatation by 1/10,000 lactic acid. Bayliss (1901) perfused the posterior extremities of the frog with Ringer and observed an increase in the rate of flow with 1/10,000 lactic acid and also when the Ringer was saturated with CO_2. Pearce (1913) reported vasoconstriction in the frog (Trendelenburg preparation) with carbonic acid using Ringer or Tyrode as perfusate. Snyder and Campbell (1920) incidentally observed that excess of OH ions in Ringer (pH 7.8) caused constriction of vessels in the frog (Trendelenburg preparation). Atzler and Lehman (1921) found constriction/

constriction of the frog's vessels below a pH of 5.0 and above a pH of 7.0, but no effect between pH 5-7. Herbst (1922) noted that in the whole frog perfused with Ringer, acid solutions in the region of pH 6 dilated, while more acid solutions (pH 4.5) constricted the vessels. Leake et al. (1923) found a maximal dilatation response in the whole frog perfused with a buffered phosphate solution at pH 7.2, whereas vasoconstriction occurred both above and below this pH. Krogh (1919-20) observed vasodilatation in the tongue of urethane anaesthetised frogs by direct application of a one per cent. acetic acid solution. Fraser (1928) also observed that a dilute solution of acetic acid with sodium acetate caused distinct dilatation between pH 3.1 - 3.8 when applied as a tiny drop on the tongue of frogs. Dilute solutions of lactic acid with sodium hydrate gave vasodilatation at a pH of 2.2. More concentrated solutions, i.e. a half normal solution of the above substances produced vasodilatation of the tongue of the frog even at a pH of 7.2. By direct microscopic observation of mesenteric vessels of the frog, Adler (1916) observed vasoconstriction by alkalies at a pH of 11 to 12, and a just recognisable constricting effect was noted at a pH of 9. Acid below pH 3 also caused vasoconstriction but between pH 3 and 9 vasodilatation occurred. Hemingway (1926-27) perfusing the hind limbs of the cat with

Ringer/

TABLE IV.

PERFUSION OF WHOLE FROG: SPINAL CORD INTACT: BRAIN DESTROYED

Initial drops per minute	Perfusion with Ringer of different pH		Concentration of ergotamine	Drops per min. after ergotamine perfusion at end of	
	pH of the Ringer	Drops per minute after 15-20 mins.		10 mins.	20 mins.
19	7.0	23	$1/1\times10^{6}$	22	28
15	7.0	20	$1/1\times10^{6}$	23	23
12	7.0	16	$1/1\times10^{6}$	15	26
13	6.5	21	$1/1\times10^{6}$	24	24
19	6.5	31	$1/2\times10^{6}$	29	27
16	6.5	26	1/500,000	38	45
15	6.5	24 (after 35 mins.)	1/500,000	34	34

Ringer pH 7.4, observed that injection of 0.5 to 1.0 cc. N/100 NaOH and 5 per cent. $NaHCO_3$ (pH 8.5) or buffered phosphate solution of pH 9 into the stream of perfusing fluid caused vasoconstriction.

During the present observations, the frog vessel was first perfused with Ringer pH 8.5 and subsequently with Ringer pH 7.0 and 6.5. It will be noticed from Table IV that decreasing the pH to 7.0 caused vasodilatation to the extent of 26 per cent., and decreasing to pH 6.5 caused vasodilatation to the extent of 63 per cent. (average of 3 experiments in each case). Subsequent perfusion with ergotamine in Ringer of the lower pH still further increased the flow although this additional increase was not so marked. Fig.3 shows the observations on a typical experiment in which a pH of 6.5 gave a moderate increase followed by a bigger increase with ergotamine. It is likely that either perfusion with acid Ringer diminished the sensitivity of the vessels to ergotamine or that the vessels having been dilated to a certain extent by the acid Ringer are unable to dilate still further.

ANTAGONISM OF ERGOTAMINE AND ADRENALINE.

Dale (1906) demonstrated that intravenous injection of 50 to 100 mgm. chrysotoxine or 2 mg. sphacelotoxine/

sphacelotoxine caused a rise in bloodpressure and injection of adrenaline before the resulting rise of pressure had subsided caused a fall in bloodpressure. This vasomotor reversal had been since then demonstrated by Barger and Dale (1907) using ergotoxine and by Dale and Spiro (1922) using ergotamine. Burn and Dale (1926) also noted during perfusion of cat's leg with defibrinated blood that if, subsequent to injection of 2 mgm. ergotamine, 0.1 mgm. adrenaline was injected into the perfusing cannula there was a prompt acceleration of flow. Cruickshank and Subba Rao (1927-28) found that adrenaline 1/50,000 failed to produce any effect after ergotoxine in bigger systemic artery rings but in smaller arteries the adrenaline effect was reversed. Observations on the vascular response of frogs to adrenaline in presence of ergotamine have also been made by a number of workers. Pearce (1913) using the Trendelenburg vessel preparation of frogs, observed that after putting in 0.1 mgm. of ergotoxine which brought about vasoconstriction, adrenaline, 1 cc. of 1/100,000, caused vasodilatation. Masuda (1925) noted that in the frog vessel preparation, perfusion with ergotamine decreased the adrenaline sensitivity and he employed this method for measuring the strength of ergot preparations, his standard being the decrease in/

in adrenaline sensitivity of the preparation to 1/10 after an hour's perfusion, or both legs were perfused separately and the activity of the ergot solution compared with that of ergotamine - ergotoxine solution perfusing the other leg and the antagonistic effect on the vasoconstriction action of adrenaline serving as a measure of activity. This method was also used by Mahn and Reinert (1925) for different commercial preparations of ergot. Forst (1926) also observed diminution of adrenaline sensitivity by Gynergin (ergotamine) and used it for finding out the quantity of the alkaloid. He took as a standard of gynergin the amount which when perfused through a Trendelenburg preparation reduced the vasoconstrictor action of adrenaline by one-half. Rothlin (1925) observed that the adrenaline contraction of perfused frog's hind limbs and rabbit's ear vessels could be prevented by ergotamine. The quantities used were 0.5 cc. adrenaline 1 in 10,000,000 and 0.5 cc. ergotamine 1 in 5,000.

In the present series of observations on the whole frog perfusion preparation, the response of the vascular system to adrenaline after ergotamine was also investigated.

As a routine procedure perfusion was carried out first with Ringer and then with ergotamine added to the above Ringer/

TABLE V.

PERFUSION OF WHOLE FROG: SPINAL CORD INTACT: BRAIN DESTROYED.

Initial drops per minute	Concentration of ergotamine	Drops per min. in 20-25 mins.	Concentration of adrenaline	Drops per min. in 20-25 mins.
27	$1/1\times10^6$	37	$1/4\times10^6$	31
17	$1/1\times10^6$	41	$1/4\times10^6$	41
13	$1/1\times10^6$	29	$1/2\times10^6$	13
18	$1/1\times10^6$	46	$1/2\times10^6$	16
13	$1/1\times10^6$	22	$1/1\times10^6$	8
15	1/500,000	27	$1/1\times10^6$	12
10	1/250,000	31	1/500,000	10
14	$1/4\times10^6$	17 (24 in 30 mins.)	$1/8\times10^6$	7

Ringer for a period of 20 to 25 minutes during which time it was considered that a maximum dilatation was obtained. The perfusion fluid was then changed to Ringer containing adrenaline, and perfusion was carried on with this for about 20 to 25 minutes. It will be noticed from Table V that adrenaline always brought about a diminution in flow after ergotamine. showing that it still caused constriction of blood-vessels. Perfusion with ergotamine for the second time after adrenaline again caused increase in the flow.

When ergotamine in concentration of $1/1 \times 10^6$ was used it was seen that $1/2 \times 10^6$ concentration of adrenaline just neutralised the effects and $1/1 \times 10^6$ and $1/4 \times 10^6$ concentrations of adrenaline always constricted the vessels either more or less than the dilatation brought about by ergotamine. It was further noticed that ergotamine in concentrations of 1/500,000 and 1/250,000 were antagonised similarly by $1/1 \times 10^6$ and 1/500,000 concentrations of adrenaline respectively. Nevertheless, a concentration of $1/8 \times 10^6$ adrenaline proved too strong for a concentration of $1/4 \times 10^6$ of ergotamine. Thus it will be noticed that adrenaline in half the concentration of that of ergotamine exactly abolished the vasodilator effect of the latter. Fig.4 shows the same effect on a kymographic/

TABLE VI.

PERFUSION OF WHOLE FROG: SPINAL CORD INTACT: BRAIN DESTROYED.

Initial drops per minute	Mixture of ergotamine and adrenaline		Drops per minute after perfusion with the mixture at intervals of			
	Concentration of ergotamine	Concentration of adrenaline	5	10	15	20 mins.
22	$1/1\times10^{6}$	$1/2\times10^{6}$	-	34	-	51
16	$1/1\times10^{6}$	$1/1\times10^{6}$	-	20	-	24
17	$1/1\times10^{6}$	$1/1\times10^{6}$	14	7	7	-
16	$1/1\times10^{6}$	$1/1\times10^{6}$	21	28	28	31
17	$1/1\times10^{6}$	1/500,000	5	-	-	-
25	$1/1\times10^{6}$	1/660,000	17	8	6	-

kymographic tracing. Fig.5 represents graphically the results of action of ergotamine and adrenaline. If adrenaline was first perfused and subsequently followed by ergotamine, the adrenaline effect was abolished by the ergotamine in approximately equal concentrations with the adrenaline, as shown in Fig.6.

Mixtures of ergotamine and adrenaline in different concentrations were also used for perfusion. It will be noticed from Table VI that concentrations of adrenaline varying from 1/500,000 to 1/660,000 along with ergotamine in a concentration of $1/1 \times 10^6$ brought about definite constriction of the vessels, whereas a concentration of $1/1 \times 10^6$ adrenaline in combination with $1/1 \times 10^6$ concentration of ergotamine either brought about a change of slight constriction or slight dilatation. Ergotamine in $1/1 \times 10^6$ concentration with adrenaline in $1/2 \times 10^6$ concentration brought about dilatation. These results show that a concentration of $1/1 \times 10^6$ ergotamine approximately antagonises a concentration of $1/1 \times 10^6$ adrenaline when both are present together in solution.

Experiments done with pithed frogs, instead of spinal frogs as in the present series, also show that ergotamine causes vasodilatation when the whole frog is perfused.

Series 2./

Series 2. In the second series of experiments, instead of perfusing the whole frog, the posterior extremities only were perfused, using the Trendelenburg method. The spinal cord of the frog was destroyed and the abdomen then opened. The bladder was tied and cut off after having ligated the vein from the bladder which empties into the abdominal vein. The rectum was similarly tied and cut off. The two renal veins were also tied and cut, and the whole intestine then removed and cut between two ligatures after having tied the mesenteric vessels. Loose ligatures were passed underneath the abdominal aorta and the abdominal vein. The frog was then fixed on the cork board with the posterior extremities upwards and a small cannula was inserted into the abdominal aorta immediately above its bifurcation, the aorta being ligated over cannula. Perfusion was started under constant pressure. When the blood was nearly washed out of the vessels, a small cannula was put into the abdominal vein. Drops were then either counted or recorded on a slow drum.

Experiments were performed in order to investigate the action of ergotamine in this preparation. The results are embodied in Figs. 7 and 8. It will be noticed from the figures that the action of ergotamine in concentrations varying from $1/10^6$ to 1/250,000 have/

have no certain effect. A moderate vasoconstriction was observed in 2 experiments; in two others a temporary but moderate vasodilatation was to be seen while in 2 more, there was practically no effect. Control experiments (Fig.7) in which perfusion was carried out with normal Ringer (pH 8.4) alone for about 2 hours showed only moderate variations within that period. From Figs. 7 and 8 it may also be noticed that changing the perfusion to Ringer containing adrenaline $1/10^6$ caused a marked vasoconstriction in three out of 4 experiments, in two of which the preparation was perfused with 1/500,000 ergotamine for 50 minutes and for 40 minutes, and in the third with 1/250,000 ergotamine for 30 minutes. Experiments were done in which the preparation was perfused with Ringer containing ergotamine for 12 to 13 minutes Before perfusing with adrenaline Ringer, to note if a shorter period of perfusion with ergotamine made any difference in the response to adrenaline. It will be noticed from Fig.9 that $1/10^6$ adrenaline caused a well marked constriction of the bloodvessels following perfusion with 1/500,000 ergotamine. From Fig.10 it will also be seen that even $1/10^7$ adrenaline caused vasoconstriction following perfusion with 1/250,000 ergotamine. From the above it is clear that irrespective of the period of perfusion and of the strength of ergotamine solution up to concentrations of 1/250,000, the/

the vasoconstrictor action of adrenaline is manifested quite clearly.

This preparation was also used to investigate the effects of injecting adrenaline into the perfusion tube before and after perfusing with ergotamine. The procedure adopted was to perfuse the vessel with Ringer solution and then to inject slowly a very small quantity of adrenaline into the perfusion tube. The preparation was then perfused with ergotamine, and adrenaline in the same quantity as above again injected. As a preliminary to the above observation, it was thought necessary to investigate the sensitivity of the preparation to the first and to the second injection of adrenaline during only a normal Ringer perfusion. As will be noticed from Fig.11, sensitivity of the preparation to the second injection of adrenaline is much less marked than to the initial injection during a normal Ringer perfusion alone. From Fig.12 it will be noticed that after perfusion with ergotamine 1/500,000, the sensitivity of the preparation to adrenaline injection is also reduced as compared with the sensitivity of the preparation to the same quantity of adrenaline before ergotamine. Thus it is obvious that although adrenaline action is less marked after ergotamine perfusion the control experiments show it is not due to the action of ergotamine.

In/

In view of uncertain effects obtained with the perfusion of the vessels of the hind limb in the frog, it was considered expedient to try the effects of injecting ergotamine in the perfusion tube while perfusion with normal Ringer was being carried on. In two experiments in which 0.1 mg. ergotamine in 0.1 cc. of Ringer solution was slowly injected, one showed no vascular change and the other showed moderate vasoconstriction. In both cases, experiments were controlled by injecting 0.1 cc. of normal Ringer. In two other experiments in which 0.02 mgm. ergotamine was injected followed by 0.2 mgm. ergotamine again after about 20 minutes, it was noticed that the larger dose caused vasoconstriction in only one of them; in the other no effect was obtained. Thus it may be inferred from the above that injection of ergotamine either has no action or has to a certain extent a vasoconstrictor action.

Series 3. In the third series of experiments, perfusion of the vessels of the stomach and intestines only were carried out. The following technique was adopted. The abdomen of a pithed frog was opened and the stomach and the intestines were isolated by cutting the rectal and the oesophageal ends between two ligatures. The coeliaco-mesenteric artery was ligated close/

close to its origin from the aorta and was then cut off from the latter. The stomach and the intestines were freed from the surrounding tissues which necessitated cutting the mesenteric attachments and the veins. The isolated piece of the alimentary canal was then put on a cork board supported by pins. A very fine cannula connected to the constant pressure perfusion outfit (as described previously) was introduced into the coeliaco-mesenteric artery and the latter was tied over the cannula. Perfusion was started and the outflow from the cut veins was allowed to flow down the cork board into a small funnel. The drops were either counted or recorded.

Fig.13 shows the results obtained in control experiments in which perfusion with normal Ringer (pH 8.4) was carried out for about an hour. The figure shows only moderate variations in the rate of flow. Figs.14 and 15 show the results obtained when ergotamine in concentrations of 1/500,000 and 1/250,000 was used as perfusate. It will be noticed from the above figures that ergotamine caused vasodilatation in each of the seven experiments and it usually took about 10 minutes for the vascular effects to be manifested. From fig.14 it will be further noticed that in one experiment injection of 0.05 mgm. adrenaline into the perfusion tube after having perfused the preparation/

preparation for about 30 minutes with 1/250,000 ergotamine caused a marked vasoconstriction. In a second experiment, instead of injecting adrenaline, the perfusate was changed to $1/10^6$ adrenaline after perfusing the preparation for about 40 minutes with 1/250,000 ergotamine and the same vasoconstriction was again noticed. From fig.15 it will be noticed that injection of 0.01 mgm. adrenaline into the perfusion tube, after perfusing the preparation with 1/500,000 ergotamine, brought about a marked vasoconstriction in one experiment (G) in which the perfusate was changed to normal Ringer before adrenaline was injected, while in the two other experiments (E,F) in which perfusion with ergotamine Ringer was continuing all the time during and after adrenaline injection the vasoconstriction was less marked in one and negligible in the other. The continued perfusion with ergotamine which has in itself a vasodilator action in this preparation might possibly explain the diminished adrenaline effect.

Perfusion with $1/10^6$ adrenaline alone in normal Ringer has a marked vasoconstricting effect in this preparation.

DISCUSSION/

DISCUSSION.

As has been pointed out before, the vasoconstrictor action of ergot alkaloids has been observed by Kobert (1884), Jacobi (1897), Dale (1906), Barger and Dale (1907), Dale and Spiro (1922), Pearce (1913), Medici (1924) and Masuda (1925), and Heymans, Bouckaert and Moraes (1932), whereas vasodilatation has been noted by Dixon (1906), Burn and Dale (1926), Rothlin (1925), Ganter (1926) and Heymans and Regniers (1927).

The results of the present investigations go to demonstrate that ergotamine causes vasodilatation in the whole frog, has an uncertain effect on the vessels of the hind limb, and causes vasodilatation of the stomach and the intestines.

With respect to the observations on the whole spinal frog, it is to be noted that a marked vascular response is brought about by ergotamine concentrations of $1/2 \times 10^6$ and higher, and is observed in from 5 to 10 minutes after changing the perfusion fluid from normal Ringer to ergotamine Ringer. A change in the calcium content of the perfusate, although in itself not causing any significant alteration in the vascular response, seems to modify the action of ergotamine. If calcium-free Ringer or Ringer deficient in calcium is perfused for 15 to 20 minutes prior to perfusion with/

with ergotamine, the latter has either no action or very little action on the vessels. The absence of any action of ergotamine in calcium-free Ringer, as observed here, agrees with the observations of Medici (1927) in the Trendelenburg preparation and contradicts those of Pearce (1913) who observed vasoconstriction in the same preparation. Decreasing the pH of the Ringer itself causes a certain degree of vasodilatation which has been observed by a number of workers, namely, Bayliss (1901), Hooker (1911), Krogh (1919-20), Fraser (1928) and others, and the results obtained here support them. The drug, nevertheless, continues to exert its vasodilator action even in the presence of acid Ringer, which action, however, is less marked.

The vasodilation observed by ergotamine in the whole frog perfusion with spinal cord and viscera intact may be a mixture of effects since the action of the drug on the limb and splanchnic vessels may be different and then the presence of spinal cord may introduce a complication if the spinal centres are influenced. From the results

obtained with perfusion of limb vessels it has been noticed that the effects of perfusion with ergotamine ~~is~~ are rather uncertain; so that the possibility of the limb vessels participating in the vasodilatation is uncertain./

uncertain. Again it has been found that the presence or absence of the spinal cord does not seem to change qualitatively the vascular action of ergotamine in the whole frog since vasodilatation is also present in the pithed frog. That the splanchnic vessels participate in the dilatation brought about by ergotamine is shown by the observations that ergotamine causes vasodilatation of the isolated stomach and the intestines. Thus the vasodilator action of ergotamine is exerted chiefly through the splanchnic vessels, the hind vessels apparently taking no significant part.

Regarding the mode of action of ergot alkaloids on bloodvessels, Dale (1906), Barger and Dale (1907) and later Dale and Spiro (1922) attribute to the drug a stimulant action on the plain muscle. Burn and Dale (1926) do not give any reason for the vasodilatation brought about by ergotamine on the perfusion of the cat's legs. Dixon (1906) attributes to ergot a vasoconstrictor action by central stimulation, also a peripheral vasodilator effect. Rothlin (1925) regards the vasoconstriction as due to sympathetic irritation. Ganter (1928) regards the vasodilatation in small doses as due to sympathetic paralysis and the vasoconstriction in larger doses due to direct action on the bloodvessels.

In/

In discussing the mode of action of the drug on the vessel wall, one has to keep in view the significant facts, namely, that the drug causes vasodilatation only when normal Ringer is employed for perfusion and there is no vasodilatation if calcium-free Ringer is used in the perfusate. This naturally leads one to the question of the part played by calcium on the vascular response to drugs as also the action of calcium itself on the vascular tone. Bayliss (1924) points out on the basis of work of Asher and Pearce that when the frog's vessels are perfused with Ringer, adrenaline causes vasoconstriction, but when perfused with isotonic saline, adrenaline causes vasodilatation and also suggests that calcium is necessary for the normal effect of adrenaline on the sympathetic nerve endings. Cow (1911) found that arterial rings in calcium-free saline solution reacted more to adrenaline and pituitrin than in normal saline. Regniers (1926) observed that excess of calcium diminished the vasoconstrictor action of adrenaline but not of pituitrin. Suppression of calcium also depressed the vasoconstrictor action of adrenaline, but mere diminution increased it. As to the action of calcium on the vascular tone, Alday Redonnet (1920) found an increase in vascular tone/

tone by calcium. Gramentski (1927) also found that high concentration of calcium increased the vascular tone and absence diminished it. Brull (1930) found that calcium augmented the tonus of smooth muscle of the vascular wall of the dog. Schretzeumayer (1931) observed that the action of calcium on vascular tone is to produce an increase in tonus preceded by a diminution, the latter effect being muscular and the former sympathetic. Schafer et al. (1933) also got increase in vascular tonus by calcium in the cat and in the rabbit.

From the above considerations one is led to suggest that certain degree of vascular tone is maintained by the presence of calcium in the perfusing Ringer, and for the vasodilatation by ergotamine, this tone is necessary. Thus it is that when calcium-free Ringer is employed for perfusion, ergotamine fails to cause vasodilatation, as a result, possibly, of lack or diminution of vascular tone.

Now the point for discussion is the mode of action of ergotamine on bloodvessels. Although it is a difficult problem to tackle, one is inclined to put forward the simplest explanation. Since Clark (1933) suggests that the effects of calcium and potassium and also of methylene blue on the frog's heart is a surface/

surface effect, it is equally probable that ergotamine too acts on the surface of the cells by being in some way fixed on it. In the light of this statement one can further suggest that deficiency of calcium in the Ringer prevents the vasodilator action of ergotamine by causing certain changes on the cell surfaces which render them incapable of fixing the drug.

Regarding the vascular response of frogs to adrenaline following ergotamine, Masuda (1925), Mahn and Reinert (1925), Forst (1926) and Rothlin (1925) found diminution in the adrenaline activity after ergotamine and Pearce (1913) observed vasodilatation with adrenaline following ergotamine. In the present series of observations it has been noticed that adrenaline alone causes vasoconstriction whether the whole frog is perfused or only the hind limbs or the stomach and the intestines. It has also been observed that the vasoconstrictor action of adrenaline is exerted even after perfusing any of the above preparations with ergotamine. In the whole frog, perfusion with Ringer fluid containing both adrenaline and ergotamine gives rise to vasoconstriction if the adrenaline concentration preponderates and vasodilatation if the ergotamine concentration preponderates. In the event of a vasodilator effect of the mixture, since the effect can be explained by the/

the more powerful vasodilator action of ergotamine over the vasoconstrictor action of adrenaline, the assumption of sympathetic paralysis by ergotamine is unnecessary.

Again, in the Trendelenburg preparation, the results of injecting adrenaline in the perfusion tube show that the sensitivity of the preparation to adrenaline after perfusion of the hind limbs with ergotamine is diminished. But since the same diminution of sensitivity is seen as a result of second injection of adrenaline even during perfusion with normal Ringer, the diminution of adrenaline sensitivity cannot be attributed to ergotamine. Thus on the whole the results as obtained with the whole frog perfusion as well as with the perfusion of isolated limbs, or of the isolated alimentary canal, give no evidence that ergotamine suppresses or reverses the vascular action of adrenaline in the frog.

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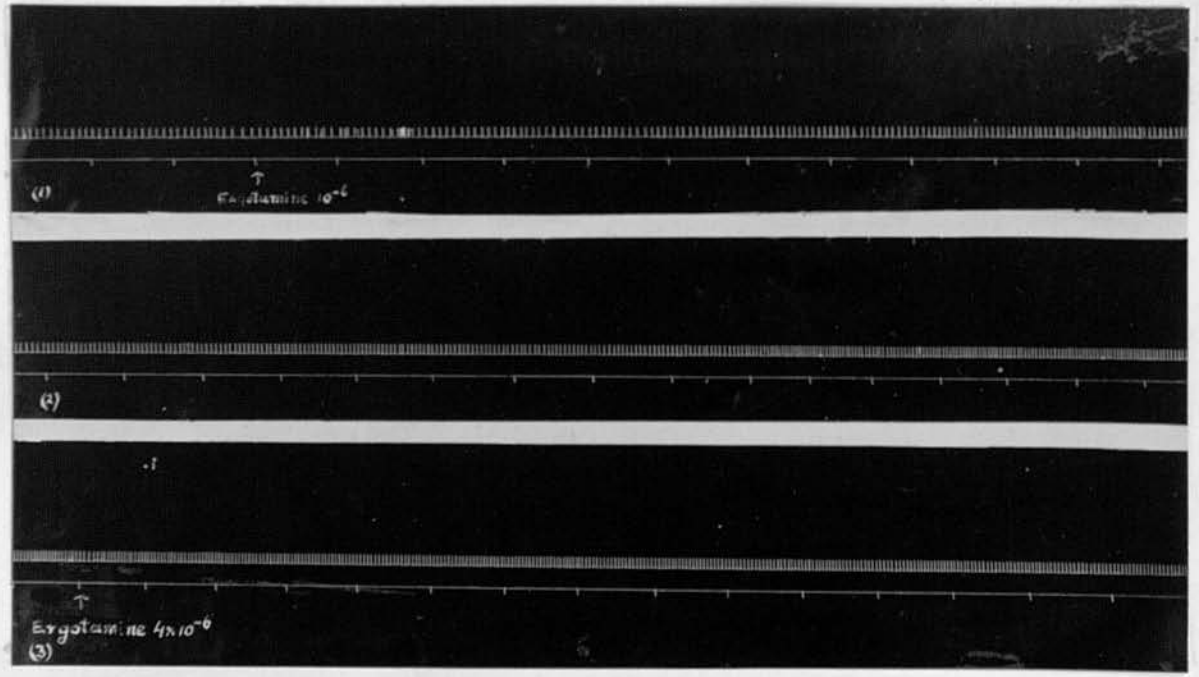

Fig. 1.

Perfusion of whole vascular system of frog, R. Esculenta.
Top tracing = outflow drops: bottom tracing = 1 minute interval.
Effect of ergotamine.
The tracings 1 to 3 are continuous. They show an increase in the outflow brought about by 1/1,000,000 ergotamine in Ringer. No further increase in the outflow is seen when perfusion fluid is changed to Ringer containing 1/250,000 ergotamine.

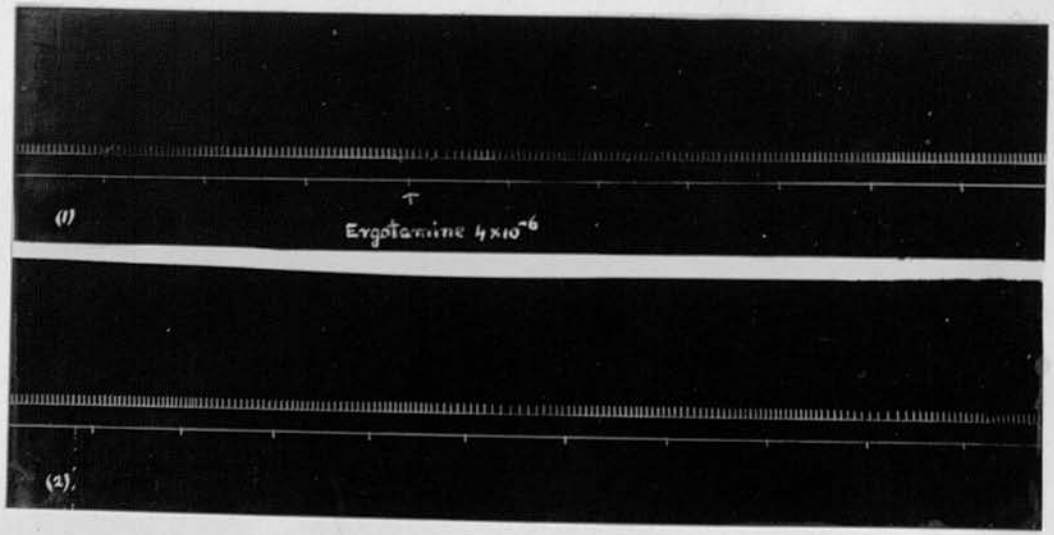

Fig. 2.

Perfusion of whole vascular system of frog, R. Esculenta, with Calcium free Ringer.
Top tracing = outflow drops: bottom tracing = 1 minute interval.
Effects of ergotamine in calcium free Ringer.
The tracings shows no increase in the outflow even with 1/250,000 ergotamine.

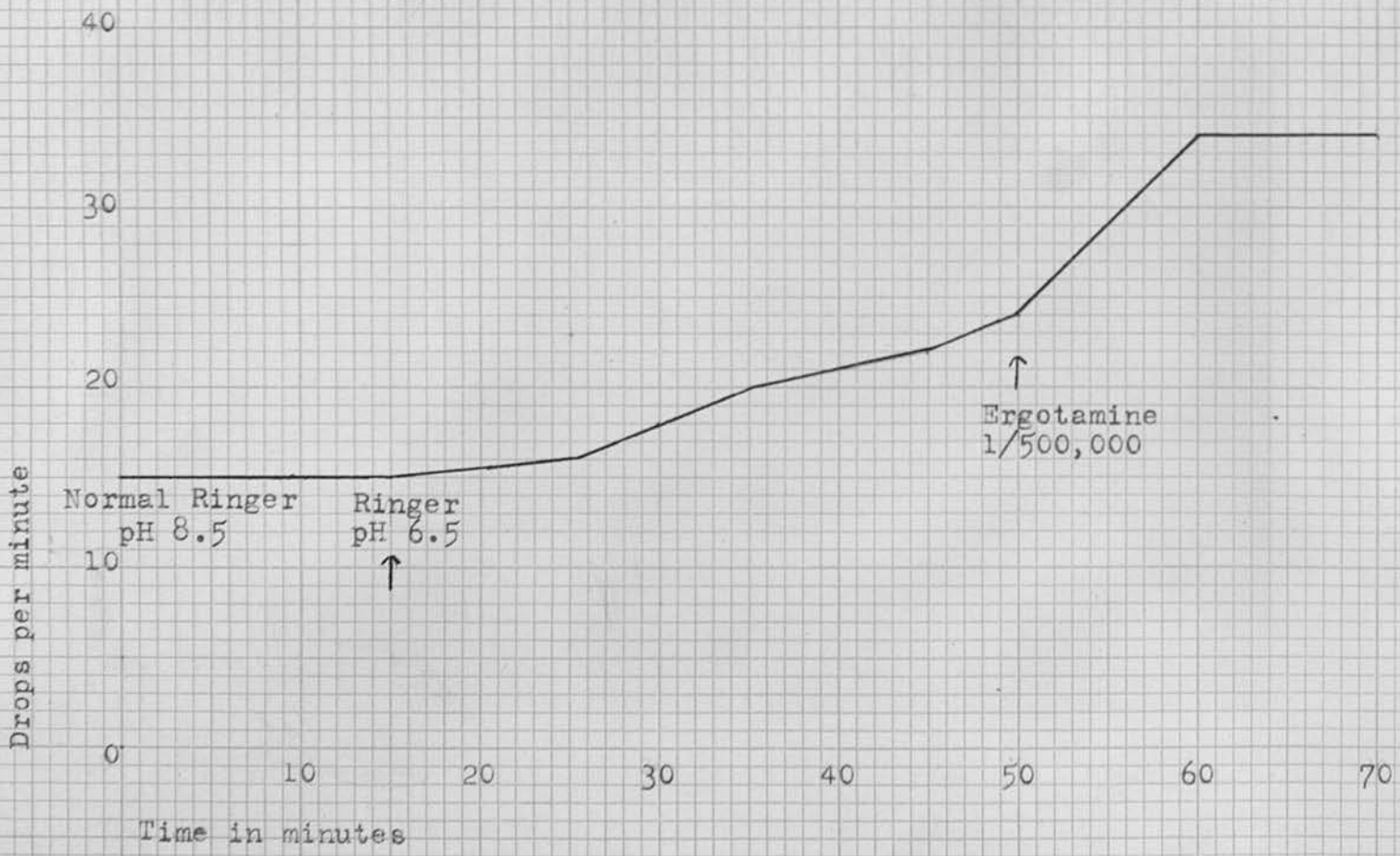

Fig. 3.

Perfusion of whole vascular system of frog, R. Esculenta.
Effect of alteration in the pH of the perfusing Ringer on the action of ergotamine.

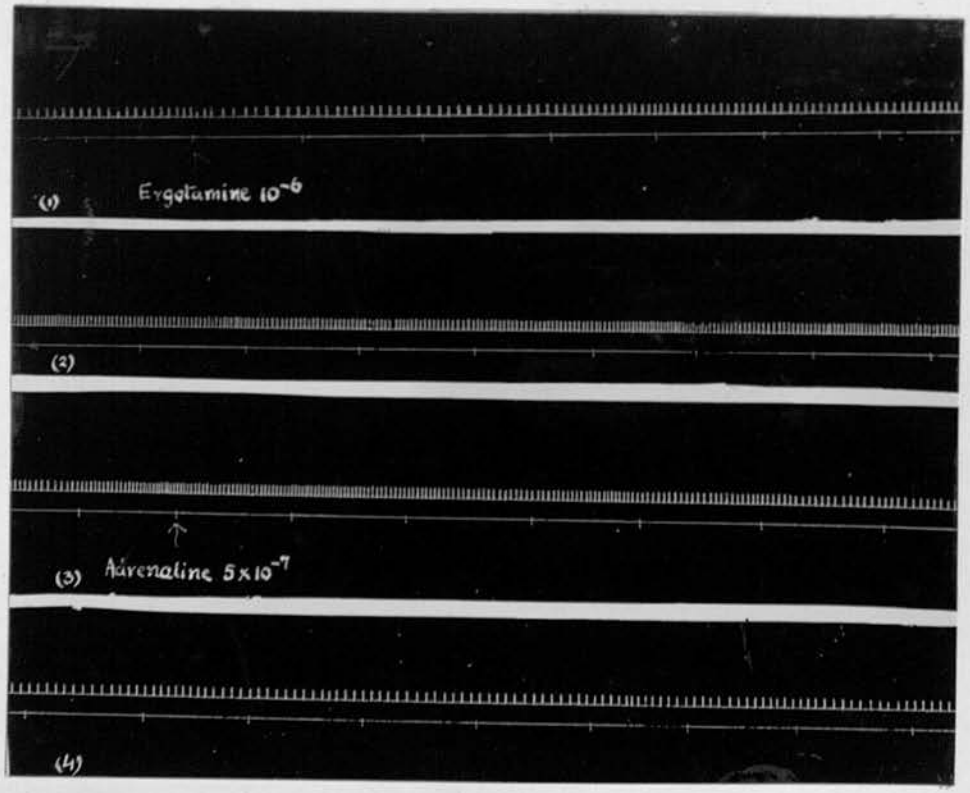

Fig. 4.

Perfusion of whole vascular system of frog. R. Esculenta.
Top tracing = outflow drops: bottom tracing = 1 minute interval.
Effects of ergotamine and adrenaline.
The tracings 1 to 4 are continuous. They show an increase in the outflow brought about by 1/1,000,000 ergotamine in Ringer and subsequent diminution in the outflow by 1/2,000,000 adrenaline.

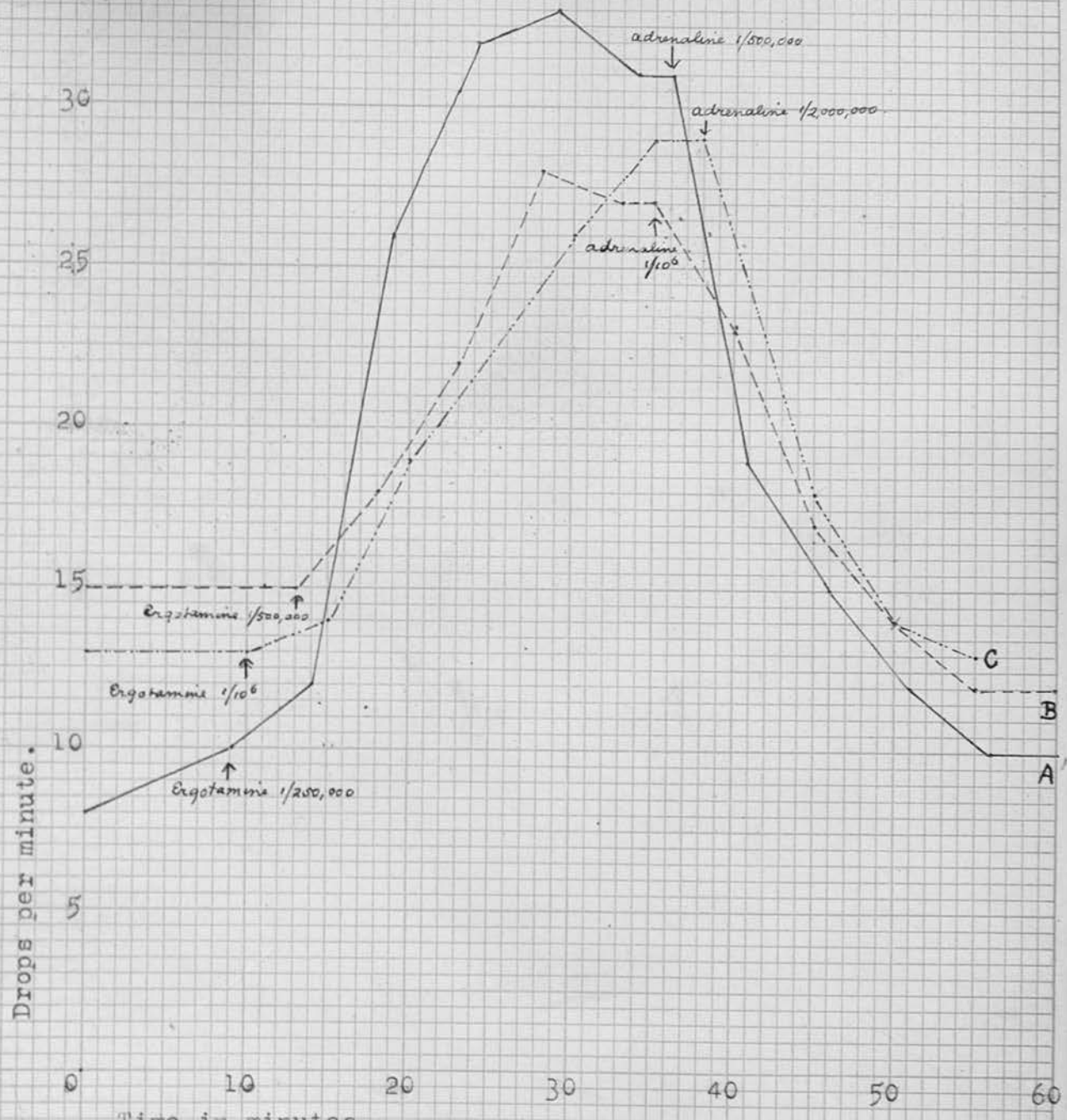

Fig. 5.

Perfusion of whole vascular system of frog, R. Esculenta. Ringer.
Effect of adrenaline after ergotamine in concentrations of half that of ergotamine.

A (——) shows the effects of ergotamine 1/250,000 and adrenaline 1/500,000
B (- - - -) shows the effects of ergotamine 1/500,000 and adrenaline 1/1,000,000
C (- - - -) shows the effects of ergotamine 1/1,000,000 and adrenaline 1/2,000,000

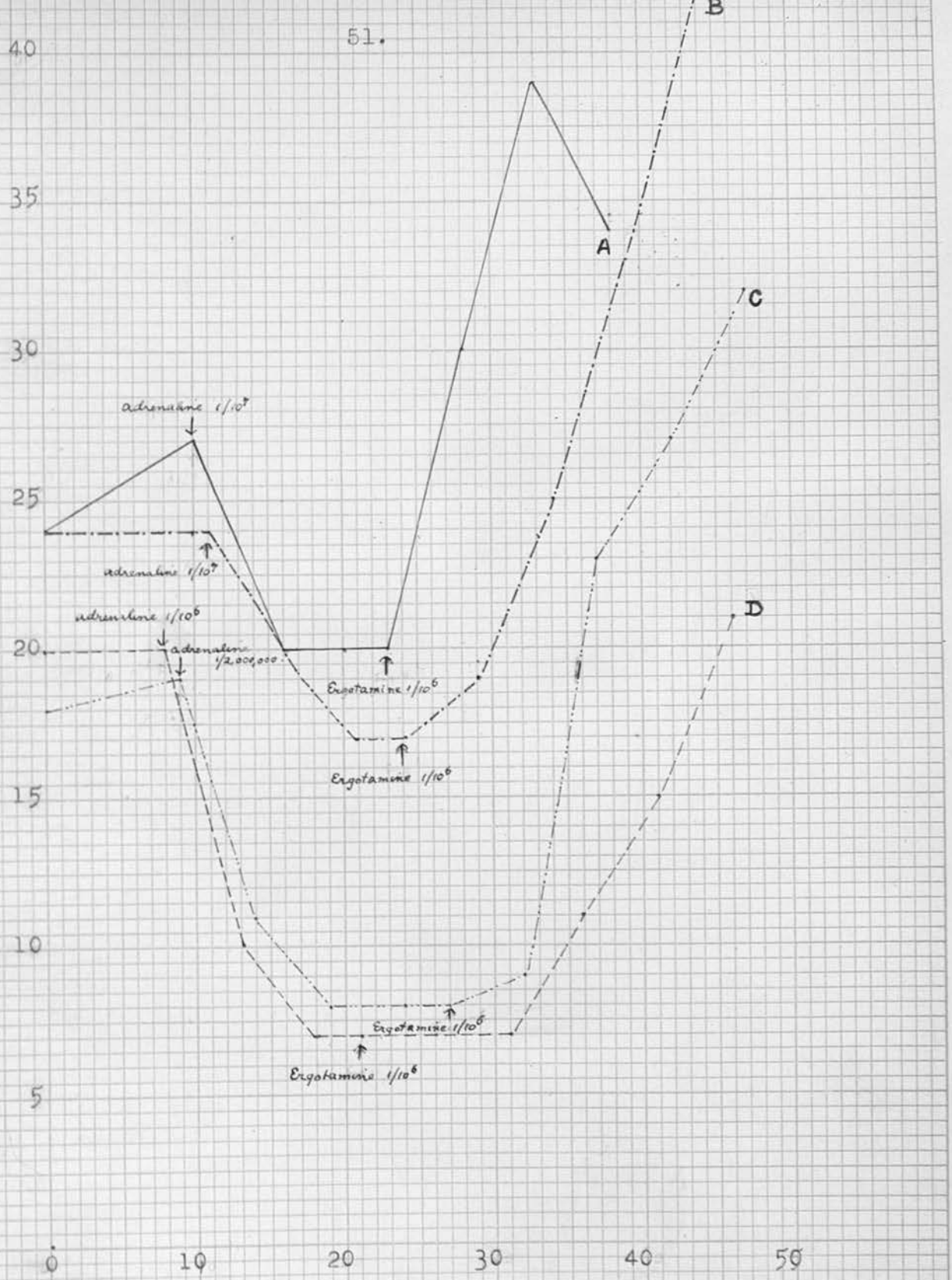

Fig. 6.

Perfusion of whole vascular system of frog R. Esculenta. Ringer.
Effect of ergotamine after adrenaline.
A(——) and B(-·-·-) show the effects of adrenaline 1/10,000,000 and ergotamine 1/1,000,000
C(-·-·-·-) shows the effects of adrenaline 1/2,000,000 and ergotamine 1/1,000,000
D(----) shows the effects of adrenaline 1/1,000,000 and ergotamine 1/1,000,000

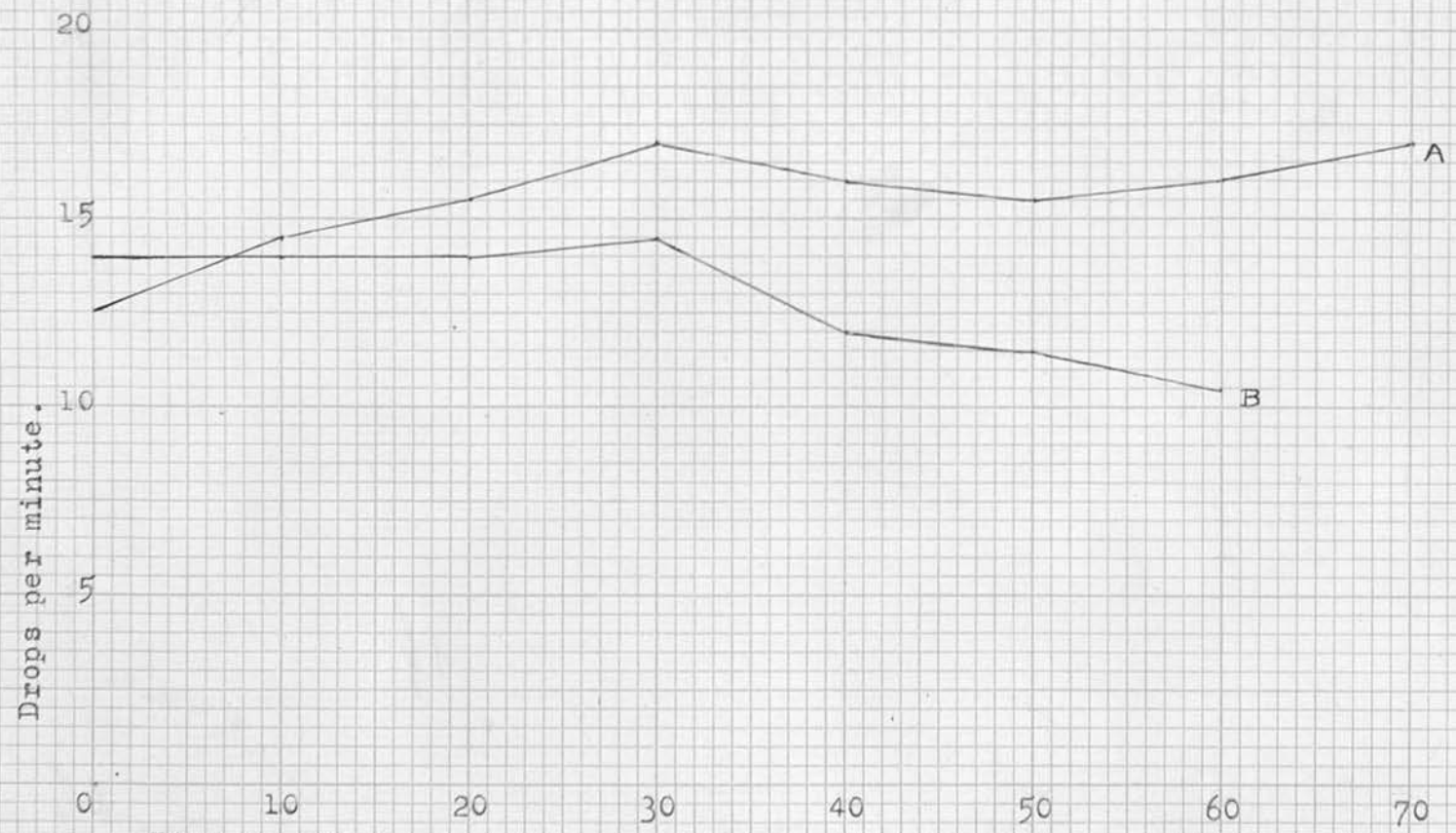

Fig. 13.

Perfusion of the blood vessels of the stomach and the intestines of frog, R. Esculenta. Ringer.
A and B show the effects of perfusion with Ringer alone.

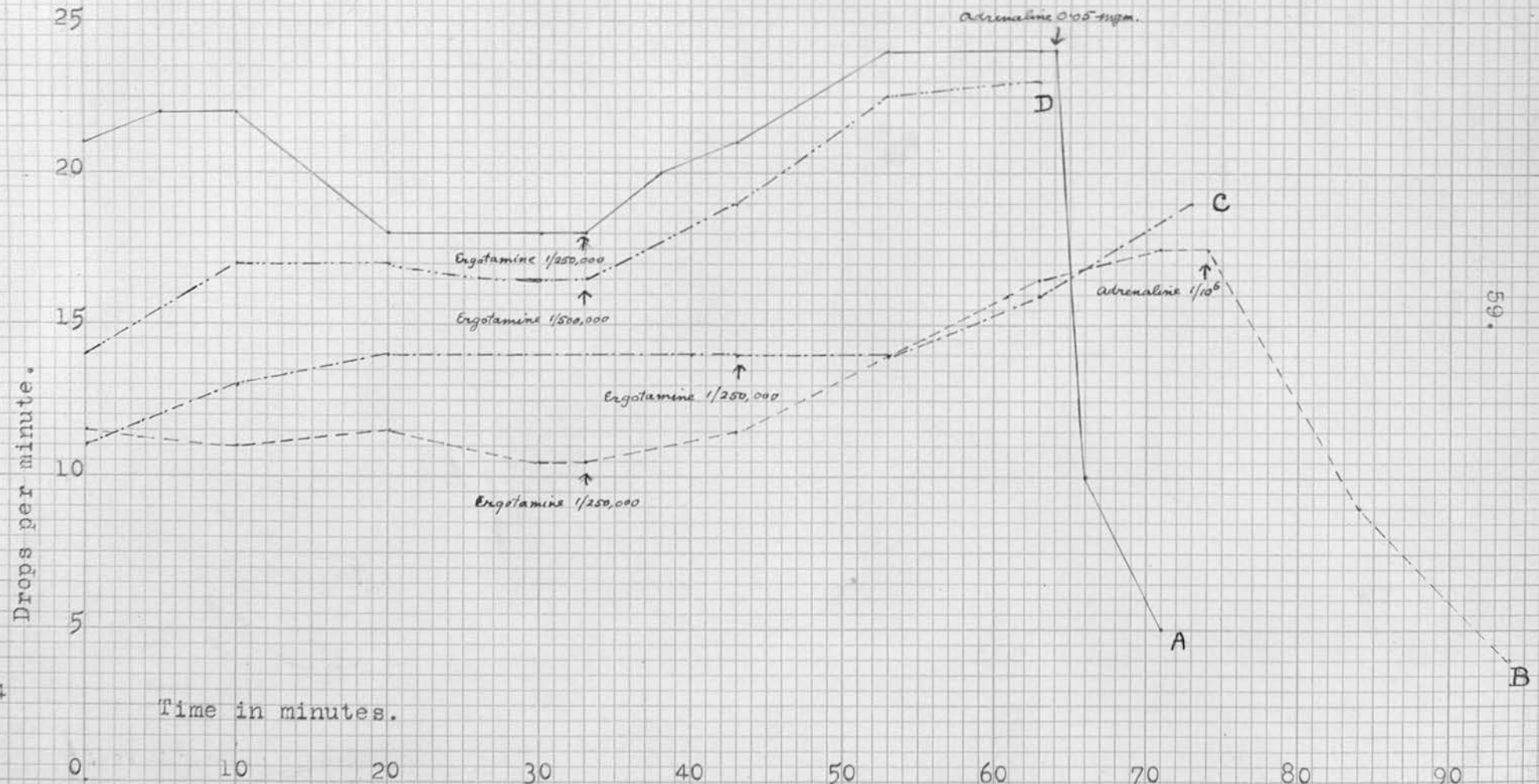

Fig. 14

Perfusion of the blood vessels of the stomach and the intestines of frog, R. Esculenta. Ringer.
A(——) shows the effects of ergotamine 1/250,000 followed by an injection of 0.05 mgm adrenaline into the perfusion tube.
B(– – –) shows the effects of ergotamine 1/250,000 followed by adrenaline 1/1,000,000
C(—·—) and D(—··—) show the effects of ergotamine 1/250,000 and 1/500,000 respectively.

HEART.

LITERATURE.

As has been pointed out before, the real pharmacology of ergot alkaloids starts with the work of Dale (1905, 1906) on chrysotoxine and that of Barger and Dale (1906-07) on ergotoxine. Dale (1906) noted that the intravenous injection of 20 mgms. chrysotoxine to cats at first quickened the beat of the heart if initially slow and often caused secondary slowing which was not abolished by atropine even in large doses. With vagi cut or medulla destroyed a frequent effect of the drug, when the heart was rapid and feeble, was to cause slowing and augmentation. Barger and Dale (1906-07) perfused the isolated heart of the cat and the rabbit with Locke Ringer containing 1/50,000 Ergotoxine hydrochloride, and noted in some cases an increase in the force of the beat, but in some cases there was no effect. In the anaesthetised animal with medullary centres and vagi intact, ergotoxine caused vagus inhibition of the heart. Dale and Spiro (1922) found slowing of the heart in intact cats by 2.5 mgm. ergotamine and attributed the result to irritation of vagal nerve endings. They also concluded that both ergotoxine and ergotamine have the same pharmacological action. Dixon (1906) observed that ergot augmented the output both on the intact

and/

and on the isolated heart. Viotti (1924) perfused the isolated guinea-pig's heart with 0.000025%, 0.0001% and 0.00036% ergotamine in Ringer solution, and noted a diminution in the frequency and in the height of contractions of the heart. Atropinisation with 0.003% solution suppressed the inhibitory action of ergotamine on the height of contraction and increased the frequency. He also perfused the isolated rabbit's heart with ergotamine in concentrations varying from 0.0001% to 0.001%, and noted a reduction in the height and in some cases in the frequency of contractions, especially with the weaker concentrations of the drug. Stronger concentrations, however, gave transitory augmentation of height and frequency of contractions followed by reduction. After atropinisation, the inhibitory action of ergotamine was suppressed. Viotti explained the inhibitory action of ergotamine as due to excitation of the vagal nerve endings and the motor action produced either normally or after atropinisation by feeble doses as due to stimulation of the sympathetic nerve endings. Rothlin (1923) noted a significant decrease in the heart rate of cats and dogs by repeated injections of 0.5 mgm. ergotamine, which was not affected by section of vagi or by atropinisation. In very small doses, however, he noted that/

that the systolic effect was increased, and in bigger doses the frequency and the systolic effect decreased. Andrus and Martin (1927) found that ergotamine, in doses from 0.25 to 1.0 mgm. injected intravenously, slowed the heart in dogs and cats under artificial respiration anaesthetised with urethane and with the vagi intact; the slowing was relieved by atropine. In animals with both vagi cut and 2.0 mgm. atropine sulphate injected intravenously, ergotamine still produced slowing of the sinus rhythm, a prolongation of the P-R interval and a delayed transmission of the excitatory process in the auricle. Subsequent injection of 1 cc. of 1 in 10,000 of adrenaline failed to cause acceleration. Otto (1928) failed to demonstrate any significant action of ergotoxine or ergotamine on the accelerator nerve mechanism of the heart of the cat or the dog in doses from 18 mgm. to 110 mgm. per kilogram. The animals he used were either narcotised with chloretone and ether or were decerebrated and maintained under artificial respiration with double vagotomy. All injections were followed by slowing of the heart and there was a variable degree of diminution of accelerator nerve response but never a complete disappearance. Edward Coelho (1928) noted immediate slowing of the cardiac rhythm and augmentation of the P-R interval with 1 mgm. per kilogram ergotamine in a chloralose anaesthetised/

anaesthetised dog but the rhythm was immediately accelerated by 0.5 cc. adrenaline (1 in 1000) injected intravenously after an interval of 15 mins. Ergotamine in doses from 1 to 2 mgm. per kilogram was also able to diminish response to a preceding intravenous injection of 0.5 to 1.0 cc. of adrenaline (1 in 1000). Moore and Cannon (1930) found that in the unanaesthetised cat 1.0 mgm. ergotoxine per kilogram reduced the basal heart rate in the normal and in the sympathectomised animal but had not much effect on the vagotomised ones. Struggling of the animal increased the heart rate both before and after ergotoxine except in the vagotomised animal where the increase was not significant. Youmans and Trimble (1930) observed that in the trained unanaesthetised dogs intravenous injection of 0.25 to 0.5 mgm. ergotamine brought about immediate slowing of the heart to about 53 per cent., which was prevented or abolished by 0.05 mgm. per kilogram of atropine. Epinephrin, 1.0 cc. of 1 in 10,000 to 1 in 3500, injected 4 to 35 minutes after ergotamine, caused no acceleration and 1 cc. of 1 in 1000 epinephrin caused a slight transient acceleration; but the above doses of epinephrin gave similar results in unergotaminised animals too. With vagi cut, however/

however, ergotamine caused definite slowing of the heart unaffected by atropine but less marked than in the intact animal. Rasolt and Walawski (1932) noted that 0.5 to 1.0 mgm. ergotamine injected into the dog caused slowing of heart with continued sinus arrhythmia. Atropinisation or section of vagus caused acceleration to the normal rate. No changes in rhythm were obtained by injecting ergotamine in atropinised or vagotomised animal. Woods, Nelson and Nelson (1932) have noted that in the anaesthetised vagotomised dogs, 0.1 mgm. ergotamine which removed splanchnic constriction to 0.00005 to 0.0001 mgm. epinephrin, was unable to prevent cardiac acceleration caused by similar doses of epinephrin. Agnoli (1927) perfused the frog heart with 0.2 to 0.3 cc. of ergotamine tartarate, 1/2000, to 1 cc. of Ringer in a Straub's cannula and observed a diminution of the systolic contractions. Ergotamine 1/8000 produced noticeable diminution in strength of systolic contractions which in a few minutes disappeared. With a concentration of 1/100,000 no diminution was observed and on the contrary there was an increase in the frequency. A concentration of 1/20,000 had a very slight effect. The addition of adrenaline 1/1000 to a heart completely stopped by ergotamine had no effect. Rothlin (1925) observed/

observed that ergotamine tart. 1/100,000 can stop the positive ino- and chronotropic action of adrenaline 1/2,000,000 on the isolated frog's heart. Amsler (1920) perfused the heart of Rana esculenta by Straub's method and noted that after nicotinisation and ergotaminisation of the ventricle, adrenaline acted inversely and caused either diastolic standstill or a negative ino- and chronotropic effect. This action of adrenaline could be removed by atropine, and was thought to be due to the parasympathetic action of adrenaline as a result of sympathetic paralysis by ergotamine. The drugs used by Amsler were 2 drops each of 0.5% nicotine, 0.05% ergotamine, 0.1% adrenaline and 0.2% atropine. Navratil (1927) perfused the hearts of the toad and Rana esculenta with ergotamine tartrate 1 in 20,000 for 40 mins. and 1 in 10,000 for 30 mins., and noted that after such a perfusion neither adrenaline 1/10^{8} nor the accelerans substance formed in the heart after 2 minutes stimulation of the sympathetic trunk had any effect.

Kolm and Pick (1921) showed that ergotamine could inhibit even the strong cardiac contractions brought about by adrenaline in presence of excess of calcium. Loewi/

Loewi and Navratil (1926) observed that ergotamine sensitised the isolated heart of the frog to the action of vagus substance and acetyl choline. They perfused the frog's heart with 1 in 10,000 ergotamine tartarate for 20 minutes and noted that the negative inotropic action of acetylcholine, 1 in 100,000,000, was considerably prolonged. They further observed that the same concentration of ergotamine which *in vivo* sensitised to the vagus substance and to acetyl choline inhibited *in vitro* the splitting of these substances by the heart extract. Asher (1925) found that the above vagus effect observed by Loewi was due to the hypodynamic condition of the heart or to the presence of ergotamine itself and he further suggested that ergotamine was apt to render the heart hypodynamic.

From the above literature on the action of ergotamine it will be clear that observations have been made mostly on mammals and comparatively fewer on frogs and the results have varied somewhat with different workers. It was thought necessary to reinvestigate the action on the frog's heart with a view to throwing further light upon the problem.

METHOD/

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METHOD/

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In the earlier part of the investigation, perfusion of the isolated frog's heart was carried out with a cannula in the sinus venosus, the fluid being ejected through the aorta. The method chiefly adopted throughout the investigation has been the double cannula method described originally by Hartung and modified by Clark (1911-12 and 1913). In this method the flow through the heart is maintained by means of heart valves and the sinus, the auricle and the ventricle are exposed equally to the action of the perfusion fluid and all parts contract against a pressure. A special advantage of the apparatus is that 1 or 2 cc. or even more of the fluid can be perfused continuously through the heart and further that the lipoids in the heart are not washed out and the heart does not become hypodynamic. The circulation of fluid provides aeration and so no oxygen is needed. Briefly speaking, the method consists in putting a cannula in the inferior vena cava and another in the left aorta. The remaining vessels are ligated and the heart excised. The apparatus, instead of being fixed upon cork by pins, is held by a clip, the latter holding the venous cannula. Movements are recorded by fine clips fixed to the auricle and/

and the ventricle and connected with light straw levers. Considering the manifold advantages, this method was preferred to Straub's method, and to the single cannula open perfusion method.

The Ringer's solution used had the following composition: NaCl 0.7%, KCl 0.014%, $CaCl_2$ anhydrous 0.012%, and $NaHCO_3$ 0.05%. It was always made in glass distilled water and the chemicals used were all B.D.H. (analytical reagents). The pH of the Ringer's fluid was determined before every experiment using Phenol red (pH 6.8 - pH 8.4) or Bromthymol Blue (pH 6.0 - pH 7.6) as indicators and matching against standard B.D.H. tubes. A 1 per cent. solution of pure HCl was employed to reduce the pH as required. Most of the experiments were done with Ringer's solution having a pH of 8.5.

Ergotamine used was always Femergin II of Sandoz and Adrenaline Chloride of Parke Davis and Co.

RESULTS.

On perfusing the heart with the single cannula perfusion method it was noted that ergotamine concentrations of $1/10^6$, 1/500,000, and 1/250,000 had no effect on either the strength or the frequency of contractions/

contractions. A concentration of 1/20,000 diminished the strength of contractions. After washing the heart with Ringer, the strength of contractions improved.

In the perfusion experiments with the double cannulae method, the heart was perfused with a known quantity of Ringer and drugs were added to the Ringer. In some of the experiments, however, the normal Ringer was pipetted off gently and replaced by the drug of a known concentration. The perfusion fluid was usually about 1 cc., rarely 2 cc. The foregoing results with this method are all expressed in terms of the concentration of the drug in the circulating fluid. At the close of the experiments the volume of the perfusing Ringer was measured with a view to be sure of the concentration of the drug perfusing the heart. Ergotamine in different concentrations was employed to perfuse the isolated heart of Rana esculenta. It was observed that concentrations of $1/10^6$ and $1/10^5$ had no obvious effect on such a heart, whereas concentrations of 1/25,000, 1/20,000 and 1/13,000 and 1/10,000 caused diminution in the height of contractions and in some isolated experiments diminution in the rate too and irregularity. The tracings/

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tracings Nos. 1 and 2 show the effects with a concentration of 1/20,000 ergotamine. From Fig.1 it will be noticed that ergotamine caused diminution in the strength of ventricular contractions and in about 10 minutes the beats became irregular. Fig.2 shows the effects of ergotamine in the same concentration and it will be noticed that after a period of 12 minutes the ventricular beats were very much reduced but they never became irregular. Fig.3 shows the negative chronotropic effect followed by irregularity in 1/10,000 of ergotamine. Fig.4 shows the effects of ergotamine on both auricular and ventricular beats in 1/20,000 concentration. From the above it will appear that the response of the isolated frog's heart to ergotamine even in the same concentrations is variable but the drug in the concentrations employed produced most frequently a negative inotropic effect on the heart and sometimes a negative chronotropic effect associated with irregularities. After washing out the ergotamine solution from the isolated heart with normal Ringer, it was noticed that the cardiac contractions improved immediately.

In the observations above mentioned, the pH of the perfusing Ringer was about 8.5. Some observations were also made when the Ringer had a pH of 7.6 and 7.0, and/

and no noticeable difference was seen in the action of ergotamine on the isolated frog's heart. Fig.5 shows a very moderate negative inotropic effect on the auricular and ventricular contractions with 1/40,000 ergotamine.

ERGOTAMINE AND ADRENALINE.

Before discussing the action of adrenaline following ergotamine on the heart, a short account of its known action will be given.

As early as 1895 Oliver and Schafer (1895) found that in the isolated frog's heart the suprarenal extract in less than 1 per cent. concentration produced spontaneous contractions in the quiescent heart, and accelerated rhythm in the infrequent heart, lengthening of the groups if the heart showed group-beating, and an increase in the strength of contractions if the heart was beating feebly. Burridge and Seth (1928-29), perfusing the heart of Rana Tigrina, noted the augmentor action of adrenaline and found that at low dilutions, $1/10^4$ to $1/10^6$, the drug also exerted an independent depressing action which was produced even in atropinised hearts. He also stated that hearts did not accelerate/

accelerate with adrenaline unless the ventricular conditions made acceleration necessary, extra effort being met primarily by a lengthened stroke of the ventricular pump, acceleration only coming in when the latter did not suffice. He further observed that the drug in $1/10^6$ concentration would revive an exhausted heart or in some cases make it worse, and would sometimes cause acceleration on an increase in tone according to the experimental conditions. Brodie and Cullis (1911) found that adrenaline in small amounts produced an increase in force without acceleration, whereas stronger solutions increased the tone and caused acceleration. Barlow (1928) observed that epinephrin in $1/10^{10}$ or $1/10^8$ concentration produced a pure depression in an isolated perfused frog's heart, but the above concentration in combination with acetyl choline $1/10^8$ to $1/10^{10}$ produced positive ino- and chronotropic effects. But $1/10^5$ to $1/10^7$ epinephrin, either alone or with acetyl choline, produced a positive reaction, either predominantly ino- or chronotropic. Higher concentrations 1/1000 of adrenaline proved toxic to the heart. Barlow and Sollmann (1926) observed that epinephrin in concentrations of $1/10^6$ or $1/10^7$ augmented the perfused frog's heart but if perfusion with this concentration was continued, the cardiac/

could paralyse the cardiac accelerator nerve endings. Otto (1928), Edward Coelho (1918), Youmans and Trimble (1930), and Woods, Nelson and Nelson (1932) found no difference in adrenaline response after ergotamine as compared with the normal.

RESULTS.

The action of adrenaline on the isolated frog's heart perfused with double cannulae was investigated first. In an experiment with 1.2 cc. of Ringer, pH 8.5, additions of 0.0001 mgm., 0.0001 mgm., 0.0002 mgm., and 0.0004 mgm. of adrenaline at intervals of about 4 minutes caused slight increases in the height of ventricular contractions with each addition. The effect lasted for a very short time. The height of auricular contraction was found to increase very slightly with the first addition of adrenaline only. Addition of 0.00001 mgm., 0.0001 mgm. and 0.001 mgm. to the perfusion cannula which contained 1 cc. Ringer, pH 8.5, gave identical results. Addition of 0.01 mgm. to 1 cc. of the perfusion fluid caused diminution in the height of contraction of the frog's ventricle and 0.05 mgm. of adrenaline brought about immediate stoppage of the ventricle after a few beats. During the investigations of the action of adrenaline after ergotamine, it was noticed that a concentration of adrenaline from $1/10^8$ to $1/10^6$ caused an increase in

the/

the heights of both the auricular and the ventricular contractions after the heart was perfused with ergotamine for variable lengths of time. In Fig.6 it will be noticed that a heart which was perfused with 1.25 cc. of Ringer, pH 7.6, 0.025 mgm. ergotamine was put in the perfusion fluid. After 14 minutes 0.025 mgm. more of ergotamine was again put in, and after about 13 minutes a further 0.05 mgm. was put in, and subsequently the perfusion continued for 10 minutes. At this stage both the auricular and ventricular beats were greatly diminished. Addition of 0.0001 mgm. adrenaline increased the strength of contractions. Fig.7 is a tracing from another experiment in which 0.15 mgm. ergotamine was added to the perfusing Ringer (about 1 cc.), pH 7.0, in 4 successive doses of 0.025 mgm., 0.025 mgm., 0.05 mgm. and 0.05 mgm. each. The total period of perfusion extended for about 70 minutes. Addition of 0.0001 mgm. adrenaline increased the strength of contraction of the ventricle. Experiments were done in which higher and lower concentrations of adrenaline were used. It was noticed that after perfusion with ergotamine for about 60 minutes ($1/10^6$ concentration for 15 minutes, 1/500,000 for 17 minutes, 1/100,000 for 16 minutes, and 1/25,000 for 12 minutes), adrenaline $1/10^8$ caused augmentation in the height of ventricular/

ventricular contraction. Adrenaline in concentrations 1/40,000 and 1/25,000 always caused further diminution in the height of ventricular contractions and even in the rate of contraction, due certainly to a toxic action.

Using also the single-cannula perfusion method it was noticed that after perfusing the heart with 1/500,000 ergotamine for about 12 minutes, adrenaline ($1/10^6$) caused a distinct and immediate augmentation in height of contractions. In none of the experiments was any significant change in the rate noticed except when adrenaline was used in toxic doses.

INFLUENCE OF CALCIUM.

Ransom (1917) perfused the heart of frogs *in situ* and noticed that calcium-free Ringer reduced the efficiency of the heart which could be restored by adding normal Ringer or adrenaline. He also noted that 0.25 per cent. caffeine, which had no action in normal Ringer, caused a distinct increase in systole and in tone in calcium-free Ringer, and 0.1 per cent. strophanthin had also a marked therapeutic action in absence of Ca when it had none in presence of calcium. Clark/

Clark and Daly (1920-21) observed that diminishing the $CaCl_2$ concentration of Ringer to 0.003 per cent. greatly reduced the force of contraction and impaired the conduction of impulses from the auricles to ventricle, the heart stopping in diastole in 30 minutes. Calcium-free Ringer arrested the heart in diastole in 5 minutes. Increasing the $CaCl_2$ percentage in the Ringer from 0.012 to 0.048 per cent. caused imperfect relaxation in diastole in a few minutes, although the heart continued to respond to stimulations indefinitely. De (1928), working with heart strips of Rana Temporaria, observed that calcium-free Ringer caused a rapid decrease in force of the ventricle amounting to 50 per cent. in less than 3 secs., but normal Ringer restored such a heart very quickly. Clark, Percival and Stewart (1928) observed that the maximum response of the frog's heart is with 0.43 millimolar Ca concentration. Reduction below this depressed the heart response and an increase of the ionic calcium produced little effect until 1.5 millimolar was attained. This and higher values caused marked depression. Da Costa (1926), while studying the action of pituitrin on frog's heart perfused by Straub's method, observed that the drug in calcium-free Ringer had no effect, whereas in normal Ringer it had a positive ino- and chronotropic effect/

effect. Burridge (1914) noted that the depressant action of adrenaline was antagonised by calcium. Kolm and Pick (1921a) observed that lack of free calcium in Ringer solution decreased the activity of the cardiac sympathetic and increased that of the vagal, moreover adrenaline acted on such hearts with the production of a negative inotrope and caused diastolic standstill which was removable by atropine. If the heart was treated with $CaCl_2$, the sympathetic activity was increased and adrenaline produced strong ventricular contractions. The same observers (1921b) noted that with a heart rendered sympathetic hypersensitive with excess $CaCl_2$, acetyl choline also increased the contraction response of the frog's heart instead of diminishing it. They thus concluded that acetyl choline, as also other parasympathetic drugs, has a sympathomimetic action under certain conditions. Agnoli(1927) observed that frog's heart perfused with calcium-free Ringer solution was very sensitive to the action of ergotamine and a concentration of 1/100,000 was effective. A concentration of 1/8000 brought about ventricular paralysis but washing with normal Ringer solution restored the heart to normal.

The part played by calcium on the response of the isolated frog's heart was investigated in the following/

following manner:-

1. The isolated frog's heart was perfused with the normal Ringer solution and then with Ringer free from calcium, followed by ergotamine in calcium-free Ringer. The drug was finally washed out and replaced by normal Ringer solution. Tracing 8 shows that the heart contractions are reduced when perfusion with calcium-free Ringer is carried out: subsequent perfusion with ergotamine (in calcium-free Ringer) in a concentration of $1/10^6$ brought about a negative inotropic effect. On changing back to normal Ringer, the height of ventricular contractions again became normal. Tracing 9 shows the marked negative inotropic effect with irregularity of ergotamine 1/50,000 in calcium-free Ringer. Changing the perfusion fluid to normal Ringer increased the strength of ventricular contractions.

2. The isolated heart was perfused with normal Ringer and then the concentration of $CaCl_2$ in the Ringer was increased and the effects noted. This fluid was then replaced by normal Ringer again. Ergotamine was then added and the effects noted. The calcium chloride percentage in the Ringer was then again increased to the same extent as before. In tracing 10 it will be noticed that when $CaCl_2$ percentage in the Ringer was increased to 0.018 per cent., the strength of ventricular/

ventricular contractions increased. After washing with normal Ringer, 0.05 mgm. ergotamine (to give concentration of 1/20,000) was added to the perfusion fluid (1 cc.) with the usual negative inotropic effect. On adding $CaCl_2$ to increase the strength to 0.018 per cent. $CaCl_2$ again, the effect was at once seen in the increased height of contractions which reached the same value as that produced by the addition of $CaCl_2$ to the normal Ringer. The addition of calcium chloride therefore overcomes the negative inotropic effect of ergotamine and also ergotamine does not prevent the full augmentation of the inotropic effect due to the addition of calcium. Tracing 11 shows similar results with the addition of $CaCl_2$ to make 0.024 per cent. of $CaCl_2$. The beneficial effect of $CaCl_2$ on the ventricular contractions is better marked than in the previous tracing. In other experiments 1/5000 and 1/10,000 concentrations of ergotamine were used in normal Ringer and after the effects of ergotamine were manifested, $CaCl_2$ in the Ringer was increased to three times its percentage in the normal Ringer (i.e. to 0.036 per cent.). The same beneficial action of calcium chloride was observed. In one experiment when the concentration of 1/10,000 ergotamine had made the ventricular contractions irregular, the added $CaCl_2$ was/

was seen to remove the irregularity.

3. The heart was perfused with Ringer containing high percentage of calcium and the effect of adding ergotamine was noted. Tracing 12 shows that addition of 0.05 mgm. to 1 cc. of perfusing fluid with 0.048 per cent $CaCl_2$ (ergotamine concentration of 1/20,000) had no effect. A further addition of 0.05 mgm. ergotamine had also no effect. Other examples in which the $CaCl_2$ concentration in the Ringer was similarly raised to 0.048 or 0.06 per cent ~~prevented~~ failed to disclose the negative inotropic action of such concentrations of ergotamine as would be effective on the hearts perfused with normal Ringer.

Thus it will appear from the above that an excess of calcium in the perfusing Ringer has an antagonistic effect to the action of ergotamine on the isolated frog's heart whereas lack of calcium favours the action.

ATROPINE AND ERGOTAMINE.

Andrus and Martin (1927) found that in anaesthetised animals (dogs and cats) with artificial respiration and vagi intact, a slowing of the heart beats caused by 0.25 - 1 mgm. ergotamine was relieved by atropine. But with vagi cut they did not observe any effect/

effect of an intravenous injection of 2.0 mgm. atropine on the action of ergotamine. Youmans and Trimble (1930) found that in the trained unanaesthetised dog 0.05 mgm. per kilo. atropine prevented or abolished slowing of the heart brought about by intravenous injection of 0.25 to 0.5 mgm. ergotamine. But when the vagi were cut, the slowing of heart by ergotamine, although less marked than in the intact animal, was unaffected by atropine, either before or after ergotamine. Dale (1906) observed that in animals with vagi cut and under artificial respiration, the secondary slowing of the heart observed by ergot alkaloids was not abolished by atropine. Agnoli (1927) observed that after treatment of the isolated heart with 1/1000 atropine, the effect of ergotamine in calcium-free Ringer disappeared almost completely, although subsequent additions of ergotamine caused a negative inotropic effect even after atropine. Viotti (1924) noted that treatment of the isolated guinea-pig's or rabbit's heart with 0.003 per cent. atropine suppressed the inhibitory action of ergotamine. Amsler (1920) noted that atropine removed the negative inotropic action of adrenaline after ergotaminisation and nicotinisation of the isolated frog's heart.

The action of ergotamine was tried on frog's isolated/

isolated heart after the heart was previously perfused with atropine solution. It will be noted from tracing 13 that after perfusion with atropine $1/5\times10^{6}$ for a period of 15 minutes, ergotamine in a concentration of 1/40,000 caused a diminution in the height of ventricular contraction to the extent of 50 per cent., there being no change in the rate. The heart was washed out and again perfused with atropine solution in a concentration of $1/2\times10^{6}$ for 11 minutes. The atropine was washed out and ergotamine 1/40,000 was used for perfusion. This caused diminution in the height of contractions to the extent of 80 per cent. in about 8 minutes. In another experiment after perfusion of the heart with atropine in a concentration of $1/10^{6}$, ergotamine 1/40,000 produced not only a negative inotropic but also a negative chronotropic effect.

The action of ergotamine, therefore, is exerted in its usual way even after atropine.

ERGOTAMINE ON THE SINUS OF THE HEART.

Besides the above observations on the response of the isolated frog's heart, it was considered necessary to study the effects of application of ergotamine on the sinus of the intact heart of the anaesthetised/

anaesthetised frog. This method of investigation was considered necessary with a view to a more thorough understanding of the action which the drug might possess on the nervous elements of the heart. Clark (1912) had observed that local application of heat to the sino-auricular junction caused loss or diminution of the inhibitory action of the medulla which the warming of no other part would do. Mines (1913-14) found that by local application of atropine to the sinus venosus of the frog's heart, the effect of stimulation of the intracranial vagus on the frequency of the beat could be eliminated. Bruno Kisch (1926) in his exhaustive treatise on the action of chemicals and drugs on the frog's heart made the following observations. He used urethane anaesthetised frogs, put pieces of filter paper soaked in drugs and chemicals over the ~~sino-auricular junction~~ sinus. He noted that m/10 KCl caused acceleration of the heart which was manifested even with vagus stimulation. M/10 $CaCl_2$ solution produced primary slowing and secondary acceleration. Filter paper pieces soaked with 1/1000 acetyl choline caused a negative inotropic effect on the heart and with 1/1000 adrenaline produced no effect except when the heart was partially damaged by experimental interference. Pieces of filter paper soaked in 1/1000 ergotamine were applied on the ~~sino-auricular~~ sinus/

sinus
sino-auricular junction of the urethane anaesthetised frog's heart. The application of the drug gave variable results. A series of 14 experiments were performed and it was noticed that in two experiments there was an immediate diminution of the heart rate from 33 per minute to 24 per minute in one experiment and from 45 to 34 per minute in the other. In the rest of the experiments diminution in rate was slight or absent. Application of filter paper pieces soaked with adrenaline solution 1/1000 had generally no effect. A very slight increase or a very slight decrease in the heart rate was, however, noticeable in some experiments. Application of m/10 KCl on the sino-auricular junction either before or after ergotamine had a well marked effect. The systolic tone of the heart increased and there was augmentation of the heart rate which was soon followed by diminution of the rate. The potassium effect was always pronounced even after ergotamine. Tracing 14 shows the ergotamine effect as also the potassium effect when applied
sinus
on the sino-auricular junction on pieces of filter paper.

In some of the experiments small doses of ergotamine were injected into the abdominal vein of a urethane anaesthetised frog and records taken. Either no/

no effect or slight diminution in the heart rate was observed. Fig. 15 attached shows that injections of 0.005 mgm. ergotamine had no effect. About 9 minutes later further injections of 0.01 mgm. ergotamine similarly failed to produce any effect. About 11 minutes later 0.2 cc. of $1/10^6$ adrenaline was injected and this caused a very slight increase in the strength of ventricular contractions.

DISCUSSION.

The investigations of Dale led him to conclude that ergot paralysed the myoneural junctions of the true sympathetic system and he (1906) observed "that the paralysis already shown to be confined to the myoneural junction of the true sympathetic system is further limited to those of motor functions leaving those concerned with inhibition relatively or absolutely unaffected". Since this memorable work a considerable number of investigations have been carried out on ergotoxine and the comparatively more recent alkaloid ergotamine which has been shown by Dale and Spiro (1922) to possess the same pharmacological action as ergotoxine. Rothlin (1929) observed that the exclusive action of ergotamine on the augmentor nerve fibres as first proclaimed by Dale is not borne out by facts. He claims that it not only paralyses the augmentor functions/

functions of the sympathetic but also the inhibitory functions. Amsler (1920) showed that ergotamine inhibited the action of the end apparatus of the sympathetic and Kolm and Pick (1921) also made the same observation. Otto (1928) and Woods, Nelson and Nelson (1932) stated that the drug had little effect upon accelerator nerve response of the heart. Coelho (1928) also noted that ergotamine did not inhibit the action of adrenaline on the heart. Youmans and Trimble (1930) concluded that the action of the drug depended on the balance between the vagal and the sympathetic mechanisms and there was also the possibility that the effect of the drug was due to its toxicity on the heart muscle. Andrus and Martin (1927) suggested that the action of ergotamine might be quite apart from vagal action in view of the fact that the drug produced slowing even after atropinisation. Moore and Cannon (1930) concluded that the slowing of the heart by ergotamine was due to central action which increased the vagal tone. Asher (1925) thought that the drug was toxic to the heart and rendered it hypodynamic. Zunz (1932) suggested that in small doses the slowing of the heart by ergotamine was due to (1) central action as a result of increased B.P.; (2) stimulation of the vagi; (3) direct action on the myocardium. In large doses there is the paralysis of the sympathetic. In view of the different suggestions given/

given as to the mode of action of ergotamine, one has to examine each of them in the light of the present work.

SYMPATHETIC NERVE ENDINGS.

As will be noticed from the results stated before, adrenaline even in very dilute solution, $1/10^8$, increases the strength of contractions of the heart which has been perfused with strong concentrations of ergotamine for as long as 1 hour or more. The negative inotropic effect observed by Amsler (1920) after ergotaminisation of the heart was never observed except in a few of the earlier experiments when very strong concentrations of adrenaline were used and this was evidently due to the toxicity of the solution. It is possible that the negative inotropic effect observed by Amsler was due to the accumulation of acid which may have caused a change in the pH of the solution. This is quite likely to take place in the Straub method of perfusion, which he used, and secondly it seems he used very strong adrenaline solution. Thus the evidence goes to prove that the sympathetic nerve endings are not paralysed in the isolated frog's heart under the conditions of the experiment and these observations further lend support to those of Otto (1928), Wood, Nelson and Nelson (1932) and Edward Coelho (1928) on mammals.

Regarding/

Regarding the action of ergotamine on the parasympathetic nerve endings, proofs to the effect that it stimulates them have been found in the work done by Andrus and Martin (1927), Youmans and Trimble (1930) and Viotti (1924). The present observations on the isolated frog's heart fail to reveal any vagal action since, as ~~it~~ has been observed, ~~that~~ ergotamine continues to exert its negative inotropic effect on the heart even if the latter has been previously treated with atropine. Nevertheless, I am inclined to believe that ergotamine does increase the vagal tone to a certain extent in the intact heart of the anaesthetised animal. As has been pointed out previously, the application of the ergotamine over the ~~sino-auricular junction~~ sinus or its injection into the abdominal vein of the frog in some cases does decrease the heart rate; the possibility of such a decrease being due to augmentation of vagal tone cannot be lost sight of. This effect is undoubtedly of a temporary nature especially when the drug is applied over the sinus ~~auricular junction~~ and is also not very pronounced.

In the isolated frog's heart, at any rate, one is inclined to conclude that the action of the drug is exerted on the cells of the heart muscles themselves. The question then arises whether the action is a surface action or whether the drug enters the interior/

interior of the cells. The rapidity with which the drug acts leads one to believe that the action is exerted on the cell surfaces. Most other drugs and chemicals are believed to act principally on cell surfaces. Lack of calcium and excess of potassium etc. all exert their actions through the cell surfaces. It has been noticed, as has been shown before, that excess of calcium in the Ringer prevents the action of ergotamine on the myocardium, and deficiency of calcium augments the action. Addition of calcium to the perfusing Ringer of the heart already depressed by ergotamine antagonises the depression. It would thus seem that the presence of calcium prevents the cell surfaces being acted upon by the drug and lack of calcium facilitates the action.

It has been remarked that the application of adrenaline on the sinus has no effect on the heart either before or after ergotamine. This is also in accordance with the findings of Bruno Kisch (1926) who observed that action of adrenaline is seen only when the heart was damaged during manipulation. Application of m/10 KCl, on the other hand, increased the tone and rate of heart beat both before and after ergotamine followed by a decrease. It is suggestive that the action of KCl solution is a local action exerted on the sinus.

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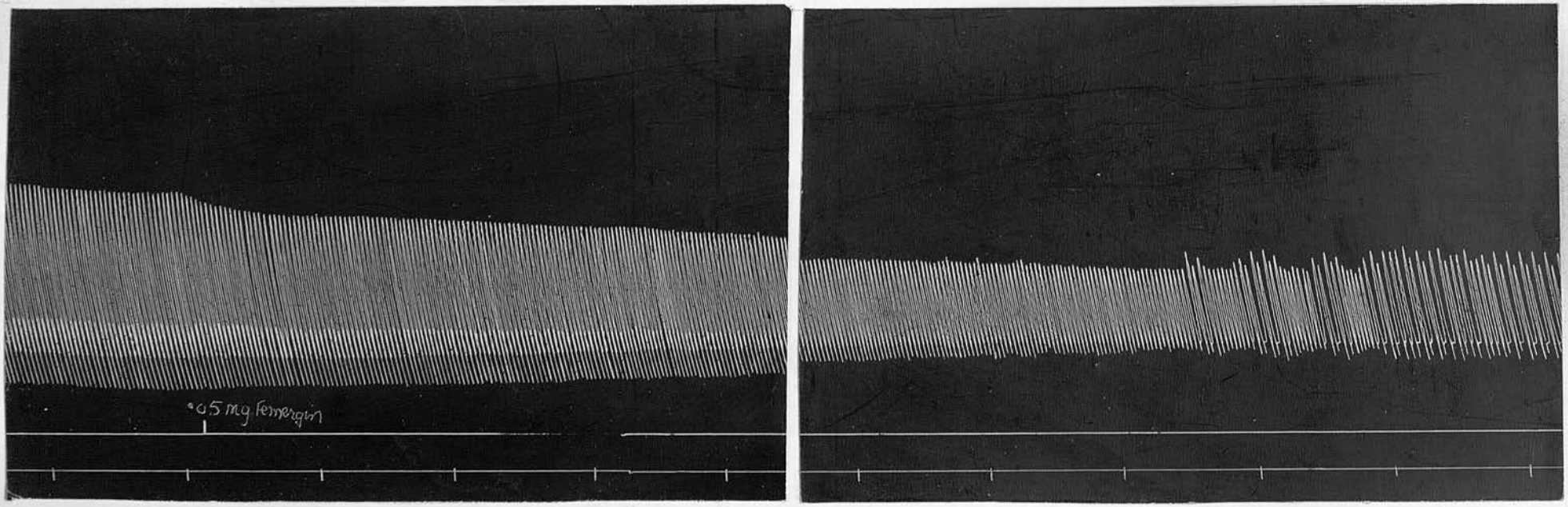
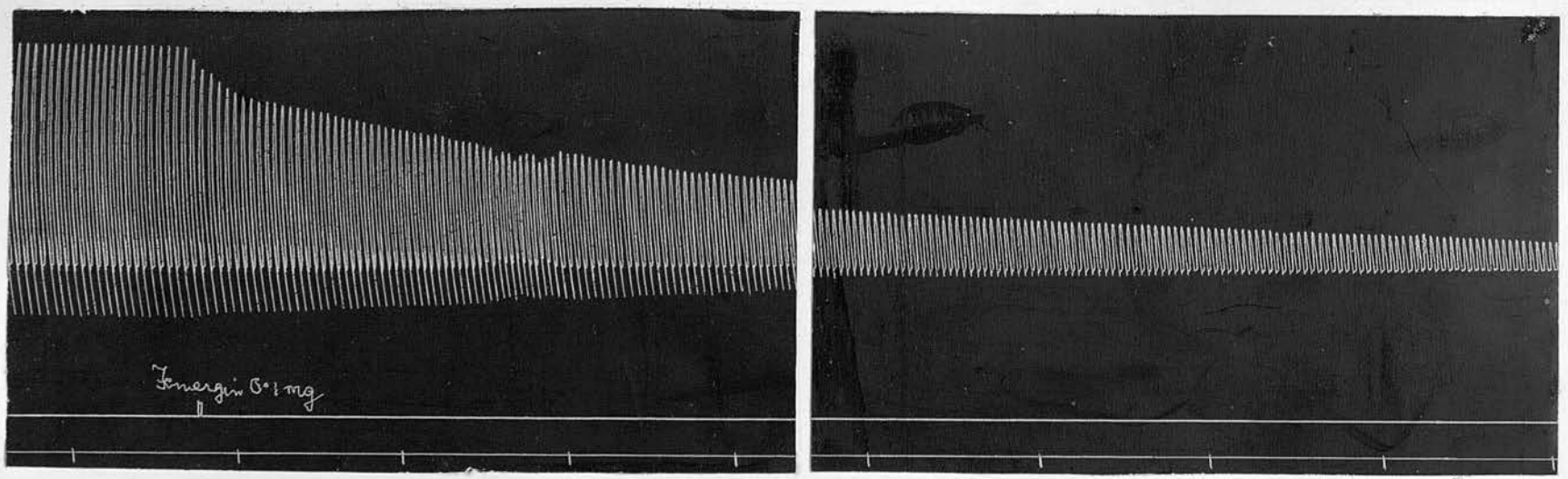
Figure 1.



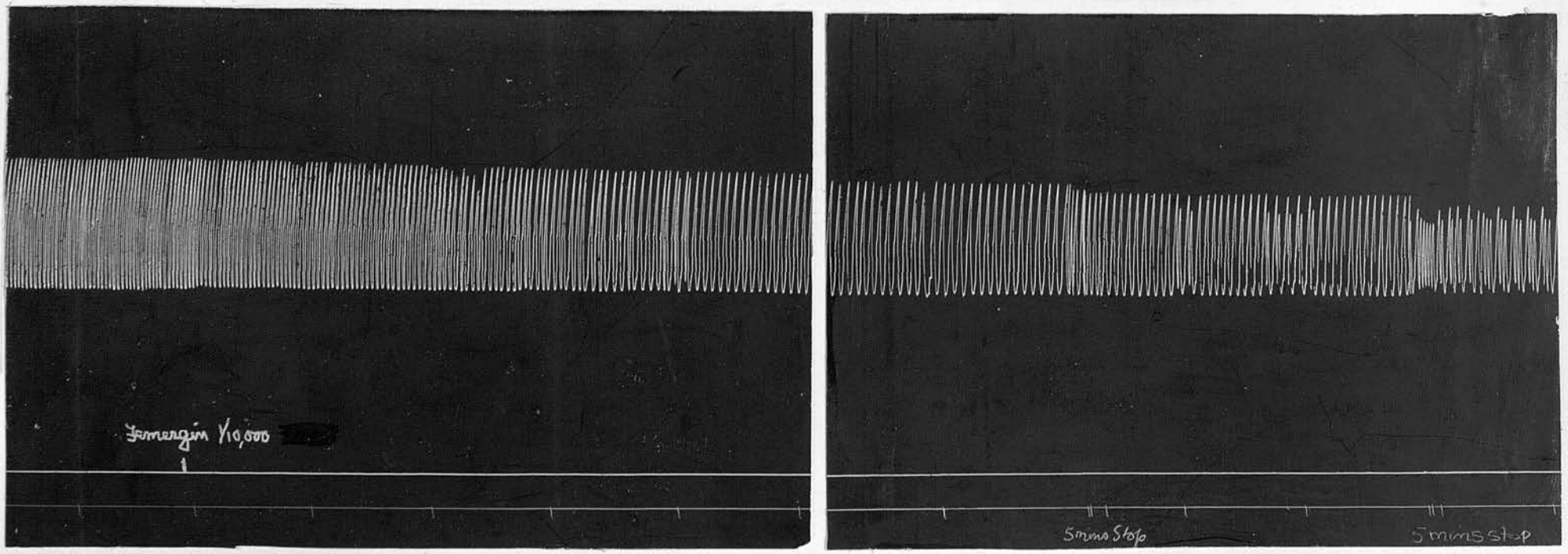
Rana Esculenta (Hungarian). Heart perfused with Clark's method.
The tracing shows the action of 1/20,000 ergotamine Tartrate in Ringer pH 8.5.
Volume of Ringer perfusing the heart was 1 c.c.
At the signal 0.05 mgm ergotamine was added to the perfusing Ringer.
Time: 1 per min.

Figure 2.



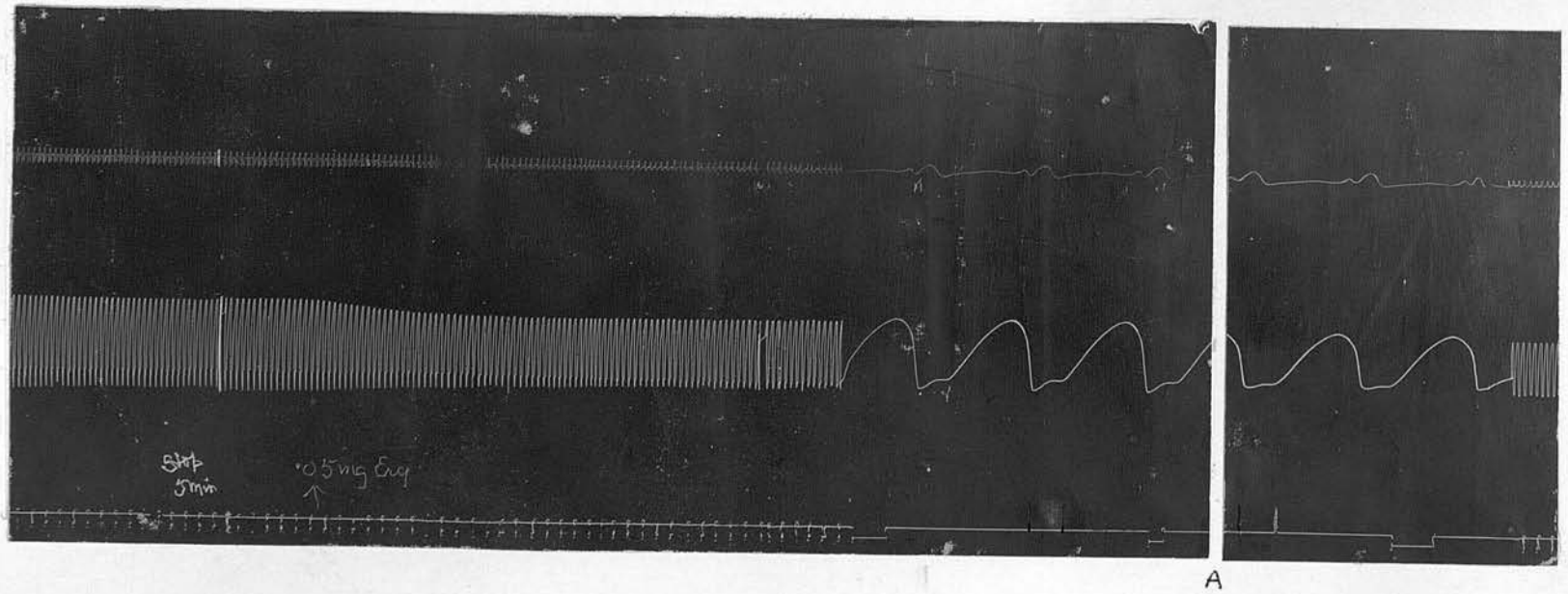
Rana Esculenta (Hungarian). Heart perfused by Clark's method.
The tracing shows the action of 1/20,000 Ergotamine tartrate in Ringer pH 8.5.
Volume of Ringer perfusing the heart was 2 c.c. At the signal 0.1 mgm ergotamine was added to the perfusing Ringer.
Time 1 per minute.

Figure 3.



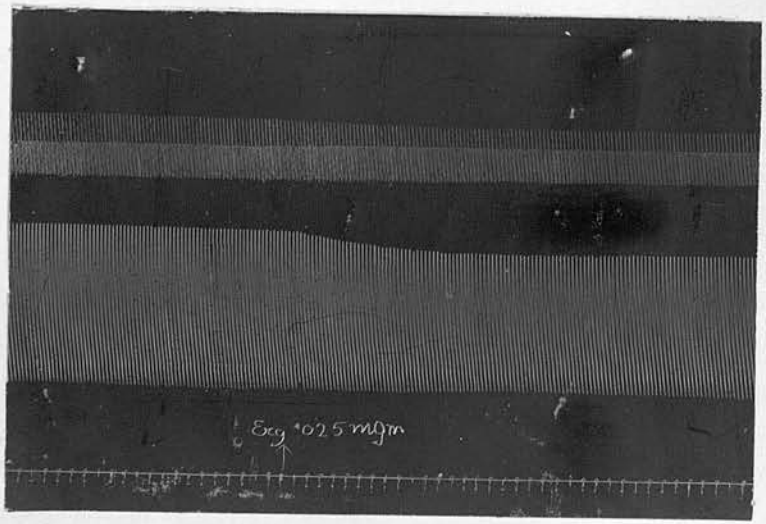
Rana Esculenta (Hungarian). Heart perfused by Clark's method.
The tracing shows the action of 1/10,000 ergotamine tartrate in Ringer pH 8.5.
Volume of Ringer perfusing the heart was 2 c.c. At the signal, the normal Ringer was replaced by Ringer containing ergotamine.
Time 1 per minute.

Figure 4.



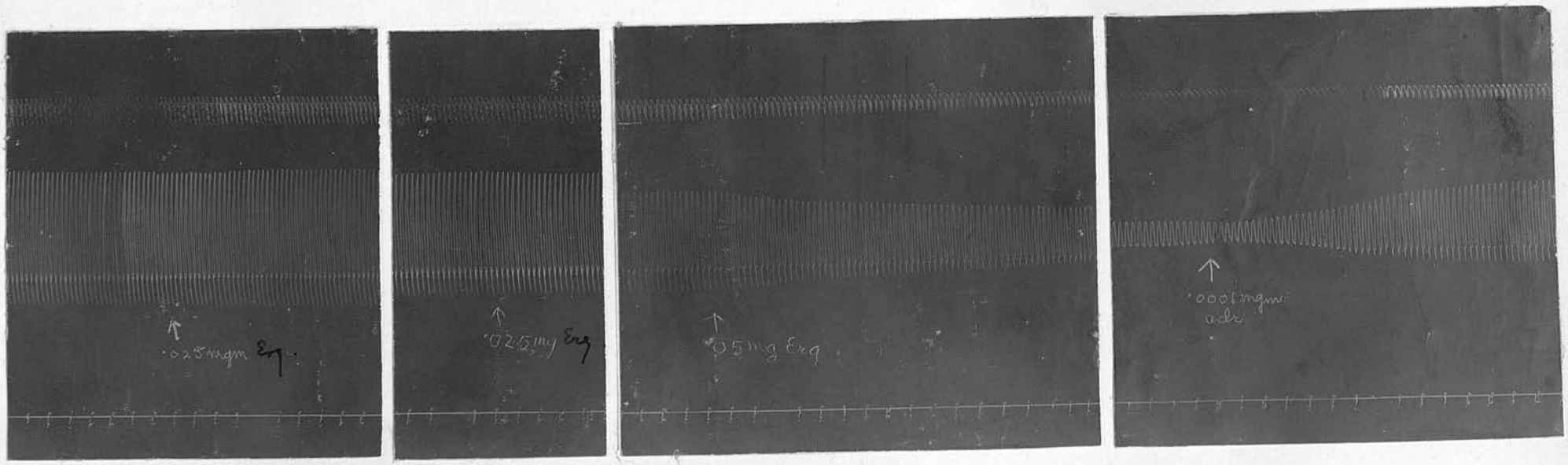
Rana Esculenta (Hungarian). Heart perfused by Clark's method.
The tracing shows the action of 1/20,000 ergotamine tartrate in Ringer pH 8.5. The upper tracing is auricular and the lower ventricular.
Volume of Ringer perfusing the heart was 1 c.c. At the signal 0.05 mgm ergotamine was added to the Ringer.
Time 12 per minute. At the mark A, tracing for 6 minutes 30 seconds have been removed.

Figure 5.



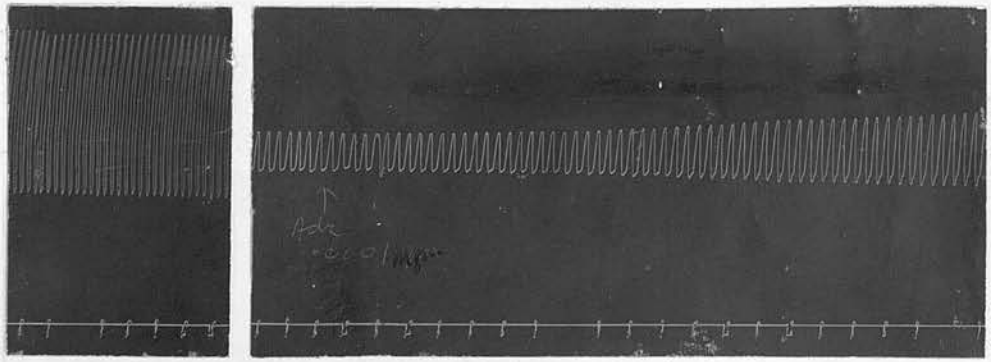
Rana Esculenta (Hungarian). Heart perfused by Clark's method.
The tracing shows the action of 1/40,000 ergotamine tartrate in Ringer pH 7.0.
Upper tracing is Auricular and the lower is Ventricular.
Volume of Ringer perfusing the heart was 1 c.c. At the signal 0.025 mgm ergotamine was added to the perfusing Ringer.
Time 12 per minute.

Figure 6.



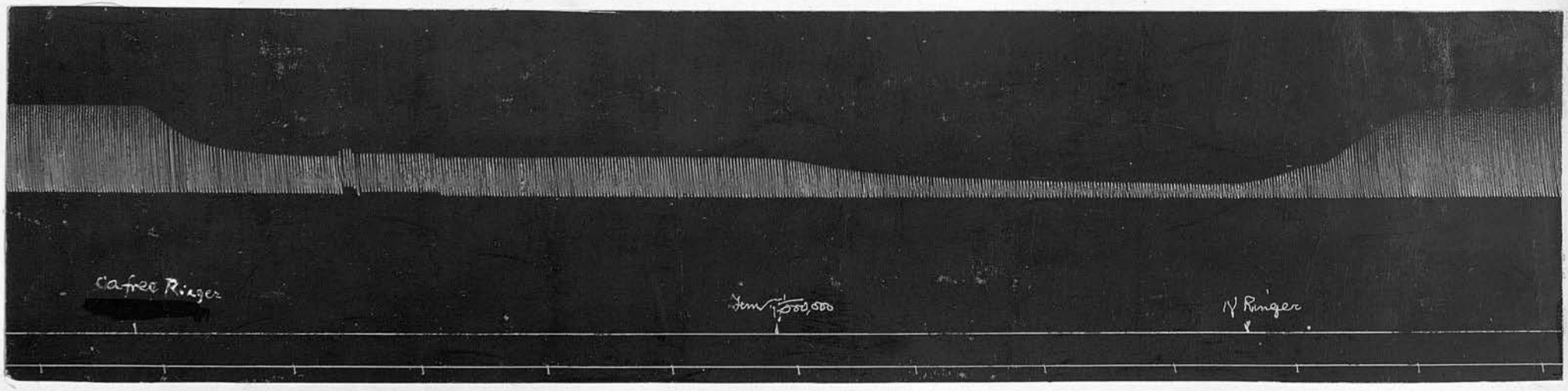
Rana Esculenta (Hungarian). Heart perfused by Clark's method.
The tracing shows the action of 1/12,500,000 Adrenaline after perfusing the heart with ergotamine tartrate for 37 minutes in Ringer pH 7.6. Upper tracing is auricular and the lower ventricular.
Volume of Ringer perfusing the heart was 1.25 c.c. At the signals, 0.025 mgm, and 0.05 mgm. ergotamine and .0001 mgm adrenaline were added to the perfusing Ringer.
Time 12 per minute.

Figure 7.



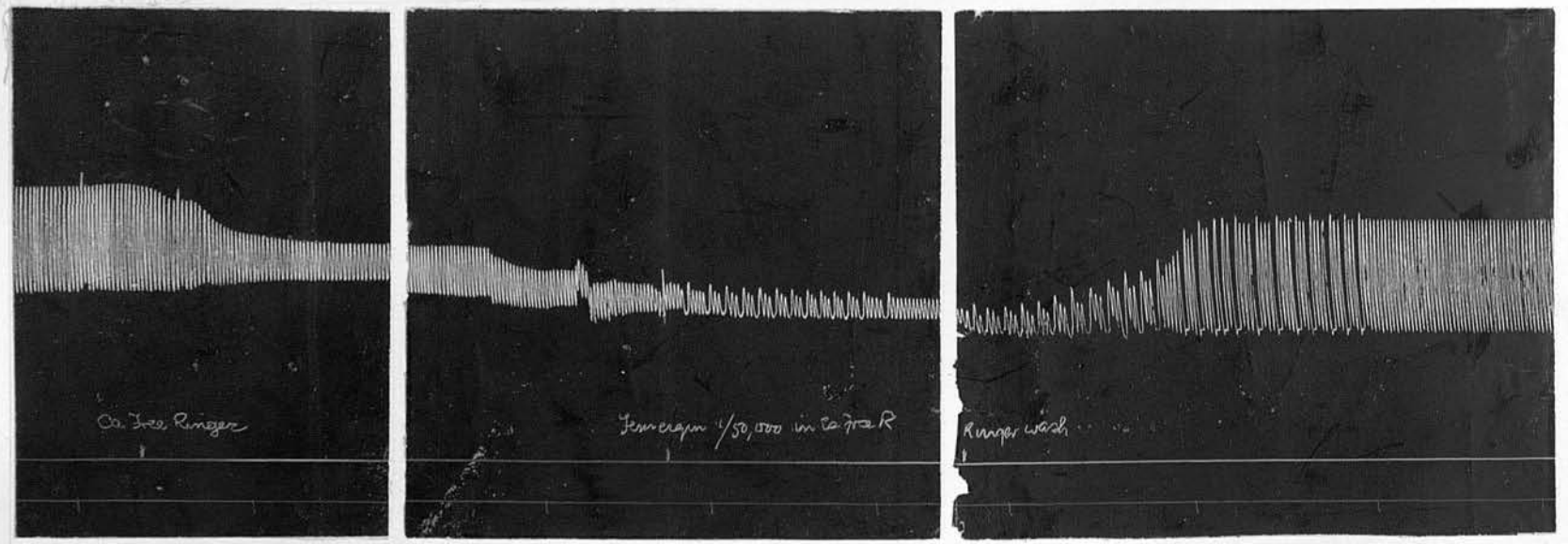
Rana Esculenta (Hungarian). Heart perfused by Clark's method.
The tracing shows the action of 1/10,000,000 Adrenaline after perfusing the heart with ergotamine tartrate for 70 minutes in Ringer pH 7.0.
Volume of Ringer perfusing the heart was 1 c.c. Ergotamine was added to the perfusing Ringer in 4 instalments (not shown) of 0.025 mgm, 0.025 mgm, 0.05 mgm, and 0.05 mgm.
Time 12 per minute.

Figure 8.



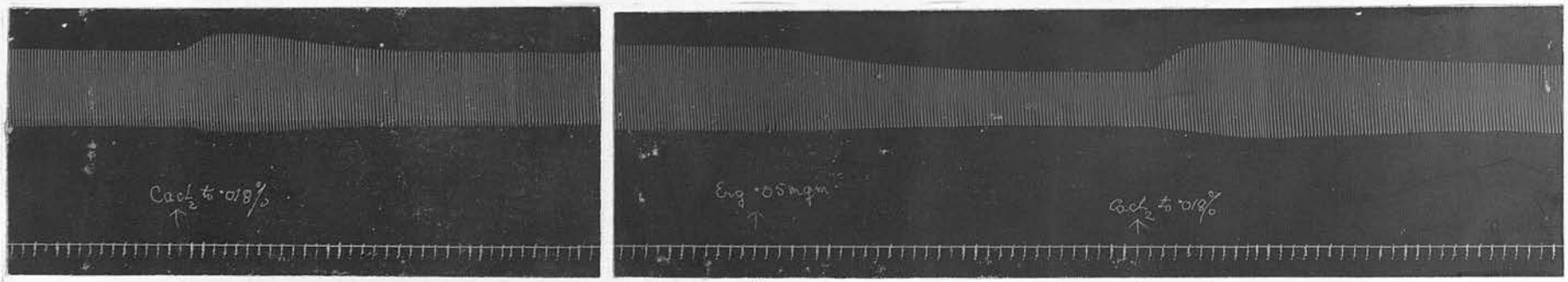
Rana Esculenta (Hungarian). Heart perfused by Clark's method.
The tracing shows the action of 1/1,000,000 ergotamine tartrate in $CaCl_2$ free Ringer pH 8.5.
Time 1 per minute. (temergin)

Figure 9.



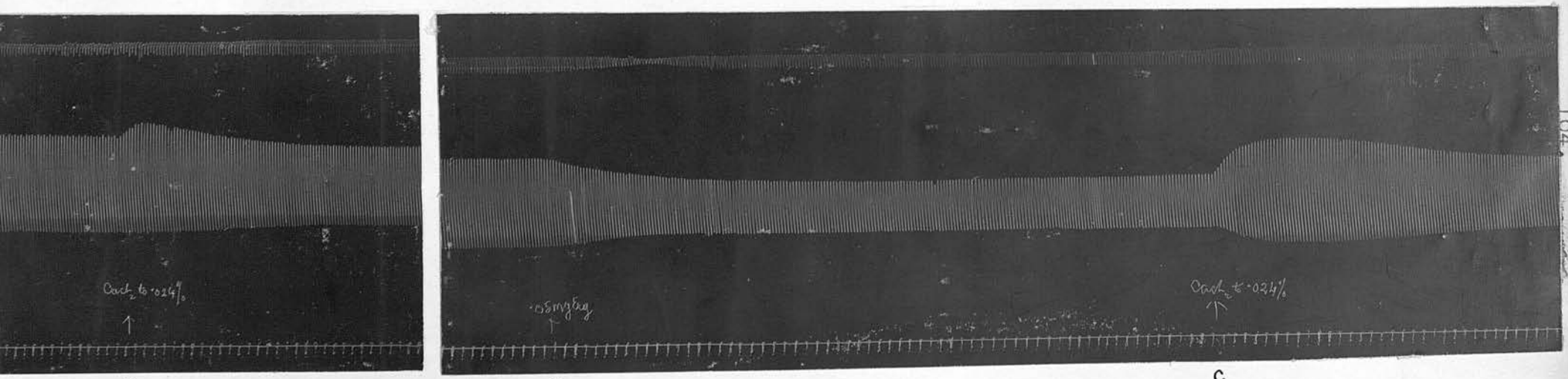
Rana Esculenta (Hungarian). Heart perfused by Clark's method.
The tracing shows the action of 1/50,000 ergotamine tartrate in $CaCl_2$ free Ringer pH 8.5.
At the signals marked, the perfusing Ringer was changed to $CaCl_2$ free Ringer, to ergotamine (Semergin) in Ca free Ringer, and to normal Ringer again.
Time 1 per minute.

Figure 10.



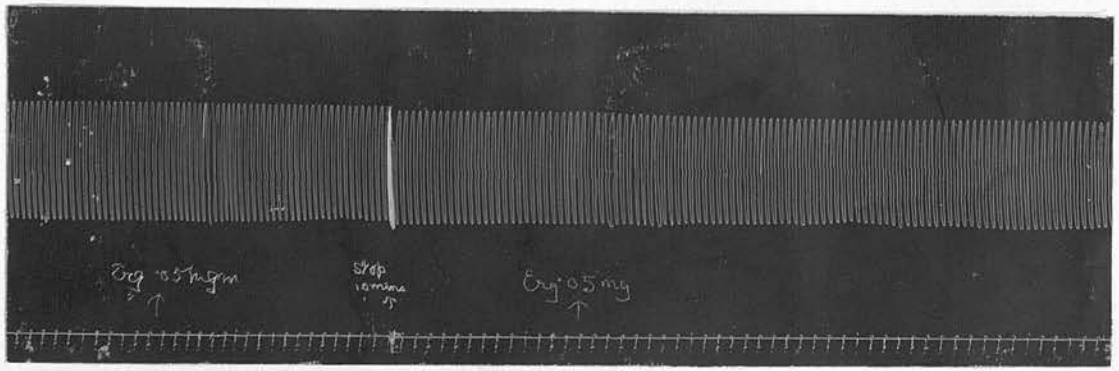
Rana Esculenta (Hungarian). Heart perfused by Clark's method.
The tracing shows the effect of increasing the $CaCl_2$ percentage of the perfusing Ringer to 0.018 both before and after ergotamine 1/20,000.
Volume of perfusing Ringer was 1 c.c. At A $CaCl_2$ solution was added to make 0.018 per cent. The perfusing fluid was then replaced by 1 c.c. normal Ringer (not shown). At B 0.05 mgm ergotamine was added. At C $CaCl_2$ solution was again added to make 0.018 per cent.
Time 12 per minute.

Figure 11.



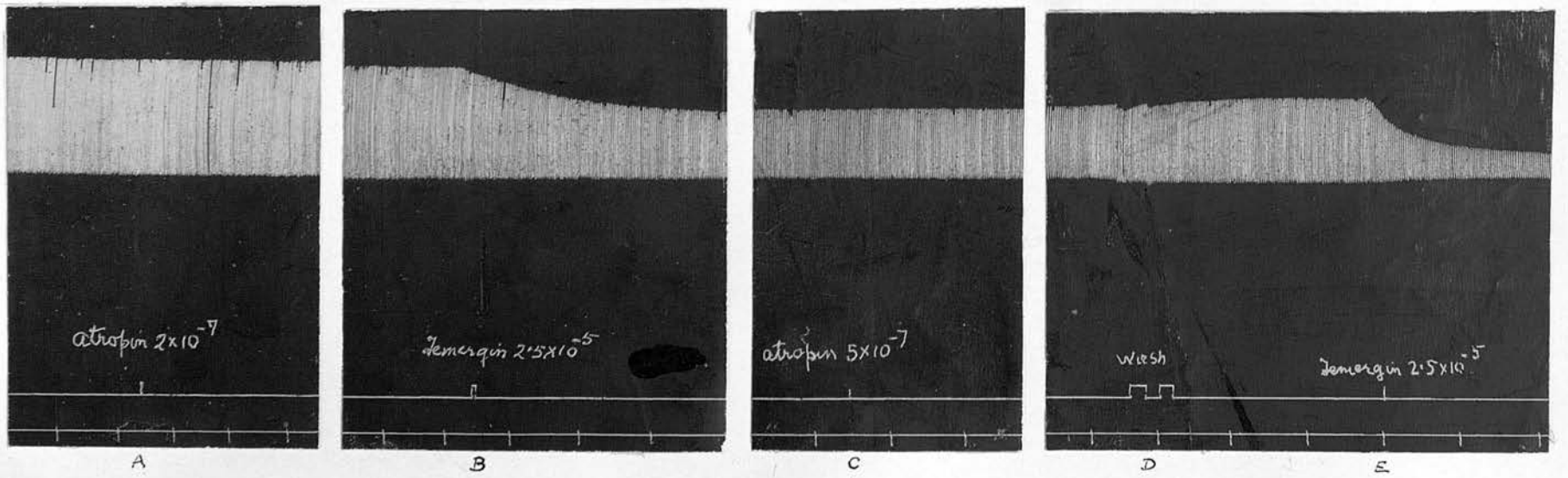
Rana Esculenta (Hungarian). Heart perfused by Clark's method.
The tracing shows the effect of increasing the $CaCl_2$ percentage of the perfusing Ringer to 0.024 both before and after ergotamine 1/20,000. Upper tracing is Auricular and the lower Ventricular. Volume of perfusing Ringer was 1 c.c. At A $CaCl_2$ solution was added to make 0.024 per cent. The perfusing fluid was then replaced by 1 c.c. normal Ringer (not shown). At B 0.05 mgm ergotamine was added. At C, $CaCl_2$ solution was again added to make 0.024 per cent.
Time 12 per minute.

Figure 12.



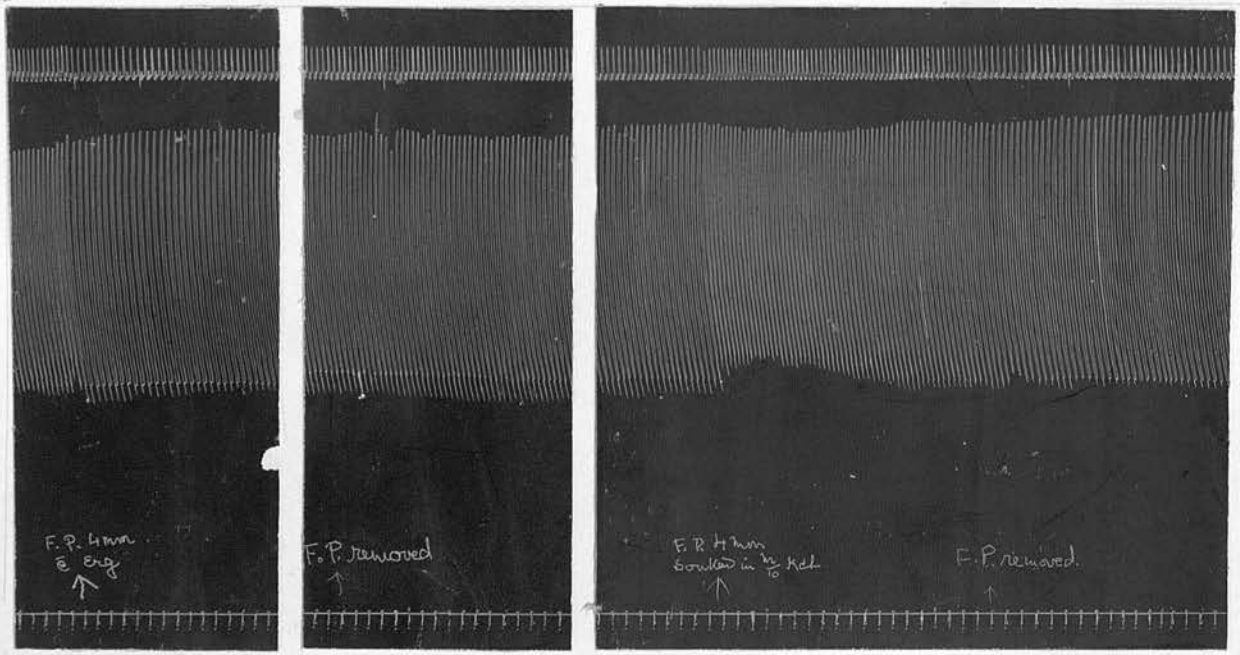
Rana Esculenta (Hungarian). Heart perfused by Clark's method.
The tracing shows the effect of ergotamine in Ringer having 0.048 per cent $CaCl_2$.
Volume of perfusing Ringer was 1 c.c. and the Ringer contained 0.048 per cent $CaCl_2$. At A, 0.05 mgm ergotamine was added into the perfusing Ringer. At B, record was stopped for 10 minutes. At C, 0.05 mgm ergotamine was again added.
Time 12 per minute.

Figure 13.



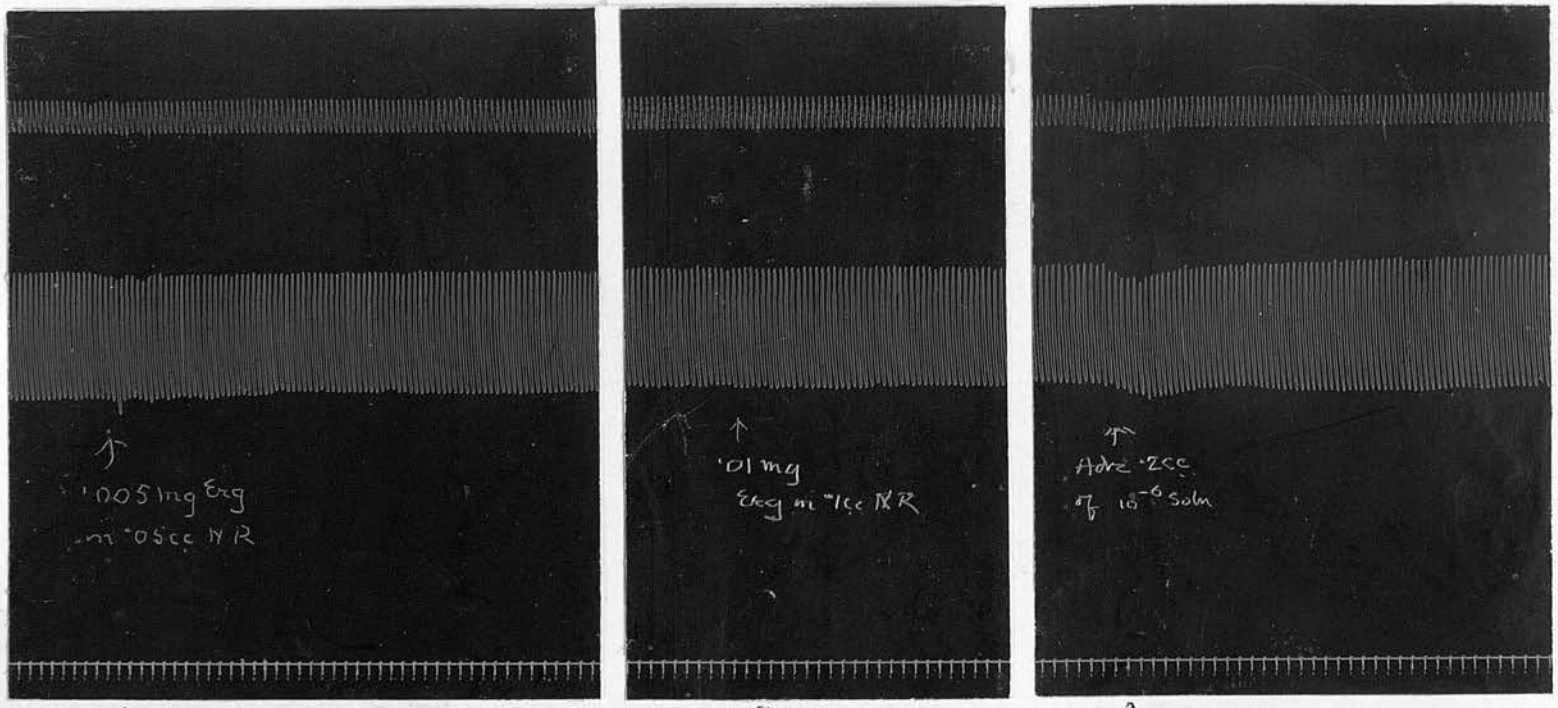
Rana Esculenta (Hungarian). Heart perfused w by Clark's method.
The tracing shows the action of ergotamine after atropin.
The perfusing solutions used were, at A, atropine 1/5,000,000; at B, ergotamine 1/40,000; at C. atropine 1/2,000,000; at D. normal Ringer wash; at E, ergotamine 1/40,000. 9 minutes tracing between A and B omitted.
Time 1 per minute.

Figure 14.



Rana Esculenta (Hungarian). Anaesthetised with urethane.
The tracing shows the effect of application of pieces of filter paper soaked in ergotamine solution, 1/1000, and in m/10 KCL solution on sinus venosus. Upper tracing is Auricular and the lower Ventricular.
At A, filter paper soaked in ergotamine was applied. At B, filter paper was removed. At C, filter paper soaked in m/10 KCL solution was applied. At D, filter paper was removed.
Time 12 per minute.

Figure 15.



Rana Esculenta (Hungarian). Anaesthetised with urethane.
The tracing shows the action of intravenous injection of ergotamine into the frog. Upper tracing is auricular and the lower ventricular.
At A, .005 mgm ergotamine in .05 c.c. Ringer was injected.
At B, .01 mgm ergotamine in .1 c.c. Ringer was injected.
At C, .2 c.c. of 1/1,000,000 Adrenaline solution was injected.
Time 12 per minute.

CORONARY CIRCULATION.

LITERATURE.

Studies on the coronary vessels have been made using many different methods of investigation. The isolated coronary vessel has been investigated by Pal (1909, 1912), Cow (1911), Campbell (1911), Barbour (1912), Park (1912), and more recently by Cruickshank and Subba Rau (1926) and Ducret (1930). Perfusion of non-beating strips of heart muscle has been used by Porter (1898) and by Elliot (1905). Perfusion of the isolated beating heart has been investigated by Langendorff (1895), Porter (1898), and Schafer (1904), and a number of other observers. Wiggers (1909) used the perfused non-beating heart as also Hammouda and Kinosita (1926). Experiments on the whole animal have been done by Porter (1898), by Wiggers (1909) and by Muller (1906) who collected blood emerging from a cut coronary vein, and by Muller, Smith and Graber (1927) who used a Morawitz cannula inserted in the coronary sinus of the intact heart of a dog. After the heart-lung preparation had been developed by Starling, most of the observations were made by the use of this preparation and also with de Barenne's modification. More recently, the innervated heart-lung preparation has also been adopted for the same purpose by Anrep and Segall (1926a). By the use of one or the other of the above methods, observations have been made

with/

with a view to studying the innervation and the action of various drugs on the coronary vessels. The main purpose of the present investigation was to study the response of the coronary vessels to ergotamine and to adrenaline with a view to understanding whether the former gave rise to paralysis of the sympathetic nerve endings in the coronary vessels.

The literature on the action of drugs on the coronary vessels is vast, but a brief review of the subject would not be out of place. The following observations on the isolated coronary vessels have been made. Pal (1909) observed relaxation of the coronary artery of the ox by 2 drops of adrenaline and the relaxation was changed to contraction by pituitary extract. He (1912) further noticed that relaxation of the coronary vessel can be brought about by atropine, adrenaline, caffeine, theobromine and nitrites, and contraction by muscarine, pilocarpine, physostigmine and pituitrin. Cow (1911) observed dilatation in the sheep's coronary arteries by adrenaline. Campbell (1911) using the isolated coronary artery of sheep obtained irregular results but in the ox he always observed relaxation with 1/40,000 hemisine and contraction with pituitrin. Barbour (1912) observed contraction of the human coronary vessels, taken out within/

within a few hours after death, by adrenaline 1/1,000,000, but he observed dilatation in the sheep's and in the pig's. Park (1912) observed relaxation in the ox by 1/50,000,000 epinephrin which he attributed to a sudden lowering of tone. Ducret (1930) observed a strong dilating effect of adrenaline on the coronary vessels and observed that the maximum effect was dependent on a definite state of tone equivalent to a load of 0.2 g. per sq.mm. of artery wall. Spalteholz and Hochrein (1931) found that adrenaline, digitalis, pituitrin and $BaCl_2$ all constricted the surviving coronary arteries and amyl nitrite had a dilator effect. The results with the perfused isolated heart have been as follows. Elliot (1905) perfused a non-beating strip of cat's ventricle by a single coronary artery and found an increase in the flow by adrenaline. He used the non-beating strip to avoid the action of metabolites on the beating heart. Schafer (1904) did not find any effect in the isolated rabbit's and cat's heart with 0.00015 to 0.0006 g. of suprarenal extract. Dale (1909) observed a vasoconstriction in the isolated heart of the rabbit by injecting 5 minims of pituitary extract into the perfusing fluid and remarked that "Coronary arteries afford an example of arterial area slightly if at all affected by/

by adrenaline but stimulated to intense constriction by pituitrin". Dale and Laidlaw (1910) noted a decrease in the coronary flow by 1.25 mgm. histamine in the rabbit. Wiggers (1909) observed that 0.1 to 3.0 mgm. adrenaline constricted the coronary vessels of the dog and dilatation was never obtained. He observed the same in the cat and the rabbit. Brodie and Cullis (1911) observed a diminution followed by augmentation with 0.00001 gm. adrenaline in the perfused rabbit's heart. Campbell (1911) observed no action or slight constriction with adrenaline in the perfused heart of the sheep, dog and cat. Barbour and Prince (1915) observed that in monkeys 0.000025 to 0.002 gm. epinephrin caused a coronary vasoconstriction. Smith, Miller and Graber (1925) obtained a coronary vasoconstriction in the perfused heart of the rabbit by injecting 0.2 cc. of 1/10,000,000 adrenaline. They further observed a decrease in flow by pituitrin and an increase by acetyl choline. Hammouda and Kinosita (1926) observed that adrenaline, 0.1 cc. of 1/50,000, caused coronary vasodilatation, the preliminary constriction being due to mechanical factors. Gunn (1926) noticed a coronary vasoconstriction in the rabbit with 1/30,000 histamine, and in the cat he obtained a pronounced vasodilatation with 1/30,000 to 1/20,000,000 histamine/

histamine. The flow returned to its initial value when the solution was replaced by normal solutions. Gruber and Robert (1926) perfused the isolated heart of cats, rabbits and rats, and observed that 1 cc. of 1/500,000 of the alkaloid adrenaline caused vasodilatation of coronary vessels and 1 cc. of 1/1000 adrenaline caused vasoconstriction. Adrenaline chloride or suprerenalin 1/1000 caused vasodilatation in all dilutions. Straub and Grassmann (1930) observed coronary vasoconstriction with $1/10^6$ pituitrin in the rabbit and vasodilatation in the cat. With histamine $1/10^6$ he observed coronary vasodilatation in the cat but vasoconstriction in the rabbit with $1/10^5$ histamine. Caffeine $1/10^3$ gave marked vasodilatation in the cat and slight vasodilatation in the rabbit. Hochrein and Keller (1931) found that the coronary flow is increased both by adrenaline and by histamine and diminished by CO_2 irrespective of changes in the general circulation. Melville (1932) observed that pituitrin (0.05 mgm. of the extract) reduced the coronary flow markedly but within 5 mins. the flow again increased. The coronary constriction observed by pituitrin could be overcome by adrenaline, ephedrine, histamine, nitrite, and chloretone.

Perfusing the heart with the isolated heart-lung preparation, Evans and Starling (1913) found an augmentation/

augmentation of the coronary flow with 1 cc. of 1/10,000 adrenaline in the blood of the feeder. Markwalder and Starling (1913-14) using the heart-lung preparation observed vasodilatation with 1 or 2 cc. of 1/10,000 adrenaline. Anrep and Stacey (1927) on the same preparation and controlling the heart by stimulation of the right auricle at 150 per min., observed that adrenaline 0.01 mgm. increased the coronary flow by 85% and 0.1 cc. of pituitrin diminished it by 75%: 0.1 g. caffeine and 7.3% CO_2 had also an augmenting effect on the coronary flow. Bodo (1928) working on the heart-lung preparation, observed a diminution in coronary flow with 0.1 cc. pituitrin which was observed only on first addition of the drug but not on subsequent additions of even larger doses; augmentation of the flow was produced by caffeine, amyl nitrite and tr. digitalis. Hausler (1929) also found a coronary constriction with 0.1 cc. of pituitary extract and vasodilatation with 1 cc. of amyl nitrite in the heart-lung preparation. Rossler (1930) observed a vasoconstriction of the coronary vessels with pituitrin and also noted that the latter could be removed by histamine and by papaverine.

The following observations have been made using ergotoxine or ergotamine. Viotti (1924) observed that in/

in the perfused rabbit's heart 0.00036 per cent. ergotamine caused a feeble constriction of the heart vessels but after atropinisation it caused dilatation. In the guinea-pig's heart 0.0001 per cent. ergotamine augmented the coronary flow feebly but it was augmented markedly after atropine. Rothlin (1925) found that the dilatation brought about by adrenaline in the isolated coronary artery of the ox can be prevented by ergotamine. Cruickshank and Subba Rau (1926), using the isolated coronary rings of the ox and the dog, observed that ergotoxine 1/500,000 to 1/100,000 either had no effect or produced transitory vasoconstriction followed by vasodilatation, and that adrenaline continued to produce vasodilatation even after ergotoxine. This was observed even when ergotoxine was added in concentrations considerably larger, i.e. 1/50,000, than those sufficient to abolish or reverse the effect of adrenaline on systemic arteries. Von Saalfeld (1931), working with the heart of reptiles, found that adrenaline caused vasoconstriction, but after ergotoxine it caused vasodilatation. Sato (1931) observed that adrenaline first constricted and then dilated the coronary vessels of the heart of the rabbit and dog. The effect could be suppressed by ergotamine but ergotamine in itself did not influence the coronary circulation.

Condorelli/

Condorelli (1932) in his monograph gave a survey of the action of drugs on coronary vessels, and observed that pilocarpine, nicotine, pituitrin, insulin, barium chloride, organic extracts, histamine and chloroform, all caused coronary vasoconstriction, whereas camphor, purine bases, nitrites, acetyl cholime, lobeline and ethylene diamine and sodium acetate caused vasodilatation.

Besides the influence which the drugs exert on the coronary flow, Anrep (1926) states that mechanical factors, namely, changes in the aortic B.P., the temperature of the circulating blood, the cardiac output (only in the innervated preparations), chemical factors, e.g. oxygen lack and increased CO_2 tension, and progressive dilatation of the heart in the prolonged heart-lung experiments, all affect the coronary flow.

METHOD.

Observations on the isolated coronary vessels rings are of uncertain value so far as negative results are concerned, but positive results are quite reliable. Further, the method is difficult to apply in the study of the smaller arteries which may behave in a different way from the larger vessels.

Perfusion/

Perfusion of the beating heart by inserting a cannula into the aorta and measurement of the fluid emerging from the venae cavae was used by Porter (1898) and Langendorff (1895). But Schafer (1904) pointed out an error in this method arising from imperfect closure of aortic valves which allowed leakage of fluid into the left ventricle. His modification consisted in passing the cannula, which is tied into the root of the aorta, through the aortic orifice into the left ventricle and ligaturing the pulmonary veins (which could be done most easily by tying the roots of the lung). The perfusion fluid, therefore, passed into the root of the aorta at a pressure which was itself enough to maintain the circulation through the coronary system; the pressure was increased and rendered intermittent by the contractions of the ventricle. Wiggers (1909) suggested that Schafer's modification was unsatisfactory. He thought that the perfusion pressure would alter considerably with changes in the cardiac contractions brought about by nerve stimulation or by drugs. Besides, he pointed out that Schafer allowed fluid to fill the right chamber and then recorded the overflow from the auricle and the ventricle by allowing the fluid to leak out of the vena cava and the pulmonary artery. This method

would/

would measure the blood returned to the right heart as long as its beat or tonus remained unaltered for as much blood would flow away as was added by the heart vessels. But when the cardiac contractions were changed by drugs or by nerve stimulation, the outflow would no longer give a true indication of coronary flow. He thus introduced the cannula into the coronary arteries directly and worked on the arrested heart. This, however, had the drawback that the arrested heart itself might enter into a state of rigor and thus compress the intramuscular vessels.

The isolated heart-lung preparation of Knowlton and Starling (1912) is a more suitable method to adopt because the heart is under more normal conditions and is performing external work. Besides, the mechanical conditions as well as the composition of blood and the heart rate are under control. De Barenne (1921) succeeded in measuring the total coronary flow by a modification of Starling's heart-lung preparation but his modification of the preparation has the drawback that only the left side of the heart performed work. The results, however, obtained with this modification have confirmed the results obtained by the Starling heart-lung preparation. In the present investigation the isolated heart-lung preparation of Starling was used/

used. Defibrinated blood was used in all the experiments. Dogs weighing 6 to 12 kilograms were usually employed anaesthetised with amytal, 0.5 cc. per kilo. (10% solution). After the preparation was made, a Morawitz cannula was inserted into the coronary sinus through the right auricular appendix. The coronary sinus outflow was measured by collecting the blood from the coronary sinus in a graduated cylinder for a period of 30 seconds. In some later experiments records were also taken of the coronary flow by using a Condon tipper. The drugs were added to the venous reservoir after the preparation had been running steadily for about half an hour. In the later experiments the drugs were injected into the venous tube. Records were kept of the coronary sinus outflow as also of the heart rate and the cardiac output. The tables of the results embody, besides the sinus outflow and the peripheral output, the total coronary flow, which is approximately 5/3 the sinus outflow, and the total ventricular output which is the observed peripheral output plus the coronary flow.

The drugs employed were either ergotamine tartrate (Sandoz) dissolved in water with the help of a crystal of tartaric acid and heat, or ergotamine methane sulphonate (Sandoz) dissolved in water by stirring with/

with a glass rod for a little while. Other drugs used were adrenaline chloride (P.D. and Co.), pituitrin (P.D. and Co.), and atropine sulphate.

RESULTS.

The results in Table I embody the changes in the coronary sinus outflow obtained by putting in 2.5 mgm. to 10 mgm. ergotamine in the heart-lung preparation. The drug was added to the reservoir except in experiment No. 7/1 (Table I) in which it was injected into the tube leading to the superior vena cava. From the results it may be noticed that ergotamine increased the coronary flow although the increase when a high average B.P. was used was less marked. The increase was also noticed when heparinised blood was used instead of defibrinated blood as in experiment 4 in the Table.

Table II shows the response of the coronary vessels to adrenaline following the additions of 5 to 10 mgm. ergotamine in the perfusion circuit. It will be noticed that even 0.01 mgm. adrenaline was sufficient to increase the coronary flow immediately. Larger doses (0.1 mgm. adrenaline) had the same effect. The response of coronary vessel to ergotamine and adrenaline with an average B.P. of 120 mm. Hg. and the heart run at 170 per min., is shown in Fig.1. Table III embodies the results obtained in a typical experiment with/

TABLE I.

Exp.	Coronary Sinus Outflow	Ergotamine used	Coronary sinus outflow per minute at intervals of				Arterial Blood Pressure
			5 mins.	10 mins.	15 mins.	20 mins.	
+1	18.4 cc.	5 mgm.	16.5 cc.	20.5 cc.	23.5 cc.	-	70 mm. Hg.
*2	20 cc.	2.5 mgm.	20 cc.	23 cc.	-	-	72 mm. Hg.
3	38 cc.	5 mgm.	38 cc.	52 cc.	62 cc.	76 cc.	86 mm. Hg.
⊕4	36 cc.	10 mgm.	38 cc.	44 cc.	48 cc.	-	88 mm. Hg.
5	46 cc.	10 mgm.	46 cc.	62 cc.	78 cc.	-	76 mm. Hg.
6	36 cc.	5 mgm.	42 cc.				120 mm. Hg.
7	90 cc.	10 mgm.	88 cc.	94 cc.	100 cc.		120 mm. Hg.

+On addition of 5 mgm. more of ergotamine, the coronary sinus outflow increased to 27 cc. and 28.5 cc. in 10 and 20 minutes respectively.

*On addition of 5 mgm. more of ergotamine, the flow increased to 28.5 cc., 30 cc. and 35 cc. in 5, 10 and 20 minutes respectively.

⊕Heparinised blood was used in the preparation.

Experiments Nos. 1 to 5 were done in the naturally beating heart, whereas in experiments Nos. 6 and 7 the heart was run by stimulating the auricle.

TABLE II.

Ergotamine and Adrenaline. (For explanation, see Text).

Exp.	Coronary Sinus outflow per minute after ergotamine	Adrenaline used	Coronary Sinus outflow per minute 1 to 2 minutes after adrenaline	Arterial Blood Pressure
1	26 cc.	.1 mgm.	66 cc.	70 mm. Hg.
2	106 cc.	.05 mgm.	148 cc.	85 mm. Hg.
3	100 cc.	.01 mgm.	160 cc.	120 mm. Hg.

The heart in all the three experiments was run by stimulating the right auricle.

TABLE III

Drug used: ERGOTAMINE.

Wt. of Dog 7 Kilos. Temp. 37° C.
Vol. of Blood 650 cc. A.B.P. 72 mm. Hg.

Time	Sinus Outflow	Total Flow A	Peripheral output as measured, B	Total Output A + B	Heart Rate
0.00	20 cc.	33.3 cc.	210 cc.	243 cc.	120
0.00	Ergotamine 2.5 mgm.				
0.05	20 cc.	33.3 cc.	210 cc.	243 cc.	120
0.10	23 cc.	38.3 cc.	210 cc.	248 cc.	120
0.11	Ergotamine 5 mgm.				
0.16	28.5 cc.	47.5 cc.	186 cc.	233.5 cc.	120
0.21	30 cc.	50 cc.	180 cc.	230 cc.	120
0.26	35 cc.	59 cc.	180 cc.	239 cc.	120

with ergotamine. The Table embodies the total coronary flow, the peripheral output as measured and the total cardiac output, as also the heart rate. It will be noticed that the total cardiac output and the heart rate did not undergo any appreciable change with ergotamine.

The effect of pituitrin was tried both before and after ergotamine. The effect of pituitrin is one of coronary vasoconstriction both before and after ergotamine. It was noted that in one experiment with an average B.P. of 76 mm. Hg., 10 mgm. ergotamine was injected into the preparation 16 minutes before adding pituitrin. Subsequent addition of 0.25 cc. of pituitrin decreased the coronary flow from 78 cc. per min. to 12 cc. per min. which was soon reduced to drops; in the other experiment, 0.25 cc. of pituitrin reduced the flow from 94 cc. per min. to 48 cc. per minute. In the latter experiment the average blood pressure was maintained at 88 mm. Hg. and 20 mgm. ergotamine was added to the blood of the preparation previous to the addition of pituitrin.

DISCUSSION.

The investigations into the coronary circulation has been facilitated by the use of a Morawitz cannula, which/

which has proved very suitable for use with heart-lung experiments. It is introduced into the coronary sinus and an estimate of the total coronary sinus outflow can be obtained by multiplying the flow obtained by 5/3. It was shown by Evans and Starling (1913) that the sinus outflow represents only 60% of the total coronary flow and that this was constant under all conditions. This was confirmed subsequently by Markwalder and Starling (1913-14). Anrep, Blalock and Hammouda (1929) further obtained evidence that the relationship between sinus outflow and total coronary flow was only slightly affected by changes in aortic pressure, output of the heart, perfusion pressure, heart rate and temperature of blood. It was the same in the beating, standstill or fibrillating heart. Anrep (1926) stated on Nakagawa's results that this ratio might only change slightly with large increases of aortic blood pressure.

Before discussing the results it is better to discuss the various factors influencing the coronary flow which have been considered by different investigators. Langendorff (1895) and Porter (1898) noticed that increase in the perfusion pressure augmented the coronary flow in the isolated heart. Markwalder and Starling (1913-14) also found that changes in the mean blood pressure in the heart-lung preparation changed the coronary flow. Anrep and Segall (1926) pointed out/

out that the only mechanical factor influencing coronary circulation in the denervated heart-lung preparation was the arterial blood pressure. Anrep and King (1928) also observed that the coronary flow depended on the mean blood pressnre and not on the systolic or the diastolic pressures alone.

Porter (1898) and Wiggers (1909) suggested that an important part in coronary circulation was played by the massaging action of heart muscles during contraction. Nakagawa (1922) observed that variations in the cardiac output had negligible effects on the coronary flow in the denervated heart-lung preparation. Anrep (1926) thought that there was no evidence in favour of the view that physiological increase in the strength of contractions increased the coronary flow and it was demonstrated that changes in the strength of the heart rate produced by changes in the stroke volume had no effect on the coronary flow. Anrep and Stacey (1927) also found that the total coronary flow was independent of the strength of cardiac contractions. In the innervated heart-lung preparation, on the other hand, Anrep and Segall (1926b) noticed that the increase in the cardiac output augmented the coronary flow.

Miller, Smith and Graber (1927), working on the heart of intact dogs under heparin, found reduction in the coronary flow by decrease in the heart rate and an/

an augmentation by an increase. Nakagawa (1922) observed that an increase or decrease in the heart rate had no effect on the coronary flow. Stella (1931) also observed that variations in coronary flow are independent of changes in the heart rate. Hammouda and Kinosita (1926) found that in the isolated perfused rabbit's heart the coronary flow was independent of the heart rate. Anrep and King (1928) observed that changes in the heart rate or in the systematic output had on the whole no effect on coronary flow within wide limits.

Nakagawa (1922) noted an increase in the coronary flow at lower temperatures and remarked that the flow was within wide limits independent of the temperature of the heart. Sassa (1923) observed that temperature changes involving the whole heart altered the cardiac frequency and the coronary flow in opposite directions but when the temperature change was confined to the sino-auricular node or when the extra cardial nerves were stimulated, the cardiac rate and coronary flow changed in the same direction. Anrep and Hausler (1929) observed that in the heart-lung preparation, cooling the blood increased the coronary flow and warming decreased it, but cooling of the heart muscle led to a diminished coronary flow. They remarked that the direct effect of cooling the blood on the coronary/

coronary vessels was made more pronounced than the indirect effect of the more forcible and prolonged contraction of the heart as a result of cooling the heart muscle.

From the above observations it would appear that, other things being equal, in the isolated heart-lung preparation the coronary flow is not at any rate appreciably affected by the strength and rate of cardiac contractions, the temperature, nor by changes in the cardiac output and the temperature. The only factor which is likely to alter the coronary flow is the average blood pressure. Under the circumstances during the observations the average blood pressure was maintained constant as also the temperature and the heart rate.

Besides the mechanical factors described before, there are chemical factors which may influence the coronary circulation. Hammouda and Kinosita (1926) observed acceleration of the coronary flow during anoxaemia. Hilton and Eichholtz (1924-25) reported a similar acceleration by diminution of the oxygen tension of the blood in the heart-lung preparation. Barcroft and Dixon (1907) showed an increase in coronary flow of the isolated heart by increasing the CO_2/

CO_2 tension in the perfusing fluid and this was subsequently confirmed by Markwalder and Starling (1913-14) on the heart-lung preparation. Anrep (1926) thought that the factor directly responsible for an increase in coronary flow was the lack of oxygen and that in asphyxia the main factor for coronary dilatation was the lack of oxygen and not an increase of CO_2.

Further it has also been observed by Anrep (1926) that in the heart-lung preparation the gradual and spontaneous increase of coronary flow was of usual occurrence. It might be thought that the metabolites are responsible for this increase. But Hilton and Eichholtz (1924-25) showed that after an increase in the coronary flow had taken place, replacing the circulating blood by fresh blood did not decrease the coronary flow. They therefore thought that metabolites were not responsible for the increased flow. Anrep (1926), however, suggested that the progressive increase in the coronary flow in the heart-lung preparation was the result of progressive dilatation of the heart in the denervated heart-lung preparation which caused weakening of the heart muscle.

Although Schafer found no effect on coronary flow with adrenaline (1905), a large number of observers/

observers, Elliot (1905), Pal (1909), Barbour (1912), Park (1912), Evans and Starling (1913), Markwalder and Starling (1914), Hammouda and Kinosita (1926), and Ducret (1930) found relaxation of coronary vessels by adrenaline. Contraction was observed by Barbour (1912) on the human coronary artery and by Barber and Prince (1915) on the monkey, by Smith, Miller and Graber (1925) in rabbits. Brodie and Cullis (1911) got relaxation preceded by diminution and Campbell (1911) observed slight constriction in the sheep, dog and cat, and relaxation in the ox. The evidence thus points to the fact that in most of the animals adrenaline produced relaxation of the coronary vessels.

Since the work of Dale (1906) on the action of ergot it has generally been accepted that ergot and its alkaloids ergotoxine or ergotamine have the power to paralyse the motor sympathetics although Rothlin (1929) believes that the inhibitors too are paralysed. If Dale's view is correct, we should expect that adrenaline should continue to cause vasodilatation even after ergotamine. The action of ergotamine on the sympathetic nerve endings of the coronary vessels have been worked out by a number of observers with varying results. Cruickshank and Subba Rao (1926) found that the vasodilator action of adrenaline on the isolated coronary vessel ring remained unaffected even after large/

large doses of ergotoxine. Rothlin (1925) and Sato (1931) on the other hand found that the adrenaline effect was suppressed by ergotamine. Sallfeld (1931) noted a reversal effect of adrenaline after ergotoxine in the heart of the reptile. The present results show that the dilating action of adrenaline on the coronary vessels is not stopped by previous addition of ergotamine to the heart-lung preparation. Thus the observations fit in with the view expressed by Dale (1906) on the general action of ergot, that the inhibitory endings of the sympathetic are not paralysed, and they also confirm the results obtained by Cruickshank and Subba Rao (1926) on the isolated rings of coronary vessels.

The next point under consideration is whether the drug has any action in itself on the coronary flow. The results obtained show that a certain degree of increased coronary flow is always brought about by ergotamine which is not so quick as that by adrenaline and takes about 5 minutes to appear. This effect has been observed when the average blood pressure employed was low, at about 80 mm. Hg., and was also observed, although less marked, with an average blood pressure of 120 mm. Hg. Thus one is justified in attributing to ergotamine a slight vasodilator action.

Rossler/

Rossler and Pascual (1932) state that the vasomotor effect of a drug may be masked, accentuated or reversed by the action which the drug may simultaneously exercise on heart beat. As far as could be observed (Table III) ergotamine had no effect on the heart muscle itself of the heart-lung preparation - and therefore the observed changes in coronary flow are due to its action on the blood vessels alone. The coronary vasodilatation action of ergotamine has also been noted by Viotti (1924) and by Cruickshank and Subba Rao (1926) who observed relaxation preceded by temporary contraction.

Pituitrin has been reported to be a coronary vasoconstrictor by Pal (1909), Campbell (1911), Dale (1909), Smith, Miller and Graber (1926), Straub and Grassman (1930) and Melville (1932), Anrep and Stacey (1927), Bodo (1928) and a number of other workers. In the present observations too, coronary vasoconstriction was obtained with pituitrin, and this vasoconstriction was also observed when pituitrin was used following ergotamine.

So far as has been gathered, no observations on the action of ergotamine have been made on the isolated heart-lung preparation which is by far the most suitable way to study the action of drugs on coronary vessels. The present investigation thus helps one to conclude that the sympathetic nerve endings of the coronary vessels are not paralysed by ergotamine.

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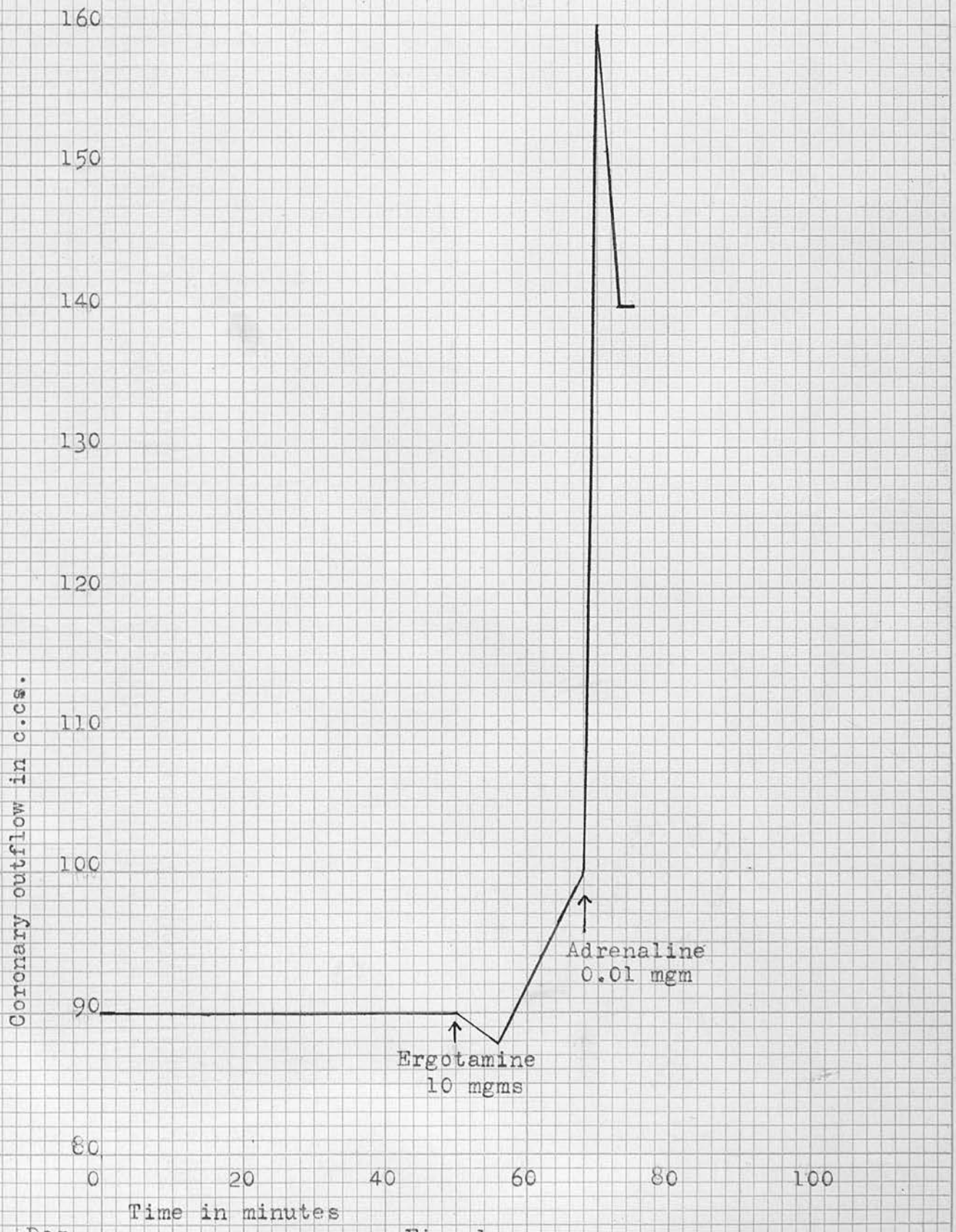

Dog

Fig. 1.

Action of ergotamine and adrenaline on the coronary sinus outflow.
Average B.P. 120 mm Hg.

APPENDIX.

A. Experimental study of the action of insulin on normal and diabetic hearts, by E.W.H. Cruickshank, B. Narayana and D.L. Shrivastava.
Reprint from the Indian Journal of Medical Research, Vol.16, Oct. 1928.

B. Studies in blood diastase, by Charles Reid and B. Narayana.
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Reprint from the Quarterly Journal of Experimental Physiology, Vol.24, 1934.

EXPERIMENTAL STUDY OF THE ACTION OF INSULIN ON NORMAL AND DIABETIC HEARTS

BY

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FROM

THE INDIAN JOURNAL OF MEDICAL RESEARCH, VOL. XVI, No. 2, OCT. 1928.

CALCUTTA
THACKER, SPINK & CO
1928

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[Received for publication, June 5, 1928.]

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A. EXPERIMENTS ON THE WHOLE ANIMAL.

THE whole question of the action of insulin upon carbohydrate metabolism is so beset with complicating factors that one is forced, in an attempt to arrive at some idea as to the mechanism of the action of insulin, to limit the bounds

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of the investigation and to eliminate as many associated factors as are compatible with the investigation of such a complex affair as sugar metabolism.

It is accepted that the liver, skeletal and cardiac muscle play the most prominent of parts in sugar metabolism and in deciding the effect of insulin upon the animal economy. So baffling at times is the interplay of ductless glands, the liver and musculature in determining the ultimate carbohydrate balance of the body that any study of the action of insulin upon the body as a whole, in the present state of our knowledge, is beset with endless difficulties.

For this reason, then, and bearing in mind the vast amount of work done on the function of the liver, glands and skeletal muscle in this connection, we considered that it would be worth while to look at the problem only in so far as the heart was concerned.

1. Methods.

Estimations of:

(1) Blood Sugar.

(2) Glycogen of heart muscle.

Operative Procedure:

(1) Pancreatectomy.

(2) Isolated heart lung preparation.

Estimations.

1. *Blood Sugar.*—This was estimated by MacLean's method.

2. *Glycogen.*—For the estimation of glycogen a modification of Pflüger's method was employed. The work of Evans(1) and that of Holmes and Holmes(2) shows that considerable difficulty may be encountered with the estimation of glycogen. Evans suggested that the greater the volume of dilute alcohol precipitating the glycogen, the smaller would be the results obtained. Holmes and Holmes, however, could recover very small amounts of glycogen very satisfactorily despite a large volume of alcohol. They used a much stronger percentage of alcohol than Evans, but in their test experiments it seems they did not heat for three hours with 60 per cent KOH as is done in the Pflüger's method. We have found considerable difficulty in obtaining good duplicate results. On experimentation with heart, skeletal and diaphragmatic muscle, it was noted that all the glycogen could not be recovered when 60 per cent KOH was used. The alkali, besides destroying proteins and reducing sugars, destroyed to some extent the glycogen also. After a few trials with 30 per cent KOH, this was found to be the most efficient as will be clear from the following typical example of our results:—

The results of experiments on glycogen estimations in the recovery of pure glycogen.

Glucose equivalent of glycogen after digesting with 60 per cent KOH.	Glucose equivalent of glycogen after digesting with 30 per cent KOH.	Glucose equivalent of glycogen expected to be present.
0.325 gramme.	0.385 gramme.	0.0402 gramme.

The alcohol in final dilution was always approximately 75 per cent and it would seem that the loss is occasioned by the use of a too strong alkali.

Accordingly the following procedure was adopted.

About 3 to 4 grammes of muscle were taken and weighed in a weighing bottle, from which it was transferred to a test-tube (18 cm. × 2·7 cm.) in which 30 per cent KOH solution had been kept hot in a boiling water-bath under a reflux condenser. The volume of KOH solution in c.c. was adjusted to equal that of the number of grammes of muscle taken. The muscle usually took 20 to 30 minutes to be disintegrated after which, digestion was continued for about two hours, by which time the fluid had become clear. The test-tube was then removed, cooled under running water, and its contents transferred to a centrifuge tube of about 55 c.cs. capacity; the test-tube was thoroughly washed out with small quantities of water and the washings added to the centrifuge tube, the quantity of water used being about 4 to 5 c.cs. Sufficient absolute alcohol was then added to precipitate the glycogen; this was usually about 40 c.cs., depending upon the quantity of glycogen present. While adding alcohol it was noted that at a certain point a flocculent precipitate makes its appearance; the addition of the alcohol should be continued till no further change in the turbidity be observed. After this the centrifuge tube was covered with a small beaker and set aside overnight for the precipitate to settle completely.

Next morning the tube was centrifuged until the supernatant liquid was quite clear. The clear liquid was filtered off by decantation through a starch-free filter paper. The residue was thoroughly stirred, by means of a glass rod, with a small quantity of alcohol, centrifuged and filtered as above. If the supernatant liquid was not colourless after the second washing, the process was again repeated. The residue in the centrifuge tube was dissolved in water and transferred to a conical flask of about 100 c.cs. capacity. Any residue left on the filter paper was also dissolved and transferred to the flask. The solution was neutralised with acetic acid and to it conc. HCl was added in the proportion of 1·1 c.cs. of acid to every 16 c.cs. of solution. The acidified solution was kept for three hours on a boiling water-bath to ensure complete hydrolysis. After hydrolysis the solution was neutralized by KOH, filtered through starch-free filter paper and the filtrate made up to 250 c.cs. for glucose estimation by MacLean's method. Usually 4 c.cs. were taken for estimation, and the results expressed as glucose equivalent of glycogen.

Operative Procedures.

1. *Depancreatization.*—In order to obtain thoroughly diabetic animals we performed a complete pancreatectomy. The original technique for the removal of the pancreas was described by Minkowski(3) in 1893, who, by that year, had completed his researches in which he corroborated the theory of Lepine(4) that the pancreas controlled carbohydrate metabolism by the secretion of a hormone

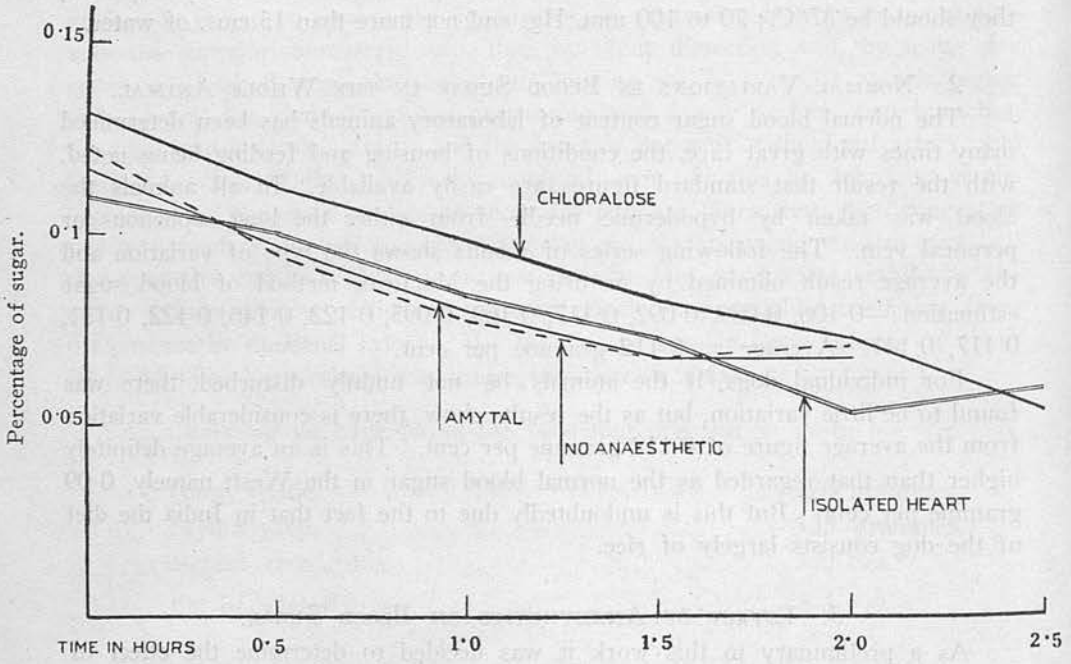

Fig. 1.—Effect of insulin on blood sugar curve with and without anæsthetics.

4. The Effect of Anæsthetics on the Glycogen Content of the Normal Heart.

As a corollary of the changes in blood sugar incident upon the administration of anæsthetics, changes in muscle sugar and muscle glycogen were to be expected. It was necessary therefore to examine this question and to determine which anæsthetic was least disturbing to the glycogen and sugar stores of the muscle with which we would be dealing. The greatest and most incontestible criticism of such experiments as are here described, is that no information can be obtained as to the normal resting content of the heart previous to the experiment. Only by repeated examinations covering a long series of experiments can one arrive at an approximate average for glycogen and free sugar content of the heart.

In repeating this type of estimation we decided that both the right and left heart should separately be examined, for several workers have shown how varied may be the distribution of glycogen in the liver and in different muscles, variations depending not only upon the nature of the animals' diet, but upon the muscle or portion of the organ from which a sample is taken for estimation. Karczag, MacLeod and Orr(9) have further shown that only by careful standardizing of animals, in this case rats, by strict attention to the methods of

feeding and caging recommended by the Wistar Institute for Anatomy in U. S. A., and after a definite and suitable period of starvation can satisfactory results be obtained. In larger animals, however, the extremely uniform results given by Karczag, MacLeod and Orr cannot be expected, at least not under the conditions for the supervision of animals obtaining in India. From approximate average normal results we have, however, been able to show to what extent the glycogen content of the right and left ventricles of the heart may be altered by the administration of anæsthetics. The following table shows that of all the anæsthetics used, amytal undoubtedly has the least effect upon muscle glycogen :—

TABLE I.

The Effect of Anæsthetics on the Glycogen Content of the Normal Heart.

Anæsthetic.	Glycogen per cent in grammes.	
	Right heart.	Left heart.
$CHCl_3$	..	0.380
$CHCl_3$	..	0.146
$CHCl_3$	..	0.281
Chloralose + $CHCl_3$	..	0.309
Chloralose + $CHCl_3$	0.117	0.322
Chloralose + ether	..	0.254
Chloralose, average of 5 experiments	0.539	0.362
Amytal, average of 9 experiments	0.654	0.571

5. THE ACTION OF INSULIN ON BLOOD SUGAR IN NORMAL ANIMALS.

It is known that the initial fall in blood sugar is, within wide limits, independent of the dose of insulin administered and is practically the same whether the insulin be given intravenously or subcutaneously. There is, however, a relationship between dosage and the hypoglycæmia produced, but such a relationship can only be shown by the use of small doses of insulin.

In determining the action of insulin upon blood sugar, the important thing is to see that the animal has a normal blood sugar, has been well fed and is not restless or apprehensive of danger. We have not studied the rate of recovery as it is not germane to our problem seeing that we are to deal with a heart cut off from all supplies of glycogen apart from its own musculature.

The following table shows the reaction of normal animals to insulin:—

TABLE II.

The Effect of Insulin on Blood Sugar with and without Anæsthetics.

Anæsthetic	Blood Sugar in grammes per cent.						Insulin.	
	Normal.	Hypoglycæmia.					½-hourly.	Total.
None ..	0.095	0.095	0.085	0.075	..	..	10 units	30 units.
None ..	0.122	0.095	0.077	0.067	0.070	..	20 „	60 „
None ..	0.144	0.105	0.058	0.032	0.046	..	30 „	120 „
$CHCl_3$ + ether ..	0.122	0.108	0.122	0.100	0.087	..	40 „	160 „
Chloralose ..	0.131	0.111	0.097	0.081	0.072	0.052	40 „	200 „
Amytal ..	0.117	0.097	0.079	0.047	0.067	..	50 „	200 „

The first figure represents the normal blood sugar determined from a sample taken previous to the administration of the anæsthetic. The others are from samples taken half-hourly. Insulin was injected after taking each sample.

The effect of insulin on the blood sugar of the whole animal is seen in the curves in Figure 1, which is a graphic representation of the results already given in Table II. It is evident that when an anæsthetic increases the blood sugar, insulin administered immediately deals with it. In fact it is well known that if the blood sugar be increased, the fall due to insulin may be much more rapid than usual even if it does not reach such a low level as is usual in cases with normal blood sugar.

That the dose of insulin has been sufficient in all cases is seen by the steady fall which reaches its lowest limits in about one and a half to two hours.

We must admit that such tests as these in the whole animal reveal merely the fact that hypoglycæmia will be successfully combated until such time that the scores of glycogen begin to be exhausted. In fact we have been surprised at the amount of insulin which a normal dog will stand, without showing signs of convulsion or tremor, but merely showing a disinclination to stand or be interested in a manner becoming an intelligent dog.

The fall of blood sugar in the isolated heart lung preparation under insulin is also shown. It is in every way comparable to that occurring in the whole animal.

B. EXPERIMENTS ON THE ISOLATED HEART LUNG PREPARATION.

1. Glycogen Content of the Normal and Diabetic Heart Muscle.

As we had to obtain a figure which might reasonably be regarded as an average for glycogen and realising that under our experimental conditions the

isolated heart was cut off from the glycogen depôts of the body, a series of estimations were made of the glycogen content of the right and left ventricles of the heart of whole animals and of hearts which had been isolated from 2 to 4 hours.

Tables III and IV give the details of these estimations.

TABLE III.

Normal Animals.

	NON-EXPERIMENTAL.		EXPERIMENTAL.		Duration of experiment in hours.
	Glycogen grammes per cent.		Glycogen grammes per cent.		
	R.	L.	R.	L.	
	0.657	0.276	0.669	0.470	2
	0.737	0.558	0.590	0.367	2
	..	0.592	0.221	0.646	3
	..	0.932	0.784	0.533	5
	0.731	0.625	0.651	0.494	2
	..	0.779	..	..	..
	0.683	0.780	..	..	..
	0.386	0.427	..	..	..
	0.736	0.568	..	..	..
Average ..	0.654	0.571	0.583	0.502	3
Average for whole heart	0.613		0.543		..

TABLE IV.

Diabetic Animals.

	NON-EXPERIMENTAL.			EXPERIMENTAL.			
	Glycogen grammes per cent.		Duration of diabetes in days.	Glycogen grammes per cent.		Duration of diabetes in days.	Duration of experiment in hours.
	R.	L.		R.	L.		
	1.000	1.181	5	0.875	0.860	5	4
	0.483	0.555	8	0.471	0.615	5	2
	0.408	0.411	4	1.090	0.760	4	2
	0.439	0.559	3	..	0.972	4	3
	..	..	..	0.525	0.410	3	3
	..	..	..	1.310	..	9	4
Average ..	0.608	0.677	5	0.854	0.723	6	3
Average for whole heart	0.643		..	0.788		..	..
Whole heart averages ..				Normal			0.578
				Diabetic			0.716

It will be seen that in the whole animal, the normal heart has an average glycogen content of 0·654 per cent for the right ventricle and 0·571 per cent for the left, while in the isolated heart under experimental conditions, the glycogen content falls to 0·583 gramme and 0·502 gramme per cent for the right and left ventricles respectively. The reason for the fall would at first sight appear to be due to the lack of the replenishing of blood sugar presumably from the liver. It is probable that the percentage of sugar in the plasma falls below normal, and the heart is forced to call upon its glycogen in order to supply the energy required for its activity. It will also be noted that the left heart contains less glycogen than the right, a condition which does not obtain in the whole diabetic animal. In the non-experimental or whole diabetic animal the glycogen contents are, for the right ventricle 0·608 gramme per cent, and for the left 0·677 gramme per cent, while in isolated diabetic hearts the averages are, 0·854 gramme and 0·723 gramme per cent for the right and left ventricles respectively. The average figures for the normal heart under experimental and non-experimental conditions are 0·578 while for the diabetic it is 0·716. While such figures show that the diabetic heart tends to store more glycogen than the normal, a difference of 70 milligrams of sugar in hearts averaging 50 grammes weight cannot be of any great significance. The marked differences in glycogen contents of normal and diabetic animals obtained by Cruickshank(10) and given here for comparison are undoubtedly due to the fact that the animals had been subjected to chloroform anæsthesia, which causes a marked disappearance of glycogen from the hearts of normal animals but seemingly did not have the same effect upon diabetic hearts.

Glycogen Content of Normal and Diabetic Hearts (Cruickshank).

Normal.		Diabetic.	
Average of 10 experiments ..	0.478	Average of 14 experiments ..	0.706
		Average of 13 experiments fed with glucose	0.774

It will be noted that in the cases where sugar has been fed to the animal in large amounts before or in smaller amounts to the heart during the experiment, the heart shows some power of storing it as glycogen. It would seem, in view of the fact that the diabetic heart shows post-mortem glycolysis, that the storage of sugar as glycogen is not due to a lack of the power of the heart muscle to break down glycogen, but to the hyperglycæmia incident upon a rich supply of glucose.

2. The Effect of Insulin on the Glycogen Content of the Normal and Diabetic Heart.

Normal Hearts.—In evaluating the effect of insulin on sugar storage one must first note what obtains in the normal and experimental animal fed with

sugar without the administration of insulin. In normal animals we find an average glycogen content of the right and left heart of 0·654 and 0·571 respectively, and in normal hearts of whole animals fed with sugar there is no noteworthy alteration in these figures. The addition of insulin to the normal whole animal has a little effect on the average glycogen content, as is seen from the figures in Table V, which are 0·668 and 0·736 per cent for the right and left ventricles respectively.

TABLE V.

Effect of Insulin on Glycogen Content of the Normal Heart.

	Without Insulin.		With Insulin.	
	R. V. grammes.	L. V. grammes.	R. V. grammes.	L. V. grammes.
Whole animal	0.654	0.571	0.668	0.736
Isolated heart not fed with sugar.	0.583	0.502	0.380	0.286
(Utilization of sugar) ..	(2.7 to 4 04 mgs.)		(5.4 mgs.)	
Isolated heart with sugar ..	0.794	0.539	1.091	0.905
(Utilization of sugar) ..	(8.70 mgs.)		(8.11 mgs)	

In the altered conditions of the isolated and perfused heart which has been shown to lose little of its glycogen during a three-hour experiment, the addition of insulin effects either a storage which depends upon the extent of the increased sugar content of the blood with which the isolated heart is perfused, an increase due to the initial effect of the anæsthetic upon the dogs taken for blood only, or these may be a distinct loss of glycogen from the heart due to the increased demand of the heart for sugar, the result of large doses of insulin. It is true that where hypoglycæmia is prevented by the addition of sugar, the usual result in these experiments of insulin administration is to effect a storage of glycogen. The fact that the isolated heart under the influence of insulin will utilize sugar until the blood sugar is reduced to 50 per cent of the original amount, shows that the heart will utilize the circulating sugar to supply its energy needs and, if possible, store sugar as glycogen. It has, however, been found in certain experiments that hearts, which with large doses of insulin utilize much sugar, do not increase their glycogen stores. The question as to what determines the storage of glycogen cannot here be settled, but it seems that storage depends not only upon a minimal amount of sugar in the circulating blood, but upon the tension of free sugar in the cardiac muscle. The apparent storage of glycogen under these conditions cannot be put to any accurate test but such results bear

out the work of McCormack and MacLeod(11) and others, who have shown that in a comparable series of rabbits insulin plus dextrose may effect a marked increase in the glycogen content of the heart but not of the skeletal muscles. Best, Hoet and Marks have shown that storage is also effected within the muscles(12).

This is further borne out by the results of our experiments upon normal isolated hearts to the circulation of which glucose has been added to maintain a blood sugar approaching a high diabetic level, namely, 0·4 per cent.

Diabetic Hearts.—In Table VI, the most noteworthy effect of insulin is the generally low figures representing glycogen storage. In the whole diabetic animal glycogen may be high as here shown, namely, 0·608 gramme and 0·677 gramme per cent. The isolated heart perfused with normal blood without the addition of insulin appears to utilize the circulating blood sugar and to spare if not store glycogen. If, however, large doses of insulin, 20 units half-hourly, be given, the glycogen content is reduced to figures which correspond with those for the normal heart. Feeding a diabetic heart with glucose plus insulin so as to maintain a blood sugar percentage of about 0·5 gramme, effects little change in the

TABLE VI.

Effect of Insulin on the Glycogen Contents of the Diabetic Heart.

	Without Insulin.		With Insulin.	
	R. V. grammes.	L. V. grammes.	R. V. grammes.	L. V. grammes.
Whole animal	0.608	0.677	0.365	0.449
Isolated heart with normal blood.	0.812	0.745	(4.90 mgs.)	
(Utilization of sugar) ..	(4.60 mgs.)		0.407	0.487
Isolated hearts with normal blood + sugar.	....	....	(8.51 mgs.)	
(Utilization of sugar) ..	....	....	....	...
Isolated heart with diabetic blood.	0.263	0.150	0.231	0.211
(Utilization of sugar) ..	(0.92 mg.)		(7.65 mgs.)	

glycogen content of a heart which has markedly increased its sugar utilization. As will be pointed out later in discussing sugar utilization, it is necessary to feed a diabetic heart with markedly diabetic blood to determine its power of glycogen storage and we see that under truly diabetic conditions the diabetic heart muscle has lost its power of retaining stores of reserve carbohydrates. When insulin is

given to such a heart, its power to utilize sugar is in marked contrast to its inability to effect a storage of glycogen. It may be concluded from such results that insulin restores the normal mechanism of glycogen utilization bringing the glycogen content down to and even below the normal level. The abnormal power of the diabetic heart to store glycogen is lost and the building up processes, enhanced in the normal heart by insulin, are not equally accelerated in the diabetic heart by the addition of insulin. The factors controlling the balance between glycogen storage and glycogen breakdown under insulin are still unknown.

3. The Utilization of Sugar by the Normal and Diabetic Hearts.

The question is sugar utilization has been the subject of much investigation. Locke and Rosenheim(13) were among the first to become engaged in this problem. Later MacLean and Smedley,(14) Mansfield,(15) and Starling with his co-workers took up the question with regard both to the normal and diabetic organism. Knowlton and Starling(16) in 1912 found a marked difference between the sugar consumption of isolated normal and diabetic hearts. MacLean and Smedley perfusing the hearts of diabetic dogs and cats with Ringer's solution concluded that the diabetic heart was unable to utilize sugar unless pancreatic extract were added to the perfusing fluid. In 1913, Patterson and Starling(17) came to the conclusion that there was little difference if any between the power of the normal and the diabetic heart to use sugar and as a result of their work they were inclined to abandon the view that the essential, or at any rate, the primary factor in diabetes is the absence of the power on the part of the tissues to consume sugar. This attitude was largely due to the disturbing element thrown into the question by the work of one of us(10) on the glycogen content of the diabetic heart. We are of opinion, however, that a more careful check upon the factors responsible for the loss of blood sugar arising from the technical disabilities of the isolated heart lung preparation, would have in all probability furnished a much greater uniformity of results. A definite step forward was made when Hepburn and Latchford(18) by carefully planned and controlled experiments upon the normal rabbit's heart perfused with Ringer's solution, showed that the addition of insulin to the perfusion fluid raised the sugar consumption from an average of 0·89 to an average of 1·89 mg. per gramme of heart muscle per hour.

The part played by insulin on the sugar utilization of the diabetic heart of the dog perfused with blood has not been subjected to investigation largely because of the difficulty in obtaining some standard or average figure for the sugar utilization of the normal.

The problem before us then was to investigate the normal utilization with and without insulin and from that to proceed to the investigation of the diabetic heart. A fact which has been overlooked in dealing with the perfused diabetic heart is, that, in order to determine whether or not the purely diabetic heart muscle will take sugar from the blood and use it for energy or store it as

glycogen, it is essential to feed such a heart with diabetic blood. To perfuse a diabetic dog's heart with normal blood is tantamount to giving it insulin and if a heart be given insulin, as we shall later show, its ability to use the sugar present in the tissue spaces, which is indicated by the plasma sugar content, is definitely enhanced. In our experiments we have taken whole blood for sugar estimations, and while aware that herein may lie a point of criticism we feel that the results obtained on whole blood are worthy of note and indicate generally the degree of sugar utilization obtaining under the conditions of the experiments.

The Method of Calculation of Sugar Utilization.—The duration of experiments of this nature is determined by the condition of the lungs. If for artificial respiration one uses a Starling Ideal Pump, in which the expiration is wholly the result of the elasticity of the lung, and maintains a moderate degree of inflation, there is no reason why a heart lung preparation should not continue to function efficiently for at least three hours. On several occasions we have had preparations which continued to maintain an output of 300 to 400 c.cs. per minute for four and even five hours. It is important to maintain a blood pressure of between 70 and 85 mm. Hg., a venous pressure of about 15 cms. of water and a constant normal temperature. Œdema of the lungs is probably due to the use of defibrinated blood, the increasing alkalinity of the blood and a lack of attention in securing and maintaining a venous inflow suitable to the size of the heart employed. In our experiments we decide upon the venous inflow or cardiac output by noting the action of the heart under different inflows and having arrived at what we consider a suitable figure, we maintain it by slight adjustments of the venous pressure. In most of our experiments we have found, by attention to these details, that the heart continues to beat actively with little change in stroke volume until the onset of œdema. Œdema once begun, continues to increase rapidly and when the loss of blood from this cause becomes marked, we terminate the experiment at the end of the ensuing half-hour period.

The utilization of sugar in the isolated heart lung schema can only be determined if certain points are noted and the necessary corrections arising therefrom made. These points are:—

1. Loss of blood through œdema of the lung.
2. Loss of blood in virtue of possible small leaks, occasioned by the use of defibrinated blood. This can be guarded against by careful operative technique.
3. The loss of blood taken for the estimations.
4. The loss of sugar by glycolysis in the artificial schema.

The method for making corrections to cover the first, second and third points will best be understood by reference to the details of one experiment, which may be set down thus:—

A. The details of the isolated heart lung experiment.
B. The method of correcting for loss of blood and the loss of sugar in calculating the sugar utilization.

C. The table of corrected and other data from which curves are made and tables compiled, giving utilization of sugar in mg. per gramme of heart muscle per hour.

TABLE VII.

Table showing Details of Experimental Procedures.

A. *The Experiment:* normal heart perfused with normal blood.
Date—22nd March, 1928.
Dog No. 64. Weight—7·25 kilos.
Anæsthetic: amytal.
Heart isolated: 10-0 a.m.

Time.	Temp. 0°C.	Heart rate per min.	Rhythm.	A. B. P. mm. Hg.	V. B. P. cms. Aq.	Output c.cs. per min.	Volume blood in Reservoir.
10-5	36	144	Normal	85	15.5	420	540
10-35	37	152	„	85	15.0	420	525
11-5	37	152	„	85	15.0	450	510
11-35	37	152	„	85	14.5	450	495
12-5	37	152	„	85	14.0	450	470
12-35	37	152	„	85	13.5	420	445
1-5	37	152	„	85	12.5	360	400
1-35	37	152	„	90	12.5	300	300
2-5	37	140	„	90	11.5	..	175

A. Table VII shows the details of the experiment in which the rate and rhythm of the heart, the temperature of the blood in the superior vena cava, the venous and arterial pressure and the minute volume as well as the amount of insulin or sugar added to the circulating blood are noted at the beginning of every half-hour period.

B. In order to determine the net amount of blood sugar utilized, it is essential that the loss of blood from the schema should be accurately noted for every period. The percentage of sugar is estimated at the commencement and termination of every half-hourly period. The total amount of the circulating blood is made up of that in the reservoir and that distributed between the heart and lungs and the rest of the schema. After several measurements, it was decided that the amount of blood contained in the heart and lungs would approximate 100 c.cs.; the capacity of the artificial schema was 250 c.cs. and therefore to the blood volume in the reservoir was added 350 c.cs. The average percentage of blood sugar for the period being known, the total sugar lost could

be calculated as can be seen from Table VIII. The figure thus obtained was substracted from the total amount of sugar obtaining at the beginning of the experiment, and from this corrected figure was substracted the total amount of sugar remaining at the end of the period.

TABLE VIII.

Table showing Details of Experimental Procedures.

B. *Method of Correction for Loss of Blood, and Loss of Sugar in calculating Sugar Utilization.*

The first period only is given as an example.

Time		
10-5	Total volume of blood in schema, heart and lungs	$= 540 + 350 = 890$ c.cs.
	Percentage of sugar in the blood	$= 0.214$.
	Therefore total amount of sugar present	$= 0.214 \times 8.9 = 1.905$ grammes.
10-35	Total volume of blood in schema, heart and lungs	$= 525 + 350 = 875$ c.cs.
	Percentage of sugar in the blood	$= 0.196$.
	Therefore total amount of sugar present	$= 0.196 \times 875 = 1.715$ grammes.
	Volume of blood lost during the interval	$= 15$ c.cs.
	Percentage of sugar in the blood lost	$= \frac{0.214 + 0.196}{2} = 0.205$ gramme.
	Therefore the amount of sugar lost	$= 0.205 \times 0.15 = 0.031$ gramme.
	Therefore amount of sugar available for utilization	$= 1.905 - 0.031 = 1.874$ gramme.
	Hence sugar utilized in the first half hour	$= 1.874 - 1.715 = 0.159$ gramme.

C. In normal hearts utilization of sugar is generally a uniform procedure, in diabetic hearts, however, it is quite irregular, depending upon the tension of sugar in the blood plasma, the avidity of the heart for carbohydrate and the ability of the heart to store or utilize sugar. Utilization figures were totalled, the sign positive or negative being taken into account, and the amount of sugar in mg. per gramme of heart muscle per hour calculated. In allowing for the utilization of sugar by the lungs we have made use of the markedly uniform figure of Starling, viz., 1·4 mg. per gramme of heart muscle. Unless this type of correction is made, the results obtained are incapable of interpretation. It is probable that the great irregularity in the results noted by Patterson and Starling are due to a lack of attention to such details. We have been unable to determine how these workers arrived at their figures for utilization as no mention is made in their paper as to blood volume in the reservoir or the minute volume of the heart. The following Table IX indicates the type of data secured from the method of correction just given.

Utilization in Normal Hearts.—A typical curve of the utilization of sugar in the blood perfusing the isolated heart is seen in Figure 2, the total figures for which are obtained from Table X. The curve does not show the small irregularities in utilization so clearly as does the table, the figures in which show

TABLE IX.

Table showing Details of Experimental Procedures.

C. *Method for Calculation of Utilization per gramme weight of Heart per hour.*

1. Period in hours.	2. Total sugar present.	3. Percentage sugar.	4. Sugar lost.	5. Total sugar available.	6. Total sugar utilized in grammes.
0	1 905	0.214	0.031	1.874	+0.159
½	1.715	0.189	0.029	1.686	+0.061
1	1.625	0.189	0.025	1.600	+0.333
1½	1.268	0.150	0.038	1.230	−0.033
2	1.263	0.154	0.038	1.225	+0.032
2½	1.193	0.150	0.063	1.130	+0.147
3	0.983	0.131	0.127	0.856	+0.063
3½	0.793	0.122	..	..	..

Weight of heart = 53 grammes.
Total sugar utilized = +0.762 gramme.
Sugar utilized by lungs per gramme of heart muscle per hour = 1.40 mgs. (Starling).
∴ Sugar utilized per gramme of heart muscle per hour = 2.72 mgs.

TABLE X.

Normal Heart with Normal Blood.

Date—22nd March, 1928.
Dog No. 64.
Weight of Heart = 53 grammes.

1. Period in hours.	2. Total sugar present.	3. Percentage sugar.	4. Sugar lost.	5. Total sugar available.	6. Total sugar utilized in grammes.
0	1.905	0.214	0.031	1.874	+0.159
½	1.715	0.196	0.029	1.686	+0.066
1	1.6254	0.189	0.025	1.600	+0.333
1½	1.2681	0.150	0.038	1.239	−0.033
2	1.263	0.154	0.038	1.225	+0.032
2½	1.930	0.150	0.063	1.130	+0.147
3	0.983	0.131	0.1265	0.856	+0.063
3½	0.793	0.122	..	....	..

Utilization per gramme of heart muscle per hour = 2.72 mgs.

that the isolated heart does not draw upon blood sugar in an absolutely regular manner.

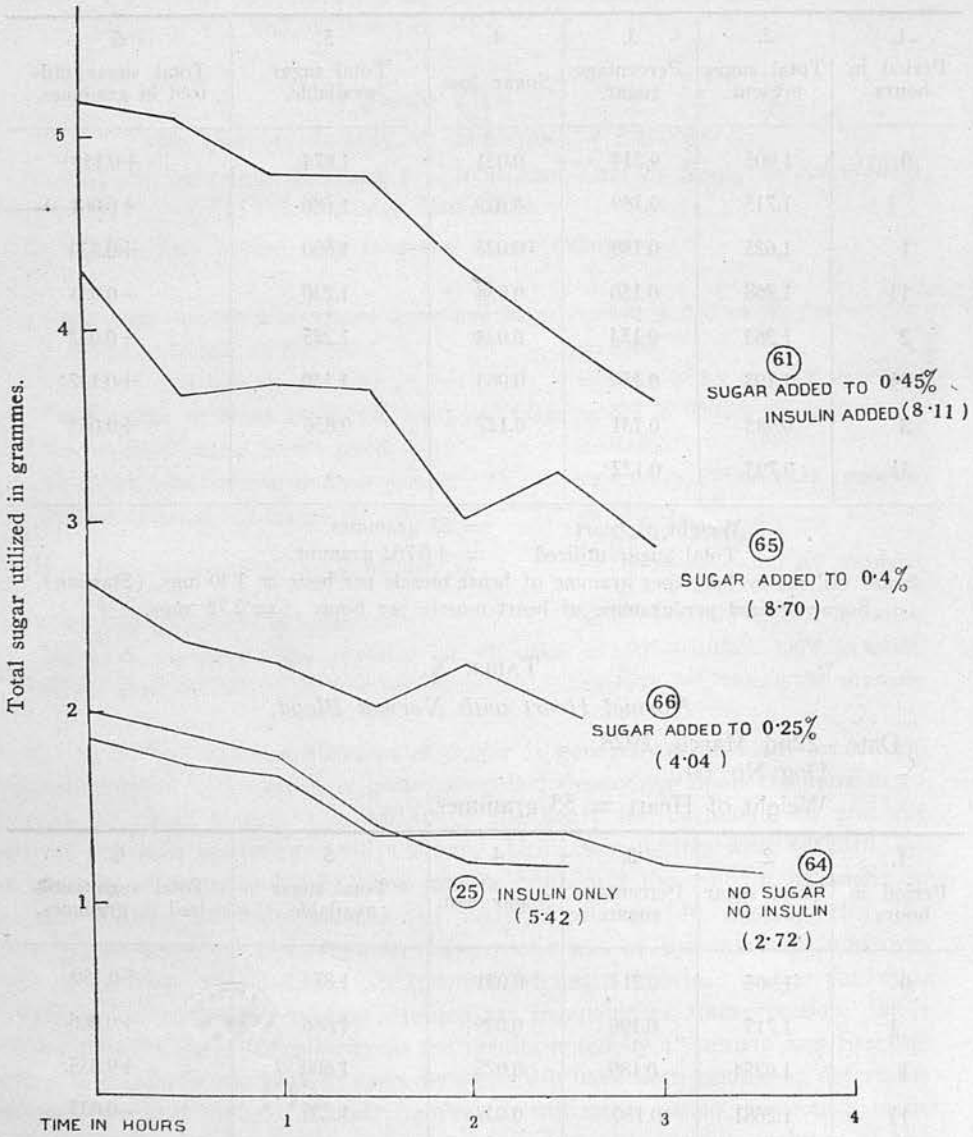

Fig. 2.—Sugar utilization of isolated normal heart.
No. of dog in circle.
Sugar utilization of mg. per gramme of heart per hour in brackets.

The extent to which the heart will utilize sugar depends on several factors which are indicated by the figures in Tables X to XIV. A survey of these

tables will show that a normal heart may use sugar to an extent varying from 1·5 to 8·7 mg. per gramme of heart muscle per hour. The reasons for such variations are evident when the details of the experiments are examined. In the first of this series shown in Table XI, the small utilization of 1·54 mgs. is due to the fact that no source of sugar supply being present apart from the glycogen stores of the heart, the blood sugar fell steadily from 0·166 to 0·109 per cent with a comparable fall in the half-hourly utilization. In the second experiment, Table X, the utilization was higher because of the higher blood sugar percentage at the beginning and the above normal percentage of blood sugar at the end of

TABLE XI.

Sugar Utilization: Normal Heart with Normal Blood.

Dog No. 40.

Weight of Heart = 64 grammes.

1. Period in hours.	2. Total sugar present.	3. Percentage sugar.	4. Sugar lost.	5. Total sugar available.	6 Total sugar utilized in grammes.
0	1.411	0.166	0.156	1.255	0.160
½	1.095	0.146	0.173	0.922	0.103
1	0.819	0.131	0.171	0.648	0.050
1½	0.598	0.122	0.017	0.581	0.063
2	0.518	0.109	..	..	..

Utilization per hour per gramme of heart muscle = 1.54 grammes.

the experiment. Table XII shows a still greater utilization due to the maintenance of the blood sugar at a level well above normal, and, as seen in Table XIII, a much greater increase is obtained by feeding the heart with glucose and maintaining the sugar level at a percentage of about 0·4 gramme. The effect of diabetic blood upon the utilization of sugar is seen from the figures in Table XIV, where an average blood sugar percentage of about 0·4 gramme was again obtained. Here the heart is dependent upon tissue hormone for its sugar consumption, and it is probable that the optimum conditions for sugar utilization are not present when diabetic blood is supplied. Figure 2 shows the curve of sugar utilization, which is typical of the isolated heart under the conditions noted against the experiments. One may conclude that for the normal functioning of the heart, it is necessary to have (1) a maintained normal blood sugar percentage which determines the tension of sugar in the tissue spaces, (2) the optimum conditions for the action of the circulating hormone which presumably are those obtaining in blood of normal reaction.

0·452 gramme per cent was perfused with normal blood. The utilization was 4·56 mgs. per gramme of heart muscle per hour, and the curve of utilization is shown in Figure 3.

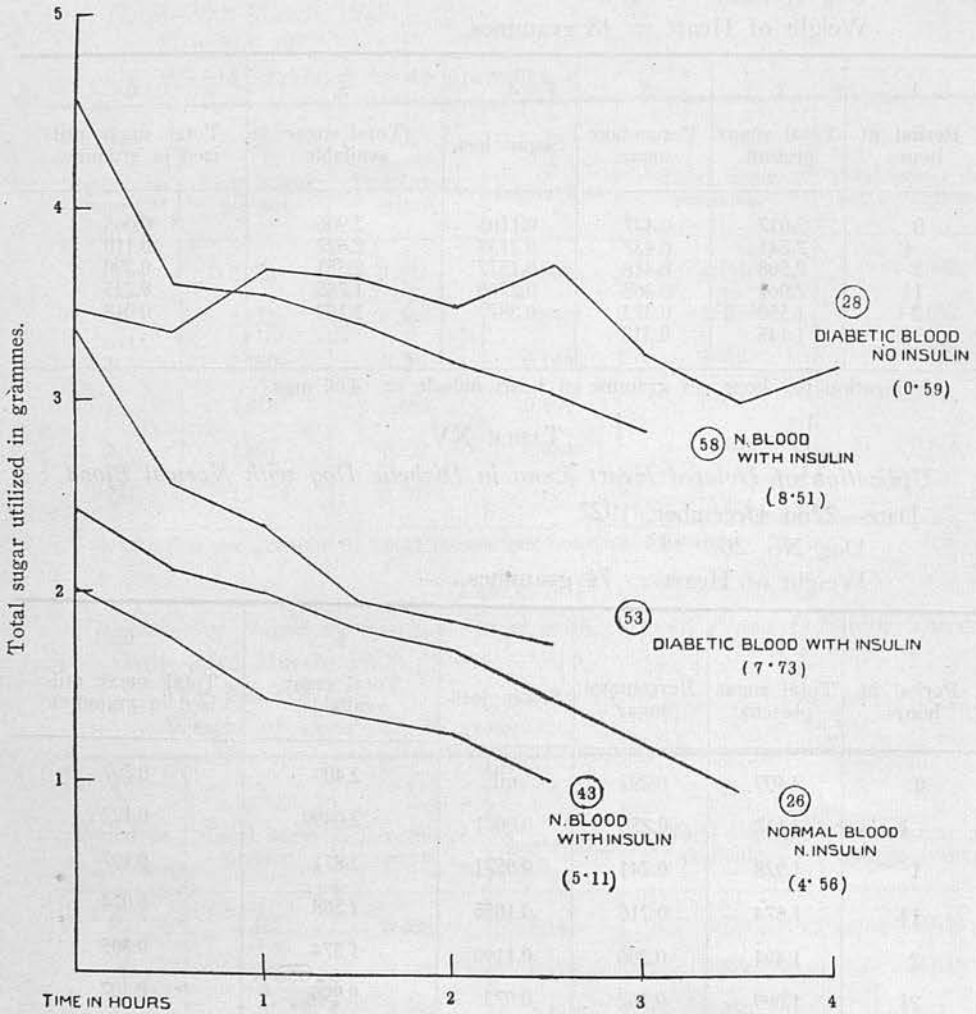

Fig. 3.—Sugar utilization of isolated diabetic heart.
No. of dog in circle.
Sugar utilization in mg. per gramme of heart per hour in brackets.

The details of an experiment on the perfusion of a diabetic heart with diabetic blood are given in Table XVI, and the utilization curve in Figure 3. The experimental dog was nine days diabetic, the dogs from which diabetic blood was taken were six and eight days diabetic, their blood sugar percentages were 0·382, 0·287 and 0·442 gramme respectively. It is clear from the figure for utilization under these conditions, namely, 0·59 mg. per gramme of heart muscle

TABLE XVI.

Diabetic Dog with Diabetic Blood without Insulin.

Date—13th January, 1928.

Dog No. 28.

Weight of Heart = 60 grammes.

1. Period in hours.	2. Total sugar present.	3. Percentage sugar.	4. Sugar lost.	5. Total sugar available.	6. Total sugar utilized in grammes.
0	3.426	0.403	0	3.424	+0.0974
½	3.332	0.392	0	..	−0.382
1	3.714	0.437	0.1086	3.605	+0.040
1½	3.565	0.432	0.104	3.461	+0.237
2	3.244	0.403	0.1038	3.120	−0.189
2½	3.309	0.427	0.0988	3.2102	+0.487
3	2.723	0.363	0.0833	2.640	+0.436
3½	2.204	0.304	0.129	2.075	−0.268
4	2.343	0.342	..	..	..

Utilization per hour per gramme of heart muscle = 0.59 mgs.

	Duration of diabetes.	Blood sugar percentage.
Exper. Dog ..	9 days	0.382
Bled Dogs ..	6 days	0.287
	8 days	0.442

per hour that the conditions necessary for sugar consumption are absent. In this case the left heart had used almost all of its presumably large glycogen stores, while the right had still a small amount present. In experiments of this type the usual, if not invariable, result is a marked loss of heart glycogen. That

the hormone is lacking in the heart muscle and in the blood of highly diabetic animals seems proved by such experiments. In an experiment in which the experimental dog, four days diabetic, had a blood sugar of 0·392 gramme per cent and the two bleeders, four and seven days diabetic, had blood sugars of 0·430 and 0·542 gramme per cent, there was an utilization of 1·25 mgs., while in another in which both the experimental and bled dog were five days diabetic and had a blood sugar percentage of 0·574 and 0·354 gramme respectively, there was an utilization of 2·4 mgs. Such results, the details of which can be seen in Tables XVI(*a*) and XVI(*b*) indicate that, up to at least a week after depancreatization, small amounts of insulin may still be present in the tissues.

TABLE XVI(*a*).

Diabetic Heart with Diabetic Blood.

Date—15th March, 1928.

Dog No. 56.

Weight of Heart = 40 grammes.

1. Period in hours.	2. Total sugar present.	3. Percentage sugar.	4. Sugar lost.	5. Total sugar available.	6. Total sugar utilized in grammes.
0	1.966	0.342	0.154	1.812	−0.001
½	1.813	0.3427	0.062	1.751	+0.313
1	1.438	0.282	0.201	1.237	−0.052
1½	1.289	0.292	0.258	1.031	+0.044
2	1.987	0.282	..	..	..

Utilization of sugar per hour per gramme in heart muscle = 2.4 mgs.

	Duration of diabetes.	Blood sugar percentage.
Exper. Dog ..	5 days	0.574
Bled Dog ..	5 days	0.354

TABLE XVI(*b*).

Diabetic Dog with Diabetic Blood without Insulin.

Date—21st January, 1928.

Dog No. 31.

Weight of Heart = 54 grammes.

1. Period in hours.	2. Total sugar present.	3. Percentage sugar.	4. Sugar lost.	5. Total sugar available.	6. Total sugar utilized in grammes.
0	2.613	0.418	0	..	—0.087
½	2.700	0.432	0.104	2.596	+0.178
1	2.418	0.403	0.0958	2.322	0.235
1½	2.087	0.363	0.0889	1.998	0.083
2	1.915	0.348	0.160	1.755	0.295
2½	1.460	0.292	0.0678	1.392	0.205
3	1.187	0.250	0.1223	1.270	0.254
3½	1.016	0.239	..	..	..

Utilization per hour per gramme of heart muscle = 2.58 mgs.

	Duration of diabetes.	Blood sugar percentage.
Exper. Dog ..	2 days	0.392
Bled Dog ..	3 days	0.332
	4 days	0.646
	5 days	0.508

4. THE EFFECT OF INSULIN ON THE UTILIZATION OF SUGAR BY THE NORMAL AND DIABETIC HEART.

Normal Hearts.—Since with insulin the rate and total amount of the fall of blood sugar during the usual three-hour period of our experiments are comparable to those in whole animals, as is seen from the curves in Figure 1, it is justifiable to regard the action of the isolated heart during the experimental period as comparable to that of the heart in the whole animal as far as its power to utilize sugar is concerned.

The action of insulin on total utilization was studied:

1. Without the addition of sugar.

and Figure 3 shows the remarkable power of the diabetic heart to use sugar in the presence of insulin, provided a high percentage of blood sugar is maintained. The figure here given, namely, 8·51 mgs. is the highest utilization figure we have obtained for the diabetic heart. In this experiment glucose was added in such amounts that the blood sugar percentage averaged 0·472 gramme. The condition is comparable to the normal heart fed with sugar plus insulin and it will be seen there is practically no difference between the functional powers of these hearts as far as sugar utilization is concerned (*see* Table XVIII). It is, however, clear, as has already been pointed out, that, in accordance with the general principle of the mass law, a position of steady equilibrium in utilization cannot be maintained when the sugar supplies are excessive.

TABLE XIX.

Diabetic Dog with Normal Blood + Insulin.

Date—9th February, 1928.

Dog No. 43.

Weight of Heart = 53 grammes.

1. Period in hours.	2. Total sugar present.	3. Percentage sugar.	4. Sugar lost.	5. Total sugar available.	6. Total sugar utilized in grammes.
0	2.032	0.254	0.125	1.907	0.137
½	1.670	0.236	0.0425	1.727	0.347
1	1.380	0.189	0.049	1.325	0.086
1½	1.239	0.177	0.0885	1.150	0.0
2	1.150	0.177	0 153	0.997	0.293
2½	0.704	0 128	..	..	..

Utilization per hour per gramme in heart muscle = 5.11 mg.

	Duration of diabetes.	Blood sugar percentage.
Exper. Dog ..	5 days	0.358

It is sufficiently clear that whether or not there be a storage of glycogen, insulin stimulates the power of the heart to metabolise sugar. The irregular rate of the disappearance of sugar which marks these experiments is similar to that which occurs in the isolated normal and diabetic heart; for such irregularities in utilization we can offer no explanation. To what extent glycogenases may be at work, stimulated or depressed by the conditions of the experiment, we know

not. It seems that in the diabetic heart a steady state of equilibrium between free sugar in blood or muscle, and the glycogen in the muscle is not maintained as it can be in the normal. The pendulum swings in a most erratic manner from utilization, either oxidation or storage, to an increase in blood sugar presumably from glycogen breakdown. But nevertheless these results of perfusion of diabetic hearts with diabetic blood would indicate that whatever the mechanism controlling carbohydrate metabolism the diabetic heart does not utilize blood sugar, either by oxidation or storage without the presence of insulin.

TABLE XX.

Diabetic Dog with Diabetic Blood + Insulin.

Date—1st March, 1928.

Dog No. 53.

Weight of Heart = 72 grammes.

1. Period in hours.	2. Total sugar present.	3. Percentage sugar.	4. Sugar lost.	5. Total sugar available.	6. Total sugar utilized in grammes.
0	3.577	0.477	0.2088	3.368	0.8162
½	2.506	0.358	0.1715	2.334	0.202
1	2.132	0.328	0.177	1.955	0.409
1½	1.546	0.262	0.2004	1.3456	0.127
2	1.219	0.239	0.1993	1.020	0.043
2½	0.9774	0.230	..	..	..

Utilization of sugar per hour per gramme in heart muscle = 7.73 mgs.

	Duration of diabetes.	Blood sugar percentage.
Exper. Dog ..	6 days	0.562
Bled Dog ..	6 days	0.412

A summary of the results of these various types of experiments will be found in Table XXII. This question must be considered in relationship to the glycogen content of the muscle and the tension of sugar in the tissue spaces of

the heart muscle. This matter is receiving attention and the results as far as they go indicate that one important factor, in sugar utilization in the presence of insulin, is the amount of free sugar in the tissue fluids bathing the muscle cells. In view of recent results upon muscle sugar further work must be undertaken upon the action of insulin in inhibiting the production of sugar from non-carbohydrate sources. It is thought better to leave further discussion of this complex subject of carbohydrate metabolism under insulin until the completion of the work referred to.

TABLE XXI.

Diabetic Heart with Normal Blood, + Insulin, + Sugar.

Date—17th March, 1928.

Dog No. 58.

Weight of Heart = 40 grammes.

1. Period in hours.	2. Total sugar present.	3. Percentage sugar.	4. Sugar lost.	5. Total sugar available.	6. Total sugar utilized in grammes.
0	4.473	0.497	0.1125	4.3605	+0.838
½	3.522	0.403	..	..	..
New Total	4.730	0.472	0.1660	3.964	—0.042
1	4.0068	0.477	..	..	..
New Total	4.133	0.492	0.2020	3.931	—0.213
1½	4.144	0.518	..	..	..
New Total	4.176	0.522	0.2460	3.930	+0.457
2	3.4725	0.463	0.3390	3.134	+0.150
2½	2.984	0.442	0.3315	2.652	0.0
3	2.652	0.442	..	..	..

Utilization per hour per gramme of heart muscle = 8.51 mgs.

	Duration of diabetes.	Blood sugar percentage.
Exper. Dog ..	4 days	0.447
Bled Dog, normal	..	0.563

TABLE XXII.

Summary of Results of Sugar Utilization of the Normal and Diabetic Heart in mg. per hour per gramme of Heart Muscle.

Normal Heart.

	With normal blood.	With diabetic blood.
Without insulin	4.04	4.66
With insulin	5.42	6.0 ?
With insulin + sugar	8.11	..

Diabetic Heart.

	With normal blood.	With diabetic blood.
Without insulin	4.59	0.59
With insulin	5.11	7.73
With insulin + sugar	8.51	..

CONCLUSIONS.

1. The blood sugar of dogs in India averages 0·112 gramme per cent, as compared with 0·09 gramme in the West.

2. The glycogen content of the heart is readily depleted by prolonged administration of volatile anæsthetics, such as, chloroform and ether, but is not materially affected by amytal.

3. The action of insulin is not affected by anæsthetics.

4. The glycogen content of the normal dog's heart is approximately 0·550 gramme per cent; the diabetic heart, unfed with sugar, is approximately 0·750 gramme per cent.

5. Insulin in the presence of excess of available sugar will effect a storage of sugar as glycogen in the heart muscle. On the other hand, insulin in excess will, by producing hypoglycæmia, effect a marked reduction in the glycogen content of the heart. In the diabetic heart, insulin invariably causes a reduction in glycogen.

6. The utilization of sugar by the normal isolated heart varies from 1·5 to 8·7 mgs. per gramme of heart muscle per hour, the variation depending upon the amount of sugar in circulation.

7. The diabetic heart perfused with diabetic blood will not utilize sugar. Its energy is derived wholly from its stored glycogen.

8. Insulin increases sugar utilization in the normal isolated heart from an average of 4 to one of 8 mg. per gramme of heart muscle per hour, depending upon the amount of sugar available.

9. In the diabetic heart insulin immediately restores and enhances the power of the heart to utilize sugar.

10. There is no great difference between the power of the normal and diabetic heart to utilize sugar, when both *sugar* and *insulin* are added to the blood circulating through them.

11. Corroboration is given to the statement that insulin may ultimately and indirectly cause a depletion of the glycogen stores of the body, by increasing utilization and inhibiting the new formation of sugar. It has been found that, in all cases where insulin effected a fall in blood sugar greater than 50 per cent, the glycogen content of the heart was very low and had suffered, presumably, a marked reduction.

ACKNOWLEDGMENTS.

Without the financial assistance given us by the Indian Research Fund Association, this work could not have been undertaken; we wish to express our appreciation of the support given us by the Association in carrying out a piece of research which, although apparently somewhat academic, is, we believe, of fundamental importance in helping towards a fuller knowledge of the diabetic problem and of its clinical handling by means of insulin.

We are indebted both to Dr. T. N. Seth, who was associated with us in the early stages of this work and to Mr. Sheonath Prosad, for much valued assistance in the course of this investigation.

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QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY

VOL. XX. No. 4
(*Issued 29th November* 1930)

STUDIES IN BLOOD DIASTASE. FACTORS WHICH CAUSE VARIATIONS IN THE AMOUNT OF DIASTASE IN THE BLOOD. By CHARLES REID and B. NARAYANA. From the Department of Physiology, Prince of Wales Medical College, Patna.

LONDON: CHARLES GRIFFIN AND COMPANY, LIMITED
42 DRURY LANE, W.C.2

Entered at New York Post Office as Second-Class Matter

1930

STUDIES IN BLOOD DIASTASE. FACTORS WHICH CAUSE VARIATIONS IN THE AMOUNT OF DIASTASE IN THE BLOOD.

By CHARLES REID and B. NARAYANA. From the Department of Physiology, Prince of Wales Medical College, Patna.

(*Received for publication* 15*th August* 1930.)

THESE studies were undertaken to elucidate the source or sources of diastase in circulating blood and to decide whether this enzyme is entirely a waste product or can be utilised by the cells of the tissues as occasion requires. Wide variations are found in the amount of diastase in the circulating blood of different species, but a complete comparative study has not yet been carried out. In the present investigation dogs were employed mainly, but certain observations have been made on human beings, rabbits, and cats.

The effects of the following procedures on the blood diastase have been observed:—

(*a*) Administration of anæsthetics.
(*b*) Ingestion of food.
(*c*) Injections of glucose, glycogen, starch.
(*d*) Injections of insulin.
(*e*) Pancreatectomy.
(*f*) Ligature of pancreatic duct.

THE METHOD OF ESTIMATING DIASTASE.

The method suggested by FYFE (1) was followed.
Into two 100 c.c. Erlenmeyer flasks are measured:

Flask.	NaCl (0·9%).	Blood.	Starch (0·1%).
1	2·8 c.c.	0·2 c.c.	—
2	1·8 c.c.	0·2 c.c.	1 c.c.

Both flasks are incubated at 37° C. for half an hour, after which period the amount of sugar in both flasks is estimated by MacLean's method. The difference in the amounts expressed in mg. and multiplied by 100 is taken as the diastatic index. Samples of blood from experimental animals were obtained by vein-puncture.

The blood diastatic index in the dogs examined ranged between 20 and 40 (most commonly 25 to 35).

Effect of Anæsthetics.

The anæsthetics used were morphine (0·1 c.c. of 10 per cent. solution per kilo body-weight), chloralose (0·1 grm. per kilo body-weight), and open chloroform and ether (duration one hour). The blood diastatic index was slightly higher (10 per cent.) for some hours after the administration of these anæsthetics, the increase not being present on the day subsequent to the administration of the anæsthetics.

The Ingestion of Food.

The animals were usually kept without food two days before the day on which the blood diastatic index was studied after the ingestion of food. Considerable decreases (up to about 30 per cent.) were observed in the blood diastatic index during several hours following the ingestion of mixed meals, the decrease being usually greatest in the course of the second or third hour. Similar results were found by one of the authors (2) in the case of man. It is noteworthy that the blood diastase content of young animals (cats and rabbits) does not change during the period (*a*) immediately after birth, (*b*) of feeding by maternal milk, and (*c*) of transition from maternal milk to ordinary adult diet.

Time of blood sample (dog). (hours)	Blood-sugar. (percentage)	Blood diastatic index.
0·00	·10	32·6
0·00	mixed meal.	—
1·00	·09	27·4
2·00	·12	25·9
3·00	·10	30·2
4·00	·13	29·4
5·00	·13	28·0

The decrease in the blood diastase following the ingestion of food does not appear to be due entirely to its increased excretion by the kidneys since the ingestion of water (up to 500 c.c.) is followed by a decrease in the blood diastase of much less extent.

Injections of Glucose.

Injections of small amounts of glucose (*e.g.* 0·05 grm. per kilo body-weight) caused no change in the amount of circulating diastase, the excess sugar having disappeared from the blood within five minutes. The blood-sugar thereafter declined steadily (from ·07 per cent. to

·06 per cent. in one and a half hours), while the diastatic index tended to rise slightly during the period of decrease. Larger amounts (0·2 grm. glucose per kilo body-weight) caused a slight fall in blood diastase.

Time. (hours)	Blood-sugar. (percentage)	Blood diastatic index.
0·00	·074	36·3
	0·2 grm. glucose injected.	
0·05	·101	33·6
0·15	·093	32·5
1·00	·070	34·8
1·45	·074	32·3
2·30	·074	35·2

Injections of still larger amounts of glucose, so as to raise the blood-sugar to nearly 0·3 per cent., *e.g.* 1·5 grm. per kilo body-weight, introduced a complicating factor, as it was found that the presence of glucose above about 0·2 to 0·25 per cent. interfered with the action of diastase on the substrate starch and gave a fictitiously low value to the diastatic index. This source of error was detected by means of a control experiment, in which the diastatic index of a specimen of blood was determined, not only in the usual way, but also after glucose had been added to a series of flasks, containing the usual amount of starch each with 0·2 c.c. blood from the same specimen, in sufficient amount to give initial percentages of glucose (·15, ·175, ... ·300) at the start of the period of incubation. Instead of the ordinary glucose solution, a protein-free and enzyme-free solution of sugar prepared from blood was tried with similar results.

Injections of Starch.

Injections of starch (0·125 grm. per kilo body-weight) did not alter appreciably the blood-sugar or the blood diastase, all the starch disappearing from the blood-stream within twenty minutes. Larger quantities (0·5 grm. per kilo body-weight) caused both a rise in the blood-sugar and blood diastatic index.

Time. (hours)	Blood-sugar. (percentage)	Blood-sugar (incubated). (percentage)	Blood diastatic index.
0·00	·089	·083	23·9
	0·5 grm. starch injected (per kilo body-weight).		
0·30	·14	·130	28·6
1·15	·10	·094	26·3
2·15	·09	·087	27·5
3·15	·085	·08	30·5
4·30	·08	·074	26·3

Injections of Glycogen.

Injections of glycogen (up to 0·5 grm. per kilo body-weight) in solution in small quantities of fluid caused a lowering of the blood diastatic index. A definite increase in the percentage of sugar in the blood occurred, but all the glycogen apparently disappeared from the circulating blood of starved dogs in the course of a few minutes, since an incubated sample of blood did not show any increased percentage of reducing sugar as would be expected from the action of blood diastase on glycogen, if present, even if allowance is made for slight glycolysis in the course of half an hour's incubation.

Time. (hours)	Blood-sugar. (percentage)	Blood-sugar (incubated). (percentage)	Blood diastatic index.
0·00	·07	·064	36·4
	4 grms. glycogen (0·5 grm. per kilo body-weight).		
0·40	·10	·094	30·3
1·40	·06	·055	31·0
2·40	·07	·062	33·0
4·10	·08	·077	35·1

Injections of Insulin.

In dogs which had been fed on the previous day with a mixed diet consisting of a large proportion of carbohydrate, injections of insulin (30 units: 8·5 kilos weight) brought about a decided fall in both the blood-sugar and the blood diastase.

Time. (hours)	Blood-sugar. (percentage)	Blood diastatic index.
0·00	·089	25·1
	30 units of insulin.	
0·40	·083	23·5
1·15	·054	19·0
1·45	·022	22·2
2·15	·015	25·5
3·15	·043	21·4

It should be observed that dogs do not show symptoms of hypoglycæmia until the blood-sugar reaches a very low level (·02 per cent.). Coincident with this fall of blood-sugar to the hypoglycæmic level, the blood diastase rises sharply and usually attains a level above or considerably above that at the beginning of the observations. It is well shown in dogs kept without food for two days previous to the injection of

insulin. This sharp rise in the blood diastase is conceivably due to the release of diastase from the liver cells under conditions of hypoglycæmia.

In the process of recovery—either natural or by means of intravenous injections of glucose in small amounts of normal salt solution (3 grm. in 10 c.c.)—from the hypoglycæmic condition the blood diastatic index falls sharply. This would appear to indicate that the diastase in the blood is being reabsorbed by the liver cells mainly to assist in the glycogen $\rightleftharpoons$ glucose function of the liver.

Injections of Diastase.

Diastase was administered by means of intravenous injections of solutions (in small quantities of warm normal salt solution) of 1 to 2 grm. of Merck's absolute diastase. The effects of the above procedure varied according to whether the animal had been fed recently or had been kept without food for at least two days—the blood-sugar rising in the former case from 0·08 per cent. to 0·10 per cent. in the course of an hour or two, and falling in the latter from about 0·072 per cent. to as low as 0·03 per cent. in the course of three hours following the injection of diastase.

Effect of Pancreatectomy.

MYERS and KILLIAN (3) state that total pancreatectomy increases, KARSNER, KOECKERT, and WAHL (4) that it may increase, and DAVIS and ROSS (5) that it decreases the diastatic activity of blood. MARKOWITZ and HOUGH (7) found a fall in blood diastase up to twenty hours after pancreatectomy, after which it returned to normal. We found that this procedure had little or no effect on the blood-diastase level in a number of dogs in which pancreatectomy was successfully performed and which survived for over ten days, the operation wound being healthy throughout.

Day of observation.	Blood-sugar. (percentage)	Blood diastatic index.
1	·106	30·1
Pancreatectomy done on first day.		
2	·092	26·2
3	·128	29·2
5	·286	23·2
6	·276	26·6
40 units of insulin given subcutaneously.		
(One hour later)	·116	30·6
(Two hours later)	·062	26·7
7	·085	26·3

The considerable lowering of the blood diastatic index about the fifth and sixth days of observation appears to be due to the interference

of the high blood-sugar level with the action of the enzyme on the substrate starch. An injection of insulin (40 units: weight of dog 11·5 kilos) caused a speedy decrease in the blood-sugar to nearly the fasting level obtained before the operation and a return of the blood diastatic index to the original figure. The subsequent decrease of the blood diastatic index is due to the action of insulin—comparable to the decrease obtained in normal animals after an injection of a similar amount of insulin.

Effect of Pancreatectomy and the Ingestion of Food.

Mixed food was administered to depancreatised dogs, insulin (2 units per kilo body-weight) having been injected (*a*) on the day previous to the meal, (*b*) immediately after the conclusion of the meal. The blood diastase following the meal taken under conditions (*a*) or (*b*) did not show variations as in normal dogs.

Ligature of the Pancreatic Duct.

Subsequent to this operation on dogs, no increase in the diastatic content of the blood was observed during a period of one to two weeks.

Discussion.

Blood diastase would not appear to be entirely a waste product circulating in the blood, since changes in the diastase content of the blood can be brought about by the ingestion of food, injection of insulin, etc. Further, the main source of this enzyme is not the pancreas, since pancreatectomy is not followed in the course of one to two weeks by a striking decrease in the blood diastatic index.

The decrease in blood diastase during the immediate hours following a meal or injections of glucose or glycogen appears to be related to the call to the liver cells in connection with the glycogen $\rightleftharpoons$ glucose reactions and probably to the release of insulin into the portal circulation, because (*a*) the injection of insulin in sufficient amount into the systemic circulation causes a decrease of similar order; (*b*) the decrease in blood diastase after ingestion of food in depancreatised dogs is much less evident. It is suggested that this decrease after insulin injections is due to absorption of diastase by the liver cells for the purpose of increasing the amount of glycogen $\longrightarrow$ glucose for the circulating blood, since injections of diastase in the amount stated, which disappear quickly from the circulating blood, increase the blood-sugar when the liver has presumably a considerable store of glycogen and possibly insulin. After a period of deprivation of food which would result in a considerable depletion of liver glycogen and also possibly a decrease in the amount of insulin in the liver or in the circulation, diastase injections cause a marked fall in the blood-sugar level, apparently accelerating the glucose $\longrightarrow$ glycogen reaction in the

liver. It would appear that insulin may help to determine, in so far as diastase is concerned, the direction of the glycogen $\rightleftharpoons$ glucose reactions in the liver—the presence of insulin, *e.g.* in the portal circulation, firstly after food, or by injection into the systemic circulation, favouring glucose $\longrightarrow$ glycogen, while in the absence or defect of insulin, *e.g.* during starvation, the glycogen $\rightleftharpoons$ glucose reactions of the liver are reduced to a minimum, as is shown by the high blood diastatic index during a period of deprivation of food. Again, the amount of diastase in the blood is greater in normal animals during periods of (*a*) *marked* insulin hypoglycæmia and (*b*) starvation (low insulin content of the blood or tissues) than during periods of normal metabolism due probably to the liver cells throwing off diastase on account of the diminished amounts of the substrates present in the liver, blood, etc. In (*a*) however, it may be entirely a terminal phenomenon, since a rise in the blood-diastase level has been observed in human subjects shortly before death from various causes (6).

Conclusions.

1. The blood diastase decreases after meals, injections of glucose, glycogen, and insulin, while anæsthetics and injections of starch cause a slight increase.

2. Since pancreatectomy causes little or no change in the amount of circulating diastase, the pancreas is not the main source of blood diastase. Ligature of the pancreatic duct does not result in any striking change in the diastase content of the blood in the course of one to two weeks.

3. Since definite variations can be induced in the blood diastase by injections of insulin, glycogen, etc., it appears likely that the circulating diastase is not entirely a waste product on its way to excretion.

4. Evidence is adduced that variations in blood diastase are probably due to its being taken up or given out by the liver cells, etc., according to the requirements of the body with respect to the glycogen $\rightleftharpoons$ glucose, probably in association with insulin.

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PRINTED IN GREAT BRITAIN BY NEILL AND CO., LTD., EDINBURGH

[From THE BIOCHEMICAL JOURNAL, Vol. XXV, No. 1, pp. 339–341, 1931]

PRINTED IN GREAT BRITAIN

XLI. STUDIES IN BLOOD GLYCOLYSIS.
PRELIMINARY OBSERVATIONS.

By CHARLES REID and BASUDEVA NARAYANA.

From the Department of Physiology and Biochemistry, Prince of Wales Medical College, Patna, India.

(*Received January 12th, 1931.*)

This study was undertaken to determine the factors concerned in glycolysis in blood incubated at 37°. It is clear from the work of a number of observers—Schmitz and Glover [1927], Stammers [1926] and Chahovitch [1926]—that (1) sterile blood loses its sugar if kept at room or body temperature; (2) the rate of glycolysis varies in different species.

Glycolysis was studied under the following conditions, *viz.* (1) in whole blood after varying periods of starvation and after feeding, (2) in laked corpuscles, (3) in serum, (4) in cyanide blood, (5) in washed erythrocytes, (6) in washed erythrocytes and glucose solution.

Method.

Our observations have been confined mainly to dog's blood. About 2 cc. of blood were drawn from one of the leg veins and incubated at 37° for a variable period; samples were taken therefrom before incubation and at intervals of 30, 60 and 120 minutes for sugar estimation by MacLean's method. During the process of taking blood asepsis was observed although it was found that this precaution was not necessary at any rate for incubation periods not extending beyond 3 hours.

It will be seen on reference to Table I that the rate of glycolysis is greatest during the first hourly period of incubation; it is greater in blood samples taken about 2 hours after the ingestion of food than after a day's starvation and it decreases considerably after 2 days' starvation.

Effect of cyanide.

To determine whether glycolysis in blood is largely or entirely an oxidative process, blood was mixed with different quantities of cyanide solution in normal saline as follows: 2 cc. blood samples were taken and mixed with 1 cc., 0·5 cc. and 0·2 cc. of 0·3 % KCN solution and the total volume was made up to 3 cc. with normal saline. A control containing 2 cc. blood and 1 cc. saline was also set up. Preliminary glucose estimations were made and the samples were incubated at 37° in small test-tubes. It will be noticed

QUARTERLY JOURNAL
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LONDON: CHARLES GRIFFIN AND COMPANY, LIMITED
42 DRURY LANE, W.C. 2

The Journal is issued at the price of £2, 2s. per volume. Subscriptions payable in advance should be sent to the Publishers, 42 Drury Lane, London, W.C. 2

Entered at New York Post Office as Second-Class matter

1934

PRINTED IN GREAT BRITAIN

QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY

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INVESTIGATIONS ON THE PHARMACOLOGY OF EVIPAN SODIUM.

By WALTER P. KENNEDY and BASUDEVA NARAYANA.
From the Department of Physiology, Edinburgh University.

(*Received for publication* 12*th July* 1933.)

A NEW barbitone derivative has recently been elaborated which, when injected intravenously, has the property of inducing surgical anæsthesia for short periods of about 15 to 20 minutes.

It has been named "evipan sodium" and is the sodium salt of cyclohexenylmethyl-N-methyl barbituric acid, which was obtained by the methylation of the mild hypnotic, phanodorm, or cyclohexenylmethyl barbituric acid. The anæsthetic effect on certain laboratory animals has been investigated by one of us and reported elsewhere [Kennedy, 1933]. The results then obtained induced us to extend the research on other pharmacological lines to find how it resembled or differed from other substances of like properties.

Experiments on Frog Heart.—The effect of the drug was tested on the frog heart (*R. esculenta*) by the closed perfusion technique originally described by Hartung and modified by Clark [1912]. This method permits of long-continued perfusion without washing the lipoids out of the heart with the consequent production of the hypodynamic state.

The first points determined were the effect of the drug on contraction, and whether or not it was fixed by the heart cells. The latter was tested by perfusing a heart for some time with a solution of evipan sodium, or more shortly evipan, and then pipetting the fluid into a second preparation.

When evipan solution was added to the perfusate of such a preparation so as to bring the concentration to 1 in 4000, the amplitude of contraction was at once reduced 10 per cent., and the rate increased. Additions of evipan at 4-minute intervals till the dilution was 1 in 500 gradually diminished both amplitude and rate till the heart stopped in diastole, and remained quiescent for over an hour when it failed to respond to stimulation.

A heart exposed to a concentration of 1 in 1000 evipan gradually stopped beating after 10 minutes, though it responded to light mechanical stimulation. The perfusate was then transferred to a second heart and a diminution of extent and slowing of the rate of contraction was again produced, and indeed to a greater degree than before. Six

minutes after the heart stopped the perfusate was washed out and 9 minutes later the contractions were normal. On transferring the solution to a third heart the same result was obtained.

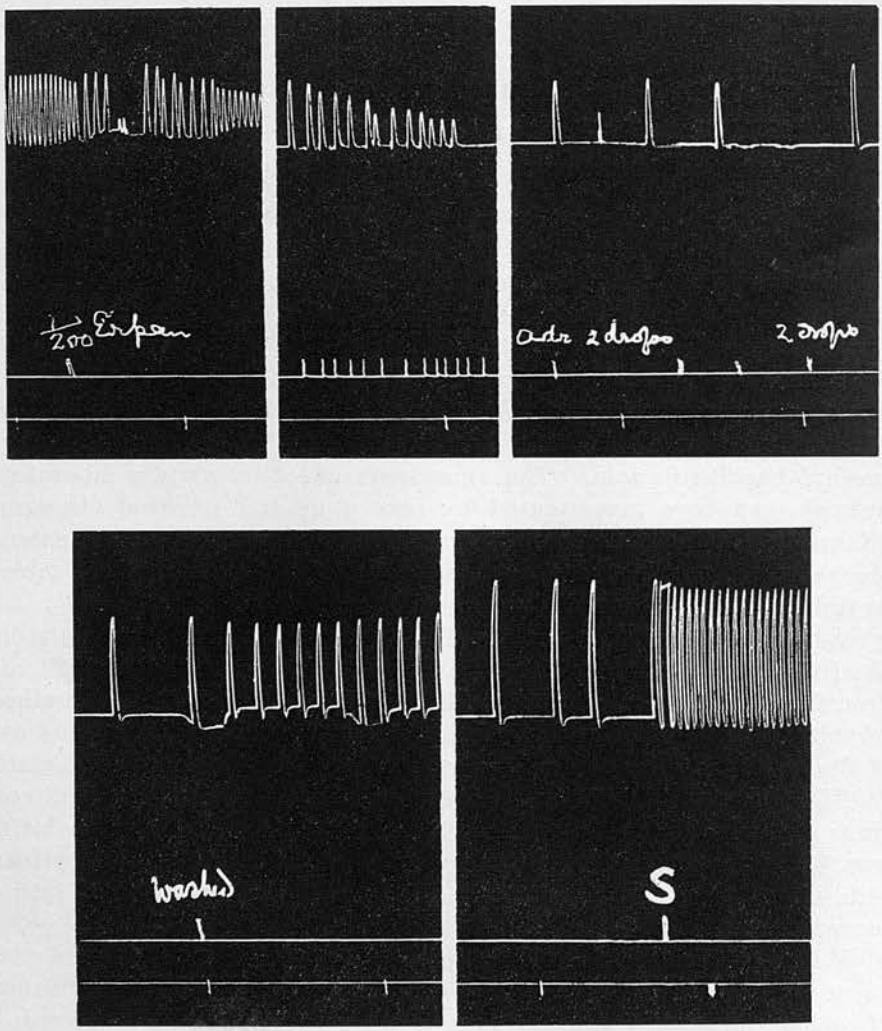

FIG. 1.—Action of evipan sodium on perfused frog heart. Portions of continuous tracing described in text.

A concentration of 1 in 200 evipan caused arrest of the frog's heart in 2 minutes, though excitability remained as shown by the response to mechanical stimuli (parts one and two of the figure). After 9 minutes 2 drops of 1/5,000,000 adrenalin were added and the heart stimulated twice to mix the fluid, and 2 more drops added (third portion of figure). Slow spontaneous contraction followed for 7 minutes, then washing

quickened the rate (fourth part). As the extent of contraction increased the rate slowed. At point S on the fifth piece of the tracing the drum was stopped for 10 minutes, after which the heart showed complete recovery. These experiments show that the depressant action of evipan on the isolated frog's heart can be reversed completely by washing out the drug.

Experiments on Blood-sugar.—The influence of an anæsthetic on the blood-sugar level may be considerable; for example, ether hyperglycæmia is well known. According to Sollmann [1932] contradictory results have been reported for the action of barbitals on blood-sugar due in part to the conditions of the experiments, especially the diet. While anæsthetic doses, according to most observers, cause no change in the level of normal fasting animals, some experimenters have found marked hyperglycæmia, *e.g.* Underhill and Sprunt [1927] with isoamylethyl-barbituric acid (amytal) on rabbits; Weiss [1925] with amytal, sodium diethyl-barbiturate (medinal), and sodium isoallylpropyl-barbiturate on cats and dogs; and Bang [1913] with diethyl-barbituric acid (veronal) on rabbits.

A series of tests were made on rabbits and guinea-pigs with evipan sodium. The animals fasted overnight, and the experiments were carried out early in the morning. Rabbit blood was obtained from the ear vein, and that of the guinea-pigs by heart puncture, the animals being treated with great gentleness to ensure as little excitement as possible. The blood-sugar was determined by the familiar Hagedorn-Jensen method. As there was a natural variation of the fasting level, the subsequent readings in each case have been calculated as percentages of the fasting level, and averaged for comparison. The duration of anæsthesia was greater in the guinea-pigs than the rabbits, so samples were taken from them after 80 minutes. The results are given in the table.

TABLE.—PERCENTAGE OF ORIGINAL BLOOD-SUGAR LEVEL.

	After 15 min.	After 25 min.	After 60 min.	After 80 min.
Guinea-pigs . . (14 experiments)	100·3	100·3	102·5	99·9
Rabbits . . . (8 experiments)	97·6	95·6	99·0	..

The dosage varied in the guinea-pigs from 45 to 90 mg. per kilo, in the rabbits from 40 to 100 mg. per kilo, but no relation between dose and effect was found. The average variation is not significant, and it can be concluded that evipan sodium has no appreciable effect on blood-sugar in rabbits and guinea-pigs.

One rabbit has been omitted from the series as the fasting level was abnormally high, probably on account of excitement; in it the blood-sugar fell from 188 mg. per cent. to 112 mg. 14 minutes after injection. A second rabbit died 18 minutes after induction of anæsthesia (dose 80 mg. per kilo) and immediately the heart ceased blood was taken by cardiac puncture. The sugar level was found to have risen from 121 mg. per cent. to 222 mg. This was also omitted as the increase was considered to be due to asphyxia, death having resulted from respiratory failure.

Respiration.—Under evipan sodium the breathing rate becomes slower and more shallow. The amount of the slowing varies considerably in different animals with the same dosage. For example, four mice of approximately the same weight were each given 1·4 mg. evipan per 10 grm. body-weight, and the lowest points of the respiratory rate were respectively 45 per cent. of the normal in 34 minutes; 64 per cent. in 40 minutes; 58 per cent. in 55 minutes; and 37 per cent. in 60 minutes. The effects on rats and rabbits were of the same order. Guinea-pigs were still more variable, and the breathing was usually so irregular that it was impossible to count. Posture influenced the rate; in one guinea-pig lying on its back the rate fell from 123 to 27 per minute 10 minutes after induction; it was then placed on its side and at once the breathing rose to 48 per minute. When death occurred as a result of high dosage it was always due to primary respiratory failure. Artificial respiration was effective in restoring some of the animals apparently about to die.

Temperature.—The effect of evipan was to lower the temperature slightly in rats, more in rabbits, and considerably in guinea-pigs. Again the results cannot be tabulated readily, as not only do the times at which the lowest points were reached vary widely, but also the amounts of the fall with the same dose. In rats the average fall was of the order of 0·3° F. In rabbits it was 1·5° F., the largest reduction being from 102·6° to 100·2° F. which occurred in 45 minutes. Guinea-pigs again showed the most variation; three which received a large but not fatal dose (80 mg. per kilo) showed falls of 102° to 97·6° F., 102·6° to 96·2° F., and 102° to 96·5° F. in 140, 110, and 120 minutes respectively. The anæsthesia lasted 250, 100, and 170 minutes. With doses of 50 mg. per kilo the temperature was lowered on the average 2·5° F., but the variations were wide.

Experiments on Cats.—Some experiments were made with cats to ascertain the effect on the blood-pressure and respiration, and the effect of repeated administration.

A cat was injected intraperitoneally with a dose of 80 mg. per kilo, and in 5 minutes was unable to stand, showing a slight degree of opisthotonos and neck rigidity. This passed off in a minute, and 10 minutes from the start, as the conjunctival reflex was still elicited, a

small amount of ether was given, and a canula inserted into the carotid artery. The pressure was 175 mm. A fatal experiment was performed on the brain which had no relation to the present work, which the animal stood well. At the end of the experiment, 70 minutes, the pressure was 120 mm. In another cat with the same dose the initial pressure was 140 mm. and after an experiment lasting 40 minutes it stood at 84 mm. In both cases the extent of respiratory movements was reduced.

The effect of additional injection of evipan was shown in another experiment. The anæsthetising dose was 70 mg. per kilo and no ether was required. The initial blood-pressure was 120 mm. mercury, and the respiratory rate 24 per minute. Ten mg. evipan per kilo were injected very slowly into the femoral vein, and within 2 minutes the blood-pressure had dropped to 93 mm. and the breathing to 20 per minute. A second 10 mg. dose was given, resulting in a further fall to 66 mm. and 18 per minute, but after 4 minutes the pressure recovered to 93 mm. Then 20 mg. per kilo were injected, with a resultant fall to 60 mm. followed by a rise to 90 mm. The respiration at this point was 14 per minute. Stimulation of the vagus caused a sharp drop of 25 mm. with temporary rise of as much above the original level. Thirty mg. evipan per kilo produced a further fall which recovered to 65 mm. and a respiratory rate of 13. Twenty minutes later 40 mg. per kilo obliterated respiration for 5 minutes though the heart continued to beat. Breathing restarted at 7 per minute and the blood pressure again recovered to 60 mm. After 10 minutes the cat was killed. In 80 minutes the animal received a total dose of 180 mg. per kilo.

Anæsthesia was induced in another cat in 6 minutes by 50 mg. evipan per kilo, and the initial blood-pressure was 110 mm. The effect of a further dose of 25 mg. (12·5 mg. per kilo) was to lower the pressure slightly and slow the breathing. The antagonistic action of coramine was tested by an intravenous dose of 0·25 c.c. with a resulting stimulation of respiration, momentary struggling, and a rise and slow fall of blood-pressure. Ligaturing the vagi deepened and slowed respiration. The peripheral end of the left vagus was stimulated, producing a sharp fall with recovery to normal on cessation. Stimulation of the right vagus produced a like result, thus showing that the vagus is not paralysed by evipan.

Discussion.

The experiments on the frog heart show that while evipan sodium exerts a depressant action on the heart, the anæsthetic is not fixed by the cells since even high concentrations are readily washed out, with a recovery of function to the original state. Further, if a quantity of evipan is perfused through a heart for some time it exerts a like action

when transferred to a second heart. If it is destroyed by the frog heart this action must be very slow. Evipan is detoxicated remarkably quickly in the intact mammal, but in the intact frog this process is much slower [Kennedy, 1933]. The ventricle is affected by the drug before the auricle. When the beats have completely ceased excitability still remains, as shown by the effect of mechanical stimulation with a pin.

The blood-sugar level is not significantly affected by this anæsthetic. Variations were found in the experiments quoted, the extreme ranges of which were +40 mg. per cent. to −20 mg. per cent., but these averaged out to a normal figure. Since asphyxia causes a rise of blood-sugar it can be said that although evipan depresses respiration, the pulmonary embarrassment is not too excessive with ordinary doses.

Evipan slows respiration and reduces temperature. The guinea-pig is more susceptible to these effects than the other animals examined. Trembling in mice was noted in the previous paper, when it was suggested this was a temperature effect as it was largely prevented by warming the animals. Trembling was not seen in cats, and only twice in rabbits, but over half the guinea-pigs exhibited some degree of tremor when anæsthetised, which supports the above suggestion.

The effect on respiration is also demonstrated in the experiments. In one case breathing ceased for about 5 minutes while the heart continued to beat, and this was followed by recovery. The stimulating effect of coramine (pyridine-β-carbonic acid diethylamide) on the respiratory centre effectively counters the depressant action of evipan, and this analeptic should be of value clinically just as it is in avertin anæsthesia. Weese [1925] gives the minimal effective dose for the cat as 20 mg. per kilo and the minimal lethal dose as 100 to 110 mg. per kilo; the third cat of this series received 180 mg. per kilo within 80 minutes, which, together with the short duration of anæsthesia with smaller doses, indicates that the substance is detoxicated in a short time.

Summary.

(1) The depressant action of evipan sodium on the frog heart is very rapidly removed by washing, and is antagonised by adrenalin. Complete recovery can be obtained in a few minutes.

(2) No significant effect is produced by evipan sodium on the blood-sugar of rabbits or guinea-pigs.

(3) Respiration is markedly slowed and temperature reduced by this anæsthetic.

(4) The detoxication of evipan sodium in mammals is very rapid.

The investigation was carried out under a grant from the Carnegie Trust, and Bayer Products kindly supplied the evipan sodium used.

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