ACTION OF ACETYLCHOLINE, NORADRENALINE AND SOME OF THE AUTONOMIC BLOCKING AGENTS ON THE PERFUSED BLOOD VESSELS OF THE FROG

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The action of noradrenaline and of acetylcholine on the perfused blood vessels of the frog was investigated using a technique slightly modified from that described by Rahman and Abhyankar (1935). Both the drugs gave a marked pressor action on the systemic vessels of the frog, the action of acetylcholine being stronger. The pressor action was only partially blocked by atropine, the blocking action being more marked for acetylcholine than for noradrenaline. The actions of the two drugs on pulmonary vessels was different from their action on the systemic vessels ; acetylcholine causing marked vasoconstriction and this vasoconstrictor action being completely, or almost completely,blocked by atropine, noradrenaline causing only a feebly vasoconstrictor action which was not blocked by atropine. The action of adrenaline was simlar to, though stronger than, that of noradrenaline. The pressor actiou of noradrenaline was completely blocked by priscol and by ergotamine, while the vasoconstrictor action of acetylcholine was not blocked by these drugs.

Certain other features of the action of noradrenaline and of acetylcholine are described. As is the case with priscol and ergotamine, *d*-tubocurarine and curalest were found to have a direct vasoconstrictor action, whereas atropine was found to cause vasodilatation. It is suggested that the vasoconstrictor actions of noradrenaline and of acetylcholine on the blood vessels of the frog are likely to be due to two different mechanisms.

Rahman and Soemowerdojo (1962, unpublished), using a technique devised by them which measured the rate of flow of the perfusion fluid through the blood vessels of the frog, found that both acetylcholine and noradrenaline caused vasoconstriction. The similarity of action of the two drugs on the vascular system of the frog suggested the possibility that the vasoconstrictor action of acetylcholine might be mediated through a mechanism which released stored noradrenaline from the vessels. With this point in view, the present series of experiments were carried out to see how the two drugs corresponded or differed in their actions under different experimental conditions.

METHODS

The experiments were carried out using a modification of the technique described by Rahman and Abhyankar (1935). The vessels were perfused with Ringer's solution with a pressure of about 20 cm of water. A decrease in the rate of flow was indicated by a rise in perfusion pressure and an increase

in the rate of flow was indicated by a fall in pressure. The modified technique was found to be sensitive enough to detect a difference in the rate of flow even as small as one per cent. It also enabled measurement of the rate of flow. Perfusion pressure changes on administration of drugs were frequently indicated in cm of water along with the graphs obtained.

The action of the drugs was investigated on the systemic and on the pulmonary vessels. The action on mesenteric vessels was investigated separately. The systemic vessels were perfused by introducing the perfusion cannula into the middle compartment of a division, usually the left, of the truncus arteriosus. The pulmocutaneous and the carotid arches of that side were ligated to ensure that the perfusion fluid did not pass through them. The systemic arch was ligated just proximal to the junction with its fellow of the opposite side. The oesophagus was ligated near its junction with the stomach and cut caudal to the ligature, while the viscera including the heart and the liver were extirpated and often the hind part of the body was also removed. The perfusion fluid entering the systemic arch flowed through the vessels supplying the larynx, the oesophagus, the occiputo-vertebral region and the forelimb on that side As perfusion continued these regions were seen to swell due to the developing oedema. The perfusion fluid, escaping out of the perfused vessels, was not allowed to accumulate on the surface of the body as such accumulation was some times found to interfere with the perfusion pressure (Fig. 6, arrows 2 & 4).

The action on mesenteric vessels was tried on only one frog at Bandong. Owing to the small size of the animal the perfusion cannula was introduced into the left systemic arch the branches of which were ligated, the right systemic arch was ligated just prior to its junction with the left arch, the dorsal aorta was ligated just beyond the origin of the coeliaco-mesentric artery. The liver was extirpated. The perfusion fluid thus flowed only through the coeliaco-mesenteric vessels. In the case of the large Indian frog (*Rana tigrina*) it was easy to perfuse the mesenteric vessels with the cannula passed into the dorsal aorta caudal to the coeliaco-mesenteric artery, the systemic vessels being ligated just proximal to their junction with each other.

The pulmonary vessels were perfused through a cannula introduced into the pulmocutaneous arch, the cutaneous branch of which was ligated. The carotid and systemic arches were also ligated and the heart and the liver extirpated. In one of the animals the pulmonary vessels were perfused via the ventricle thus avoiding the difficulty of passing the cannula into the small pulmocutancous arch ; the heart was ligated at the auriculo-ventricular junction and the sinus venosus and the liver were extirpated.

RESULTS

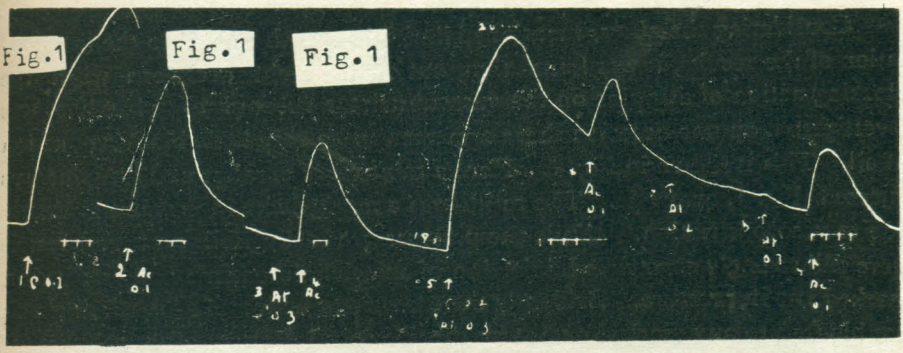
Action on systemic vessels

(a) *Action of adrenaline, noradrenaline and acetylcholine.*—All the three drugs were found to decrease the flow of the perfusion fluid through the systemic vessels. This decrease in flow was interpreted as due to the vasoconstrictor action of the drugs. However, the decrease in the rate of flow could also result from the blocking effect of the contracting skeletal muscles. Of the three drugs used, acetylcholine was known to have stimulating action on the skeletal muscles. Injection of acetylcholine, via the perfusion fluid, occasionally resulted in spasmodic contractions of the skeletal muscles, but asynchronous fasciculation was more commonly observed. The marked decrease in the rate of flow of the perfusion fluid on injection of small quantities of acetylcholine could be due both to its vasoconstrictor action and to its stimulating action on skeletal muscles. This was suggested by previous experiments by Rahman and Soemowerdojo (unpublished) who found that the effect of acetylcholine in reducing the rate of flow was diminished if the skeletal muscles were paralysed by previous administration of curare. In the present series, *d*-tubocurarine was used on a few occasions only. As further supply of this drug was not available, curalest (succinylcholinechloride) was used instead. Small quantities of these drugs were injected through the perfusion cannula. Both of them gave vasoconstrictor action, the action of curalest being stronger than that of *d*-tubocurarine. As acetylcholine still caused decrease in the rate of flow even after the administration of *d*-tubocurarine or of curalest (Fig. 1) it evidently had a marked vasoconstrictor action on the systemic vessels of the frog. The vasoconstrictor action was almost invariably followed by a vosodilator effect (Fig 2, arrow 6).

Of the three drugs used, the vasoconstrictor action of acetylcholine and of adrenaline was found to be stronger than that of noradrenaline when used in similar concentrations.

(b) *Blocking action of atropine.*—That atropine blocks the action of injected acetylcholine on the blood vessels of the mammal has been reported by many workers. In the present series the blocking action was investigated on eight occasions. Several complicating factors made it difficult to interpret the results. Injection of the same dose of acetylcholine gave different degrees of response from time to time even in the same animal. Often the vasoconstrictor effect of the drug became less marked as perfusion with Ringer's solution was continued. Also, atropine by itself gave a marked vasodilator action. This was invariably observed (Fig. 1, arrows 3. 7 & 8. Fig. 2, arrow 2 , Fig. 3, arrows 2 & 4., Fig. 7, arrow 4). So that when acetylcholine or

noradrenaline were injected soon after the injection of atropine, their vasoconstrictor action tended to be masked by the vasodilator action of atropine (Fig 2, arrow 3). Further, while investigating the blocking action of atropine,



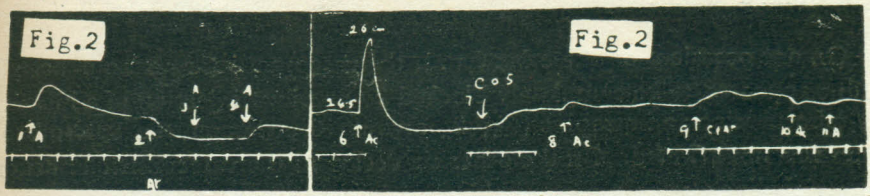


Fig. 1. The systemic vessels were perfused with Ringer's solution. Upper record, perfusion pressure. Lower record, time in 30 sec.

The record shows the effect of injection, via the perfusion fluid, of the following drugs; at arrow 1, 0.2 ml of curalest (3 mg/ml); at arrow 2, (15 min after the first injection and when the pressure had come down almost to the original level), 0.1 ml of 0.001% acetylcholine; at arrow 3, 0.3 ml (0.25 mg/ml) atropine; at arrow 4, 0.1 ml of 0.001% acetyloholine; at arrow 5, a mixture of 0.2 ml curalest and 0 3 ml atropine; at arrow 6, 0.1 ml acetylcholine; at arrows 7 & 8, 0.2 ml and 0.3 ml respectively of atropine; at arrow 9, 0.1 ml of 0.001% acetylcholine.

Fig. 2. The systemic vessels were perfused with Ringer's solution. Upper record, perfusion pressure. Lower record, time in 30 sec.

The record shows the effect of injection of the following drugs; at arrow 1, 0.1 ml of 0.0002% adrenaline; at arrow 2, 0.3 ml (0.25 mg/ml) atropine; at arrows 3 & 4 adrenaline injection repeated; at arrow 6, 0.1 ml of 0.001% acetylcholine; at arrow 7, 0.5 ml of *d*-tubocurarine (3 mg/ml); at arrow 8, acetylcholine injection repeated; at arrow 9, a mixture of 0.5 ml *d*-tubocurarine and 0.3 ml atropine in the same concentration as mentioned above; at arrow 10, 0.1 ml acetylcholine and at arrow 11, 0.1 ml adrenaline injected, acetylcholine causing only a slight vasodilatation and adrenaline still causing vasoconstriction. The record also shows fluctuations in basic perfusion pressure the cause of which is discussed in the text.

it was considered necessary to paralyse the contraction of skeletal muscles by injection of *d*-tubocurarine or of curalest. The two drugs, curalest (or *d*-tubocurarine) and atropine, were injected prior to the injection of acetylcholine,

either together or one after the other. Curalest by itself was found to be strongly vasoconstrictor and this vasoconstrictor action lasted a considerable time.

Consideriag the above mentioned limitations with regard to the interpretation of the results, it was found that, out of the eight occasions when the blocking action of atropine on the vasoconstrictor effect of acetylcholine was investigated, on one occasion there appeared to be very little blocking action at all. This is shown in Fig. 1. Here a mixture of curalest and atropine was injected (Fig. 1, arrow 5). The result was a marked rise in pressure evidently due to vasoconstriction. While the pressure gradually fell and the vessels were still strongly constricted, injection of acetylcholine (arrow 6) caused a marked rise in pressure. Evidently the constrictor action of acetylcholine was not blocked to any marked extent by the previous injection of a mixture of curalest and atropine.

On one occasion, on the other hand, the vasoconstrictor action of acetylcholine appeared to be completely blocked by previous injection of a mixture of *d*-tubocurarine and atropine (Fig. 2, arrow 10 , though the vaso-dilator action was not affected. On the other six occasions the vasoconstrictor action of acetylcholine was only partially blocked by atropine.

The blocking action of atropine on the vasoconstrictor effect of adrenaline and of noradrenaline was less marked. In no case was the constrictor action found to be completely blocked by atropine. In cases where atropine injection appeared to block markedly the constrictor action of acetylcholine injection of adrenaline or of noradrenaline still caused vasoconstriction (Fig. 2, arrow 11).

(c) *Perfusion with curalest.*—Similar results as above were obtained when, instead of injecting small doses of curalest, the systemic vessels were perfused with weak solutions of the drug. This was done on two occasions, the systemic vessels being perfused, on one occasion, with 0.0015 per cent curalest in Ringer's solution and, on the other occasion, with 0.00075 per cent curalest solution. On both the occasions the vessels were perfused first with Ringer's solution which was then replaced with curalest solution. On both the occasions when the Ringer's solution was replaced with curalest solution, the pefusion pressure rose rapidly to a miximum and this level was maintained as long as the perfusion with curalest solution was continued. While the perfusion with curalest solution continued, injection of acetylcholine or of noradrenaline caused a marked rise in pressure and this action was only partially blocked by atropine, the block being more marked in the case of acetylcholine than in the case of noradrenaline.

(d) *Blocking action of priscol.*—The blocking action of priscol on the pressor action of adrenaline, noradrenaline and of acetylcholine was studied on three animals. On two of these occasions the systemic vessels were perfused with 0 002 per cent solution of priscol, i.e. Ringer's solution containing 0.002 per cent priscol. As a control, 0.1 ml of 0.01 per cent noradrenaline was injected prior to the start of perfusion with priscol when the vessels were being perfused with Ringer's solution. This gave a marked pressor action. When the same dose of noradrenaline was injected during the period of perfusion of the vessels with priscol solution, no pressor action developed. Similar results were obtained with injection of adrenaline. Injection of weak doses of acetylcholine under similar circumstances resulted in marked pressor action. On one occasion the vessels were perfused with 0.001 per cent priscol solution. Even in this concentration priscol blocked the effect of injected noradrenaline and of adrenaline. But the pressor action of acetylcholine was not blocked even when this was proceeded by the injection of *d*-tubocurarine.

(e) *Blocking action of ergotamine.*—This was investigated in eight frogs and the results obtained were uniformly consistent. Perfusion with ergotamine tartrate in 0.001 per cent solution blocked the pressor action of adrenaline and of noradrenaline The pressor action of acetylcholine and of curalest was not blocked. Fig. 4 gives a typical record of the results obtained. In this experiment perfusion of the systemic vessels was started with Ringer's solution. While the perfusion was continued 0.1 ml of 0.003 per cent curalest was injected (not shown in Fig. 4). This caused a marked rise in pressure. When the pressure had fallen to the original level, the Ringer's solution was replaced with one containing 0.001 per cent ergotamine. This caused a rise in pressure which reached a maximum and tended to remain at that level while perfusion with ergotamine was continued. The pressure was brought down to the original level of 21 cm by tightening the screw-clamp which controlled the perfusion pressure. The subsequent events in the experiment are shown by Fig. 4. The record shows fluctuation in basic perfusion pressure. The record also indicates that ergotamine perfusion did not block the pressor action of curalest even after atropine injection. The pressure action of noradrenaline was blocked ; in fact noradrenaline caused decrease in pressure. The pressor action of acetylcholine was not blocked by ergotamine.

Fig 5 shows similar results; perfusion of the systemic vessels with ergotamine completely blocked the pressor action of adrenaline while the pressor action of acetylcholine was not blocked Injection of acetylcholine resulted in a marked rise in pressure which reached a level of 28 cm and then rapidly fell. When the same dose of acetylcholine was injected soon after an injection of *d*-tubocurarine, the rise in pressure, though still considerable, was not so

marked as before. This could be interpreted as due to the fact that *d* tubocurarine paralysed the skeletal muscles and the rise in pressure on injection of acetylcholine was then due only to its vasoconstrictor action.

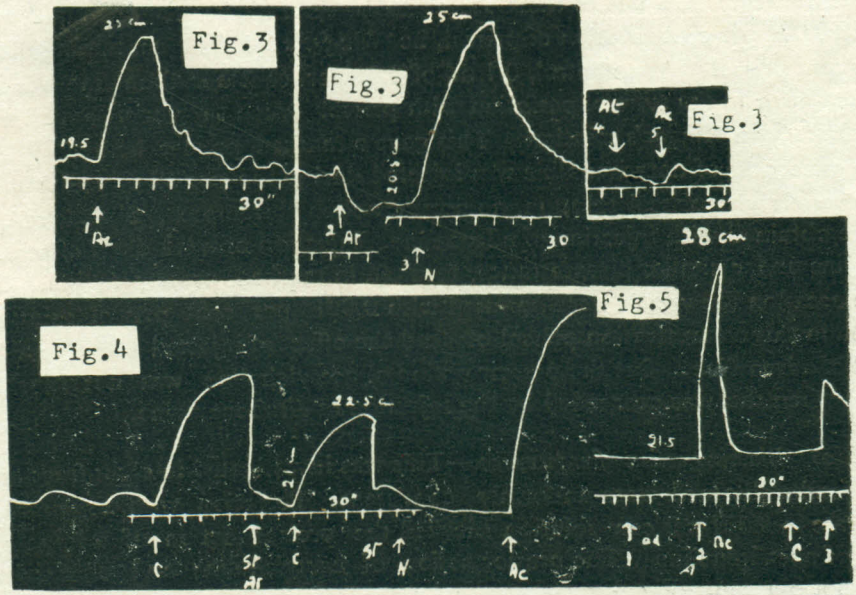


Fig. 3. The mesentric vessels were perfused with Ringer's solution. Upper record, perfusion pressure. Lower record, time in 30 sec. The basic perfusion pressure record shows a wavy character. The following drugs were injected ; at arrow 1, 0.1 ml of 0.001% acetylcholine; at arrow 2, 0.3 ml atropine (0.25 mg/ml) ; at arrow 3, 0.1 ml of 0.1% noradrenaline; at arrow 4, 0.3 ml atropine injected as above ; at arrow 5, 0.1 ml of 0.001% acetylcholine.

Fig. 4. The systemic blood vessels were perfused with 0.001% ergotamine tartrate in Ringer's solution. Fluctuations are noted in the basic pressure record Upper record, perfusion pressure. Lower reeord, time in 30 sec. The record shows the effect of injection of the following drugs ; at the first arrow C, 0.1 ml of 0.003% curalest ; at the second arrow St, the drum was stopped while the pressure fell, then the drum was re-started and 0.4 ml atropine sulphate (0.25 mg/ml) injected ; at the third arrow C, curalast injected as above ; at second St, the drum was stopped again as the pressure fell. Then it was restarted ; at arrow N, 0.1 ml of 0.001% noradrenaline ; at arrow Ac, 0.1 ml of 0.001% acetylcholine.

Fig. 5. The systemic blood vessels were perfused with ergotamine tartrate in the same way as mentioned in the case of Fig. 4. The following drugs were injected while the perfusion continued ; at arrow 1, 0.1 ml of 0.001% adrenaline ; at arrow 2, 0.1 ml of 0.001% acetylcholine ; at arrow C, 0.7 ml of *d*-tubocurarine (3 mg/ml) ; at arrow 3, 0.1 ml acetylcholine again injected.

Action on mesenteric vessels.

The investigations were made on one animal at Bandung and on three large frogs (*Rana tigrina*) at Hyderabad, India. The results of experiments at Bandung are indicated by Fig. 3. Similar results were obtained at Hyderabad

The mesenteric vessels behaved like the other systemic vessels. After atropine, injection of noradrenaline still resulted in a pronounced rise in pressure, whereas the pressor action of acetylcholine was much reduced after atropine Atropine injection itself caused a marked fall in pressure. The irrigular fluctuations in pressure observed in the basic record were evidently due to the effect of gastro-intestinal peristalsis.

Action on Pulmonary vessels

The action of the drugs on pulmonary vessels was investigated on three animals at Bandung and on three large Indian frogs at Hyderabad, India. In one of the animals at Bandung the pulmonary vessels were perfused via the ventricle (Fig. 7). In the other five animals the vessels were perfused through a cannula introduced into the pulmocutaneous arch as described earlier.

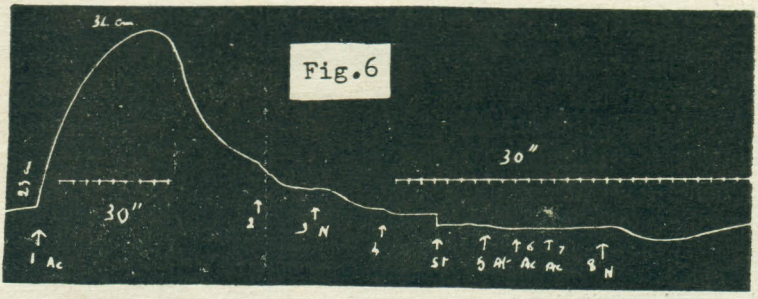
The results of these experiments indicated that the action of noradrenaline on the pulmonary vessels differed markedly from the action of acetylcholine; noradrenaline had only a slight pressor action which was usually followed by a depressor action (Fig 6, arrows 3 & 8. Fig. 7 arrow 6), whereas acetylcholine had a strong pressor action which was completely or almost completely blocked by atropine (Fig. 6, arrows 1, 6 & 7. Fig 7, arrows 2 & 5). The pressor action of noradrenaline, though slight, was not blocked by atropine.

Injection of curalest resulted in dilatation of the pulmonary vessels (Fig. 7, arrow 3). This was in marked contrast to the powerful vasoconstrictor action of the drug on the systemic vessels. This experiment, however, was not repeated.

DISCUSSION

The actions of acetylcholine and of noradrenaline on the perfused blood vessels of the frog, as revealed in the present investigations, differ markedly from their action on the mammalian vessels. Whereas, in the mammal, acetylcholine is a generalised vasodilator (Dale and Richards 1918) including the coronary vessels (Eckenhoff *et al*, 1947. Folkow and Uvnas, 1949), it has a marked vasoconstrictor action on all the vessels of the frog including the pulmonary vessels.

A feature of the action of the two drugs on the perfused blood vessels of the frog was vasodilatation which followed their vasoconstrictor action. This was more consistently seen in the case of noradrenaline (Fig 6, arrows 3 & 8, Fig. 7, arrow 6). And in conditions where the vasoconstrictor action was blocked, noradarenaline caused only vasodilatation (Fig 4, arrow N).



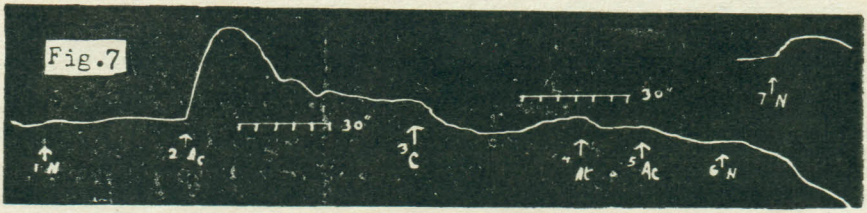


Fig. 6. The pulmonary vessels were perfused with Ringer's solution. Records indicate perfusion pressure and time in 30 sec. The following drugs were injected; at arrow 1, 0.1 ml of 0.001% acetylcholine; at arrows 2 & 4, the perfusion fluid which had collected on the surface of the body was mopped up with cotton wool resulting in a slight fall in pressure; at arrow 3, 0.1 ml of 0.02% noradrenaline; at arrow St, the drum was stopped for 8 min while the pressure continued to fall slowly. Then the drum was re-started. At arrow 5, 0.4 ml atropine (0.25 mg/ml) at arrows 6 & 7, 0.1 ml of 0.001% acetylcholine; at arrow 8, 0.1 ml of noradrenaline was again injected.

Fig. 7. The pulmonary vessels were perfused with Ringer's solution via the ventricle. Records indicate perfusion pressure and time in 30 sec. The following drugs were injected; at arrow 1, 0.1 ml of 0.002% noradrenaline; at arrow 2, 0.1 ml of 0.002% acetylcholine; at arrow 3, 0.2 ml curalest (3 mg/ml); at arrow 4, 0.2 ml atropine (0.25 mg/ml); at arrow 5, 0.1 ml acetylcholine again injected; at arrow 6, 0.1 ml noradrenaline again injected; at arrow 7, 0.1 ml noradrenaline injection repeated.

In the mammal atropine appears to block completely the vasodilator action of acetylcholine, whereas the vasoconstrictor action of noradrenaline is not blocked, though large doses of atropine are stated to antagonize the constrictor effect of adrenaline (Regniers, 1926).

In the present series of experiments atropine was found to have a marked vasodilator action on the blood vessels of the frog including the pulmonary circulation. Whether it has similar action on the perfused blood vessels of the mammal is not clear. According to Goodman and Gilman (1956) toxic amounts of atropine in man, and therapeutic doses occasionally, cause an active vasodilatation of the cutaneous blood vessels specially those of the blush area the mechanism of which is stated to be unknown. In the frog, atropine was found to decrease the vasoconstrictor action of acetylcholine. But the action was seldom completely blocked by atropine except in the case of pulmonary vessels where the vasoconstrictor action of acetylcholine was completely, or almost completely, blocked (Fig 6 & 7). The pressor action of noradrenaline was only slightly blocked by atropine.

The action of the two drugs, acetylcholine and noradrenaline, on pulmonary vessels was markedly different from their action on systemic vessels. Acetylcholine caused a marked vasoconstriction of pulmonary vessels but this action was completely, or almost completely, antagonized by atropine. The vasoconstrictor action of acetylcholine on systemic vessels was not so makedly blocked by atropine. On the other hand noradrenaline caused only a slight constrictor action on the pulmonary vessels of the frog, and this slight constrictor action was not blocked by atropine, The vasoconstrictor action of noradrenaline on the pulmonary vessels was followed by a marked vasodilator action Fig. 6, arrows 3 & 8, Fig. 7, arrow 6) which was also observed in the case of the large Indian frog.

An interesting feature of the pulmonary circulation was a marked vasodilator effect of injected curalest (Fig. 7, arrow 3), whereas this drug was found to have a profound vasoconstrictor action on systemic vessels.

Even in the mammal acetylcholine does not appear to have a uniform action on all the blood vessels. It was shown by Foggie (1938) that acetylcholine caused constriction of the pulmory vessels of the rat and guineapig. Also in the ear of cat it was noticed by Kottegoda (1953) that the skin vessels responded differently from the vessels covering the ear cartilage ; that the skin vessels gave a constrictor response under certain conditions while the vessels covering the cartilage always gave a dilator response.

As the injection of acetylcholine, like that of noradrenaline, causes vasoconstriction in the frog, the question arises whether this action of injected acetylcholine is not due to a release of stored noradrenaline in the walls of the vessels of the frog in the same way as has been suggested might happen in the case of perfused rabbit's ear under certain conditions (Burn and Robinson,

1951; Kottegoda, 1953; Burn 1961). This, however, does not seem to be the case. If this were so, it would be reasonable to expect the vasoconstrictor action of acetylcholine to show similar variations in different experimental conditions as shown by the vasoconstrictor action of noradrenaline. This was, however, not the case, the two drugs differing in several ways in their actions. The pressor action of acetylcholine on the systemic vessels of the frog was blocked by atropine to a more marked extent than the pressor action of noradrenaline. Acetylcholine had a marked pressor action on the pulmonary vessels whereas noradrenaline showed only a feeble pressor action. The pressor action of acetylcholine on the pulmonary vessels was completely or almost completely blocked by atropine, whereas the pressor action of noradrenaline was not so blocked. The pressor action of noradrenaline was completely blocked by priscol and by ergotamine whereas the pressor action of acetylcholine was not markedly affected by these drugs.

It is, therefore, likely that the vasoconstrictor action of acetylcholine on the blood vessels of the frog is not mediated through a mechanism which releases noradrenaline or a noradrenaline-like substance. The vasoconstrictor actions of the two drugs are likely to be the results of two different mechanisms.

Fluctuations in the basic perfusion pressure, i.e. fluctuations not due to the action of injected drugs, were frequently observed (Figs. 2. 3, 4, & 7). Such fluctuations were described by Rahman and Abhyankar (1935) who considered them to be possibly due to the mechanical effects of gastro-intestinal peristalsis. However, they observed fluctuations in the perfusion pressure even when the animal was deviscerated and only the hind limb vessels were perfused. Therefore they suggested that the fluctuations in the perfusion could be due to the rhythmic changes in the tone of the blood vessels. This suggestion was subsequently withdrawn when it was noticed that the fluctuations in pressure in such cases were due to the mechanical effects of the rhythmic contractions of the last part of the gut which had escaped excision and had sunk deep in the pelvis and was out of view.

In the present series of experiments fluctuations in the basic perfusion pressure were observed even when almost all the viscera were removed. The fluctuations in pressure, then, were probably due to the rhythmicity of the viscera still left intact, viz the pharynx and the oesophagus and bits of cardiac tissue after incomplete excision of the heart.

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REFERENCES

Burn, J.H. (1961). *Brit. Med. J.*, **1**, 1623.

Burn, J.H. and Robinson, J.R (1951). *Brit. J. Pharmacol.*, **6**, 110.

Dale, H.H. and Richards, A N. (1918). *J. Physiol.*, **52**, 110.

Eckenhoff, J.E., Hafkenchiel, J.H. and Landmesser, C.M. (1947). *Am. J. Physiol.*, **148** 582.

Foggie, P. (1938). *Quart. J. Exp. Physiol.*, **26**, 225.

Folkow, B. and Uvnas, B. (1949). *Acta. Physiol. Scand.*, **17**, 201.

Goodman, L.S. and Gilman, A. (1956). *The Pharmacological Basis of Therapeutics*, 2nd ed., p. 547. New York, MacMillan.

Kottegoda, S.R. (1953). *Brit. J. Pharmacol.*, **8**, 156.

Rahman, S.A. and Abhyankar, R.N. (1935). *Indian J. Med. Res.*, **22**, 687.

Regniers, (1926). Quoted by Sollmann, T. (1957). *A Manual of Pharmacology*, 8th. ed. p. 389., Phaildelphia & London, Saunders.