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SOME FACTORS INFLUENCING THE RECOVERY OF ISOLATED MYOCARDIUM FROM ACUTE ANOXIA

BY

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The heart is fundamentally aerobic in contrast to skeletal muscle. This is obvious from the rich supply of oxidative enzymes, the numerous mitochondria and the high ratio of one capillary to each muscle fibre (Olson & Schwartz, 1951). It has been previously held that the myocardium is unable to support an oxygen debt. Recent work (Coffman & Gregg, 1961) has modified this view but still indicates that, although the myocardium can derive a considerable portion of its energy requirements anaerobically during transient periods of oxygen deprivation, the capabilities of the normal heart are very limited in this respect. It is a fundamental concept that, for normal function, the heart must be adequately oxygenated.

In some circumstances the heart may, for a time, be partially or completely deprived of its usual oxygen supply. These circumstances include coronary insufficiency and some modern heart surgery. The determination of the safe length of myocardial anoxia and the factors influencing the length of this period are important problems. Some workers have also emphasized the possibility of treating or preventing the oxygen deficit by means of compounds which affect the myocardial metabolism directly.

The work described in this paper was performed in an attempt to delineate some of the factors involved in the recovery of isolated myocardium from acute anoxia and to see how this recovery could be modified by the application of such drugs as adrenaline, noradrenaline, reserpine, iproniazid, dipyridamole and sodium nitrite.

METHODS

The preparations used were the isolated heart and the isolated atria of the rabbit.

Isolated hearts were perfused by the Langendorff technique with Locke solution at 50 cm of water pressure. The constitution (g/100 ml.) of the Locke solution was: NaCl 0.9, KCl 0.042, $CaCl_2$ 0.024, $NaHCO_3$ 0.05 and glucose 0.1. It was gassed with 97% oxygen and 3% carbon dioxide. The rabbits were mainly albino and averaged between 1 and 1.2 kg body weight.

The apparatus was based on that of Baker (1951). The coronary input was measured as drops making contact across silver wires connected to a Thorp impulse counter. The amplitude of the beat was recorded by a spring-loaded Brodie Universal lever writing on a smoked drum. The lever was attached by thread to a small hook in the right ventricular apex. Drugs were given by continuous perfusion of a fixed concentration.

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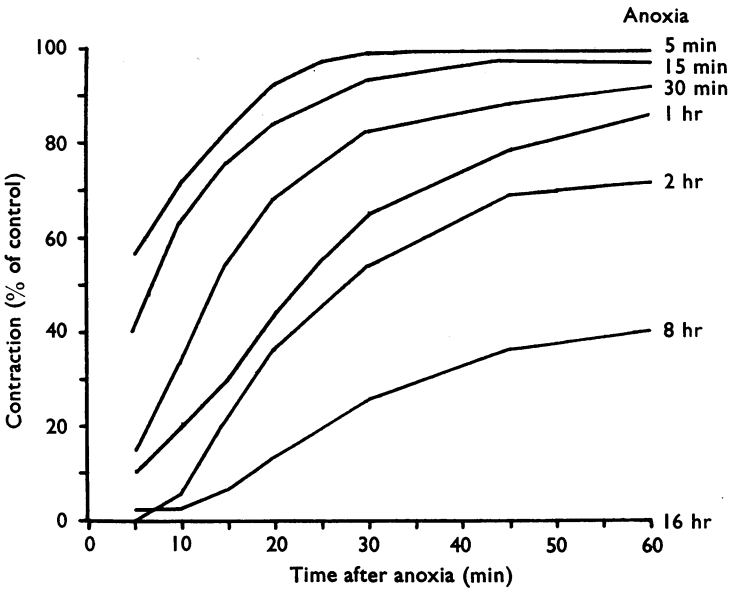

Fig. 1. Recovery of rabbit isolated atria (means of fifty-four experiments) from different periods of anoxia (values on right) at 30° C. Recovery plotted as percentages of the original amplitude of contraction at 5-min intervals over 1 hr from the end of the anoxic phase. The curve for 16-hr anoxia was at zero and, hence, is not shown.

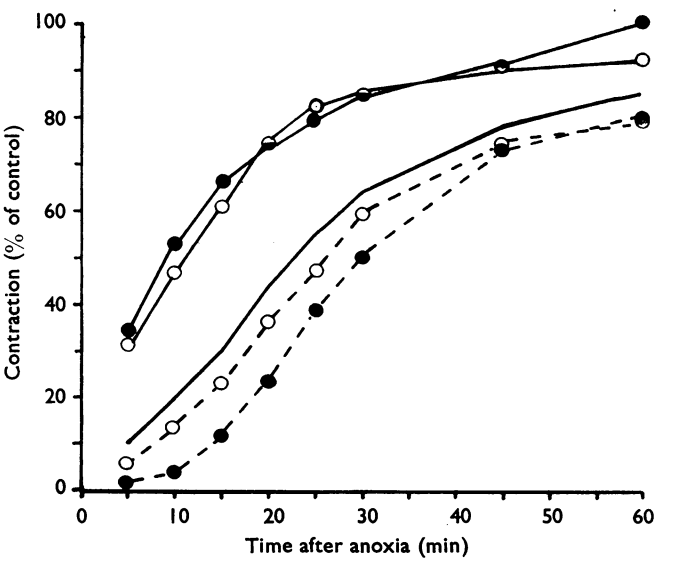

Fig. 2. Recovery of rabbit isolated atria (means of forty experiments) from 1 hr of anoxia at 30° C when either noradrenaline (0.05 μg/ml.) or adrenaline (0.05 μg/ml.) was present in either the anoxic or the recovery phase. ——, Control atria; – –○– –, noradrenaline present in the anoxic phase; —○—, noradrenaline present in the recovery phase; – –●– –, adrenaline present in the anoxic phase; —●—, adrenaline present in the recovery phase.

At least 30 min were allowed for the heart to become steady in rate and amplitude of beat before any experiments were made with periods of anoxia or with drugs.

Isolated atria were set up in Locke solution gassed with 97% oxygen and 3% carbon dioxide at 30° C. Rabbits weighing 700 to 800 g were used and were killed by stunning before removal of the atria. The atria were set up three pairs at a time in the same organ-bath. This provided an identical environment as far as possible. The contractions were recorded by isotonic levers writing one above the other on a kymograph.

After being in the bath for at least 1 hr, the atria became constant in rate and amplitude of beat and no experimental work was done till they were in this steady state.

Experimental procedures

Hearts subjected to asphyxia at 37° C for 15 min. In these hearts the coronary flow was shut off without prior arrest of the heart. At the end of 15 min, the coronary flow was again started and the rate and amplitude of the contraction were measured (and subsequently plotted as percentages of the original values) at intervals of 5 min over the next 45 min.

The values from six hearts were compared with those from six hearts where iproniazid (10 μg/ml.) was present throughout the perfusion or from six hearts where the animal had been given reserpine before the experiment. The dosage of reserpine was that recommended by Burn & Rand (1958); 1.5 mg/kg was given intraperitoneally on the first day, 3.0 mg/kg intravenously on the second day and the rabbits were used on the third day.

Atria subjected to anoxia at 30° C without prior arrest. Anoxia for periods from 5 min to 16 hr was produced by changing the oxygenated Locke solution to that which had been previously gassed with 97% nitrogen and 3% carbon dioxide. The atrial rate and contraction quickly declined and diastolic arrest occurred within a few minutes. At the end of the required period of anoxia (called the anoxic phase) the anoxic Locke solution was replaced by oxygenated solution and the rate and amplitude of the contraction were measured (and subsequently plotted as percentages of the original values) at intervals of 5 min over the next hour (recovery phase).

The recovery of control atria subjected to 1 hr of anoxia was selected as a standard with which to compare the effects of drugs upon the recovery of the atria from anoxia. If the application of a drug moved the recovery curve to the left, this would be the equivalent of exposing the atria to less anoxia, and, if to the right, the equivalent of exposing the atria to more. This provided a qualitative and quantitative assessment of the drug under investigation.

The drugs used were adrenaline (0.05 μg/ml.), noradrenaline (0.05 μg/ml.), iproniazid (5 or 10 μg/ml.), dipyridamole (1 or 10 μg/ml.) and sodium nitrite (1 or 10 μg/ml.).

RESULTS

The effect of the length of exposure to anoxia. The recovery of the rate and amplitude of contraction depended markedly on the length of the anoxic period to which the atria or hearts were subjected. Fig. 1 shows the mean recovery of the contractions of fifty-four atria which had been subjected to various periods of anoxia at 30° C ranging from 5 min to 16 hr. Atria made anoxic for 16 hr at 30° C showed no signs of recovery of rate and contraction for up to 1 hr after the end of the anoxic phase, though there was a very minor degree of recovery apparent in some of the atria several hours later.

The effect of adrenaline or noradrenaline. Fig. 2 shows the mean recovery of the contraction of forty atria from 60 min anoxia at 30° C when adrenaline or noradrenaline was added at the start of either the anoxic or the recovery phase to give a final concentration of 0.05 μg/ml. In atria not subjected to anoxia, adrenaline (0.05 μg/ml.) gave a positive inotropic effect of 36% and noradrenaline (0.05 μg/ml.) a positive inotropic effect of 32%. It will be seen that adrenaline and noradrenaline, when present in the recovery phase only,

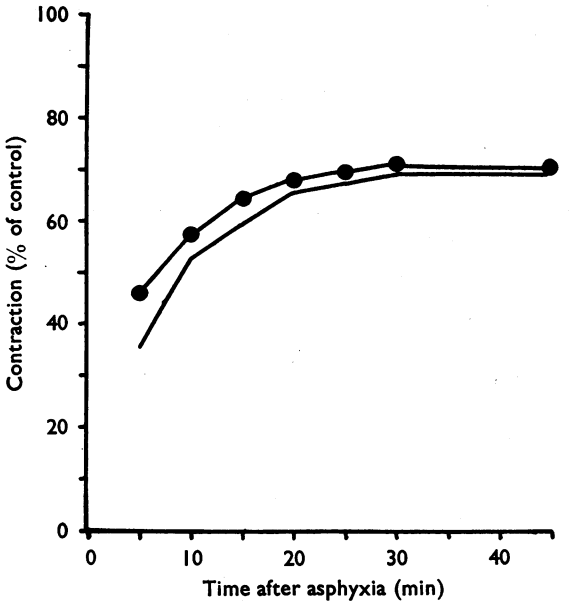

Fig. 3. Recovery of rabbit isolated hearts (means of six experiments) from 15 min of asphyxia at 37° C, compared with hearts from rabbits where the perfusate also contained iproniazid, 10 μg/ml. Recovery is plotted as percentages of the original amplitude of contraction at 5-min intervals over 45 min from the end of the asphyxic phase. ——, Control hearts; —●—, iproniazid-treated hearts.

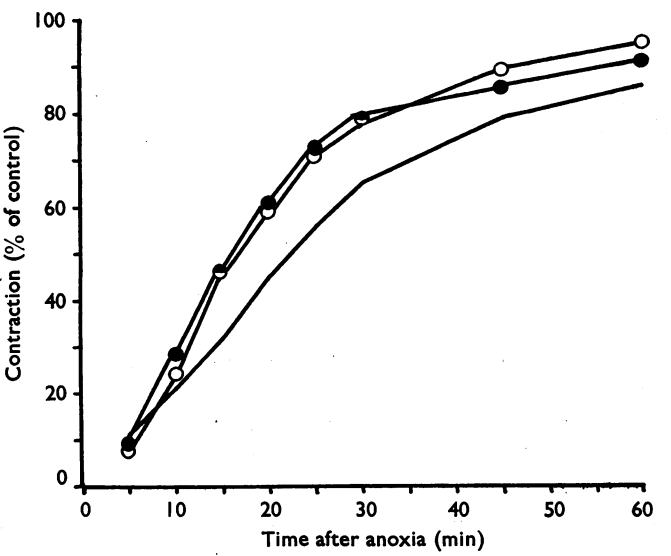

Fig. 4. Recovery of rabbit isolated atria (means of six experiments) from 1 hr of anoxia at 30° C, compared with atria under the influence of iproniazid (1 or 10 μg/ml.). Recovery is plotted as percentages of the original amplitude of contraction at 5-min intervals over 1 hr from the end of the anoxic phase. ——, Control atria; —○—, iproniazid (1 μg/ml.); —●—, iproniazid (10 μg/ml.).

greatly enhanced the recovery of the force of contraction, especially during the first 45 min. When noradrenaline or adrenaline was present in the anoxic phase only, subsequent recovery of the contraction was depressed, that induced by adrenaline being the greater.

The effect of iproniazid. Iproniazid, in the same concentration as used by Setnikar & Ravasi (1960), 10 μg/ml., was used throughout the perfusion of a series of six isolated hearts. The initial stabilizing period of 30 min with pure Locke solution was followed by a further 30 min of perfusion with Locke solution with iproniazid before any experimental results were taken. Locke solution with iproniazid was then used throughout the remainder of the experiment. During the initial stabilizing period with Locke solution with iproniazid there were no consistent effects on coronary flow, heart rate or amplitude of contraction.

The hearts were asphyxiated for 15 min as described above. The prior perfusion of iproniazid had a very small but significant ($P<0.05$) effect on the recovery of the contraction, there being a slight improvement during the first 20 min (Fig. 3). There was also a slight decrease in the recovery of the heart rate.

Fig. 4 shows the recovery of atria that had been subjected to 1 hr of anoxia at 30° C when iproniazid in concentrations of 1 μg/ml. (six atria) or 10 μg/ml. (six atria) was present in both the anoxic and the recovery phase. These concentrations of iproniazid had no obvious effect on the rate or the amplitude of contraction of atria that had not been exposed to anoxia. A clear enhancement of recovery can be seen, though there seems to be no difference in the degree of protection given with the higher concentration. For instance, the recovery of the contraction after 20 min of the recovery phase was 60%, compared with 44% for the control. This difference was highly significant, $P<0.01$.

The effect of reserpine. Administration of reserpine enhanced the recovery of the amplitude of contraction of nine atria from 1 hr of anoxia (Fig. 5) and of six isolated hearts from 15 min of asphyxia (Fig. 6). The effects were statistically highly significant, for example after 20 min of the recovery phase, the recovery of contraction of the reserpinized atria was 65%, compared with 44% for the control ($P<0.01$). The recovery of rate of contraction was not significantly altered.

The effect of dipyridamole. Fig. 7 shows the recovery of contraction of atria that had been subjected to 1 hr of anoxia at 30° C when dipyridamole, in concentrations of 0.1 μg/ml. (six atria) or 1.0 μg/ml. (six atria), was present in both the anoxic and the recovery phase. These concentrations of dipyridamole had no obvious effect on the rate or the amplitude of contraction of atria that had not been exposed to anoxia.

An obvious and significant enhancement of the recovery of contraction could be seen for the anoxia hearts, somewhat less with the lower concentration of dipyridamole; for instance, after 20 min of the recovery phase, the recovery of the contraction of atria in dipyridamole (1 μg/ml.) was 64% compared with 44% for the control, and this difference was highly significant, $P<0.01$.

The effect of sodium nitrite. Fig. 8 shows the recovery of atria that had been subjected to 1 hr of anoxia at 30° C when sodium nitrite, 1 μg/ml. (six atria) or 10 μg/ml. (six atria), was present in both the anoxic and the recovery phase. These concentrations of sodium nitrite had no obvious effect on the rate or the contraction of atria that had not been exposed to anoxia.

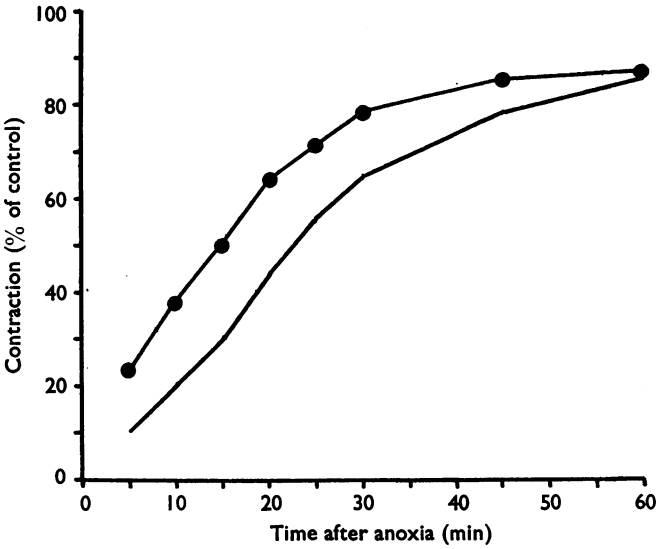

Fig. 5. Recovery of rabbit isolated atria (means of nine experiments) from 1 hr of anoxia at 30° C, compared with atria from rabbits that had also been reserpinized. Recovery is plotted as percentages of the original amplitude of contraction over 1 hr from the end of the anoxic phase. ——, Control atria; —●—, reserpinized atria.

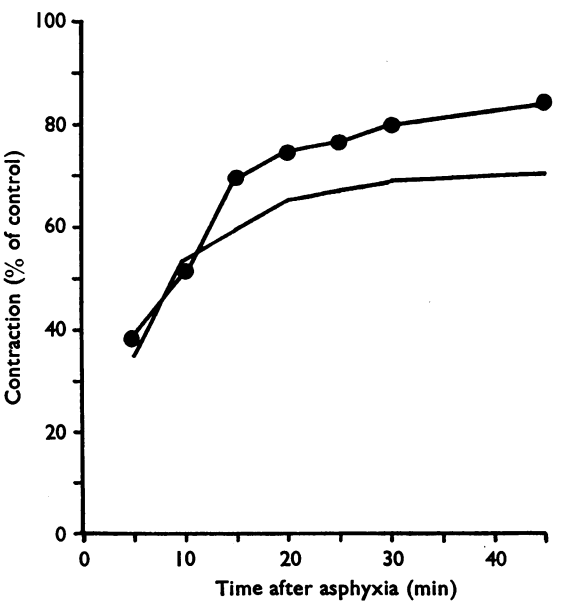

Fig. 6. Recovery of rabbit isolated hearts (means of six experiments) from 15 min of asphyxia at 37° C, compared with hearts from rabbits that had also been reserpinized. Recovery is plotted as percentages of the original amplitude of contraction at 5-min intervals over 45 min from the end of the asphyxic phase. ——, Control hearts; —●—, reserpinized hearts.

There was an obvious enhancement of the recovery of the contraction for the anoxic hearts, somewhat less with the lower concentration of sodium nitrite; for instance, after 20 min of the recovery phase, the recovery of the contraction in sodium nitrite (10 μg/ml.) was 60% compared with 44% for the control, and this difference was highly significant, $P<0.01$.

DISCUSSION

Criteria and recovery curves of controls

Some workers using techniques similar to those described in this paper have taken the time from the onset of anoxia to the occurrence of cardiac arrest as an index of the degree of anoxia. Thus if a drug lengthens this time then the drug is said to be protective in anoxia (Setnikar & Ravasi, 1960; Siess, 1961). However, it is felt that recovery of the myocardium is more important, especially from a clinical viewpoint, and consequently this was the criterion used in this paper.

As might be expected, the recovery of the isolated myocardium from acute anoxia was very dependent on the length of time for which it had been exposed to anoxia. This does not appear to be a linear relationship but seems to be more related to the log of the length of exposure and, if the percentage recovery of contraction for the different periods of anoxia is plotted against the log of the time of exposure, then for each instant of the recovery phase a linear log dose/response curve can be constructed.

It is interesting to note that the recovery of the rate is much faster than that of the amplitude of contraction even after long periods of anoxia. For example, after 20 min of the recovery phase of atria after 8 hr of anoxia, the recovery of the rate was 60% compared with 12% for the contraction. Theoretically the reason for this could be either that the rate depends on a different metabolic pathway which is more resistant to anoxia or that the metabolic level necessary to maintain the impulse to contract is less than that required for the contractile process itself.

Webb (1950) investigated the action of various metabolic inhibitors and substrates on the rabbit atria. He found that changes in rate and amplitude were relatively independent of each other. Inhibitors usually depressed amplitude more than rate but fluoride, fluoroacetate and phloridzin sometimes depressed the rate more than the amplitude. He felt that this showed that the usual resistance of the rate to inhibitors was not solely because the rate-maintaining mechanism needed less energy.

Gardner & Farah (1954) subjected rabbit isolated atria to anoxia and found measurable electrical activity for up to 30 to 40 min of anoxia. They also found that the contractile mechanism was more sensitive than was rate of beating to substrate depletion and to metabolic inhibitors such as dinitrophenol. This has been found in the whole animal by other workers: Coffman, Lewis & Gregg (1960) found that, in dogs subjected to anoxia, atrioventricular conduction was maintained even after severe and obvious damage to the myocardium.

The effects of noradrenaline and adrenaline

The relative stimulant effect of noradrenaline and adrenaline on isolated atria is very different in various species, so much so that Garb, Penna & Ganz (1956) have suggested

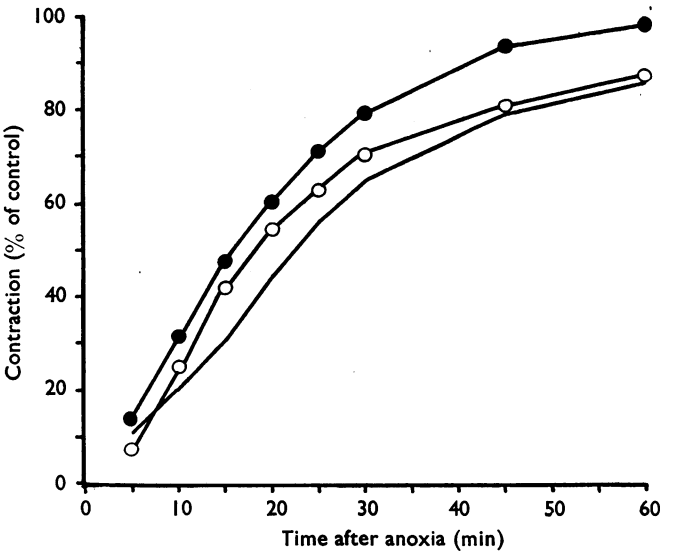

Fig. 7. Recovery of rabbit isolated atria (means of six experiments) from 1 hr of anoxia at 30° C, compared with atria also under the influence of dipyridamole (0.1 or 1 μg/ml.). Recovery is plotted as percentages of the original amplitude of contraction at 5-min intervals over 1 hr from the end of the anoxic phase. ——, Control atria; —○—, dipyridamole (0.1 μg/ml.); —●—, dipyridamole (1 μg/ml.).

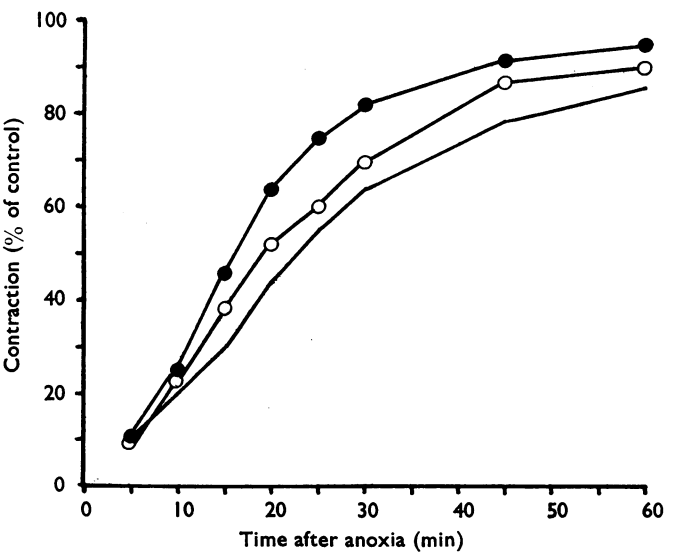

Fig. 8. Recovery of rabbit isolated atria (means of six experiments) from 1 hr of anoxia at 30° C, compared with atria also under the influence of sodium nitrite (1 or 10 μg/ml.). Recovery is plotted as percentages of the original amplitude of contraction at 5-min intervals over 1 hr from the end of the anoxic phase. ——, Control atria; —○—, sodium nitrite (1 μg/ml.); —●—, sodium nitrite (10 μg/ml.).

that this might prove to be a useful bioassay technique to distinguish between the two amines in dilute solution. Results collected by Graham (1963) suggested that, on the rabbit isolated atria, if the stimulant effect of adrenaline was taken to be 1, that of noradrenaline was 0.8. Preliminary experiments for the work described in this paper agree closely with this figure. As might be expected, therefore, there is no great difference in the power of noradrenaline or adrenaline to enhance the recovery of contraction of rabbit atria from anoxia.

Results collected by Ellis (1956) show that figures given by various workers for the relative stimulation of oxygen consumption by these amines in different species vary from noradrenaline being 0.9 of that of adrenaline to being equal. Lee (1953) recorded the oxygen consumption of cat papillary muscle and found the two drugs indistinguishable in their effects.

Yang (1963) subjected rabbit atria to 3-hr periods of anoxia. He found that there was a rapid decline of contractile force and a slower and much smaller decrease in atrial rate and, even when no movement was visible, electrical potentials could still be recorded. He felt that the rapid decline of contractile force was best explained by the reduced availability of aerobic energy and that the sino-atrial node could still generate impulses owing to its much smaller energy requirements which could be supplied anaerobically.

The effect of iproniazid

Although no longer used clinically for this purpose owing to severe toxic effects, iproniazid will relieve the pain of angina pectoris but this is thought by many to be a central nervous effect rather than a myocardial one. Previous workers have stated that iproniazid protects the heart from anoxia. Setnikar & Ravasi (1960) perfused rabbit isolated hearts with Locke solution containing 10 mg/ml. of iproniazid and found that it protected the heart to some extent from anoxia produced by perfusing the heart with anoxic Locke solution. They used the criterion of measuring the time interval from the commencement of the perfusion with the anoxic solution until the amplitude of contraction had diminished to 25% of the original. On finding that the hearts took longer to reach 25% of the original value of amplitude of contraction if iproniazid was either in the anoxic perfusing fluid or given to the animal for several days before the experiment, they said that this showed that iproniazid had some protective value. This protection was not due to depression of the heart rate or to coronary dilatation as neither was observed with the doses used. They postulated some metabolic effect as the likely cause. The mechanism of this metabolic effect, if present, is not known. Pletscher (1961) noted an increase in the blood pyruvate and lactate levels when monoamine oxidase inhibitors were given to human subjects.

Fawaz & Tutunji (1960) also found that adrenaline and noradrenaline were approximately equal in their effect on the oxygen consumption of the dog heart-lung preparation. In the work described in this paper, both these amines depressed the recovery of contraction when present in the anoxic phase. There was a small quantitative difference between the degrees of depression, that due to adrenaline being the greater. This is in keeping with the similarity of adrenaline and noradrenaline in their stimulation of oxygen consumption if it is assumed that the deleterious effects of anoxia are due solely to lack of oxygen. That this latter assumption is not necessarily true in the whole animal has been shown by Bagdonas, Stuckey, Piera, Amer & Hoffman (1961) who showed that, when canine hearts were subjected

to ischaemia and/or hypoxia *in situ*, the change in *p*H and retention of metabolites were more important than the lack of oxygen *per se*. However, at least in the isolated atria supplied solely by diffusion and where the *p*H of the medium did not change measurably over 1 hr of anoxia, it seems reasonable to assume that the deleterious effects of anoxia are due to oxygen lack.

In the work described in this paper, there was no apparent difference in the degrees of enhancement of the recovery of the amplitude of contraction given with the two doses of iproniazid used. Perhaps this enhancement of recovery could be due to the monoamine oxidase inhibiting action of iproniazid, since Axelrod, Hertting & Patrick (1961) found that amine oxidase inhibitors increase the levels of catechol amines in the tissue by " locking up " the catechol amine stores present and thus preventing them from having any effect. It has already been shown in this paper that, when adrenaline or noradrenaline was added in the anoxic phase, the recovery of the contraction after anoxia was slower. If the endogenous catechol amines play some part in depressing the recovery of the myocardium from anoxia, their inhibition might enhance recovery. This hypothesis is supported by the work described on the recovery from anoxia of atria and hearts from reserpinized rabbits. These also show an enhanced recovery from anoxia.

The effect of reserpine

That reserpine should exert a beneficial effect in the recovery of isolated myocardium from anoxia was at first surprising. Withrington & Zaimis (1961) observed that there was a striking similarity between the heart " poisoned " with reserpine and that in conditions in which the oxygen supply or the utilization of oxygen by the myocardium was substantially decreased. They thought that this might either be a defect in the myocardial metabolism or an abnormality of the contractile protein.

It is interesting to try to correlate the loss of catechol amines induced by reserpine, their postulated non-availability after monoamine oxidase inhibitors such as iproniazid (Axelrod *et al.*, 1961) and the degree of enhancement of the recovery of contraction after anoxia when either of these drugs had been given.

That the sympathomimetic amines should have an adverse effect on the anoxic myocardium is not surprising; sympathetic stimulation has been thought by several workers to play a key part in the onset of angina pectoris (Raab & Lepeschkin, 1950; Darby & Addinger, 1960).

The effect of sodium nitrite

It appears generally accepted that the basic action of the nitrites is relaxation of smooth muscle, especially that of the arterioles, capillaries and venules. The major effect is on the postarteriolar bed (Goodman & Gilman, 1955).

The use of nitrites and organic nitrates in angina pectoris has been of undisputed value. To quote Charlier (1961), ". . . nitroglycerin is probably at the moment the only coronary vasodilator substance to collect unanimously favourable reports from therapeutic trials . . ." It is also widely accepted that anginal pain arises from an imbalance in the heart between the supply of oxygen and the demand. This does not necessarily infer a reduction in coronary blood flow but an insufficiency of flow in relation to metabolic requirements.

However, agreement is far from being reached on the mode of action of nitroglycerin and the significance of experimental findings. That nitrites cause an increase in coronary blood flow in normal healthy subjects seems to be generally accepted, but Gorlin, Brachfeld, MacLeod & Bopp (1959) found that in patients with atherosclerotic coronaries there was no change or even a reduction in the coronary flow after nitrites had been taken and relief from pain achieved. Their work has been criticized owing to the nitrous oxide method used to measure the coronary flow (Charlier, 1961), but it does give rise to speculation that factors other than pure coronary vasodilatation are involved in the relief of angina by nitroglycerin. Honig, Tenney & Gabel (1960) observed almost complete inhibition of phosphorylation at high concentrations of nitroglycerin and slight but definite inhibition at " therapeutic concentrations."

These findings might revive the older theory of Raab & Humphreys (1947) that nitroglycerin interferes with the " oxygen-consuming, anoxia-producing effects of epinephrine on the contractile substance of the heart." Although this view was later criticized by Eckstein, Newberry, McEachen & Smith (1951) and Popovich, Roberts, Crislip & Menges (1956), who were unable to confirm Raab & Humphrey's findings, recent work on the mode of action of adrenaline is interesting. Belford & Feinleib (1962) and Hess, Shanfeld & Haugaard (1962) found that inotropic catechol amines increase phosphorylase activity and the order of potency in increasing this activity closely parallels their inotropic activity, adrenaline equalling noradrenaline. If, as Honig *et al.* (1960) say, nitroglycerin inhibits phosphorylase activity, this agrees with Raab & Humphrey's (1947) findings of an anti-adrenergic action.

In the work described in this paper, the enhancement by sodium nitrite of the recovery of contraction from anoxia was certainly not due to any haemodynamic change and must have been metabolic in nature, since the preparation was isolated and not perfused through its blood vessels. Taken in conjunction with the findings with iproniazid and reserpine described above, there certainly appears to be a reason for assuming a metabolic action as being part of the effectiveness of the nitrites in angina pectoris.

The effect of dipyridamole

Dipyridamole was introduced by Kadatz in 1959 as a coronary vasodilator. Its action of coronary vasodilatation has been confirmed recently in the human by Kinsella, Troup & McGregor (1962), and by West, Bellet, Manzoli & Müller (1962) in the dog, where it was said to give a marked increase in the coronary blood flow (159%) with no increase in contractility or work of the heart. Some workers have suggested that, apart from this recognized action of increasing the coronary blood flow, dipyridamole has a direct effect on myocardial metabolism. Siess (1961) ascribed to dipyridamole the role of a hydrogen acceptor. He reported that it hastened the recovery of guinea-pig atria after subjection to periods of overloading in the presence of ouabain. Recovery was accompanied by the restitution of high-energy phosphates. Laudahn (1961) examined the effect of dipyridamole on isolated myocardial mitochondria that had been damaged by lyophilization to simulate the damage caused by hypoxia. Owing to this damage, oxidative phosphorylation was decreased but on suspension in dipyridamole the efficiency of coupling was increased. These findings suggest that dipyridamole may act metabolically at cellular level allowing

increased oxidative phosphorylation, that is it may protect the tissues temporarily from the consequences of oxygen deficiency.

SUMMARY

1. The recovery of contraction of rabbit isolated atria and hearts was recorded after fixed periods of anoxia.

2. Adrenaline or noradrenaline during the anoxic phase decreased the recovery of contraction but, if present after the anoxic phase, enhanced the recovery.

3. Prior reserpinization or the presence of iproniazid enhanced the recovery from anoxia. This might possibly be caused by the abolition of the anoxia-producing effects of endogenous catechol amines.

4. Both dipyridamole and sodium nitrite enhanced the recovery from anoxia and this supports the claims of previous workers that these compounds have a beneficial effect in coronary insufficiency apart from any vasodilatation that may occur.

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