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THE EFFECTS OF AMYL NITRITE INHALATION ON MYOCARDIAL BLOOD FLOW AND METABOLIC HEAT PRODUCTION

BY

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It is a hundred years since T. Lauder Brunton introduced the nitrites for the treatment of angina pectoris (Brunton, 1867). Although there is no doubt of their value and effectiveness in this condition, the means by which this effectiveness is achieved is still in considerable doubt. Since anginal pain arises from an imbalance in the heart between oxygen supply and demand, it follows that the nitrites could act in at least one of three main ways: by improving coronary blood flow; by an effect on the pathways by which pain from the myocardium is transmitted or appreciated; and by a reduction in the oxygen requirements of the myocardium or an improvement in the efficiency of oxygen utilization. Recently attention has been turned to possible metabolic effects of the nitrites. Thus in a recent text on clinical pharmacology it is stated that " the possibility that nitrites and nitrates have a direct useful effect on myocardial metabolism has not been excluded " (Laurence, 1966). Inorganic and organic nitrates and nitrites inhibit phosphorylase activity (Hunter, Kahana & Ford, 1953; Honig, Tenney & Gabel, 1960) and, in an interesting recent paper, Penn (1965) describes the enhancing effects of sodium nitrite on the recovery after anoxia of contraction of isolated rabbit hearts and atria. The present paper describes the effects of amyl nitrite on myocardial metabolic heat production in dogs and rabbits. A preliminary account of these results has been communicated to the Physiological Society (Grayson, Irvine, Mendel & Parratt, 1965).

METHODS

Rabbits were anaesthetized with urethane (1.5 g/kg, intravenously) and dogs with pentobarbitone sodium (45 mg/kg, intraperitoneally). Blood flow in the left myocardium was determined by the method of Grayson & Mendel (1961) in which thermal conductivity is measured using heated thermocouples implanted in the muscle mass. The implications of the method have recently been subjected to detailed analysis (Grayson & Parratt, 1966) and it has been demonstrated that conductivity increment—that is, the difference in apparent thermal conductivity between tissue with blood flowing and tissue with no blood flowing—is a linear function of local flow in the vicinity of the heated thermocouple. Details of the operative procedure and analysis of the results have been fully described elsewhere (Parratt, 1964; Grayson & Parratt, 1966).

In the present work the records were also analysed for the determination of " corrected temperature." This is the temperature difference between the aorta and the myocardium to which a

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correction has been continuously applied for changes in blood flow during an experiment. Changes in blood temperature are automatically compensated by the position of the reference junction in the aorta. The experiments were carried out under conditions of controlled ventilation in a constant temperature room and heat losses may be assumed to be constant. Since blood flow fluctuations are compensated in the calculation, corrected temperature may be used as a measure of local heat production in the myocardium (Dosekun, Grayson & Mendel, 1960).

Further experiments were also carried out in which simple measurements of temperature were made in the aorta, myocardium and rectum using unheated copper-constantan thermocouples and an Electric Universal Thermometer (Elektrolaboratoriet, Copenhagen) (Parratt & Grayson, 1963).

Systemic arterial blood pressure was recorded in both species from a polyethylene cannula in the right femoral artery using a standard mercury manometer (for dogs) or a Condon manometer (for rabbits). In a few experiments blood pressure was also recorded using a Shillingford-Muller transducer with one of the two oscillator channels of the Cambridge recording camera. In these experiments mean blood pressure was estimated as diastolic plus one-third of the pulse pressure.

Amyl nitrite (3–5 minims) was administered through a side arm connected to the tracheal cannula. The duration of inhalation varied from 30 sec to 10 min but was usually 5 min. In all experiments inhalations were repeated from two to five times at half-hourly intervals. In some experiments on dogs the effect of amyl nitrite inhalation was also studied after blockade of sympathetic β-receptors with propranolol (0.25 mg/kg, intravenously).

RESULTS

Effect of amyl nitrite inhalation on myocardial blood flow

Rabbits. The results from the seven experiments that were performed are summarized in Table 1. The effect on mean systemic blood pressure was very marked, for it was found difficult in this species to control the dose of amyl nitrite inhaled. In nearly all the experiments myocardial blood flow also fell, though not as much as would be expected from the extent of the depression of the blood pressure. This means that there was a reduction in calculated myocardial vascular resistance (mean of 46%).

Dogs. Table 2 shows the results of the effects of amyl nitrite inhalation in 14 dogs. In every experiment mean systemic blood pressure fell (from the pre-inhalation level of 119 ± 6 mm Hg, mean and standard error) and myocardial blood flow usually increased (from the control conductivity increment of $4.70 \pm 0.62 \times 10^{-4}$ c.g.s. units). This indicates a marked reduction in myocardial vascular resistance. This reduction was maintained throughout the period of inhalation (Fig. 1). These effects were accompanied by reflex

TABLE 1

THE EFFECT OF AMYL NITRITE INHALATION ON MEAN SYSTEMIC BLOOD PRESSURE AND ON MYOCARDIAL BLOOD FLOW, VASCULAR RESISTANCE AND METABOLIC HEAT PRODUCTION IN RABBITS

The values are the means from three experiments in each animal. Percentages are in terms of pre-inhalation values.

	Maximal effect during inhalation			
Animal No.	Blood pressure (%)	Myocardial blood flow (%)	Vascular resistance (%)	Corrected temperature (°C)
1	−70	−23	−45	−0·24
2	−67	Nil	−66	−0·14
3	−50	Nil	−52	−0·12
4	−56	−16	−48	−0·03
5	−62	−35	−31	−0·24
6	−60	−23	−48	−0·17
7	−50	−19	−34	−0·12
Mean	−59	−17	−46	−0·15

TABLE 2

THE EFFECT OF AMYL NITRITE INHALATION ON MEAN SYSTEMIC BLOOD PRESSURE AND ON MYOCARDIAL BLOOD FLOW, VASCULAR RESISTANCE AND METABOLIC HEAT PRODUCTION IN DOGS

The values are the means from three experiments in each animal. Percentages are in terms of pre-inhalation values.

Animal No.	Maximal effect during inhalation: Blood pressure (%)	Myocardial blood flow (%)	Vascular resistance (%)	Corrected temperature (°C)
1	−39	−43	Nil	−0·43
2	−33	+27	−41	—
3	−65	+6	−62	−0·27
4	−21	+144	−58	−0·23
5	−12	+26	−22	−0·14
6	−32	Nil	−29	−0·22
7	−15	+40	−39	−0·04
8	−21	+12	−29	−0·18
9	−34	+83	−64	−0·28
10	−20	+36	−42	−0·25
11	−46	Nil	−46	−0·14
12	−27	+63	−56	−0·18
13	−10	+52	−42	−0·16
14	−32	+24	−46	—
Mean	−29	+34	−41	−0·21

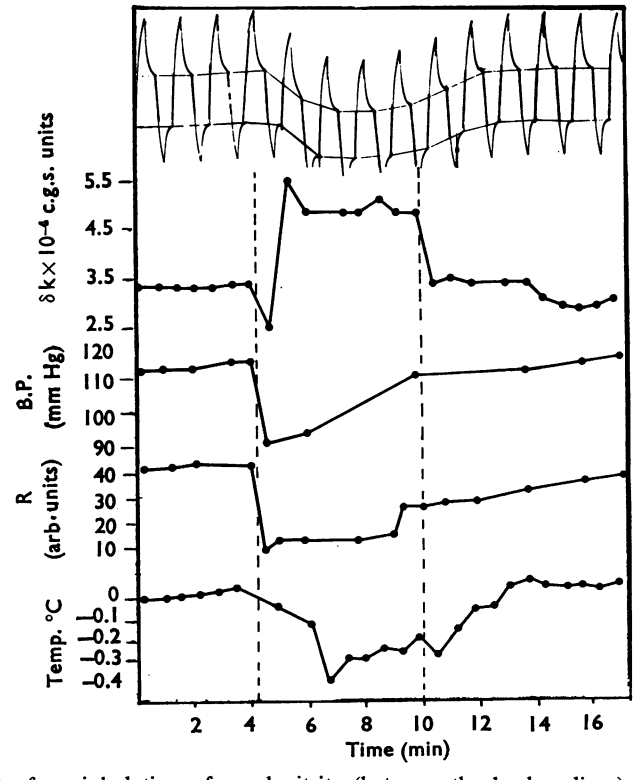

Fig. 1. The effect of an inhalation of amyl nitrite (between the broken lines) on the temperature record obtained from a heated thermocouple implanted in the myocardium of the left ventricle of a dog. This record is used to calculate myocardial blood flow and heat production. From the top, temperature record, blood flow (as conductivity increment), mean systemic blood pressure, myocardial vascular resistance and metabolic heat production (as " corrected temperature ").

increases in heart rate of from 6 to 26 beats/min from the initial level of 149 ± 3 beats/min. No clear evidence was obtained of the occurrence of tachyphylaxis after successive inhalations in the same animal. This is in contrast to what was often observed in the studies on metabolic heat production.

In order to investigate the possible contribution of the sympathetic vasodilator nerves to the decrease in myocardial vascular resistance which followed amyl nitrite inhalation, a further series of experiments (7 dogs) was performed in which amyl nitrite was administered before and after blockade of sympathetic β-receptors. No difference in the cardiovascular effects of amyl nitrite could be detected except that the reflex rise in heart rate was abolished or markedly reduced.

Effect of amyl nitrite inhalation on metabolic heat production

When myocardial " corrected temperature " was calculated by the method of Dosekun *et al.* (1960), it could be clearly demonstrated that this was depressed during amyl nitrite inhalation both in dogs and in rabbits. The extent of this depression was similar in both species (Tables 1 and 2) and occurred whether myocardial blood flow increased (as in dogs) or decreased (as in rabbits) as a result of the inhalation. The validity of this

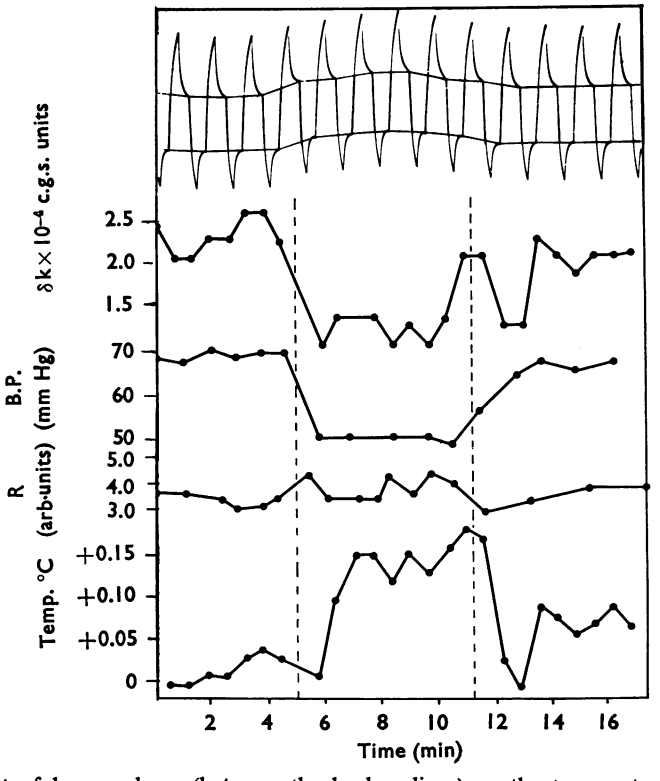

Fig. 2. The effect of haemorrhage (between the broken lines) on the temperature record obtained from a heated thermocouple implanted in the myocardium of the left ventricle of a dog. From the top, temperature record, myocardial blood flow (as conductivity increment), mean systemic blood pressure, myocardial vascular resistance and metabolic heat production (as " corrected temperature ").

measurement as an indication of myocardial metabolic heat production has been discussed fully in a recent paper (Grayson & Parratt, 1966). The calculation gives a temperature difference between the beginning of an experiment and the period of observation, corrected for blood flow change. Since the experiments were carried out in a temperature controlled room, with animals under constant positive pressure respiration, any difference in " corrected temperature " is regarded as indicating a metabolic effect.

Since it is conceivable that a lowering of blood pressure might itself decrease metabolic heat production, experiments were also performed in these dogs in which the blood pressure lowering effect of amyl nitrite was simulated by haemorrhage. In confirmation of a previous study (Grayson & Parratt, 1966) it was observed (Fig. 2) that lowering the blood pressure by haemorrhage increased " corrected temperature." The effect of amyl nitrite on metabolic heat production in the myocardium cannot therefore result from the changes induced in myocardial blood flow or in systemic blood pressure.

Effect of amyl nitrite inhalation on myocardial-aortic temperature difference in the dog

In 10 experiments the temperature of the left myocardium was compared to that of the blood in the arch of the aorta and of the rectum using direct reading thermocouples. The myocardium was always hotter than the blood flowing through it (difference $0.71 \pm 0.07°$ C, mean and standard error) but during amyl nitrite inhalation this difference was reduced by a mean of 0.19° C (Table 3). On several occasions it was noted that this effect was reduced with succeeding inhalations of amyl nitrite, a particularly clear example being shown in Fig. 3. Although a decrease in the myocardial-aortic temperature difference would result from an increase in blood flow within the myocardium, tachyphylaxis to the blood flow increasing effects of amyl nitrite inhalation were very seldom observed. This tachyphylactic effect on myocardial-aortic temperature difference with successive inhalations therefore probably indicates a definite effect of amyl nitrite on myocardial metabolic heat production.

Rectal-aortic temperature differences were also made for comparison. As shown in Table 3, there was no significant difference in temperature between these two regions and amyl nitrite had no effect on this temperature difference. This result perhaps indicates the specificity of the myocardial effects observed.

TABLE 3

EFFECT OF AMYL NITRITE INHALATION ON MYOCARDIAL-AORTIC AND RECTAL-AORTIC TEMPERATURE DIFFERENTIALS (°C) IN DOGS

	Myocardium–Aorta			Rectum–Aorta		
Animal No.	Before inhalation	During inhalation	Maximum change	Before inhalation	During inhalation	Maximum change
15	+0·31	+0·18	−0·13	+0·32	+0·28	−0·04
16	+0·70	+0·47	−0·23	+0·40	+0·24	−0·16
17	+0·40	+0·26	−0·14	−0·60	−0·60	Nil
18	+0·80	+0·40	−0·40	−0·20	−0·20	Nil
19	+0·61	+0·46	−0·15	+0·12	+0·09	−0·03
20	+1·00	+0·65	−0·35	+0·10	+0·10	Nil
21	+0·90	+0·65	−0·25	Nil	−0·05	−0·05
22	+0·97	+0·86	−0·11	Nil	Nil	Nil
23	+0·75	+0·70	−0·05	+0·30	+0·25	−0·05
24	+0·70	+0·60	−0·10	+0·10	+0·05	−0·05
Mean	+0·71	+0·52	−0·19	+0·05	+0·02	−0·04

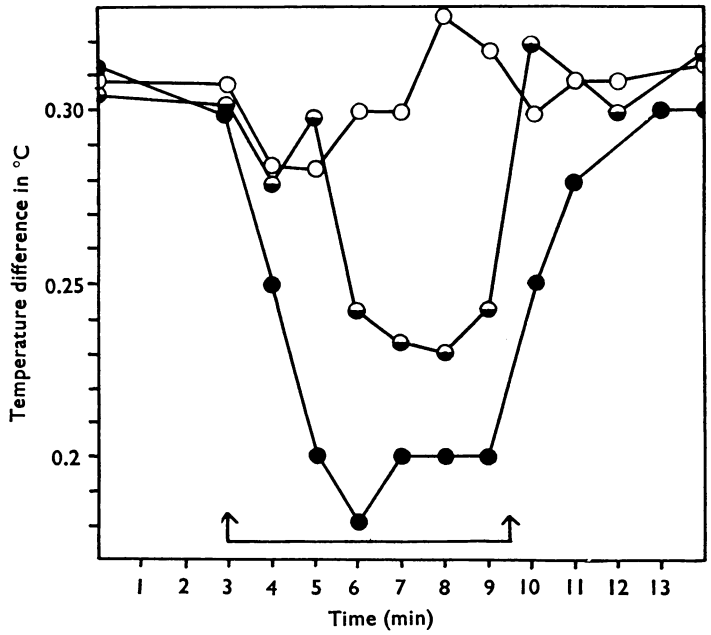

Fig. 3. The effect of three successive inhalations of the same dose of amyl nitrite on the myocardial-aortic blood temperature differential. Myocardium initially hotter than the aortic blood by 0.31° C. First inhalation ●——●, second inhalation ◒——◒, third inhalation ○——○. Note the reduced effect with successive inhalations.

DISCUSSION

Since their introduction into clinical practice the nitrites, in particular nitroglycerine, have proved to be the most consistently effective therapeutic agents in the treatment of angina pectoris. Although there can be little doubt about their clinical efficacy there is still wide disagreement about their mode of action. The classical view regards the nitrites as highly effective dilators of the coronary vessels (François-Franck, 1903), producing their effects by improving the irrigation of the myocardium. This simple view of the action of the nitrites has been challenged frequently in recent years. Gorlin and his colleagues, for example (Gorlin, Brachfeld, MacLeod & Bopp, 1959), observed a reduction in flow in patients with ischaemic heart disease though a slight increase in flow in normal patients (Brachfeld, Bozer & Gorlin, 1959). It is certainly doubtful whether the increase in coronary blood flow, which is often observed after the administration of the nitrites or nitroglycerine, alone can account for the efficacy of these substances. Increases in flow, even when they occur, are often restricted to the period immediately after administration (Rees, Redding, Ashfield, Gibson & Gavey, 1966) and in any case are not usually very marked (Ross, Ueda, Lichtlen & Rees, 1964).

There are other views regarding the possible mode of action of these drugs. One, which appears to receive little support, suggests that the syndrome of angina is the result of the accumulation of catecholamines in the myocardium and that the nitrites produce their effects through an " anti-adrenergic " action (Raab & Lepeschkin, 1950). Another view is that the nitrites cause a reduction in energy expenditure of the ventricular muscle as a

result of the decreased blood pressure and left ventricular work (Fronek & Ganz, 1960; Marchetti, Merlo & Antognetti, 1964). In the resting human subject, however, this reduction in left ventricular work is quite small (Hoeschen, Bousvaros, Klassen, Fam & McGregor, 1966).

The experiments reported here deal with flow states and heat production in the myocardium of the left ventricle. The blood flow responses to amyl nitrite inhalation were variable. In the rabbit the usual response was a reduction in flow, in the dog there was a rise in flow. This species difference is probably related to the blood pressure response. In the rabbit it was difficult to control the dose of amyl nitrite and the reduction in the blood pressure was usually greater than in the dog. In both species, however, when the vascular events were interpreted in terms of resistance, the results were similar. There was always a decrease in myocardial vascular resistance which was of the same extent in both species.

In the dog it has been shown that a decline in blood pressure is usually accompanied by a nervously mediated reflex vasodilation in the myocardium (Grayson & Parratt, 1966). It might be argued that this could be the basis of the vasodilatation observed when the blood pressure is lowered by amyl nitrite. Against this is the observation that flow was actually increased by amyl nitrite, which it never was when the blood pressure was lowered. Against it, too, is the observation that the blood flow responses were unaffected by β-adrenergic blockade with propranalol, which has been shown to block the myocardial vasodilatation which results from a reduced coronary perfusion pressure (Parratt & Grayson, 1966). In any case none of these arguments could apply to the experiments on rabbits, since, in this species, lowering the blood pressure by haemorrhage does not change resistance to flow in the myocardial vascular bed (Grayson & Parratt 1966). It must be concluded therefore that amyl nitrite has a direct vasodilator effect on the vessels of the myocardium both in the dog and in the rabbit.

Perhaps the most interesting observation arising out of the present experiments, however, is that amyl nitrite has a marked effect in reducing heat production in the myocardium both of rabbits and of dogs. This reduction in metabolic heat production is not related to the decrease in blood pressure, since it has been clearly shown that lowering the blood pressure alone has the opposite effect (Grayson & Parratt, 1966, and confirmed in the present studies). Neither is it related to a decrease in coronary vascular resistance. This is clear from Tables 1 and 2 where it can be seen that there is no obvious correlation between these two phenomena. Actual changes in myocardial blood flow cannot be responsible since, in the first place place, heat production effects in rabbits and dogs were the same, yet the blood flow effects were different. Moreover, in previous work (Parratt, 1964) it has been shown that bradykinin increases myocardial blood flow and, at the same time, increases metabolic heat production. Flow and heat production as measured in this work are thus not directly linked phenomena.

A number of workers have suggested that nitroglycerine may exert its effect through an increased efficiency of oxygen utilization by the heart. Thus Klensch & Južnič (1964) found that the proportion of the oxygen consumption dissipated as heat was reduced by up to 17.4% by the administration of nitroglycerine. Coronary perfusion was reduced in their experiments by only 3.5% and they concluded that the economy of oxygen consumption always outweighs the reduced coronary blood supply. Similar conclusions

had previously been reached by Fronek & Ganz (1960) and by Honig *et al.* (1960). The latter authors expressed the view that this beneficent effect of nitroglycerine is connected with its ability to depress ATPase activity (see Krantz, Carr & Bryant, 1951).

We have recently made the point (Grayson & Parratt, 1966) that the free energy of metabolism is utilized in two ways, one in the performance of work, the other in the production of heat; in a sense the latter may be regarded as an inverse function of cardiac efficiency. When external cardiac work is reduced, and if metabolism is proceeding, more of the available free energy must be liberated as heat. Heat production in the myocardium can thus be regarded as a measure of waste energy and a decrease in heat production as evidence of more efficient utilization of metabolic free energy. When amyl nitrite is administered heat production falls and this, in the absence of drastic changes in oxygen consumption, can only mean an increase in efficiency of utilization of metabolic free energy. It is tempting to conclude that this, as much as the vasodilator action of the nitrites, is a factor in the clinical efficacy of these preparations.

SUMMARY

1. The inhalation of amyl nitrite in closed chested anaesthetized dogs usually resulted in an increase in left myocardial blood flow, as measured using a heated thermocouple method. In anaesthetized rabbits amyl nitrite, in the doses used, decreased myocardial blood flow, but in both species when myocardial vascular resistance was calculated there was a reduction throughout the period of inhalation. The cardiovascular effects in dogs were unchanged by β-adrenergic blockade with propranolol.

2. The temperature differential between the aortic blood and the left myocardium, to which a correction factor was continuously applied for changes in blood flow, was markedly reduced during the period of amyl nitrite inhalation. This reduction in "corrected temperature" was not related to either a change in blood pressure or of myocardial vascular resistance.

3. Similar reductions in the aortic-myocardial temperature differential were observed during amyl nitrite inhalation using direct recording unheated thermocouples.

4. It is concluded that amyl nitrite reduces myocardial metabolic heat production with a resultant increase in the efficiency of utilization of metabolic free energy. It is suggested that this is a factor in the clinical efficacy of the nitrites in angina pectoris.

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REFERENCES

BRACHFELD, N., BOZER, J. & GORLIN, R. (1959). Action of nitroglycerin on the coronary circulation in normal and in mild cardiac subjects. *Circulation*, **19**, 697–704.

BRUNTON, T. L. (1867). On the use of nitrite of amyl in angina pectoris. *Lancet*, **2**, 97–98.

DOSEKUN, F. O., GRAYSON, J. & MENDEL, D. (1960). The measurement of metabolic and vascular responses in liver and muscle with observations on their responses to insulin and glucose. *J. Physiol.*, **150**, 581–606.

FRANÇOIS-FRANCK, C. A. (1903). Effet vasodilateur du nitrite d'amyle sur les vaisseaux de l'écorce cérébrale et sur les vaisseaux du myocarde. *C.R. Soc. Biol.*, **55**, 1448–1450.

FRONEK, A. & GANZ, V. (1960). The effect of nitroglycerine on the coronary and systemic haemodynamics. Proceedings of the IIIrd European Congress of Cardiology, Rome, pp. 1069–1071. Pars Altera B.

GORLIN, R., BRACHFELD, N., MACLEOD, C. & BOPP, P. (1959). Effect of nitroglycerin on the coronary circulation in patients with coronary artery disease, or increased left ventricular work. *Circulation*, **19**, 705–718.

GRAYSON, J., IRVINE, M., MENDEL, D. & PARRATT, J. R. (1965). A comparison of the effects of haemorrhage and amyl nitrite inhalation on myocardial metabolic heat production. *J. Physiol.*, **181**, 68–69P.

GRAYSON, J. & MENDEL, D. (1961). Myocardial blood flow in the rabbit. *Am. J. Physiol.*, **200**, 968–974.

GRAYSON, J. & PARRATT, J. R. (1966). A species comparison of the effects of changing perfusion pressure on blood flow and metabolic heat production in the myocardium. *J. Physiol.*, **187**, 465–488.

HOESCHEN, R. J., BOUSVAROS, G. A., KLASSEN, G. A., FAM, W. M. & MCGREGOR, M. (1966). Haemodynamic effects of angina pectoris, and of nitroglycerin in normal and anginal subjects. *Br. Heart J.*, **28**, 221–230.

HONIG, C. R., TENNEY, S. M. & GABEL, P. V. (1960). The mechanism of cardiovascular action of nitroglycerine. *Am. J. Med.*, **29**, 910–923.

HUNTER, F. E., KAHANA, S. & FORD, L. (1953). Effect of inorganic and organic nitrites and nitrates on aerobic phosphorylation in liver mitochondria. *Fed. Proc.*, **12**, 221–222.

KLENSCH, H. & JUŽNIČ, G. (1964). Ausmass und Relation von Blutdrucksenkung, peripherhämodynamischer Entlastung des Herzens und Verminderung der koronaren Perfusion nach therapeutischen Gaben von Nitroglycerin. *Arzneim. Forsch.*, **14**, 982–984.

KRANTZ, J. C., CARR, C. J. & BRYANT, H. H. (1951). Alkyl nitrites XIV. The effect of nitrites and nitrates on arterial adenosine triphosphatase. *J. Pharmac. exp. Ther.*, **102**, 16–21.

LAURENCE, D. R. (1966). *Clinical Pharmacology*, 3rd edn., p. 386. Churchill, London.

MARCHETTI, G. V., MERLO, L. & ANTOGNETTI, R. M. (1964). The effects of nitroglycerin on the coronary blood flow and oxygen consumption of the myocardium in anesthetized dogs. *Am. J. Cardiol.*, **13**, 51–57.

PARRATT, J. R. (1964). A comparison of the effects of the plasma kinins, bradykinin and kallidin, on myocardial blood flow and metabolism. *Br. J. Pharmac. Chemother.*, **22**, 34–46.

PARRATT, J. R. & GRAYSON, J. (1963). The effect of bradykinin on blood flow and heat production in the myocardium. *Experientia*, **19**, 161–162.

PARRATT, J. R. & GRAYSON, J. (1966). Myocardial vascular reactivity after beta-adrenergic blockade. *Lancet*, **1**, 338–340.

PENN, R. G. (1965). Some factors influencing the recovery of isolated myocardium from acute anoxia. *Br. J. Pharmac. Chemother.*, **24**, 253–265.

RAAB, W. & LEPESCHKIN, E. (1950). Anti-adrenergic effects of nitroglycerin on the heart. *Circulation*, **1**, 733–740.

REES, J. R., REDDING, V. J., ASHFIELD, R., GIBSON, D. & GAVEY, C. J. (1966). Myocardial blood flow measurement with ^{133}Xenon. Effect of glyceryl trinitrate in dogs. *Br. Heart J.*, **28**, 374–381.

ROSS, R. S., UEDA, K., LICHTLEN, P. R. & REES, J. R. (1964). Measurement of myocardial blood flow in animals and man by selective injection of radio-active inert gas into the coronary arteries. *Circulation Res.*, **15**, 28–41.