Br. J. exp. Path. (1980) **61**, 242

PROTECTION AGAINST ADRENOCHROME-INDUCED MYOCARDIAL DAMAGE BY VARIOUS PHARMACOLOGICAL INTERVENTIONS

J. C. YATES, G. M. L. TAAM, P. K. SINGAL, R. E. BEAMISH AND N. S. DHALLA

From the Division of Experimental Cardiology, Department of Physiology and Medicine, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3

Received for publication November 20, 1979

Summary.—Perfusion of the isolated rat heart with Krebs–Henseleit solution containing adrenochrome (25 or 50 mg/l), an oxidation product of catecholamines, resulted in contractile failure and myocardial necrosis. Various pharmacological agents known to protect the myocardium against catecholamine-induced necrosis were also found to be effective against adrenochrome-induced changes in the ultrastructure of the isolated perfused rat heart. The α-receptor-blocking drugs tolazoline and Dibenamine (dibenzylchlorethamine), and the adrenergic neurone-blocking agents guanethidine and bretylium did not alter the development of contractile failure and necrosis due to adrenochrome. The β-receptor-blocking compounds propranolol and practolol effectively protected the heart from adrenochrome-induced necrotic damage, and partially prevented contractile failure. The hydrazine-type monoamine oxidase inhibitor iproniazid completely prevented ultrastructural damage and partially maintained contractile-force development in adrenochrome perfused hearts. The non-hydrazine-type monoamine oxidase inhibitor tranylcypromine partially protected the isolated rat heart against adrenochrome necrosis, but disruption of mitochondrial structure was still seen. Tranylcypromine did not significantly improve contractile force development during adrenochrome perfusion. The calcium antagonist D-600 reduced the severity of adrenochrome-induced ultrastructural damage. These results provide strong support for the view that catecholamine-induced cardiotoxicity is mediated through the formation of adrenochrome.

A VARIETY of pharmacological agents are known to alter the extent and severity of myocardial lesions induced by the administration of catecholamines in experimental animals. The β-adrenergic-receptor-blocking compounds propranolol, pronethalol and dichloroisoprenaline reduce the incidence and severity of lesions due to isoprenaline (Lehr, Krukowski and Colon, 1966; Mehes, Rajkovits and Papp, 1966; Dorigotti *et al.*, 1969; Kahn, Rona and Chappel, 1969; Lehr, 1969; Bloom and Davis, 1974). The α-adrenergic-blocking drugs such as azapetine, phentolamine, dibenamine, dihydroergocryptine, phenoxybenzamine and tolazoline are ineffective against isoprenaline (Mehes *et al.*, 1966; Mehes, Papp and Rajkovits, 1967; Wenzel and Lyon, 1967; Dorigotti *et al.*, 1968; Zbinden and Moe, 1969), but reduce somewhat the incidence and severity of lesions caused by α-receptor agonists such as phenylephrine (Mehes *et al.*, 1967; Lehr, 1969), adrenaline (Waters and deSuto-Nagy, 1950; Mehes *et al.*, 1967; Wenzel and Lyon, 1967; Lehr, 1969), and noradrenaline (Mehes *et al.*, 1967; Wenzel and Lyon, 1967). The postganglionic neurone-blocking agent guanethidine has been reported to be ineffective against (Mehes *et al.*, 1967; Zbinden and Moe, 1969) or even to increase the severity of (Leszkov-

Address for reprints: Dr N. S. Dhalla, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3

sky and Gal, 1967) necrosis caused by isoprenaline injection. Monoamine oxidase inhibitors of the hydrazine type have been found to decrease the extent and severity of myocardial lesion following administration of catecholamines (Zbinden, 1960, 1961; Rona, Chappel and Kahn, 1963; Zbinden and Bagdon, 1963; Muller, 1966; Stanton and Schwartz, 1967; Kahn *et al.*, 1969; Zbinden and Moe, 1969), whereas the non-hydrazine-type monoamine oxidase inhibitors have been reported to be ineffective (Zbinden, 1960, 1961; Zbinden and Moe, 1969). The calcium antagonists verapamil, D-600, prenylamine, and Vascoril (cinepazet maleate) have also been found to reduce the severity of isoprenaline-induced lesions (Fleckenstein, 1971; Fleckstenstein *et al.*, 1974; Sigel, Janke and Fleckenstein, 1975).

Earlier studies on the effects of catecholamines have shown that perfusion of the isolated rat heart with spontaneously oxidized isoprenaline resulted in extensive ultrastructural damage, impairment of contractile function and subcellular changes in the myocardium, and these changes have been shown to be concentration-dependent (Yates and Dhalla, 1975; Dhalla *et al.*, 1978). Since necrotic or contractility changes could not be demonstrated when the hearts were perfused with fresh isoprenaline, it was suggested that the cardiotoxic effects of catecholamines may be at least partly due to the oxidation products of catecholamines formed in the blood or tissues (Yates and Dhalla, 1975; Dhalla *et al.*, 1978). Furthermore, adrenochrome, an oxidation product of catecholamines, has been shown to depress the contractile activity in the isolated perfused rat hearts, and the sarcolemmal membrane in these drug-perfused hearts was also found to be affected (Takeo *et al.*, 1979). It was therefore decided to study the effects of adrenochrome on the ultrastructure of the isolated perfused rat heart. Furthermore, it was thought to be of considerable interest to study the effects of adrenochrome in the presence of various pharmacological agents known to influence the catecholamine-induced myocardial necrosis *in vivo*. For this purpose, the drugs employed in the present study included propranolol and practolol (β-adrenergic blockers), tolazoline and Dibenamine (α-adrenergic blockers), guanethidine and bretylium (neurone blockers), iproniazid (hydrazine-type monoamine oxidase inhibitor), tranylcypromine (non-hydrazine-type monoamine oxidase inhibitor), and D-600 (calcium antagonist). In order to have some quantitative information about the effects of these pharmacological agents on the adrenochrome-induced changes in the myocardium, developed tension in the isolated hearts was also recorded.

METHODS

Male Sprague–Dawley rats, body wt 300–350 g, were decapitated and the heart quickly removed and arranged for perfusion by the conventional Langendorff technique. The perfusion medium was Krebs–Henseleit solution containing NaCl, 120mM; $NaHCO_3$, 25mM; KCl, 4·8mM; KH_2PO_4, 1·2mM; $MgSO_4$, 1·2mM; $CaCl_2$, 1·25mM; and glucose, 8mM. The perfusion solution, pH 7·4, was continually oxygenated with a mixture of 95% O_2 and 5% CO_2, and the perfusion temperature was maintained at 38°. The perfusion rate (7·8 ml/min) was controlled by using a Harvard peristaltic pump in all experiments.

Contractile force development and resting tension were monitored throughout each experiment by means of a steel hook in the apex of the heart connected *via* a short length of silk thread to a Grass force displacement transducer. The hearts were electrically paced at the rate of 360 beats/min by applying pulses of 1–3 volts and 2msec duration using a square wave stimulator. One of the stimulating electrodes was located in the apex of the heart and the other was in the intraventricular septum at the base of the heart. The atria were removed and the atrioventricular node crushed to facilitate external control of the heart rate.

The pharmacological agents which were utilized in this study were the hydrochloride salts and were dissolved directly in the perfusion medium. These were iproniazid (25 mg/l), tranylcypromine (25 mg/l), propranolol (1 mg/l), practolol (1 mg/l), dibenamine (25 mg/l), tolazoline (25 mg/l), D-600 (0·5 mg/l), guanethidine (2 mg/l) and bretylium (2 mg/l). The hearts were permitted a 20-min period for equilibration after the perfusion was started and

these substances were present from the beginning of the perfusion in order that the hearts would be equilibrated to these factors before adrenochrome was given. Small volumes of perfusion medium previously oxygenated with 95% O_2–5% CO_2 were used to prepare solutions of adrenochrome (25 or 50 mg/l) immediately before use.

At the end of each experiment, the hearts were abruptly switched to perfusion with a solution of 2% glutaraldehyde in 0·1M phosphate buffer, pH 7·4, for 5 min as a preliminary fixation for electron microscopic studies. Small portions of the left ventricle of each heart were subsequently dissected out, and left in the same fixative solution for 1 hr. The tissue pieces were washed for 4–6 h in 0·1M phosphate buffer, and post-fixed for 1 h in a solution of 1% OsO_4 in 0·1M phosphate buffer. These specimens were then dehydrated in a graded ethanol series and embedded in Epon. Sections were cut using diamond or glass knives on a Sorvall MT-2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined using a Zeiss EM9S electron microscope. At least 4 different hearts were used for each observation. The results were analysed by Student's *t* test.

RESULTS

The ultrastructural details of the rat heart fixed after 30 min perfusion with Krebs–Henseleit medium were quite similar to those reported earlier (Muir, 1967; Singal, Matsukubo and Dhalla, 1979). Electron microscopic examination of thin sections from the left ventricle of isolated rat hearts perfused for 30 min with 25 or 50 mg/l of adrenochrome revealed ultrastructural changes in the myocardium (Fig. 1). These changes included mitochondrial swelling and vesiculization of the cristae. Disruption of the contractile elements due to the loss of myofilaments, occurrence of contracture band and separation of the myofibrils were also evident. The damage to the myocardium was also accompanied by intracellular oedema indicated by the presence of empty spaces. Since these structural changes were more pronounced and widespread in hearts per-

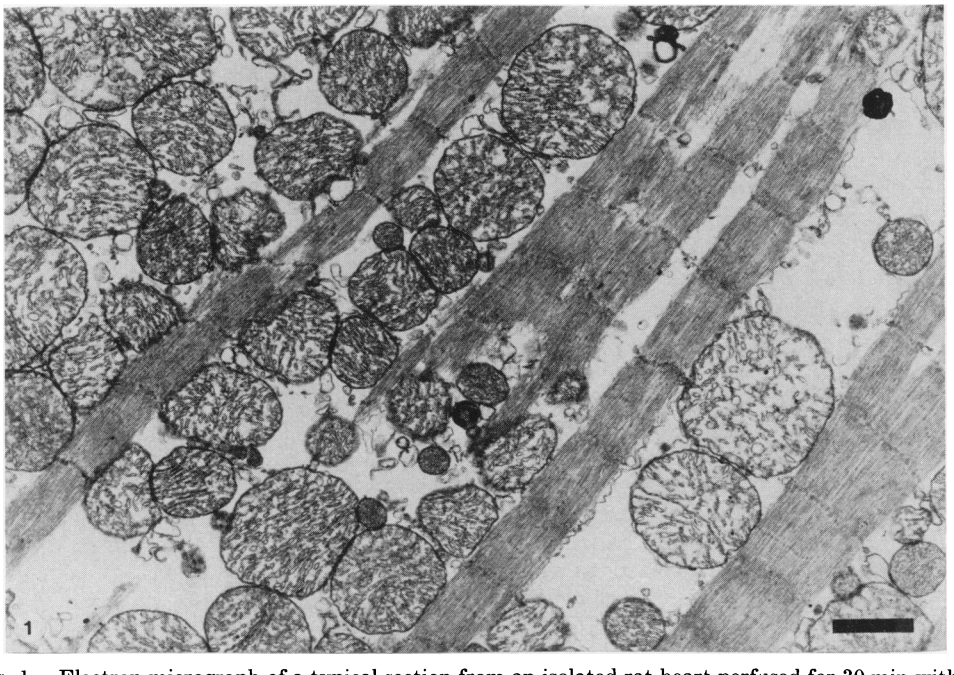

FIG. 1.—Electron micrograph of a typical section from an isolated rat heart perfused for 30 min with adrenochrome (50 mg/l). This and the following micrographs are representative of sections examined from at least 4 hearts, each perfused under the conditions described. Black bar in all micrographs indicates 1 μm.

fused for 30 min with 50 mg/l of adrenochrome, in the subsequent ultrastructural studies employing different pharmacological interventions this dosage and perfusion time with adrenochrome was adopted.

A small transient increase in contractile force, although not statistically significant, was consistently noted during the first few min of perfusion with 25 mg of adrenochrome (Fig. 2). This was followed by a gradual decline in the contractile force for up to 30 min, at which time the active tension was reduced to about 5% of the control value. Although perfusion of hearts with 50 mg/l of adrenochrome had more or less similar effect on the contractile force (Figure not shown), some differences between the effects of 25 and 50 mg/l of adrenochrome are noteworthy. The initial transient positive effect of perfusion with 25 mg/l of adrenochrome was absent in the heart perfused with 50 mg/l of adrenochrome. Furthermore, the drop in the contractile force due to perfusion with 50 mg/l of adrenochrome was quite rapid and a complete loss of contractile activity was observed within 25–30 min. Since the contractile failure produced by the higher concentration of adrenochrome was too rapid for the potentially important modifications to be readily evident, the subsequent data concerning the influence of pharmacological interventions on the contractile failure due to adrenchrome have been derived from experiments in which a concentration of 25 mg/l of adrenochrome was used.

Addition of tolazoline (25 mg/l) or dibenamine (25 mg/l), which act as α-adrenergic-receptor-blocking agents, to the perfusion medium in the absence of adrenochrome did not produce any detectable effect on the contractile activity of the hearts. Furthermore, addition of these drugs in combination with adrenochrome (25 mg/l) to the perfusion medium did not modify the pattern of contractile failure due to adrenochrome alone. The presence of tolazoline (Fig. 3A) or Dibenamine (Fig. 3B) in the perfusion was also ineffective in protecting isolated rat hearts from necrotic changes due to 30 min of perfusion with 50 mg/l of adrenochrome.

The presence of 1 mg/l of practolol, a β-adrenergic-receptor-blocking agent, in the medium was found to augment the contractile force of isolated rat hearts on starting perfusion with 25 mg/l of adrenochrome, and force of contraction remained significantly ($P < 0{\cdot}05$) above that of hearts perfused with 25 mg/l of adrenochrome alone throughout the 30-min perfusion period (Fig. 4). Another β-receptor-blocking agent, propranolol (1 mg/l), did not significantly ($P > 0{\cdot}05$) alter the course of failure of contractile force during the first 10–15 min of perfusion with 25 mg/l of adrenochrome, but significantly ($P < 0{\cdot}05$) reduced the rate of decline of contractile force during the remainder of the perfusion period (Fig. 4). These β-blocking agents did not show any significant effect on the contractile force

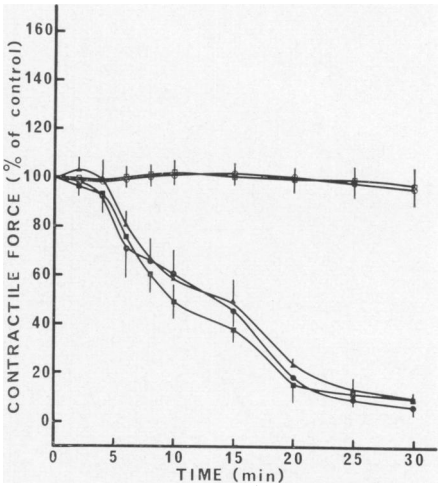

FIG. 2.—Effect of tolazoline (25 mg/l) and dibenamine (25 mg/l) on the time course of failure of contractile force development of the isolated rat hearts perfused with adrenochrome (25 mg/l). Tolazoline □—□, tolazoline plus adrenochrome ■—■, dibenamine ○—○, dibenamine plus adrenochrome ●—●, adrenochrome ▲—▲. Each point is the mean ± s.e. of 4 experiments. Mean initial contractile force values in grams were: tolazoline, 8·8 ± 0·5; dibenamine, 8·5 ± 0·1; and control, 9·6 ± 0·9 at a resting tension of 2·5 g.

FIG. 3A.—Electron micrograph of a typical section from an isolated rat heart perfused for 30 min with adrenochrome (50 mg/l) in the presence of tolazoline (25 mg/l). 3B: Electron micrographs of a typical section from an isolated rat heart perfused for 30 min with adrenochrome (50 mg/l) in the presence of dibenamine (25 mg/l).

of the hearts perfused in the absence of adrenochrome (Fig. 4). Upon electron microscopic examination of hearts perfused for 30 min with 50 mg/l of adrenochrome in the presence of either 1 mg/l of propranolol (Fig. 5A) or 1 mg/l of practolol (Fig. 5B), myocardial ultrastructure was found to be well preserved, and no evidence of necrotic damage was observed. Only occasional swelling of T tubules in hearts perfused with practolol and adrenochrome was noticed (Fig. 5B).

The time course of failure of contractile force development of isolated rat hearts perfused with adrenochrome (25 mg/l) was not different ($P > 0{\cdot}05$) from that in the presence of either of 2 adrenergic neurone-blocking agents, guanethidine (2 mg/l) and bretylium (2 mg/l) (Fig. 6). Normal contractile activity of the hearts in the absence of adrenochrome was also not affected by these neurone-blocking agents (Fig. 6). Guanethidine (Fig. 7A) and bretylium (Fig. 7B) failed to protect the isolated heart from necrotic changes due to 30 min of perfusion with 50 mg/l of adrenochrome.

The hydrazine-type monoamine oxidase inhibitor iproniazid at a concentration of 25 mg/l was found to improve contractile force development significantly ($P < 0{\cdot}05$) with effect from 6–30 min of perfusion with 25 mg/l of adrenochrome (Fig. 8). At 30 min, mean contractile force was over 40% of control level in the presence of iproniazid, whereas with 25 mg/l of adrenochrome alone the contractile force was only 5% of control at 30 min (Fig. 8). On the other hand, tranylcypromine (25 mg/l), which is non-hydrazine monoamine oxidase inhibitor, was not effective ($P > 0{\cdot}05$) in altering the time course of contractile force changes due to adrenochrome (25 mg/l). Iproniazid (25 mg/l) was also found to be quite effective in protecting myocardial structure from necrotic changes due to 30 min of perfusion with 50 mg/l of adrenochrome inasmuch as the appearance of mitochondria and myofibrils in the iproniazid-protected hearts (Fig. 9A) was comparable to that of control hearts. Although tranylcypromine (25 mg/l) greatly reduced the severity of ultrastructural damage and completely prevented development of contracture and disruption of contractile elements due to adrenochrome (50 mg/l), it was not as effective in preserving the integrity of mitochondria (Fig. 9B). Mitochondrial swelling in these hearts was found to be less pronounced, but disruption of cristae was evident throughout.

The presence of the calcium antagonist D-600 at a concentration of 0·5 mg/l in the perfusion medium resulted in a decline and disappearance of contractile activity of the isolated rat heart in less than 10 min of perfusion. This fact made it impossible to investigate the effect of this agent on the time course of failure of contractile activity caused by adrenochrome. The influence of 0·5 mg/l of D-600 on the morphological changes induced by adrenochrome 50 mg/l was studied following 30 min of perfusion of these non-contracting

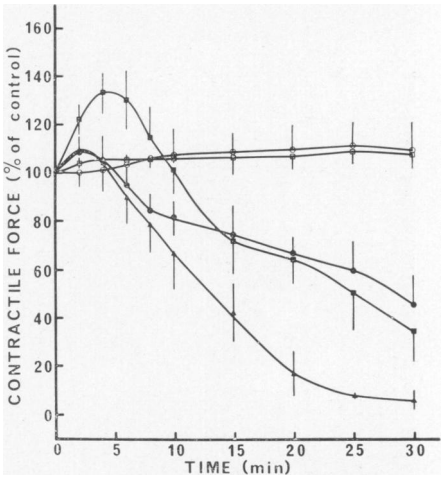

FIG. 4.—Effect of propranolol (1 mg/l) and practolol (1 mg/l) on the time course of failure of contractile force development of isolated rat hearts due to perfusion with adrenochrome (25 mg/l). Propranolol ○—○, propranolol plus adrenochrome ●—●, practolol □—□, practolol plus adrenochrome ■—■, adrenochrome ▲—▲. Each point is the mean ± s.e. of 4 experiments. Mean initial contractile force values in grams were: propranolol, 7·5 ± 0·6; practolol, 6·5 ± 0·7; control, 8·6 ± 0·8 at a resting tension of 2·5 g.

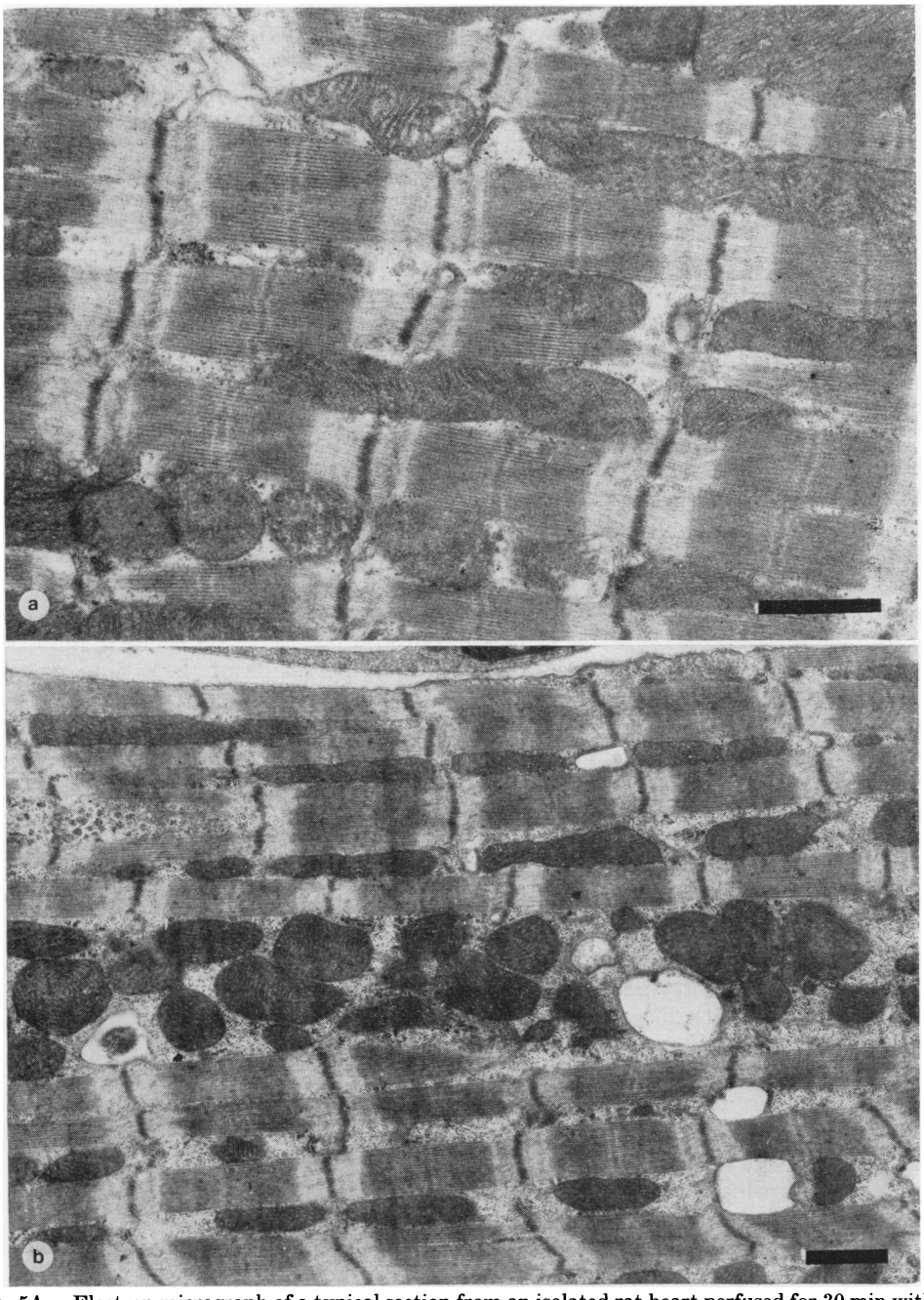

FIG. 5A.—Electron micrograph of a typical section from an isolated rat heart perfused for 30 min with adrenochrome (50 mg/l) in the presence of propranolol (1 mg/l). 5B: Electron micrograph of a typical section from an isolated rat heart perfused for 30 min with adrenochrome (50 mg/l) in the presence of practolol (1 mg/l).

but electrically stimulated (360 pulses/min) hearts. Electron microscopic examination of sections from the left ventricles of these hearts revealed ultrastructural changes which were quite different from those usually produced by 50 mg/l of adrenochrome. The most striking feature was a disalignment of the contractile filaments within the sarcomere (Fig. 10). This alteration was seen in most but not all of the sections examined. Mitochondria, sarcoplasmic reticulum and transverse tubules were in general well preserved, although large vacuoles of uncertain origin were frequently observed. None the less, the contracture and dissolution of contractile elements, and swelling and disruption of mitochondria characteristic of perfusion with 50 mg/l of adrenochrome (Fig. 1) were completely absent in hearts perfused with 50 mg/l of adrenochrome in the presence of 0·5 mg/l of D-600. The ultrastructure of hearts perfused for 30 min with 0·5 mg/l of D-600 in the absence of adrenochrome was not different from controls.

None of the pharmacological interventions employed in this study produced any noticeable increase in the severity of ultrastructural changes induced by perfusion with adrenochrome at concentrations of either 25 or 50 mg/l.

DISCUSSION

Although structural as well as functional changes in rat hearts perfused with oxidized isoprenaline have been reported (Yates and Dhalla, 1975; Dhalla *et al.*, 1978) and the presence of adrenochrome and related compounds in the oxidized isoprenaline solution has also been shown, the present study demonstrates for the first time that perfusion of isolated rat hearts with highly purified and freshly prepared solution of adrenochrome produces ultrastructural changes and causes contractile failure. Thus the results of the present study provide further evidence in support of the hypothesis that adrenochrome and related oxidation products are involved in the catecholamine-induced cardiac necrosis (Yates and Dhalla, 1975; Dhalla *et al.*, 1978). In this regard it is conceivable that in the case of unusually high levels of catecholamines, exceeding the capacity of their conventional inactivating systems such as monoamine oxidase and catechol-o-methyltransferase, the excess amounts get rerouted into other enzyme systems known to convert adrenaline to adrenochrome (Derouaux, 1943; Slater, 1949; Iisalo and Rekkarinen, 1958; Axelrod, 1964; Odajima, 1971; Valerno and McCormack, 1971). The formation of adrenochrome from catecholamines could occur through spontaneous oxidation and autocatalysis (Kisch, 1930), and catalytically *via* cytochrome C (Falk, 1949).

Although α-adrenergic-receptor-blocking agents have been reported somewhat to reduce the severity of lesions due to adrenaline injection (Waters and deSuto-Nagy, 1950; Mehes *et al.*, 1967; Wenzel and

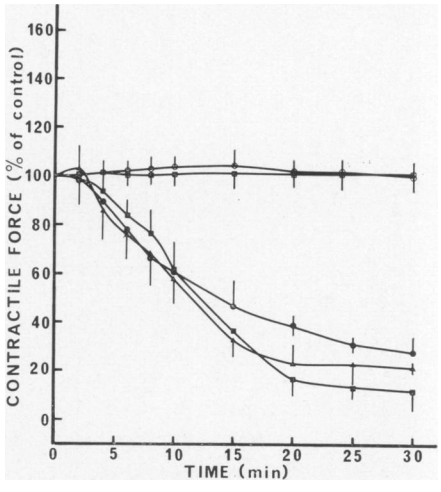

FIG. 6.—Effect of guanethidine (2mg/l) and bretylium (2 mg/l) on the time course of failure of contractile force development of isolated rat hearts due to perfusion with adrenochrome (25 mg/l). Guanethidine □—□, guanethidine plus adrenochrome ■—■, bretylium ○—○, bretylium plus adrenochrome ●—●, adrenochrome ▲—▲. Each point is the mean ± s.e. of 4 experiments. Mean initial contractile force values in grams were: guanethidine, 8·5 ± 0·6; bretylium, 8·5 ± 0·5; and control 7·8 ± 0·6 at a resting tension of 2·5 g.

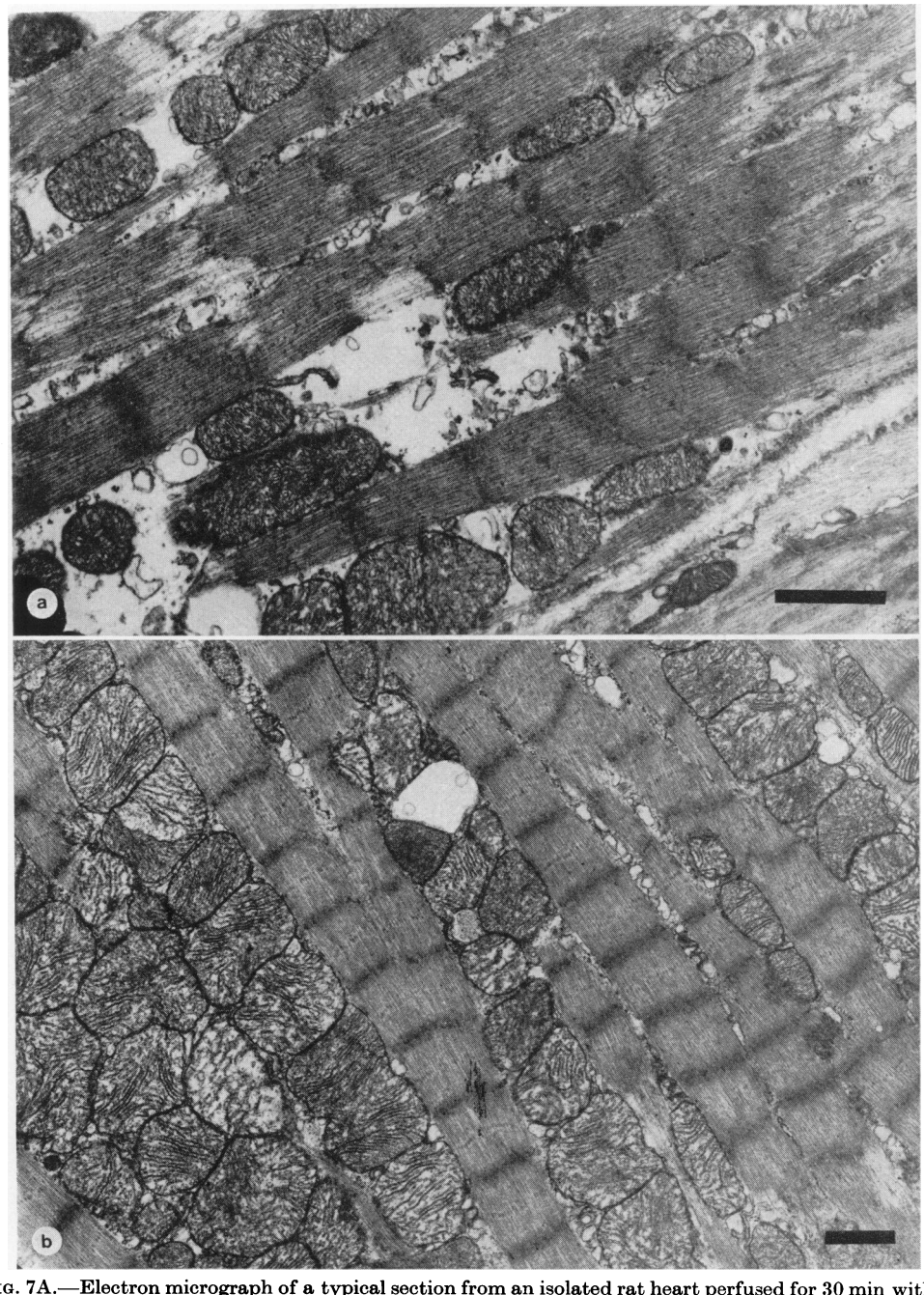

FIG. 7A.—Electron micrograph of a typical section from an isolated rat heart perfused for 30 min with adrenochrome (50 mg/l) in the presence of guanethidine (2 mg/l). 7B: Electron micrograph of a typical section from an isolated rat heart perfused for 30 min with adrenochrome (50 mg/l) in the presence of bretylium (2 mg/l).

Lyon, 1967; Lehr, 1969), they were completely ineffective in protecting the myocardium from adrenochrome-induced damage in this study. This suggests that the moderating influence of these agents in intact animals is due to their prevention of α-receptor stimulation by adrenaline and its consequences on myocardial workload and oxygen demand, whereas the direct toxic influence of adrenochrome is not mediated by α-receptor activation. It should be noted that α-receptor-blocking compounds are not very potent by themselves in preventing myocardial necrosis due to adrenaline injection, and were more effective when used in combination with a β-blocker (Mehes *et al.*, 1967; Lehr, 1969; Lehr *et al.*, 1969). Furthermore, the α-blocking agents are ineffective against necrosis induced by injection of the β-agonist isoprenaline (Mehes *et al.*, 1966, 1967; Wenzel and Lyon, 1967; Dorigotti *et al.*, 1969; Zbinden and Moe, 1969). Adrenergic neurone-blocking agents are also ineffective against adrenochrome-induced necrosis, just as they have been reported to be against necrosis produced by catecholamine injection. Just like α-adrenergic-receptor-blocking agents, neurone-blockers, which act through a totally different mechanism, were also ineffective in alleviating the cardiotoxic effects of adrenochrome.

In contrast to the α-blockers as well as neurone-blockers, the β-adrenergic-receptor-blocking agents propranolol and practolol were very effective in preventing necrosis due to adrenochrome. These results do not necessarily indicate that adrenochrome acts *via* β-receptors, since these drugs are not highly specific as to their site of action. Propranolol has been previously shown to have a negative inotropic effect on human, dog and rabbit cardiac muscle (Nayler *et al.*, 1969*a*,*b*), to decrease lipid-facilitated transport of Ca^{++} (Nayler *et al.*, 1969*a*) and to reduce or prevent increased myocardial Ca^{++} content due to isoprenaline (Bloom and Davis, 1974) and ischaemia–reperfusion (Ziegelhoffer *et al.*, 1979). It is thus not unlikely

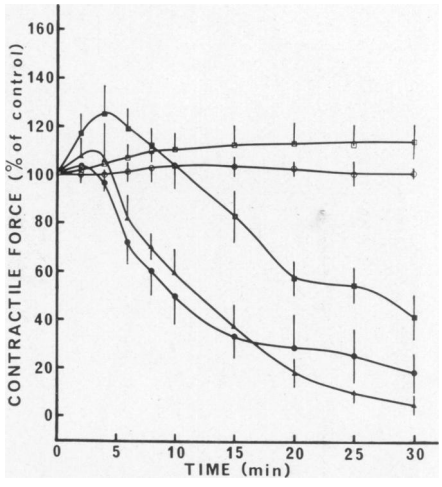

FIG. 8.—Effect of iproniazid (25 mg/l) and tranylcypromine (25 mg/l) on the time course of failure of contractile force development of isolated rat hearts due to perfusion with adrenochrome (25 mg/l). Iproniazid □—□, iproniazid plus adrenochrome ■—■, tranylcypromine ○—○, tranylcypromine plus adrenochrome ●—● adrenochrome ▲—▲. Each point is the mean ± s.e. of 4 experiments. Mean initial contractile force values in grams were: iproniazid, 7·8 ± 0·6; tranylcypromine, 8·3 ± 0·5; and control, 8·4 ± 0·3 at a resting tension of 2·5 g.

that the protective effect of β-blockers against the necrotizing influence of both adrenochrome perfusion and catecholamine injection (Lehr, Krukowski and Colon, 1966; Mehes *et al.*, 1966; Dorigotti *et al.*, 1969; Kahn *et al.*, 1969; Bloom and Davis, 1974) is a function of their influence on Ca^{++} movements. This point is further supported by the protective effect of the Ca-antagonist D-600 against adrenochrome-induced structural damage observed in this present study. In this regard it should also be noted that D-600, a methoxy derivative of verapamil, in the concentration employed by us in this study has been shown to reduce Ca^{++} conductivity in the heart muscle and the drug was found to be effective in preventing isoprenaline-induced tissue damage (Fleckenstein *et al.*, 1974). The unusual disalignment of contractile filaments within the sarcomeres of hearts perfused

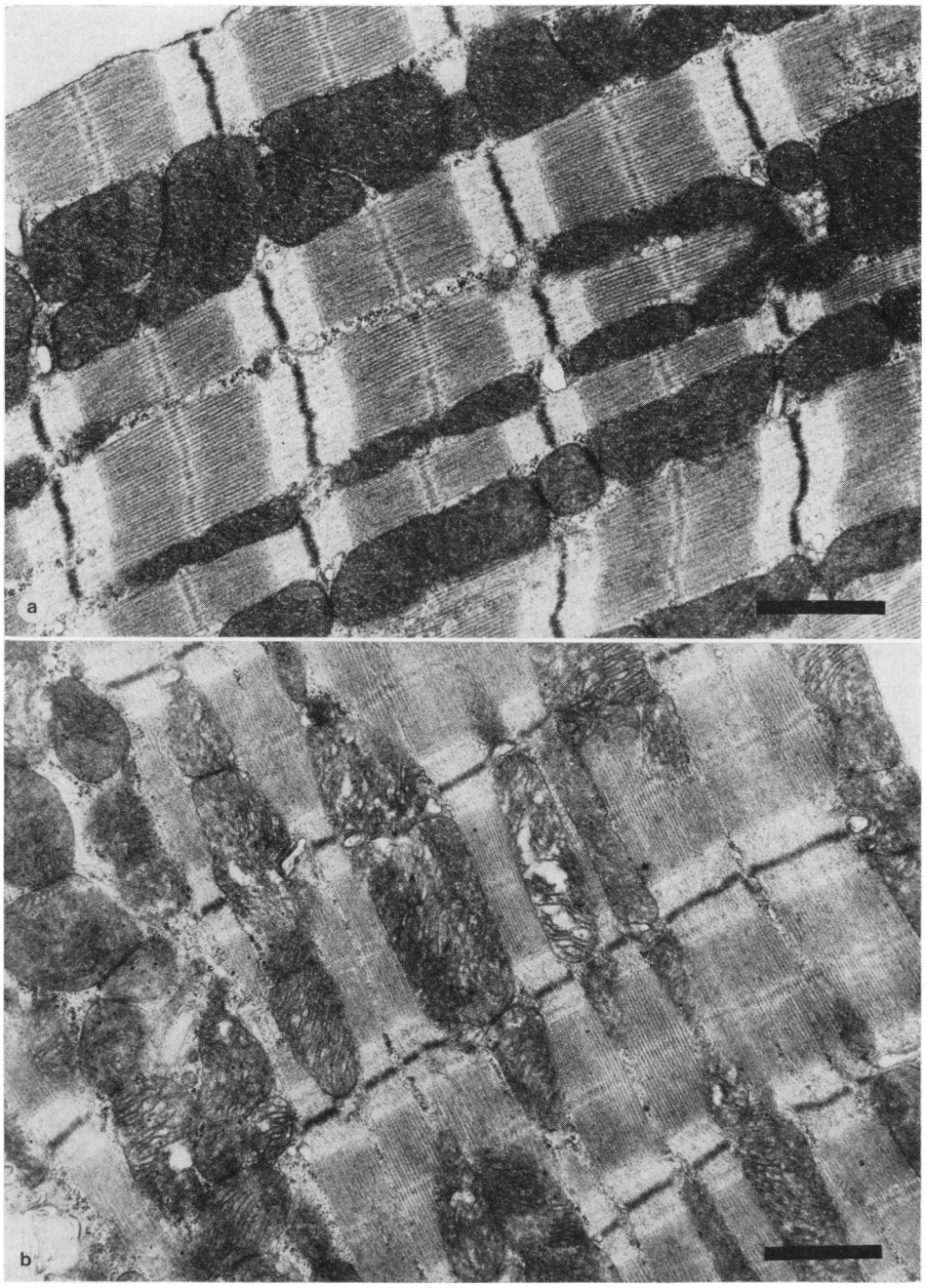

FIG. 9A.—Electron micrograph of a typical section from an isolated rat heart perfused for 30 min with adrenochrome (50 mg/l) in the presence of iproniazid (25 mg/l). 9B: Electron micrograph of a typical section from an isolated rat heart perfused for 30 min with adrenochrome (50 mg/l) in the presence of tranylcypromine (25 mg/l).

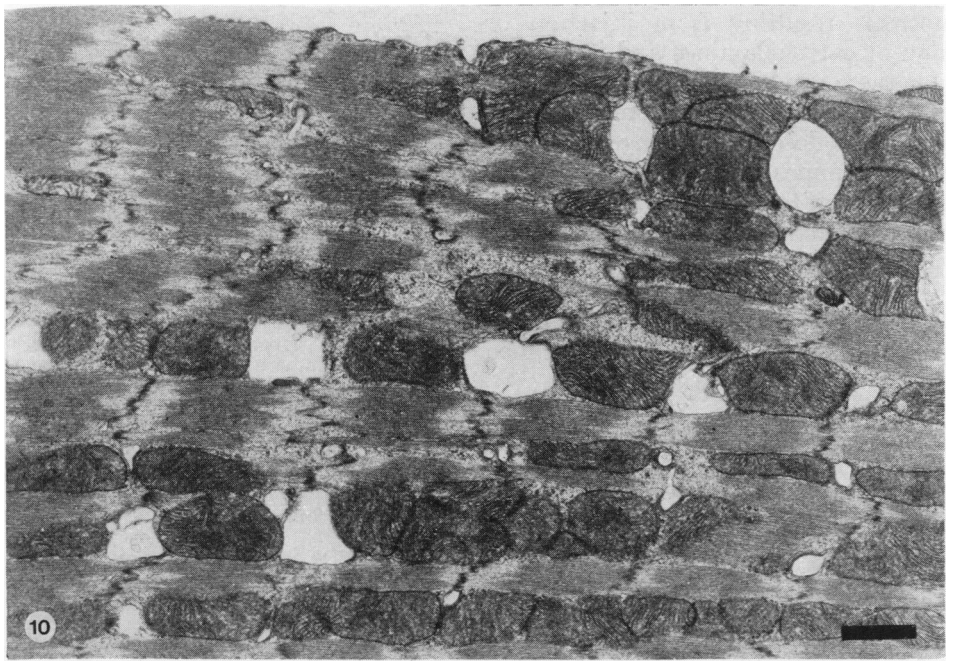

FIG. 10.—Electron micrograph of a typical section from an isolated rat heart perfused for 30 min with adrenochrome (50 mg/l) in the presence of D-600 (0·5 mg/l).

with adrenochrome in the presence of D-600 was quite different from any of the ultrastructural changes which usually characterize adrenochrome-induced necrosis. Whether these changes are indicative of an incomplete prevention of certain effects of adrenochrome or represent some unique effect of adrenochrome on the D-600-arrested heart is not clear and further studies on the effects of adrenochrome on various types of non-contracting hearts are warranted.

Experiments with tranylcypromine and iproniazid clearly demonstrate that monoamine oxidase inhibitors can prevent necrotic damage due to adrenochrome, although the non-hydrazine-type inhibitor tranylcypromine was found to be less effective than the hydrazine-type inhibitor iproniazid. These results parallel those obtained in studies on catecholamine-induced necrosis in intact animals (Zbinden, 1960, 1961; Rona *et al.*, 1963; Zbinden and Bagdon, 1963; Müller, 1966; Stanton and Schwartz, 1967; Kahn *et al.*, 1969; Zbinden and Moe, 1969). Although overt evidence of ultrastructural damage was not seen with iproniazid, practolol or propranolol, some degree of contractile failure was apparent, suggesting that all the effects of adrenochrome were not prevented. These contractile force changes may be indicative of alterations of cellular function which precede development of structural damage and are not wholly prevented by any of the above-mentioned pharmacological interventions. It is interesting to note in this regard that the monoamine oxidase inhibitor tranylcypromine failed to alter the time course of contractile-force changes even though the only subcellular structures in which damage was evident were the mitochondria. This observation may suggest that mitochondrial changes play an important role in the loss of contractile function during perfusion with adrenochrome.

In conclusion, our results on the toxic influence of adrenochrome on the myocardium, and their modification by various pharmacological agents show striking similarities with the data in the literature

on necrosis resulting from injection or infusion of catecholamines and its alteration by these same agents. It is therefore suggested that cardiac lesions resulting from massive doses or prolonged infusion of catecholamines are the result of an accumulation of toxic quantities of adrenochrome and related catecholamine oxidation products within the myocardium.

This work was supported by grants from The Great-West Life Assurance Company.

REFERENCES

AXELROD, J. (1964) Enzymatic Oxidation of Epinephrine to Adrenochrome by the Salivary Gland. *Biochim. biophys. Acta.*, **85**, 247.

BLOOM, S. & DAVIS, D. (1974) Isoproterenol Myocytolysis and Myocardial Calcium. In *Myocardial Biology: Recent Advances in Studies on Cardiac Structure and Metabolism.* Dhalla, N. S., ed. Baltimore: University Park Press. Vol. 4, 581.

DEROUAUX, G. (1943) The *in Vitro* Oxidation of Adrenalin and Other Diphenol Amines by Phenol Oxidase. *Archs. int. Pharmacodyn. Thér.*, **69**, 205.

DHALLA, N. S., YATES, J. C., LEE, S. L. & SINGH, A. (1978) Functional and Subcellular Changes in the Isolated Rat Heart Perfused with Oxidized Isoproterenol. *J. mol. cell. Cardiol.*, **10**, 31.

DORIGOTTI, L., GAETANI, M., GLASSER, A. H. & TUROLLIA, E. (1969) Competitive Antagonism of Isoproterenol-induced Cardiac Necrosis by β-Adrenoreceptor Blocking Agents. *J. Pharm. Pharmacol.*, **21**, 188.

FALK, J. E. (1949) The Formation of Hydrogen Carriers by Haemitin-catalyzed Peroxidations. *Biochem. J.*, **44**, 369.

FLECKENSTEIN, A. (1971) Specific Inhibitors and Promoters of Calcium Action in the Excitation–Contraction Coupling of Heart Muscle and their Role in the Prevention or Production of Myocardial lesions. In *Calcium and the Heart.* Harris, P. and Opie, L. H., eds. New York: Academic Press.

FLECKENSTEIN, A., JANKE, J., DOERING, H. J. & LEDER, O. (1974) Myocardial Fiber Necrosis due to Intracellular Ca Overload. A New Principle in Cardiac Pathophysiology. In *Recent Advances in Studies on Cardiac Structure and Metabolism.* Dhalla, N. S., ed. Baltimore: University Park Press. Vol. 4, 563.

IISALO, E. & REKKARINEN, A. (1958) Enzyme Action on Adrenaline and Noradrenaline, Studies on Heart Muscle *in Vitro*. *Acta Pharmacol. Toxicol.*, **15**, 157.

KAHN, D. S., RONA, G. & CHAPPEL, C. I. (1969) Isoproterenol-induced Cardiac Necrosis. *Ann. N.Y. Acad. Sci.*, **156**, 285.

KISCH, B. (1930) Die Autokatalyse der Adrenalinoxydation. *Biochem. Z.*, **220**, 84.

LEHR, D. (1969) Tissue Electrolyte Alteration in Disseminated Myocardial Necrosis. *Ann. N.Y. Acad. Sci.*, **156**, 344.

LEHR, D., KRUKOWSKI, M. & COLON, R. (1966) Correlation of Myocardial and Renal Necrosis with Tissue Electrolyte Changes. *J. Am. med. Ass.*, **197**, 105.

LEHR, D., KRUKOWSKI, M. & CHAU, R. (1969) Acute Myocardial Injury produced by Sympathomimetic amines. *Israel J. med. Sci.*, **5**, 519.

LESZKOVSKY, G. P. & GAL, G. (1967) Observations on Isoprenalin-induced Myocardial Necroses. *J. Pharm. Pharmacol.*, **19**, 226.

MEHES, G., PAPP, G. & RAJKOVITS, K. (1967) Effect of adrenergic α- and β-Receptor Blocking Drugs on the Myocardial Lesions induced by Sympathomimetic amines. *Acta physiol. hung.*, **32**, 175.

MEHES, G., RAJKOVITS, K. & PAPP, G. (1966) Effect of Various Types of Sympathicolytics on Isoproterenol-induced Myocardial Lesions. *Acta physiol. hung.*, **29**, 75.

MUIR, A. R. (1967) The Effects of Divalent Cations on the Ultrastructure of the Perfused Rat Heart. *Am. J. Anat.*, **101**, 239.

MÜLLER, E. (1966) Histochemical Studies on the Experimental Heart Infarction in the Rat. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **254**, 439.

NAYLER, W. G., STONE, J., CARSON, V., MCINNES, I., MACK, V. & LOWE, T. E. (1969*a*) The Effect of Beta Adrenergic Antagonists on Cardiac Contractions, Myofibrillar ATPase Activity, High-energy Phosphate Stores and Lipid-facilitated Transport of Calcium Ions. *J. Pharmac. exp. Ther.*, **165**, 225.

NAYLER, W. G., CHIPPERFIELD, D. & LOWE, T. E. (1969*b*) The Negative Inotropic Effect of Adrenergic Beta-receptor Blocking Drugs on Human Heart Muscle. *Cardiovasc. Res.*, **3**, 30.

ODAJIMA, T. (1971) Myeloperoxidase of the Leucocyte of Normal Blood. II. Oxidation Reduction Reaction Mechanism of the Myeloperoxidase. *Biochim. biophys. Acta*, **235**, 52.

RONA, G., CHAPPEL, C. I. & KAHN, D. S. (1963) The Significance of Factors Modifying the Development of Isoproterenol-induced Myocardial Necrosis. *Am. Heart J.*, **66**, 389.

SIGEL, H., JANKE, J. & FLECKENSTEIN, A. (1975) Restriction of Isoproterenol-induced Myocardial Ca Uptake and Necrotization by a New Ca-antagonistic Compound (Ethyl-4(3,4,5,-trimethoxycinnamoyl) Piperazinyl Acetate) (Vascoril). In *Recent Advances in Studies on Cardiac Structure and Metabolism.* Fleckenstein, A. and Rona, G., eds. Baltimore: University Park Press. Vol. 6, 121.

SINGAL, P. K., MATSUKUBO, M. P. & DHALLA, N. S. (1979) Calcium-related Changes in the Ultrastructure of Mammalian Myocardium. *Br. J. exp. Path.*, **60**, 96.

SLATER, E. C. (1949) The Measurement of Cytochrome Oxidase Activity of Enzyme Preparations. *Biochem. J.*, **44**, 305.

STANTON, H. C. & SCHWARTZ, A. (1967) Effects of Hydrazine Monoamine Oxidase Inhibitor (Phenelzine) on Isoproterenol-induced Myocardiopathise in the Rat. *J. Pharmac. exp. Ther.*, **157**, 649.

TAKEO, S., FLIEGEL, L., BEAMISH, R. E. & DHALLA, N. S. (1980) Effects of Adrenochrome on the Rat Heart Sarcolemmal ATPase Activities. *Biochem. Pharmacol.*, **29**, 559.

VALERNO, D. M. & MCCORMACK, J. J. (1971) Xanthine Oxidase-mediated Oxidation of Epinephrine. *Biochem. Pharmacol.*, **20**, 47.

WATERS, L. L. & DESUTO-NAGY, G. I. (1950) Lesions of the Coronary Arteries and Great Vessels of the Dog following Injection of Adrenalin. Their Prevention by Dibenamine. *Science*, **111**, 634.

WENZEL, D. G. & LYON, J. P. (1967) Sympathomimetic Amines and Heart Lactic Dehydrogenase Isoenzymes. *Toxic. appl. Pharmac.*, **11**, 215.

YATES, J. C. & DHALLA, N. S. (1975) Induction of Necrosis and Failure in the Isolated Perfused Rat Heart with Oxidized Isoproterenol. *J. mol. cell. Cardiol.*, **7**, 807.

ZBINDEN, G. (1960) Inhibition of Experimental Myocardial Necrosis by the Monoamine Oxidase Inhibitor Isocarboxazid (Marplan). *Am. Heart J.*, **60**, 450.

ZBINDEN, G. (1961) Effects of Anoxia and Amino Oxidase Inhibitors on Myocardial Necrosis Induced by Isoproterenol. *Fed. Proc.*, **20**, 128a.

ZBINDEN, G. & BAGDON, R. E. (1963) Isoproterenol-induced Heart Necrosis, an Experimental Model for the Study of Angina Pectoris and Myocardial Infarct. *Rev. Can. Biol.*, **22**, 257.

ZBINDEN, G. & MOE, R. A. (1969) Pharmacological Studies on Heart Muscle Lesions Induced by Isoproterenol. *Ann. N.Y. Acad. Sci.*, **156**, 294.

ZIEGELHOFFER, A., DAS, P. K., SHARMA, G. P., SINGAL, P. K. & DHALLA, N. S. (1979) Propranolol Effects on Myocardial Ultrastructure and High energy Phosphates in Anaesthetized Dogs subjected to Ischemia and Reperfusion. *Can. J Physiol. Pharmacol.*, **57**, 979.