Br. J. exp. Path. (1982) **63**, 167

MYOCARDIAL CELL DAMAGE AND CARDIOVASCULAR CHANGES DUE TO I.V. INFUSION OF ADRENOCHROME IN RATS

P. K. SINGAL*, K. S. DHILLON, R. E. BEAMISH, N. KAPUR AND N. S. DHALLA

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

Received for publication November 16, 1981

Summary.—*In vivo* effects of adrenochrome (1–32 mg/kg), an oxidation product of catecholamines, on the heart ultrastructure, ECG and blood pressure were studied in rats over a period of 60 min following a single i.v. injection of the drug. One milligram of the drug had no influence on the myocardium or the cardiovascular system, whereas maximum changes in these parameters were recorded at 32 mg/kg of adrenochrome. The maximum structural damage, reached within 5–10 min, included marked swelling of mitochondria and sarcotubular system, intracellular and perinuclear oedema, hypercontraction of myofibrils and partial separation of the intercalated disc. Ultrastructural changes in the myocardium due to 4 and 8 mg of adrenochrome were not accompanied by any cardiovascular effects and the changes were fully reversed within 60 min of the injection of the drug. However, at 16 and 32 mg/kg of adrenochrome both heart rate and blood pressure were depressed within 5 min of drug administration. At these concentrations of adrenochrome arrhythmias, mainly due to premature ventricular contractions, were also noticed. Ultrastructural and cardiovascular changes seen at these higher concentrations of adrenochrome showed only a partial recovery. The data indicates that adrenochrome-induced ultrastructural changes in the heart are due to a direct myocardial effect of the drug which may not involve haemodynamic changes and the latter are most probably a consequence of this effect. However, the present study has not been able to rule out direct vascular effects at higher concentrations of adrenochrome.

IT IS NOW well established that excessive amounts of catecholamines in the blood produce myocardial cell damage and change heart function and metabolism (Pearce, 1906; Szakacs and Cannon, 1958; Rona *et al.*, 1959; Handforth, 1962; Sobel *et al.*, 1966; Bloom and Cancilla, 1969; Singal *et al.*, 1981*a*). Several mechanisms, such as increased cardiac work and peripheral vasodilation (Chappel *et al.*, 1959; Rona *et al.*, 1959), depletion of high-energy phosphates (Fleckenstein *et al.*, 1974; Takenaka, 1975), intracellular build-up of calcium (Bloom and Davis, 1974; Fleckenstein *et al.*, 1974), mobilization of free fatty acids (Kjekshus, 1975) and imbalance of cell electrolytes (Waddell, 1961) have been proposed to explain catecholamine-induced structural and functional changes in the heart. Recently, another concept in the study of catecholamine-produced myocardial necrosis has been introduced by the observation that isolated rat hearts perfused with spontaneously oxidized isoprenaline showed structural damage quite similar to that seen after isoprenaline administration in animals (Yates and Dhalla, 1975; Dhalla *et al.*, 1978). Since no myocardial cell damage was seen in isolated hearts perfused with fresh catecholamines, it was suggested that the cardiotoxic effects of catecholamines may be due, at least partly, to the oxidation products such as

Correspondence to: Pawan K. Singal, Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3.

* Scholar of the Murphy Foundation and The Great West Life Assurance Company, Winnipeg.

adrenochrome. Role of oxidation products of catecholamines in catecholamine-induced myocardial necrosis has also been indicated by many other studies (Carlsten and Poupa, 1977; Severin, Sartore and Schiaffino, 1977).

Although in isolated rat hearts adrenochrome has been shown to depress the contractile function and cause myocardial cell damage in a dose-dependent manner (Yates *et al.*, 1980*a*, *b*; Yates, Beamish and Dhalla, 1981), information about *in vivo* effects of adrenochrome on the myocardium is lacking. The present study was therefore undertaken to examine whether i.v. injection of adrenochrome has any cardiovascular and/or myocardial ultrastructural effects in rats.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 250–300 g were anaesthetized with a single i.p. injection of Nembutal (50 mg/kg). After intubation of the trachea, the right carotid artery was cannulated for recording the blood pressure, and the right femoral vein was cannulated for making i.v. injections of adrenochrome. The latter was either obtained commercially (Sigma Chemical Co., St Louis) or freshly synthesized by the method of Heacock, Nerenberg and Payza (1958), and the purity was checked by infra-red spectrophotometric and thin-layer chromatographic examinations of the samples. Adrenochrome was dissolved in saline immediately before use and was administered at doses of 1, 4, 8, 16 or 32 mg/kg body wt in a total volume of 0·25 ml. Control rats received saline without any drug. ECG was recorded by standard Lead II. Both ECG and blood pressure were continuously monitored and data analysed by Student's *t* test. The animals were killed by decapitation 5, 10 and 60 min after the injection of adrenochrome.

At the end of each experiment hearts were fixed in 0·1M phosphate buffer (pH 7·4) containing 2% glutaraldehyde at 4°. Small tissues pieces 4–6 mm in size were taken from 4 different areas of the mid-myocardial layer of the free left ventricular wall between the mid-region and apex of the heart. These tissue samples were immersed for 15 min in the aldehyde fixative solution. This briefly fixed tissue was further cut into pieces smaller than 1 mm^3 and allowed to stand in the buffered glutaraldehyde for a total of 2 h for further fixation. In each experimental group, for comparison, heart was also fixed by the procedure of perfusion-fixation described earlier (Singal, Matsukubo and Dhalla, 1979). The tissue pieces were washed overnight in the cold phosphate buffer containing sucrose, postfixed for 1 h with 1% osmium tetroxide, dehydrated in a graded alcohol series and embedded in Epon 812 according to the method of Luft (1961). Sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined by Zeiss electron microscope.

RESULTS

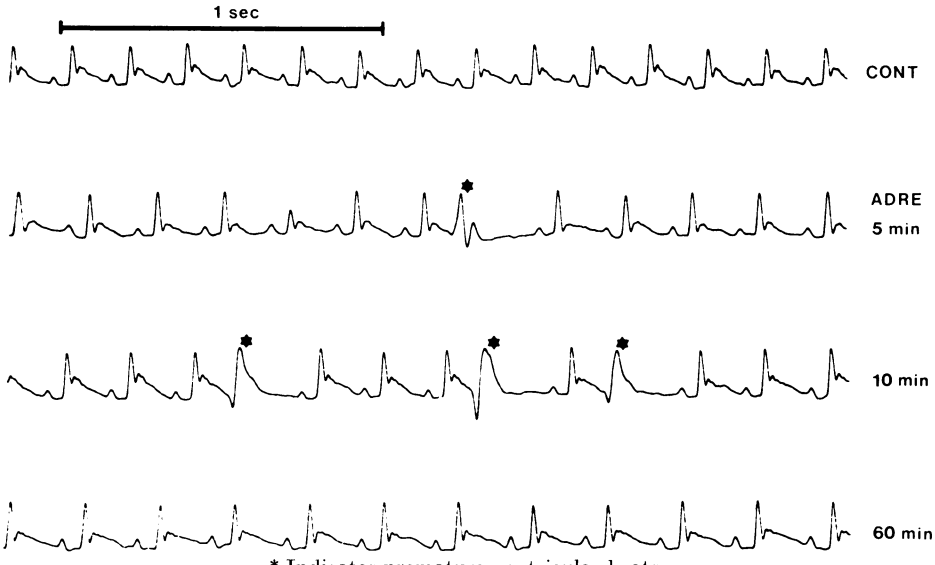
Cardiovascular effects

Since anaesthetized animals were used in this study, it was decided to study the effects of anaesthesia alone on the heart rate, ECG and ultrastructure. Although anaesthesia always reduced the heart rate (average heart rate in 4 rats: conscious, 480 ± 40; anaesthetized 405 ± 20), it did not alter the ECG. Effects of anaesthesia

TABLE.—*Time-course study of the effects of different concentrations of adrenochrome on the heart rate (HR) and blood pressure (BP) in anaesthetized rats.*[a]

Time after injection	1 mg/kg		4 mg/kg		8 mg/kg		16 mg/kg		32 mg/kg	
	HR (beats/min)	BP (mmHg)	HR (beats/min)	BP (mmHg)	HR (beats/min)	BP (mmHg)	HR (beats/min)	BP (mmHg)	HR (beats/min)	BP (mmHg)
Control (12)	410 ± 22	135 ± 10	392 ± 22	124 ± 9	424 ± 20	140 ± 11	351 ± 18	126 ± 8	357 ± 23	128 ± 7
5 min (12)	415 ± 23	140 ± 11	400 ± 20	126 ± 8	411 ± 21	152 ± 12	310 ± 12	93 ± 4[b]	297 ± 21	90 ± 5[b]
10 min (8)	402 ± 21	126 ± 12	396 ± 21	117 ± 6	404 ± 18	132 ± 9	299 ± 21	89 ± 9[b]	294 ± 23	89 ± 3[b]
30 min (4)	395 ± 21	133 ± 11	410 ± 19	128 ± 9	410 ± 23	140 ± 11	320 ± 28	88 ± 12	279 ± 31	100 ± 11
60 min (4)	418 ± 22	139 ± 12	426 ± 18	136 ± 10	400 ± 19	138 ± 10	344 ± 13	99 ± 14	308 ± 32	97 ± 9
Incidence of arrhythmias (%)	0		0		0		41·7		75·0	

( ) number of animals in each group.
[a] mean values ± s.e. mean.
[b] Significantly different ($P < 0.05$) from their respective controls.



* Indicates premature ventricular beats.

FIG. 1.—Time-related effects of adrenochrome (32 mg/kg) on the ECG recorded from Lead II.

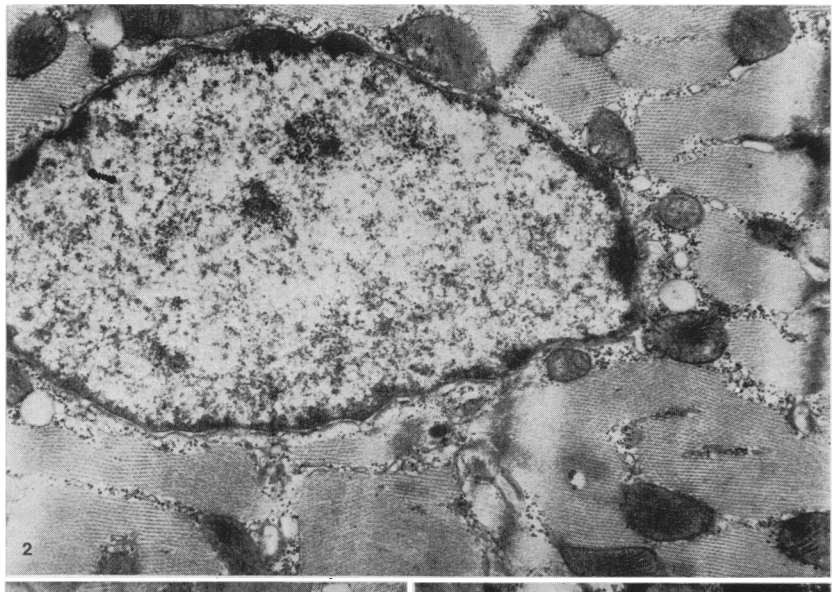
alone on the blood pressure were not defined, owing to the difficulty of obtaining this information in conscious rats by cannulation for an ideal comparison.

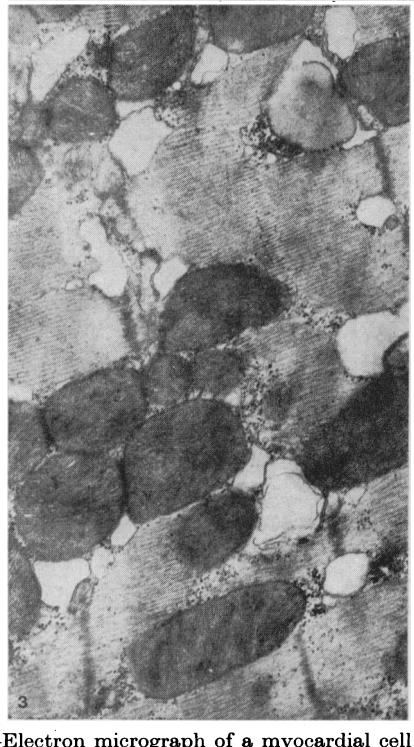
Time- and dose-related effects of i.v. administration of adrenochrome (1–32 mg/kg) on the heart rate as well as blood pressure have been summarized in the Table. Immediately after the injection of adrenochrome, a transient increase in heart rate and blood pressure, lasting only a few seconds, was seen in all rats at all the concentrations used in this study. However, no relationship could be established between these changes and adrenochrome concentration because of the large variations in response. After this fleeting change, the heart rate and blood pressure in rats injected with 1, 4 or 8 mg/kg of adrenochrome returned to normal and remained unchanged for the duration of the experiment. Rats injected with 16 or 32 mg/kg of adrenochrome within 5 min showed a depression in their sinus rates as well as pressure readings. A partial recovery of these changes was seen at the end of 60-min period.

The ECG pattern remained unaffected at 1–4 mg/kg of adrenochrome. During the 60-min experimental period, 1–2 premature ventricular beats were observed in rats injected with 8 mg/kg of adrenochrome. However, reversible arrhythmias were seen in 5/12 rats injected with 16 mg/kg of adrenochrome and in 9/12 rats injected with 32 mg/kg of adrenochrome. These adrenochrome-induced arrhythmias were mainly due to premature ventricular contraction (Fig. 1). However, 2 of the animals injected with 32 mg/kg of adrenochrome also showed occasional heart block and there was one episode of ventricular fibrillation.

Ultrastructural effects

The ultrastructure of hearts (Fig. 2) from rats anaesthetized for 60 min with Nembutal (50 mg/kg) was found to be quite similar to that of hearts from unanaesthetized rats as well as to that of isolated hearts perfused by the Langendorff technique (Muir, 1967; Singal *et al.*, 1979). A moderate dilation of the intracellular space in those portions of the intercalated disc running parallel to the myofibrils appears to be a normal feature in these electron-microscope preparations of the heart (Singal *et al.*, 1979).





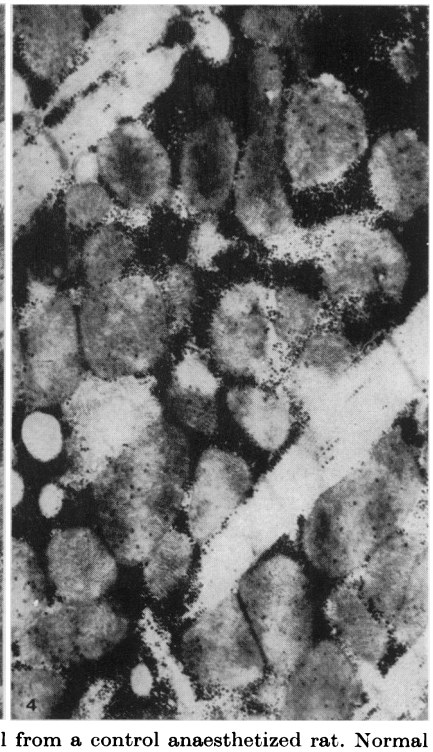


FIG. 2.—Electron micrograph of a myocardial cell from a control anaesthetized rat. Normal distribution of the nuclear chromatin and structures in the perinuclear area is shown. × 10,667.

FIG. 3.—Ten minutes after adrenochrome (4 mg/kg). Myocardial cell showing marked swelling of the sarcotubular membrane system. × 10,667.

FIG. 4.—Sixty minutes after adrenochrome (4 mg/kg). Striking accumulation of glycogen granules is apparent in the myocardial cell. × 10,667.

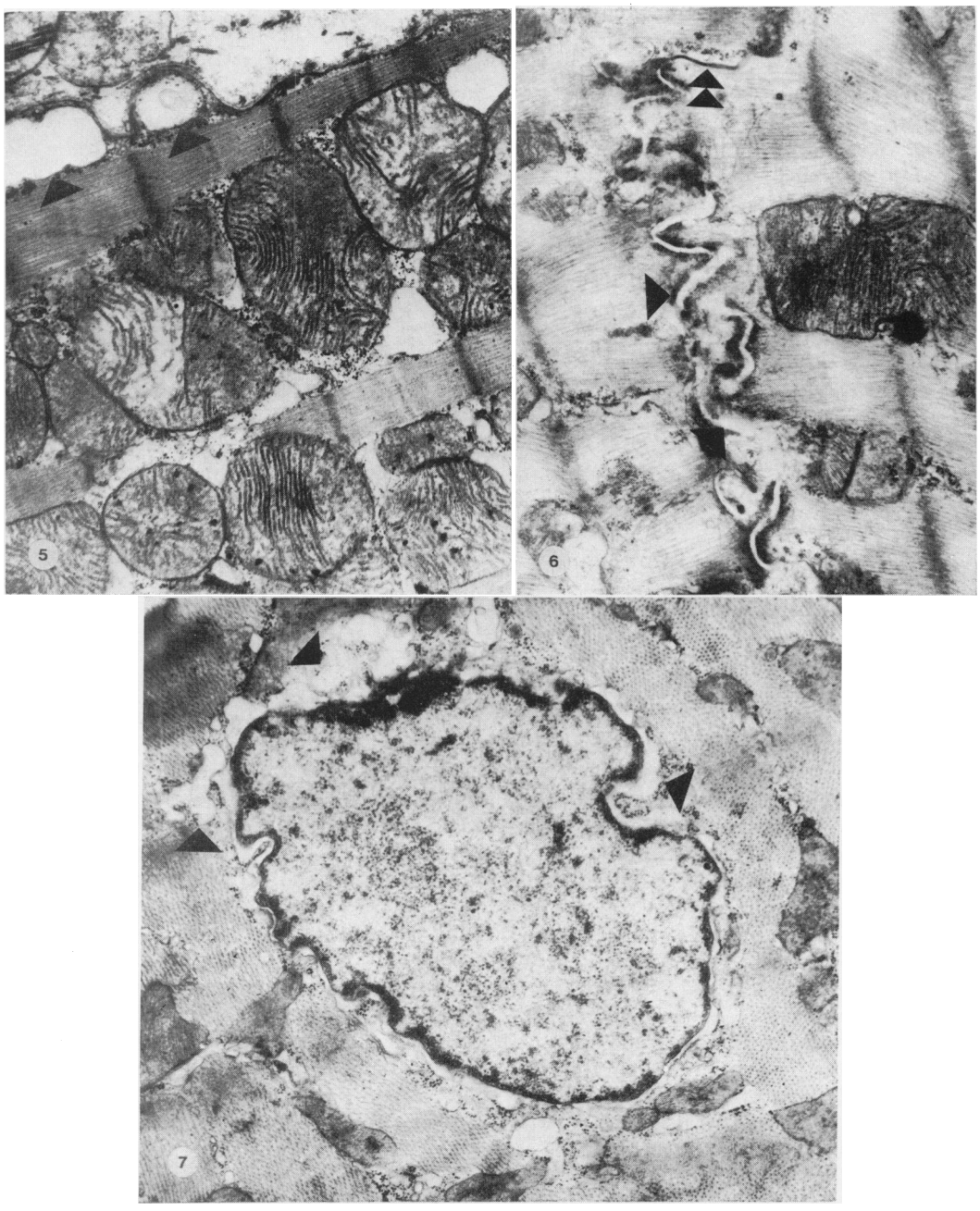


FIG. 5.—Five minutes after adrenochrome (8 mg/kg). Myocardial cell showing swelling of the mitochondria. Formation of vacuoles in the subsarcolemmal regions (arrows) as well as in other areas of cytoplasm is also apparent. × 12,800.

FIG. 6.—Five minutes after adrenochrome (8 mg/kg). Derangement of myofilaments, swelling of the mitochondria and a moderate separation (arrows) of the intercalated disc had occurred. An intact gap junction in the intercalated disc can also be noticed (double arrow). × 12,800.

FIG. 7.—Ten minutes after adrenochrome (8 mg/kg). Electron micrograph showing moderate perinuclear oedema (arrows). × 8,000.

One mg/kg of adrenochrome did not cause any ultrastructural changes in the heart. Hearts fixed 5 min after the injection of 4 mg/kg adrenochrome also did not show any significant changes in their subcellular details except for a minor change in the appearance of mitochondria. The outer margins of these organelles appeared angular in shape. However, hearts fixed after 10 min of adrenochrome administration (4 mg/kg) showed a dramatic swelling of the sarcotubular system (Fig. 3). Although some enlargement of the mitochondria was also seen, it was not accompanied by any intramitochondrial structural changes. Sarcomeres and other structures such as nucleus, Golgi body and intercalated disc were normal in appearance. By 60 min the ultrastructure of the cell was normal except for a striking accumulation of glycogen granules (Fig. 4).

Most of the cellular structures in hearts fixed at 5 min after injection of 8 mg/kg of adrenochrome (Figs 5 and 6) were affected. The sarcomeres were in a contracted state and the arrangement of myofibrils was disrupted in more than 50% of the cells in these hearts. Swelling of the mitochondrial as well as sarcotubular system was quite noticeable. In some of the mitochondria swelling was also accompanied by disruption of cristae. Nuclear chromatin was condensed and marginated. Perinuclear as well as subsarcolemmal oedema was also noticed. Although some separation of the cells at the intercalated disc was apparent., the specialized junctions such as nexus and desmosomes were still maintained (Fig. 6). After 10 min of drug injection, swelling of the sarcotubular system was present, whereas swelling of the mitochondria was not as pronounced. Perinuclear (Fig. 7) as well as subsarcolemmal oedema was also present in these 10-min hearts. Except for a sporadic accumulation of glycogen granules, the ultrastructure of the hearts after 60 min of 8 mg/kg of adrenochrome was comparable to the normal.

When the animals were injected with 16 and 32 mg/kg of adrenochrome, changes in mitochondria, sarcotubular system, perinuclear region and intercalated disc were more severe compared to those in the rats injected with 8 mg/kg of adrenochrome. All these structural changes were reversed by 60 min in hearts from rats injected with 16 mg/kg except for the sarcotubular system, which was still oedematous (Fig. 8). In hearts from rats given 32 mg/kg of adrenochrome, changes in the nuclear region as well as in the sarcotubular system did not revert to normal at 60 min (Fig. 9). Marked perinuclear oedema seen after 60 min of administration of the drug was comparable to that seen after 5 min. There was no difference between the effects of commercially obtained and freshly synthesized adrenochrome samples. The ultrastructural changes described here were also confirmed in hearts fixed by perfusion-fixation procedures.

DISCUSSION

The results obtained in this study show that i.v. administration of adrenochrome (4–32 mg/kg) produces ultrastructural changes in the myocardium and these changes become apparent within 5 min of injection. Lack of these changes in simultaneously processed controls as well as their complete or partial reversal after 60 min in experimental hearts rules out the possibility that observed structural changes are fixation artefacts. This contention is also supported by the fact that similar structural changes were noted in hearts fixed by 2 different fixation procedures. Furthermore, an increase in the severity of structural changes with an increase in the amount of adrenochrome administered and an incomplete reversal of these changes at higher concentrations of the drug indicate a dose-dependency of adrenochrome effects. Occurrence of arrhythmias and changes in the heart rate as well as blood pressure seen only at higher concentrations of adrenochrome also confirmed that these changes were dose-dependent. Our observation of glycogen accumulation after 60 min of ad-

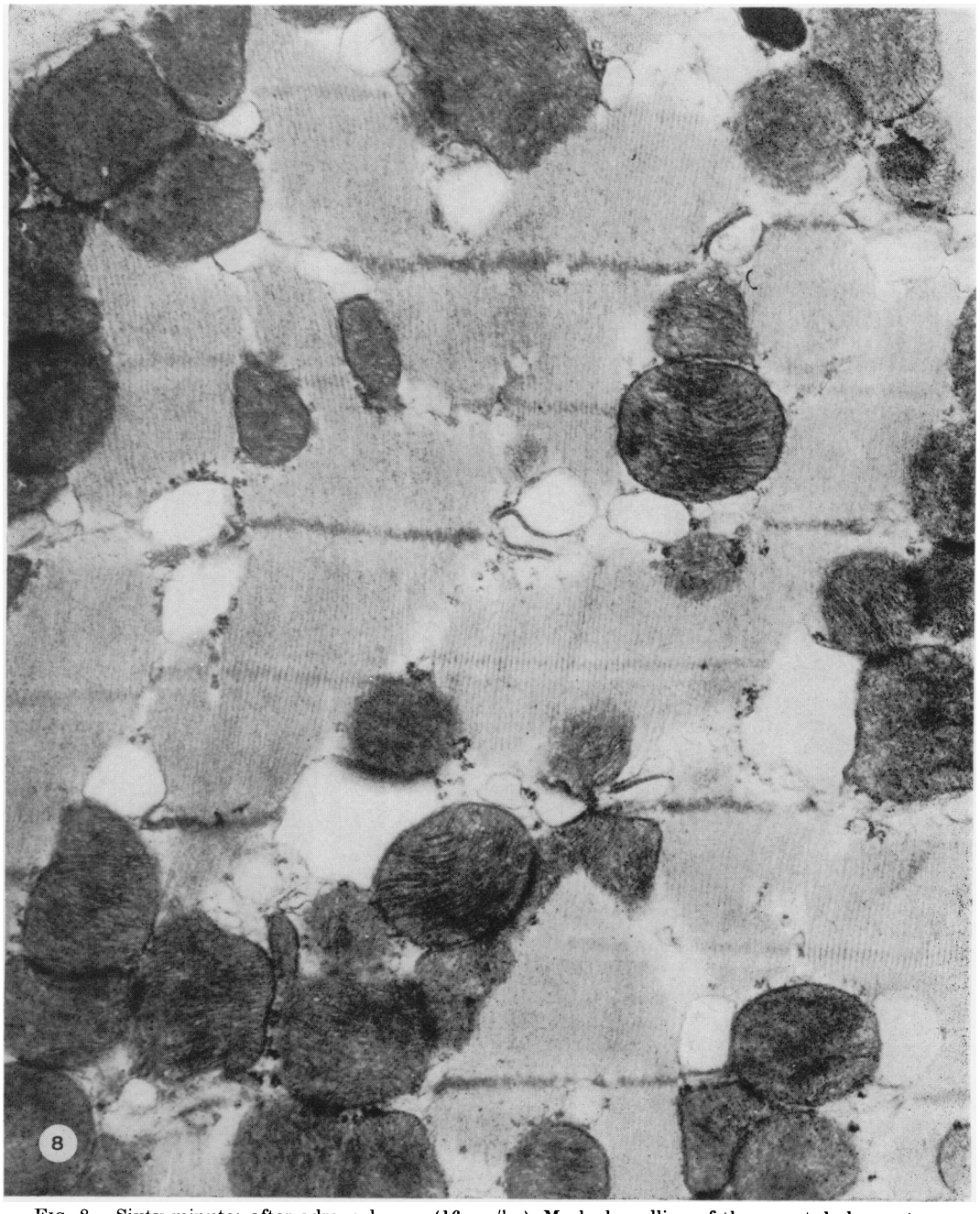


FIG. 8.—Sixty minutes after adrenochrome (16 mg/kg). Marked swelling of the sarcotubular system throughout the cell is apparent. × 16,000.

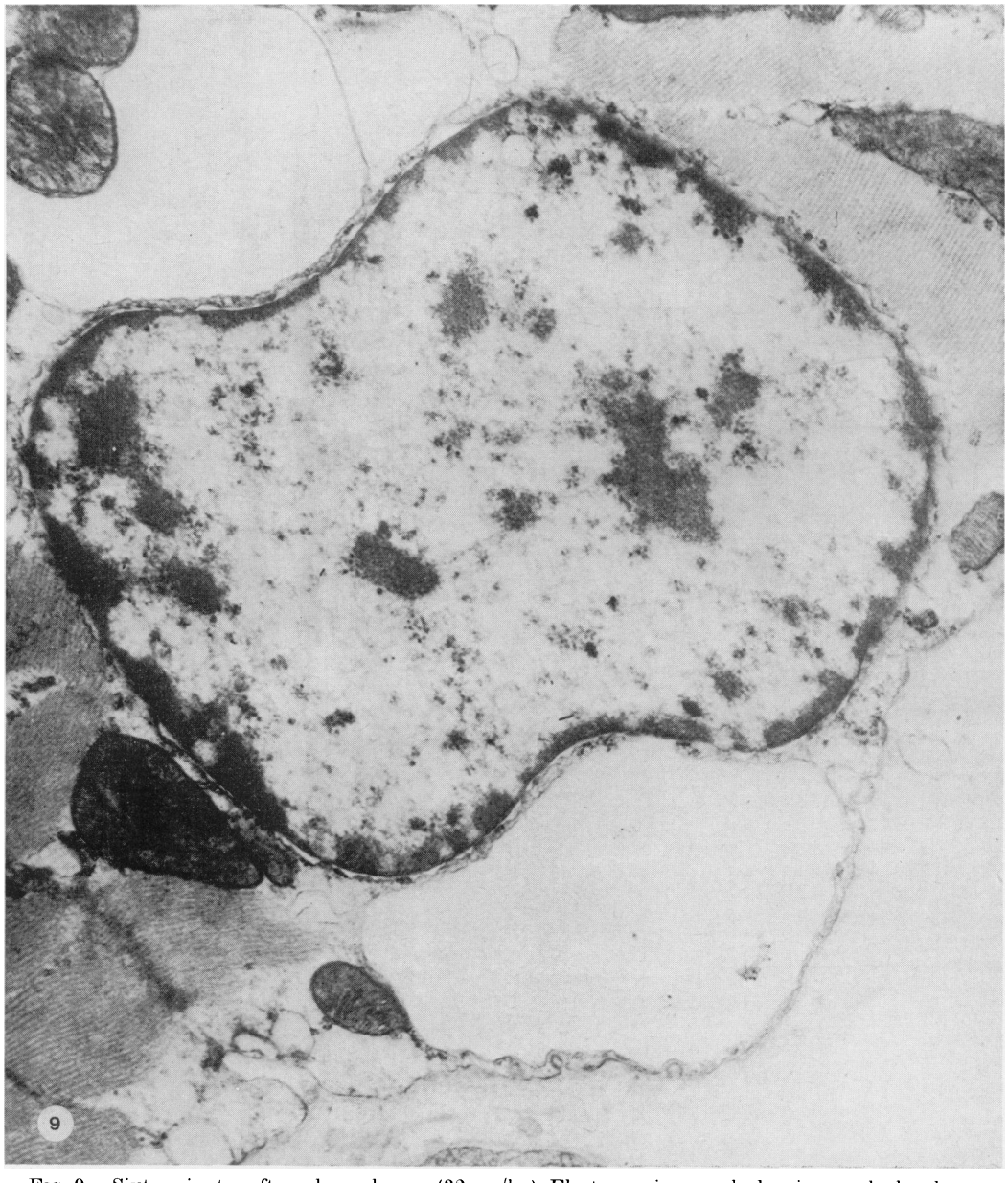


FIG. 9.—Sixty minutes after adrenochrome (32 mg/kg). Electron micrograph showing marked oedema in the juxtanuclear region. × 14,400.

renochrome administration is supported by *in vitro* studies on liver homogenate in which adrenochrome has been shown to stimulate the synthesis of glycogen by suppressing the formation of hexose diphosphate (Wajzer, 1947). However, it is likely that this effect of adrenochrome is not dose-dependent because at 8–32 mg/kg of adrenochrome the accumulation of glycogen was less apparent than that seen after 4 mg/kg of adrenochrome.

It is important to note that the ultra-

structural changes observed in the isolated rat heart perfused with oxidized isoprenaline or adrenochrome (Yates and Dhalla, 1975; Yates *et al.*, 1980*a*, *b*, 1981; Dhalla *et al.*, 1978; Singal *et al.*, 1981*b*) were qualitatively similar to those reported after the administration of excessive amounts of isoprenaline to a variety of experimental animals including rats (Ferrans *et al.*, 1964; Maruffo, 1967; Csapo, Dusek and Rona, 1972; Kutusuna, 1972; Bloom and Davis, 1974; Singal *et al.*, 1981*a*). Bloom and Cancilla (1969) reported swelling of the mitochondria as well as hypercontraction of the myofibrils in some of the myocarcial cells as early as 2 min after the administration (i.p. 5 mg/kg) of isoprenaline. Similar structural changes were seen after *in vivo* administration of adrenochrome in our study. Furthermore, the intracellular oedema due to adrenochrome observed in this study resembles that reported after isoprenaline injections (Bloom and Cancilla, 1969). The similarities between the adrenochrome- and isoprenaline-induced ultrastructural changes provide support for the view that catecholamine-induced myocardial necrosis may also involve catecholamine oxidation products such as adrenochrome (Yates and Dhalla, 1975; Dhalla *et al.*, 1978). Under normal conditions the *in vivo* formation of adrenochrome is checked by preventing the formation of the 2,3-dihydroindole-5,6 quinone ring system by deamination and O-methylation. However, consistently high levels of catecholamines in the blood under abnormal conditions, exceeding the capacity of monamine oxidase and catechol-O-methyl transferase system, can lead to the formation of adrenochrome *via* both spontaneous (Kisch, 1930), and enzyme-catalysed (Derouaux, 1942; Slater, 1949; Iisalo and Rekkarinen, 1958; Axelrod, 1964; Odajima, 1971; Valerno and McCormack, 1971) oxidations.

It is known that administration of catecholamines causes cardiovascular and haemodynamic changes, and it has been contended that these changes cannot completely account for the ultrastructural damage to the myocardium (Rosenblum, Wohl and Stein, 1967*a*, *b*; Maruffo, 1967; Ostadal, Rychterova and Poupa, 1968; Regan *et al.*, 1972). The data presented here clearly show that at least adrenochrome-induced ultrastructural changes in the myocardium can occur without any change in the ECG or blood pressure, indicating that some degree of structural disorganization of the myocardium can be tolerated without functional consequences. Thus it appears that structural changes in the heart and haemodynamic changes to a limit can occur independently of each other.

In a recent study on the isolated perfused rat hearts, it has been suggested that cardiotoxic effects of adrenochrome may involve increased free radical activity as well as interaction of these oxidation products with biological active sulphydryl groups (Singal *et al.*, 1981*b*). It is conceivable that a similar mechanism may also be responsible for the *in vivo* effects of adrenochrome. This is also consistent with the suggestion that catecholamine-induced cardiac necrosis may involve the formation of superoxide radicals (Singal *et al.*, 1981*a*). Although direct myocardial effects of adrenochrome may explain the fall in blood pressure seen in this study, the possibility of vascular changes at higher concentrations of this drug cannot be ruled out.

This study was supported by grants from The Great West Life Assurance Company and Sellers Foundation, Winnipeg.

REFERENCES

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

BLOOM, S. & CANCILLA, P. A. (1969) Myocytolysis and Mitochondrial Calcification in Rat Myocardium after Low Doses of Isoproterenol. *Am. J. Path.*, **54**, 373.

BLOOM, S. & DAVIS, D. (1974) Isoproterenol Myocytolysis and Myocardial Calcium. *Rec. Advanc. Stud. Card. Struct. Metabol.*, **4**, 581.

CARLSTEN, A. & POUPA, O. (1977) Effect of Time on the Response of Cyanide Anoxia of Frog Heart Damaged by Isoproterenol. *Pharmacol. Toxicol.*, **41**, 18.

CHAPPEL, C. I., RONA, G., BALAZS, T. & GAUDRY, T. (1959) Comparison of Cardiotoxic Actions of Certain Sympathomimetic Amines. *Can. J. Biochem. Physiol.*, **37**, 35.

CSAPO, Z., DUSEK, J. & RONA, G. (1972) Early Alterations of Cardiac Muscle Cells in Isoproterenol induced Necrosis. *Arch. Path.*, **93**, 356.

DEROUAUX, G. (1942) The *in Vitro* Oxidation of Adrenaline and Other Diphenol Amines by Phenol Oxidase. *Arch. Internat. Pharmacodyn*, **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.

FERRANS, V. J., HIBBS, R. G., BLACK, W. C. & WEILBAECHER, D. G. (1964) Isoproterenol-induced Myocardial Necrosis. A Histo-chemical and Electron Microscopic Study. *Am. Heart J.*, **68**, 71.

FLECKENSTEIN, A., JANKE, J., DÖRING, H. J. & LEDER, O. (1974) Myocardial fiber necrosis due to intracellular Ca overload—A new principle in cardiac pathophysiology. *Rec. Advanc. Stud. card. Struct. Metabol.*, **4**, 563.

HANDFORTH, C. P. (1962) Isoproterenol-induced Myocardial Infarction in Animals. *Arch. Path.*, **73**, 161.

HEACOCK, R. A., NERENBERG, C. & PAYZA, A. N. (1958) The Chemistry of the "Amino-chromes". I. The Preparation and Paper Chromatography of Pure Adrenochrome. *Can. J. Chem.*, **36**, 853.

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

KISCH, B. (1930) Die Autokatalyxe der Adrenalin-oxydation. *Biochem. Z.*, **220**, 84.

KUTUSUNA, R. (1972) Electron Microscopic Studies on Isoproterenol-induced Myocardial Lesions in Rats. *Jap. Heart J.*, **13**, 168.

KJEKSHUS, J. K. (1975) Role of Free Fatty Acids in Catecholamine-induced Cardiac Necrosis. *Rec. Advanc. Stud. Card. Struct. Metabol.*, **6**, 183.

LUFT, J. H. (1961) Improvements in Epoxy Resin Embedding Method. *J. Biophys. Biochem. Cytol.*, **9**, 409.

MARUFFO, C. A. (1967) Fine Structural Study of Myocardial Changes Induced by Isoproterenol in Rhesus Monkeys (*Macaca mulatta*). *Am. J. Path.*, **50**, 27.

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

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

OSTADAL, B., RYCHTEROVA, V. & POUPA, O. (1968) Isoproterenol-induced Acute Experimental Cardiac Necrosis in the Turtle. *Am. Heart J.*, **76**, 645.

PEARCE, R. M. (1906) Experimental Myocarditis: A Study of the Histological Changes Following Intravenous Injections of Adrenalin. *J. exp. Med.*, **8**, 400.

REGAN, T. J., MARKOV, A., KAHN, M. I., JESSANI, M. J., OLDEWURTEL, H. A. & ETTINGER, P. O. (1972) Myocardial Ion and Lipid Exchange during Ischemia and Catecholamine induced Necrosis: Relation to Regional Blood Flow. *Rec. Advanc. Stud. Card. Struct. Metabol.*, **1**, 656.

RONA, G., CHAPPEL, C. I., BALAZS, T. & GAUDRY, R. (1959) An Infarct-like Myocardial Lesion and Other Toxic Manifestations Produced by Isoproterenol in the Rat. *Arch. Path.*, **67**, 433.

ROSENBLUM, I., WOHL, A. & STEIN, A. A. (1965*a*) Studies in Cardiac Necrosis. I. Production of Cardiac Lesions with Sympathomimetic Amines. *Toxicol. appl. Pharmacol.*, **7**, 1.

ROSENBLUM, I., WOHL, A. & STEIN, A. A. (1965b) Studies in Cardiac Necrosis. II. Cardiovascular Effects of Sympathomimetic Amines producing Cardiac Lesions. *Toxicol. appl. Pharmacol.*, **7**, 9.

SEVERIN, S., SARTORE, S. & SCHIAFFINO, S. (1977) Direct Toxic effect of Isoproterenol on Cultured Cardiac Muscle Cells. *Experientia*, **33**, 1489.

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.

SINGAL, P. K., DHILLON, K. S., BEAMISH, R. F. & DHALLA, N. S. (1981*a*) Protective Effect of Zinc against Catecholamine-induced Myocardial Changes: Electrocardiographic and Ultrastructural Studies. *Lab. Invest.*, **44**, 426.

SINGAL, P. K., YATES, J. C., BEAMISH, R. E. & DHALLA, N. S. (1981*b*) Influence of Reducing Agents on Adrenochrome-induced Changes in the Heart. *Arch. Path. Lab. Med.* **105**, 664.

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

SOBEL, B., JEQUIER, E., SJOERDSMA, A. & LOVENBERG, W. (1966) Effect of Catecholamines and Adrenergic Blocking Agents on Oxidative Phosphorylation in Rat Heart Mitochondria. *Circ. Res.*, **19**, 1050.

SZAKACS, J. E. & CANNON, A. (1958) Norepinephrine Myocarditis. *Am. J. Path.*, **30**, 425.

TAKENAKA, F. (1975) Effects of isoproterenol and some other drugs on high energy phosphate metabolism in rat myocardium. *Rec. Advan. Stud. card. Struct. Metabol.*, **6**, 151.

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

WADDELL, A. W. (1961) Adrenaline, Noradrenaline and Postassium Fluxes in Rabbit Auricles. *J. Physiol.*, **155**, 209.

WAJZER, J. (1947) Synthèse du Glycogène en Présence d'Adrenochrome. *Bull. Soc. Chim. Biol.*, **29**, 237.

YATES, J. C., BEAMISH, R. E. & DHALLA, N. S. (1981) Ventricular Dysfunction and Necrosis Produced by Adrenochrome Metabolite of Epinephrine: Relation to Pathogenesis of Catecholamine Cardiomyopathy. *Am. Heart J.*, **102**, 210.

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.

YATES, J. C., TAAM, G. M. L., SINGAL, P. K., BEAMISH, R. E. & DHALLA, N. S. (1980*a*) Protection against Adrenochrome-induced Myocardial Damage by Various Pharmacological Interventions. *Br. J. exp. Path.*, **61**, 242.

YATES, J. C., TAAM, G. M. L., SINGAL, P. K., BEAMISH, R. E. & DHALLA, N. S. (1980*b*) Modification of Adrenochrome induced Cardiac Contractile Failure and Cell Damage by Changes in Cation Concentrations. *Lab. Invest.*, **43**, 316.