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ECTOPIC VENTRICULAR RHYTHMS AND MYOCARDIAL INFARCTION IN THE DOMESTIC PIG AND THEIR RESPONSE TO NIALAMIDE, A MONOAMINE OXIDASE INHIBITOR

BY

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The development in Indian domestic pigs of ectopic ventricular rhythms and a low-frequency tachycardia after the two-stage ligature of the anterior descending branch of the left coronary artery is described. When the ligature was tied 26±3 mm from the left auricular margin, the change in rhythm developed after 2 to 2.5 hr, reached a maximum after 6 hr and persisted up to 28 hr. The histopathological character of the resultant acute myocardial infarction is presented. In all, 52 pigs were used. Pretreatment of 9 pigs, with 3 as controls, with a monoamine oxidase inhibitor, nialamide, 20 mg/kg daily for 2 to 4 days, suppressed the development of experimental ventricular arrhythmia and favourably influenced the ischaemic myocardium by reducing the oedema and the formation of microthrombi. A possible mode of action involving monoamine oxidase inhibition is suggested.

The use of the dog for experimental cardiac arrhythmia has been criticized because it has a highly variable coronary circulation (Wiggers, 1952) and has more coronary anastomoses than man (Craig & Learned, 1954); this contrasts with the uniformly regular pattern in the pig (Garamella, Hay & Anderson, 1956). Among the numerous advantages of the pig are the basic genetic similarity among pigs of the same strain (Johns & Olson, 1954) and a blood lipoprotein pattern similar to the human (Barr, 1953). The pig is adaptable to extreme changes of diet, and, while the vagus plays no part in the innervation of the coronary arteries in the dog (Juhasz-Nagy & Szentiranyi, 1961), it has a pronounced effect in the pig (Dukes, 1955). Further, angina pectoris, coronary failure and acute myocardial infarction in man have been shown to be closely related to coronary artery narrowing and occlusion in the pig (Paul, Norman, Zoll & Blumgart, 1957).

Experimental narrowing of a coronary artery in the pig so frequently causes fibrillation (Zoll & Norman, 1952) that this animal has been utilized for testing drugs for antifibrillatory activity (Garamella *et al.*, 1956). Blumgart, Zoll, Freedberg & Gilligan (1950) reported that sudden marked narrowing or complete ligation of a major coronary artery promptly resulted in death of pigs in 15 min. This is not so in dogs, because of extensive anastomoses (Beck & Leighninger, 1954).

Lowenfels, Neumann, Wade, Wedel, Lord & Hinton (1956) found that, unlike the porcine heart, the canine heart withstood a high degree of coronary occlusion. Coronary arteries in man and pig are functional end-arteries, and, out of 23 pigs in which the left circumflex or one of its branches was ligated, 17 animals lived for 2 to 29 days (Paul, Norman, Zoll & Blumgart, 1957), but the incidence of arrhythmia was not studied. During a study of arrhythmia in "miniature pigs," Winbury, Hausler, Prioli & Zitowitz (1960) observed no ectopic tachycardia, but only ectopic beats, nodal rhythm or heart block. This did not favour the use of pigs for experimental arrhythmias. However, Arora & Sivappa (1962) have since developed a successful method of inducing in domestic pigs a stable ectopic ventricular low-frequency tachycardia due to acute myocardial infarction caused by a 2-stage ligation of the coronary artery. This preparation was used in the following experiments. Nialamide, a monoamine oxidase inhibitor, was used in these experiments, as hydrazide amine oxidase inhibitors are reported to reduce the extent and severity of myocardial necrosis induced by isoproterenol (Zbinden, 1961).

METHODS

Domestic pigs, bought from the local pig breeders, were bred, raised and maintained in our laboratory. A total of 52 pigs, of both sexes aged between 3 and 4 months (15 to 25 kg body weight), were used; 4 of them were used for sham-operations and 12 for the nialamide drug trials (3 as controls).

Two-stage coronary ligation. Intravenous pentobarbitone sodium (35 mg/kg body weight) anaesthesia was used for the operation. A transglottal self-retaining endotracheal rubber tube connected to an automatic respirator utilizing the ambient room air was employed for maintaining intermittent positive pressure respiration. A skin incision 10 cm long was made over the anterior part of the left fourth rib and thoracotomy performed through a corresponding incision in the pleuro-periosteal layer, a piece of rib 5 cm long being removed from the anterior end of the fourth rib. The pericardial sac was opened parallel to and in front of the phrenic nerve; the margins were retracted with stay sutures. By gentle blunt dissection the anterior descending branch of the left coronary artery was freed for a few millimetres from its deeply embedded position in the anterior interventricular sulcus, just above and behind the visible vein.

The method of ligature was based on that of Harris (1950). The first ligature of cotton thread was tied snugly over the artery and a bent steel probe (0.45 mm diameter; 26 BWG) placed alongside it, so that the artery was partially occluded to the same extent every time. The duration of this procedure averaged 20 sec. During a period of half an hour the retraction of the thoracic wall was relaxed and the opening in the chest kept covered with moist sterile gauze. The second ligature was tied tight adjacent to the first, causing complete occlusion. The wound was closed in layers in proper sequence. In order to cause consistently an ectopic ventricular low-frequency tachycardia, the ligature was invariably tied 26 ± 3 mm from the free diastolic margin of the left auricular appendage (Arora & Sivappa, 1962). Neomycin-bacitracin-sulphacetamide (Ne-Ba-Sulf, Dumex) powder was dusted over the pericardium and the layers of the chest wall before the closure of the chest, and the pneumothorax was reduced before opposing the subcutaneous tissues. The animal was allowed to recover after removal of the endotracheal tube.

After recovery, the pig was kept isolated from the others for a few days. A 4-channel ink-writing Officine Galileo electrocardiograph was used to record standard limb lead electrocardiograms during the operative procedures, particularly at the time of dissection, during the tying of the two ligatures and in the dangerous intervening half-hour. After recovery from the anaesthetic, electrocardiographic observations were made at frequent intervals, as

judged necessary from the Levinthal-Duo Trace Cardioscope, till the arrhythmia ceased completely. Four pigs were subjected to the same pre-operative and operative procedures except for the actual tying of the knots over the artery.

Nialamide trials. Nialamide (Niamid) was given intramuscularly in a daily dose of 20 mg/kg body weight. The solution was made by slight acidification with hydrochloric acid. Three groups of 3 pigs each, chosen at random, received the drug for 2, 3, and 4 days, respectively, before the operation. Another group of 3 acted as a control. All the 4 groups underwent the standard 2-stage ligation procedure. The last dose of the drug was given 4 hr before ligation. Electrocardiographic records were taken at appropriate times.

Pathological studies of the hearts. Histopathological studies were done on the hearts of the animals dying of ventricular fibrillation after the operation. The hearts of all the other pigs were removed under pentobarbitone sodium anaesthesia, immediately examined and preserved in neutral formol-saline. Histological studies were made on sections stained with haematoxylin and eosin; serial sections were taken at the level of the ligature to judge the extent of occlusion. The hearts of the nialamide-treated pigs were removed 48 hr after ligation for a similar study.

RESULTS

Ectopic ventricular arrhythmia. Sudden closure of a large branch of the left coronary artery in the pig resulted in the development of a characteristic arrhythmia of ectopic ventricular origin. The pattern of events varied to a very small degree from one pig to another, and was broadly predictable. The observed electrocardiographic changes were those generally recognized as representing varying degrees of myocardial irritability, ischaemic injury and necrosis. The genesis of arrhythmia is illustrated in Fig. 1 by representative sections clipped from continuous electrocardiographic tracings taken prior to and at different intervals after complete occlusion. Premature ventricular systoles were not common after the first partially-occluding ligature, and rarely exceeded 1% when they occurred. Very soon after the completely occluding ligature was tied, depression of the S-T segment, with an increasingly upward coving and an inversion and profound alteration in the contour of the T wave, were observed. The Q-T interval was prolonged with progressive deepening of the Q wave. The heart rate gradually increased and reached a maximum by about the sixth hour, after which it started declining in spite of the ectopic beats replacing the normal sinus beats.

The appearance of extrasystoles, increasing in frequency within the first 5 min of occlusion, invariably foreshadowed ventricular fibrillation. This was more likely if the ligature had been placed nearer the origin of the left coronary artery or if the second ligature was tied within half an hour of the first.

During the succeeding secondary phase, that is, between 0 min and 2 hr after the second ligature, there was little or no ectopic activity. This is typically represented in Fig. 2. The essential phase of ectopic rhythm began slowly 2 to 2.5 hr after the ligature with the rapid build-up of an ectopic ventricular arrhythmia. By about 4 hr all the beats could be seen to be of ectopic idioventricular origin. With few exceptions the maximum total heart rate, 140 to 172 beats/min, was attained by about the sixth hour or earlier. The fully developed extrasystolic rhythm appeared to be entirely of ventricular origin; it was difficult to find a single normal beat even in long strips.

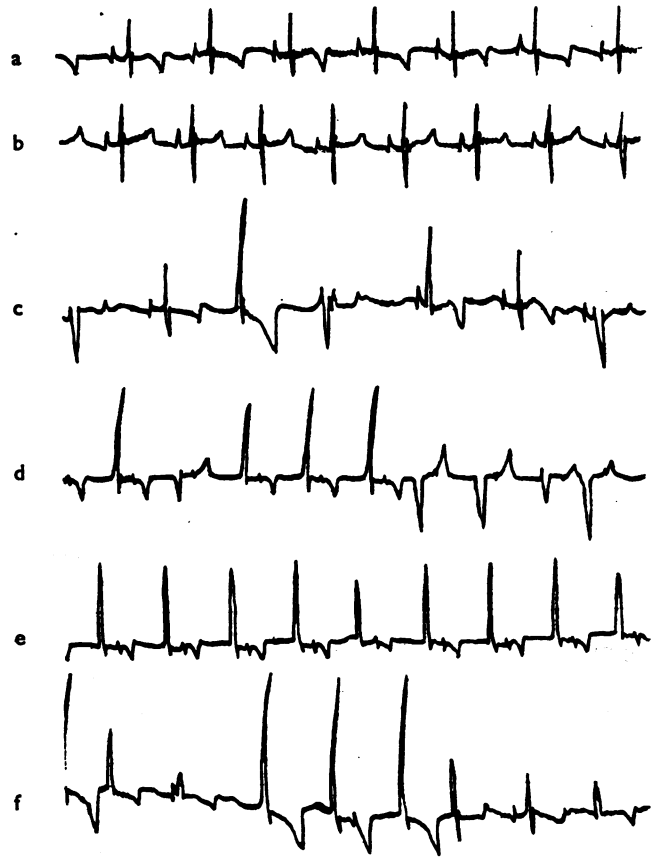

Fig. 1. Electrocardiographic development of ectopic ventricular rhythms in the pig after coronary ligation. The control electrocardiogram (*a*) was taken while the pig was under pentobarbitone sodium anaesthesia; the rest were taken after recovery from anaesthesia at varying intervals after a complete ligation; (*b*) 1.05 hr; (*c*) 4.15 hr; (*d*) 8.14 hr; (*e*) 20.30 hr; and (*f*) 48.05 hr. Time base 0.2 sec between thick vertical lines. Lead 2.

During the earlier stages of the arrhythmia it was essential to follow the cardiographic or electrocardiographic oscillations for minutes at a time. There would be no extrasystoles for a few min, but then they would appear in quick succession up to even 40 to 50/min. However, 20 to 30/min was the commonly observed ectopic rate. Sometimes, a definite sequence in the changes could be discerned. A multifocal type of rhythm was usual in the first 12 hr and gave the appearance of a ventricular focus gaining or losing the lead over the S-A node. In general, it was a low-frequency ectopic ventricular tachycardia.

However, in some hearts occasional beats of nodal or supraventricular origin were also seen. An almost completely ectopic rhythm persisted throughout a minimum period of 28 hr. By the second post-operative day the total heart rate was reduced and there were increasing intervals of sinus rhythm. It was the first 24 hr that showed the most intense ectopic activity. Even at the thirtieth hour the extrasystoles

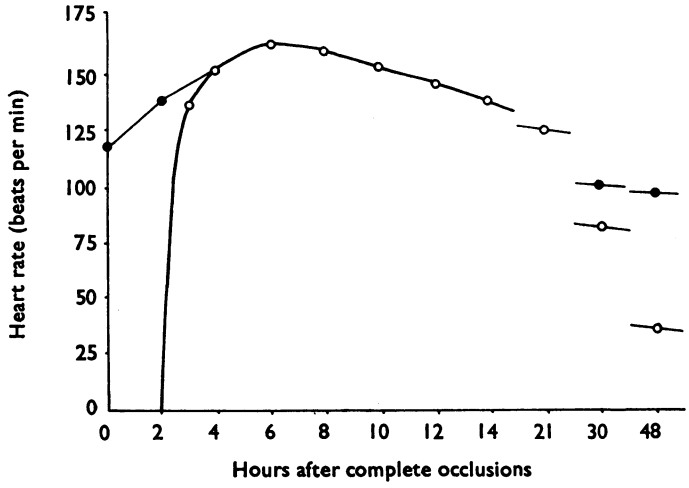

Fig. 2. Changes in the heart rate and frequency of ectopic beats after a complete coronary ligation. Ordinate: heart rate (beats/min). Abscissa: hr after complete occlusion. The total heart rate (sinus beats – ● –, premature systoles – ○ –) is plotted against the time interval after complete coronary occlusion.

were coming in groups accounting for more than 75% of the total heart rate. During the later stages an intermittent appearance of outbursts of ectopic beats reproduced a pattern apparently similar to that noticed at the earliest period between the second and fourth hours.

The level of occlusion. The chances of provoking a reproducible and constant ectopic ventricular rhythm, after ligation of the anterior descending branch of the left coronary artery, depended upon the site of occlusion, as shown in Table 1. This determined the calibre of the vessel ligated, and thus the extent of the involved part of the heart.

Ventricular fibrillation. Of the four pigs with the ligature placed very near the auricles, as used by Harris (1950) in the dog, none lived for more than 5 min, most of them dying between 1.2 and 3.1 min after ligation. The importance of duration

TABLE 1

EFFECT OF THE SITE OF LIGATURE OF THE ANTERIOR DESCENDING BRANCH OF THE LEFT CORONARY ARTERY ON CARDIAC RHYTHM AND MORTALITY

No. of pigs	Distance of ligature from the atrial margin (cm)	Result	Deaths	Mortality (%)
4	1·1–2·0	Irreversible quick ventricular fibrillation	4	100
8	2·1–2·5	Reversible ventricular fibrillation	3	37·5
12	2·5–3·0	Complete ectopic ventricular rhythm	1	8·3
6	3·1–3 5	Ectopics: 30 to 42%	0	0
4	3·5–4·0	Ectopics: 1 to 6%	0	0
2	4·1–4·5	Occasional ectopics	0	0

of occlusion on the development of ventricular fibrillation has also been highly impressive. The period of susceptibility to early fibrillation was between 2 and 15 min after complete occlusion. It was initiated by an increasing number of ventricular extrasystoles, gradually progressing to paroxysmal ventricular flutter and frank fibrillation. With the set of 8 pigs ligated at the higher level between 2.1 and 2.5 cm, ventricular fibrillation could be reversed by promptly untying the ligature. Out of 8 pigs ligated, 5 were saved by removing the occlusion within this critical period.

Influence of nialamide on the ectopic rhythms. The influence of nialamide on the pigs undergoing the 2-stage ligation was briefly one of a graded restraint on the development of arrhythmia. This is shown in Fig. 3 and Table 2. The control

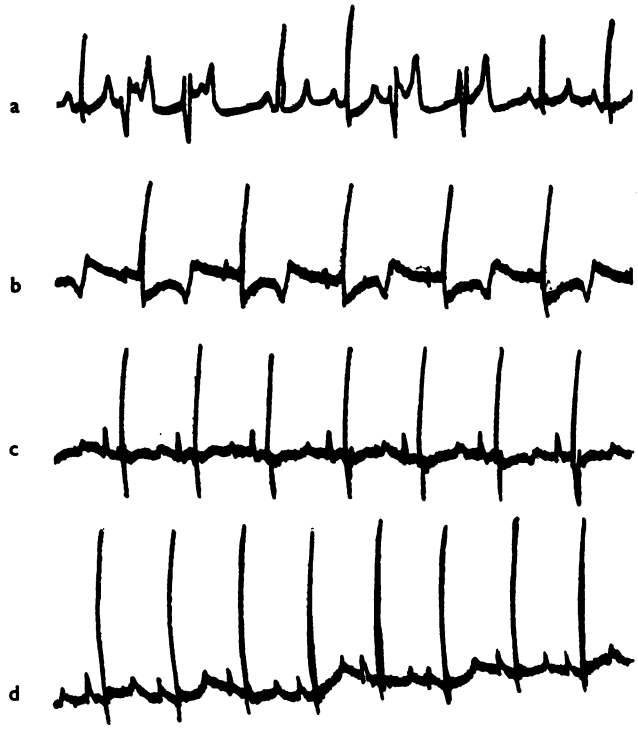

Fig. 3. Influence of pretreatment with nialamide on experimental ectopic ventricular rhythms. Lead 2 electrocardiograms taken 24 hr after coronary ligation in control pigs and in pigs treated with nialamide 20 mg/kg body weight injected intramuscularly daily, (*a*) control, untreated, (*b*) treated for 2 days, (*c*) treated for 3 days, (*d*) treated for 4 days. Time base 0.2 sec between thick vertical lines.

group generally showed the fastest total heart rate, the greater proportion of ectopic beats and the most marked myocardial changes. The group receiving the drug for 2 days prior to ligation showed a definite discernible response by a reduction in the number of ectopic beats and in the severity of the myocardial changes. In the group which had received the drug for 3 days before operation, the improvement

TABLE 2

INFLUENCE OF PRETREATMENT WITH NIALAMIDE ON THE POST-OCCLUSIVE HEART RATE, NUMBER OF ECTOPIC BEATS AND MYOCARDIAL NECROSIS IN THE PIG

No. of pigs	No. of daily doses of nialamide 20 mg/kg	Average heart rate (range) /min	Average ectopics (%)	Myocardial necrosis grading
3	0	152 (140–160)	100·0	++++
3	2	140 (131–159)	19·6	+++
3	3	131 (129–138)	1·6	+++
3	4	126 (120–128)	0	++

was more marked; this was shown by a drastic shortening in the duration of arrhythmia or by its complete suppression, as occurred in the 4-day treated group.

Pathological studies of the hearts. (1) Macroscopic appearances. The hearts of all the ten pigs dying shortly after ligation were available for study. None of the remaining animals died except the one which died 6 days after developing arrhythmia. This pig showed, *post mortem*, some pulmonary congestion and a little fluid in the pericardium. The other pigs were killed at different intervals after ligation.

The earliest detectable gross alteration was seen, 3 hr after ligation, as a pale, swollen and irregular area of ischaemia. After 4 hr occlusion the heart had a clay-coloured appearance with an outer yellow margin, which became more pronounced after a week. Roughening of the pericardium occurred 20 hr after occlusion together with a dimpling of the central part of the infarcted area. Ventricular rupture was

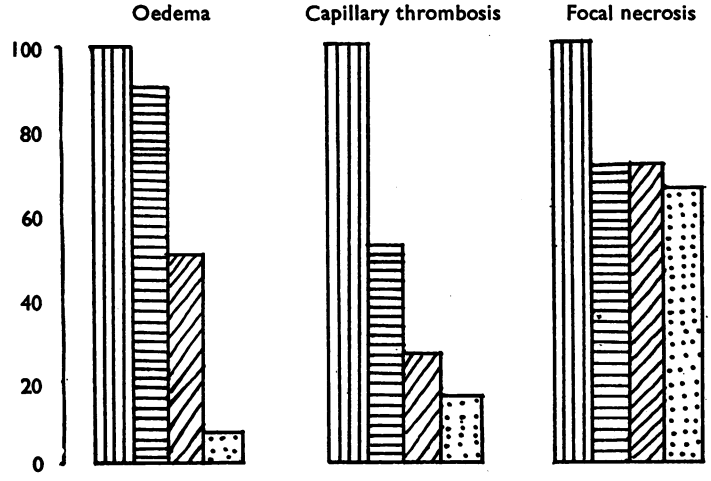

Fig. 4. The effect of nialamide pretreatment on oedema, focal necrosis and capillary thrombosis in myocardial infarction in the pig. Quantitative estimates of various changes observed from a histopathological study of the infarcted area 48 hr after coronary ligation in the pigs. Vertical-lined columns denote the control untreated pigs, the full height representing a 100% lesion; similarly, the horizontal-lined columns denote the 2-days treated, diagonal-lined columns the 3-days treated, and the dotted columns the 4-days treated pigs (mean of 3 pigs for each experiment).

not seen even in those extensive infarcts which were followed later by fibrosis. Total infarction of the wall was observed in the pigs surviving sufficiently long, usually a day. The entire heart, including the infarcted muscle, was stiff and contracted in those dying with ventricular fibrillation, but in others the infarct was very soft and almost liquefied. The line of demarcation between the necrotic and normal tissue

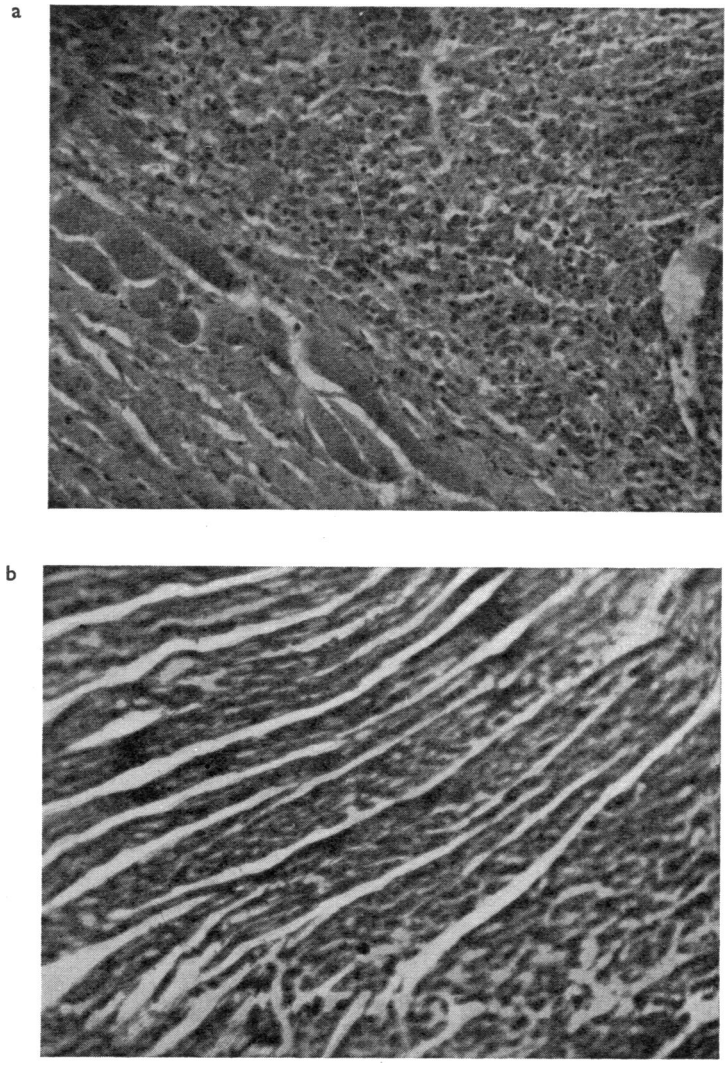

Fig. 5. (*a*) Pig. Sections from the heart removed 24 hr after ligature of the coronary artery. Upper right: infarcted area showing myocardial necrosis and leucocytic infiltration. The muscle fibres are deeply stained with eosin and the outlines of the muscle bundles are indistinct. Lower left: edge of the infarct containing a few normal muscle fibre bundles. H. and E. Magnification ×250. (*b*) Pig. Section of ventricular musculature taken at the periphery of the infarct. Note the almost normal appearance of the myocardial fibres. Nialamide-treated for 4 days. H. and E. Magnification ×250.

was most clearly seen on the endocardial surface, and where sections were taken across both infarcted and healthy tissue. The inner part of the wall was less affected than that lying beneath the pericardium. Hearts from pigs surviving more than 6 weeks after occlusion showed very thin infarcted areas, sometimes as little as about 3 mm thick, consisting of tough fibrous tissue. The pericardial surface showed an organized pericarditis.

(2) Microscopic appearances. The microscopic changes largely corresponded with the age of the infarct. The hearts of pigs dying within 1 or 2 hr generally presented an almost normal appearance. Those dying after 3 hr of permanent occlusion consistently showed leucocytic infiltration, increasing acid staining of fragmented and hyaline muscle fibres and vacuolation of the nuclei. The earliest change noted was a hyalinization of the cytoplasm, loss of striation in the muscle fibres and pyknosis, karyorrhexis and karyolysis of the nuclei. The more centrally situated part of the infarct showed the most severe changes, including loss of muscle striations together with vacuolation, swelling and smudging of the outline of the cells.

Influence of nialamide on myocardial infarction. (1) Macroscopic appearances. It was remarkable that, while the pigs without the drug showed macroscopic thinning and infarction of the wall of the left ventricle, none of the drug-treated animals showed any external changes; though changes due to infarction were seen microscopically. The difference in the macroscopic appearances of the hearts of the treated and untreated animals was reflected in the fact that the former showed complete ventricular beats, and did not bulge passively as those in the control group, even though a slightly cyanotic area of myocardium appeared.

(2) Microscopic appearances. The microscopic appearances of the heart muscle in the treated group showed negligible oedema in the marginal area, less capillary dilatation and the pericardium was free from leucocytic infiltration. It was difficult to detect microthrombi in the peripheral area, and haemorrhage and vascular congestion were almost absent. At 4 days, when changes due to infarction should be most severe, the myocardial fibres in the treated group retained almost their original structure. A quantitative assessment of the histopathological appearances in the infarcted areas in the treated and control series is illustrated in Figs. 4 and 5.

DISCUSSION

Many methods have been employed for causing slow coronary occlusion in laboratory animals (Garamella *et al.*, 1956). Multiple variables in a seemingly simple procedure influence the mortality (Blumgart, 1959; Garamella *et al.*, 1956). The degree and the duration of narrowing are the most vital factors (Zoll & Norman, 1952). In order to minimize the variables the 2-stage ligation technique was convenient as it could be carried out at a single sitting with the ligature at a precise level. From preliminary experiments (Arora & Sivappa, 1962) it was found that the time interval could be kept down to 30 min, but the site of the ligature had to be not less than 26 ± 3 mm from the free diastolic margin of the atrium; fatal ventricular fibrillation occurred when the ligature was placed above this. The fact that the ectopic rhythms developed were slower when the ligature was placed more

peripherally favours the conception that a critical ischaemia, a state created by a disturbed myocardial vascular demand–supply relationship, may be a determining factor.

The post-ligation heart rate rarely exceeded 150 to 172/min, about 15% more than the control rate. It was thus a low-frequency tachycardia, and even this continued only for a brief period averaging 4 hr. The " miniature pig " did not show a tachycardia after such a ligation (Winbury *et al.*, 1960), probably due to the species difference and to a greater narrowing of the artery in the first stage in our study. The lack of marked post-occlusion tachycardia in the pig could be due to the considerable vagal control, similar to what occurs in surviving human coronary thrombosis. The changes in the S-T segments and T waves in the electrocardiograms usually continued until 9 to 48 hr after ligation.

Free wall and bundle branch blocks were frequently met with, showing bigeminal, trigeminal and other rhythms. The ectopic ventricular low-frequency tachycardia in the domestic pig was quicker in onset, short-lived in duration and subsided earlier.

Although the porcine experimental arrhythmia is similar to its human counterpart in the sequence of infarction followed by arrhythmia, it is multifocal and possesses a rate close to the sinus rate. Further, it is self-limiting and does not often prove fatal if the occlusion is left unreleased.

No gross changes were seen in the heart for periods of ligation under 15 min. Maximum changes occurred when the occlusion lasted for 20 min or more. The infarcted area was definitely demarcated from the normal on the tenth day. In the five and six weeks infarcts the scar was remarkably thinned out and fibrosed. Usually by about the sixth week, the infarct was replaced by granulation tissue and finally by a collagenous scar.

Pretreatment of pigs with nialamide (20 mg/kg daily for 4 days) before the 2-stage coronary ligation strikingly diminished the severity and duration of ectopic arrhythmia. However, the beneficial influence on the heart rate and rhythm was not equally reflected in the extent of the infarct. A quantitative estimate of the infarct showed an average reduction of 93% in oedema and 84% in capillary thrombosis in 4-day nialamide-pretreated groups, while under the same conditions the average reduction in focal necrosis was only 34%.

A number of possibilities have been suggested for the beneficial effect of monoamine oxidase inhibitors in ischaemic heart disease. Shimamoto, Ishioka & Fujita (1961) have suggested a new type of antithrombotic effect of nialamide on the rabbit ear vessels, and Shimamoto, Yama-Zaki, Tsuchihashi, Sunaga & Ishioka (1961) report that nialamide saved all of 12 patients with transmural myocardial infarction without relapse.

Thus the drug may act by preventing the process of infarction itself. A lack of significant reduction in the extent of the infarct observed in our series of experiments argues against vasodilatation being the only mode of action. Further, in this study isoniazid, a hydrazide diamine oxidase inhibitor (Boyer, Lardy & Myrback, 1960), was not found to afford any protection of the myocardium from damage in pigs with a ligated coronary artery. Possibly nialamide acts by causing

an accumulation of monoamines in cardiac muscle, and it is this which protects the myocardium from further damage.

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REFERENCES

ARORA, R. B. & SIVAPPA, D. S. (1962). Ectopic ventricular rhythm following coronary occlusion in the pig. *J. Pharm. Pharmacol.*, **14**, 315–316.

BARR, D. P. (1953). Some chemical factors in the pathogenesis of atherosclerosis. *Circulation*, **8**, 641–654.

BECK, C. S. & LEIGHNINGER, D. S. (1954). Operations for coronary artery disease. *J. Amer. med. Ass.*, **156**, 1226–1233.

BLUMGART, H. L. (1959). Anatomy and functional importance of intercoronary arterial anastomoses—Editorial. *Circulation*, **20**, 812–815.

BLUMGART, H. L., ZOLL, P. M., FREEDBERG, A. S. & GILLIGAN, D. R. (1950). The experimental production of intercoronary anastomoses and their functional significance. *Circulation*, **1**, 10–27.

BOYER, P. D., LARDY, H. & MYRBACK, K. (1960). *The Enzymes*, Vol. 2. New York: Academic Press.

CRAIG, R. L. & LEARNED, B. B. (1954). Patterns of the anterior descending branch of the left coronary artery in the dog. *Amer. Heart J.*, **48**, 455–458.

DUKES, H. H. (1955). *Physiology of Domestic Animals*, 7th ed., p. 131. New York : Comstock Publishing Co.

GARAMELLA, J. J., HAY, L. J. & ANDERSON, J. G. (1956). Studies in ventricular fibrillation in the pig: Evaluation of an antifibrillatory agent tested by closed chest coronary occlusion. *S. Forum*, **7**, 298–301.

HARRIS, A. S. (1950). Delayed development of ventricular ectopic rhythms following experimental coronary occlusion. *Circulation*, **1**, 1318–1328.

JOHNS, T. N. P. & OLSON, B. J. (1954). Experimental myocardial infarction. I. A method of coronary occlusion in small animals. *Ann. Surg.*, **140**, 675–682.

JUHASZ-NAGY, A. & SZENTIRANYI, M. (1961). Separation of cardio-accelerator and coronary vasomotor fibres in the dog. *Amer. J. Physiol.*, **200**, 125–129.

LOWENFELS, A. B., NEUMANN, C. G., WADE, W. H., VAN WEDEL, J., LORD, JR., J. W. & HINTON, J. W. (1956). The effects of gradual occlusion of the coronary arterial circulation in dogs and pigs. *S. Forum*, **7**, 302–306.

PAUL, M. H., NORMAN, LEONA R., ZOLL, P. M. & BLUMGART, H. L. (1957). Stimulation of inter-arterial coronary anastomoses by experimental coronary occlusion. *Circulation*, **16**, 608–614.

SHIMAMOTO, T., ISHIOKA, T. & FUJITA, T. (1961). New type of antithrombotic effect of monoamine oxidase inhibitor compared with anticoagulant. Abstracts 34th Sci. Session, Amer. Heart Ass. *Circulation*, **24** (Pt. II), 1050.

SHIMAMOTO, T., YAMA-ZAKI, H., TSUCHIHASHI, H., SUNAGA, T. & ISHIOKA, T. (1961). Application of antithrombotic effect of nialamide in arteriosclerotic disorders including myocardial infarction. Abstracts 24th Sci. Session, Amer. Heart Ass. *Circulation*, **24** (Pt. II), 1040.

WIGGERS, C. J. (1952). The functional importance of coronary collaterals. *Circulation*, **5**, 609–615.

WINBURY, M. M., HAUSLER, L. M., PRIOLI, N. A. & ZITOWITZ, L. (1960). Effects of coronary occlusion on the chronotropic action of epinephrine in the miniature pig. *Fed. Proc.*, **19**, 88.

ZBINDEN, G. (1961). Effect of anoxia and amine oxidase inhibitors on myocardial necrosis induced by isoproterenol. *Fed. Proc.*, **20**, 128.

ZOLL, P. M. & NORMAN, LEONA R. (1952). The effects of vasomotor drugs and of anaemia upon intercoronary arterial anastomoses. *Circulation*, **6**, 832–842.