Creatine Kinase Isoenzymes in Serum of Pigs Having Myocardial and Skeletal Muscle Necrosis

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ABSTRACT

Serum creatine kinase (CK) and lactic dehydrogenase (LD) isoenzyme activities were measured in blood serum of pigs having myocardial damage and skeletal muscular lesions. Myocardial and muscular damage was induced by restraint stress provoked by intravenous infusion of a pharmacological restraint (succinylcholine-chloride) during 12 minutes.

Pigs of Swedish Landrace and Swedish Landrace × Yorkshire breed, stress-susceptible (halothane-sensitive) and nonreacting pigs were studied. Severe myocardial damage and slight to moderate skeletal muscle necrosis were found 24 hours after restraint stress in the stress-susceptible pigs whereas in nonreacting pigs generally only myocardial lesions of moderate extent were registered. No significant increase was detected in the serum CK-BB (CK-1) or CK-MB (CK-2) activity whereas a pronounced elevation of the CK-MM (CK-3) activity was found, particularly in the stress-sensitive animals.

In the myocardial tissue of pigs only a low CK-MB activity was found (about 4-5% CK-MB in addition to CK-MM) and this may explain the low CK-MB activity in serum of pigs subjected to severe myocardial damage. This is further supported by the pronounced increase in the anodal serum fractions LD 1-2 in animals free from skeletal muscular lesions. In the halothane-sensitive pigs skeletal muscle necrosis besides the myocardial lesions contributed to the high levels of CK-MM activity in serum.

RÉSUMÉ

Cette expérience visait à mesurer l'activité des isoenzymes de la créatine kinase (CK) et de la déshydrogénase lactique (LD), dans le sérum de porcs dont le myocarde et les muscles squelettiques affichaient des lésions dégénératives imputables à un stress de contrainte provoqué par l'infusion intraveineuse de chlorure de succinylcholine, durant 12 minutes.

L'expérience portait sur des porcs Landrace et Landrace-Yorkshire suédois, sensibles au stress à l'halotane, et sur des témoins. Vingt-quatre heures après le stress de contrainte, on constata, chez les porcs susceptibles, la présence d'une nécrose marquée du myocarde, mais seulement légère ou modérée, dans les muscles squelettiques; les témoins ne présentaient par ailleurs qu'une nécrose modérée du myocarde. On ne décela pas d'élévation appréciable de l'activité sérique de CK-BB (CK-1) ou de CK-MB (CK-2); on enregistra toutefois une élévation marquée de l'activité sérique de CK-MM (CK-3), surtout chez les porcs sensibles au stress de contrainte.

Dans le myocarde des porcs, on enregistra une faible activité de CK-MB d'environ 4-5%, en plus de celle de CK-MM; cette constatation pourrait expliquer la faible activité de CK-MB dans le sérum des porcs dont le myocarde affichait beaucoup de nécrose. L'augmentation prononcée des fractions sériques anodiques LD 1 et 2, chez les témoins, appuierait cette hypothèse. Chez les porcs sensibles à l'halothane, la nécrose des muscles squelettiques, en plus de celle du myocarde, contribua à élever le niveau de l'activité du CK-MM sérique.

INTRODUCTION

The diagnosis of myocardial infarction in man has been based, classically, on a history of chest pain, electrocardiographic documentation and elevated serum enzymes. The most frequently measured enzymes are lactic dehydrogenase (LD) and creatine kinase (CK). The search for specific myocardial markers has led to improved techniques for the quantitative analysis of the isoenzyme of LD and CK.

Elevated serum-LD activity is a sensitive but nonspecific indicator of myocardial and skeletal muscle damage in man since LD enzymes are found in many organs and tissues. Nevertheless the stable increase in the anodal LD fractions (LD 1-2), (due to the long half-life of these isoenzymes), detectable in acute myocardial infarction (AMI) in man is advantageous and normally useful in the diagnosis of such disorders. The CK-MB is highly specific for the heart's mus-

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cular tissue and a relative CK-MB activity of about 20-30% of the total CK activity has been demonstrated in man (16, 20). Quantitation of the cardio-specific enzyme CK-MB is therefore the most sensitive and specific indicator available for the diagnosis of an acute myocardial infarction in man.

Restraint stress may be one of the major stress factors in the porcine stress syndrome (PSS) in connection with the transporting and slaughter handling procedures of the pigs. Sudden death is not exceptional among pigs exposed to such stress situations and severe acute cardiomyopathy has been demonstrated in these pigs (9). Severe mental stress can be produced in animals subjected to different kinds of immobilization. Restraint stress under experimental conditions provoked by a pharmacological restraint (succinylcholine) also caused myocardial and skeletal muscular damage in pigs (10, 11, 12). Pigs sensitive to halothane anaesthesia (animals classified as sensitive to halothane-anaesthesia reacting with malignant hyperthermia, pathological lactate production and skeletal muscular rigidity) show a higher ability to develop PSS than non-sensitive pigs. In stress-susceptible pigs, more pronounced myocardial and skeletal muscular lesions were occasioned by experimental restraint stress than in halothane-negative pigs (nonreactants) (12).

The purpose of the present investigation was to evaluate the changes in CK and LD isoenzyme activities in serum of pigs exposed to restraint stress. The influence of myocardial damage on the isoenzyme activities during a 24-hour period following the stress procedure and difference between stress-susceptible (halothane-sensitive) and nonreacting pigs in this respect was investigated.

MATERIALS AND METHODS

ANIMALS

The pigs used were of Swedish Landrace and crossbreeds of Swedish Landrace and Yorkshire, both females and castrated males, 70-80 kg in bodyweight (five to six months of age). The animals were tested with halothane-anaesthesia at ten weeks of age and nine of the pigs were classified as stress-susceptible (HP);[1] six pigs were classified as nonreactors (HN).[1]

To produce restraint stress in the animals, synthetic short-acting succinylcholine-chloride, Celocurin® was administered by intravenous injection for 12 min according to Johansson *et al* (10). Gross muscle relaxation was achieved with respiratory activity maintained. The animals were slaughtered with a bolt pistol 24 hours after the restraint procedure and necropsy took place immediately. Tissue samples were taken from the heart and skeletal muscles for examination by both light and electron microscopy.

BLOOD SAMPLING

A vein catheter was inserted two days before the experimental procedures to avoid exciting the animal when blood was collected. The catheter was inserted via an ear vein into the vena cava cranialis by means of a Seldingar guidewire (Stille, Stockholm). Blood samples for CK and LD analysis were drawn at rest prior to the restraint stress, and six, 12 and 24 hours after the procedure.

Blood was collected in glass tubes and stored at +8°C for up to three hours. Then the serum was separated by centrifugation at 6000 rpm for 10 min and stored at -70°C until analysed. Serum from human subjects taken during the course of AMI was kindly supplied by the department of Clinical Chemistry at Huddinge Hospital, Huddinge, Sweden. Human serum was also obtained from healthy volunteers at the Faculty of Veterinary Medicine in Uppsala.

TISSUE SPECIMENS

Fresh tissue samples for CK and LD isoenzyme separation were obtained from porcine heart and skeletal muscles (M. longissimus dorsi and M. semitendinosus) and human heart muscular tissue[2] and further prepared and homogenized according to the methods for CK isoenzyme assay described earlier (22).

DETERMINATION OF LD AND CK ISOENZYMES

In serum and tissue homogenates the CK activities were assayed and electrophoretic separation of the CK isoenzymes was performed on prepared gel plates (Corning, Contron, Stockholm) according to methods described earlier (22). The electrophoretic separation of LD isoenzymes was performed in agarose gel according to Hyldegaard-Jensen (7). The total LD activity was measured spectrophotometrically in an LKB reaction analyser 8600 at 37°C by means of LD-A enzyme reagents (ADA, Stockholm), as recommended by the enzymes committee of the Scandinavian Society for Clinical Chemistry.

In order to obtain optimal conditions for the electrophoretic procedures, the total CK activity of samples applied to the gel plates did not exceed 30 μkat/L.[3]

The detection limit for the CK isoenzymes was determined by electrophoretic separation of dilutions of known activity from CK-MM preparations from porcine skeletal muscular tissue and from purified CK-MB preparations from human heart tissue (kindly supplied from the Dept. of Clinical Chemistry, Huddinge Hospital, Sweden).

MORPHOLOGICAL EXAMINATION

Macroscopic evaluation of the hearts was performed by dissecting the myocardium and coronary vessels. For the histopathological examination, several tissue blocks were taken from the papillary muscles of the left ventricular wall

[1]HP = Halothane-positive, HN = Halothane-negative.
[2]Human tissue was obtained from the Department of Pathology, University of Uppsala, Sweden.
[3]According to the System International (SI-system) enzyme activities are expressed in μkat/L (1 μkat/L = 60 U/L).

and the lower and the upper halves of the interventricular septum. Representative samples were also taken from the right ventricular wall. These samples were fixed in 10% neutral formalin. Other tissue blocks were snapfrozen in a cryostat for succinic dehydrogenase reaction. The fixed tissue was embedded in paraffin wax and cut into 3 μm thick sections. These were stained with hematoxylin and eosin, Mallory's phosphotungstic acid hematoxylin (PTAH) and Masson's trichrome. The degrees of myocardial damage were graded on a scale of 0 to 5 using the following criteria: 5, presence of grossly visible pale areas of myocardial damage of varying size including severe necrotic changes at histological examination; 4, extensive confluencing foci of necrosis represented by foci of weak discoloration; 3, isolated foci of necrotic myocardial cells; 2, necrosis of individual myocardial cells in more than 50% of the slides examined; 1, necrosis of individual myocardial cells in less than 50% of the slides; 0, no necrosis.

In the HP pigs (nos. 1 to 6) and in the HN animals (nos. I to III) (Table I) six to ten specimens from the two semimembranous muscles were used for histological examination. In the rest of the pigs six to ten samples of 21 skeletal muscles of the following muscle groups were examined: muscles of the neck, pectoral girdle, flexors and extensors of the shoulders, forelimbs and thighs. Specimens were also taken from psoas muscles, diaphragm, muscles of the abdominal and thoracic walls and muscles of the back. The formalin-fixed tissues were embedded in paraffin wax, cut, and stained with haematoxylin-eosin and Masson's trichrome. Microscopic evaluation followed the scheme of Bethlem (1) and grading ranging from no change (representing no discernible morphological alteration), slight, moderate, to severe (representing severe changes) was applied to each specimen (Table I).

STATISTICAL METHODS

The severity of the myocardial and skeletal muscle necroses and the LD and CK activities in serum 24 hours after the restraint stress were correlated according to the Spearman rank correlation test.

Differences between mean values for LD, CK and related isoenzymes in different groups were evaluated by Student's t-test. The statistical calculations are based upon the logarithmic values (^{10}ln) for CK activity (μkat/L).

RESULTS

MORPHOLOGICAL FINDINGS

The degree of myocardial and skeletal muscular damage in nine HP and six HN pigs 24 hours after restraint stress is demonstrated in Table I.

In the HP pigs, myocardial lesions varying from isolated foci of necrosis (graded 3) to grossly visible pale areas (graded 5) were seen after the stress procedure (Figs. 1-3). In the HN animals confluencing foci of necorsis of the myocardial tissue was demonstrated in only two pigs (graded 4). In the other HN pigs only necrosis of individual cells (graded 1-2) were detected (Table I).

At macroscopical examination of the myocardium, multiple pale and yellow-brown patches and areas of necrosis were observed in the ventricular myocardium of three HP pigs (grade 5) (Figs. 1 and 2, Table I). Tissue samples, graded 4, revealed disseminated and confluencing small foci of weak discoloration of the cardiac muscle. Myocardial alterations of lower grade were only seen at the histopathological examination (Fig. 3). The appearance of the myofibrillar damage is not dealt with here, as this has been described previously (11).

Among the skeletal muscular groups examined, the muscles of the back and the flexor muscles of the shoulders showed the most pronounced lesions. In the skeletal muscles, morphological changes varying from slight to severe were demonstrated in the halothane-sensitive pigs (Table I, Figs. 4-6). In the HN pigs no muscular lesions could be demonstrated in three animals (specimens were taken only from M. semimembranosus),

TABLE I. Extent of Cardiac and Skeletal Muscle Necrosis in Halothane-sensitive and Nonreacting Pigs, 24 Hours After Restraint Stress

Halothane-sensitive pigs (HP)	Pig no.	1	2	3	4	5	6	7	8	9
	Extent of cardiac necrosis	4	4	5	4	5	3	5	3	3
	Extent of skeletal muscle necrosis	slight[a]	slight[a]	slight[a]	moderate[a]	slight[a]	severe[a]	severe	severe	severe
Nonreacting pigs (HN)	Pig. no.	I	II		III	IV	V		VI	
	Extent of cardiac necrosis	4	2		2	1	1		4	
	Extent of skeletal muscle necrosis	no[a]	no[a]		no[a]	slight	moderate		slight	

[a]Only M. semimembranosus examined

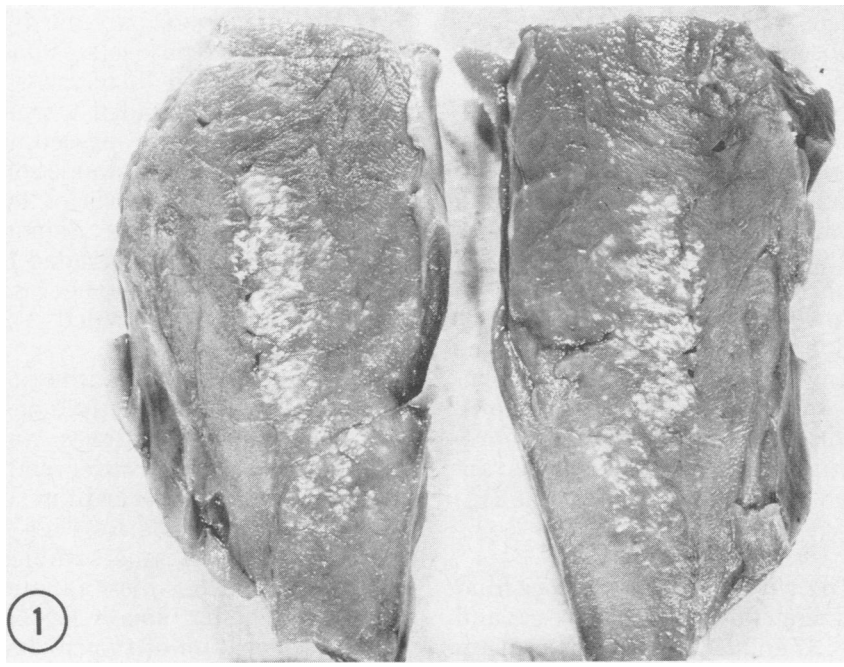

Fig. 1. Multiple confluencing pale foci in the left ventricular myocardium of pig number 7.

whereas slight to moderate alterations were demonstrated in various skeletal muscle groups in the other HN pigs (Table I). Muscle specimens graded slight showed mild focal alterations. In most examples of this group the only recognizable changes were nuclear alterations and loss of striation in association with either granular or hyaline degeneration. Animals showing more pronounced muscular changes in either distribution or severity of alterations with more advanced myofibral damage characterized by myolysis, calcifications and signs of reparative processes, were graded moderate (Fig. 5). Specimens in four cases designated severe displayed extreme changes (Fig. 6).

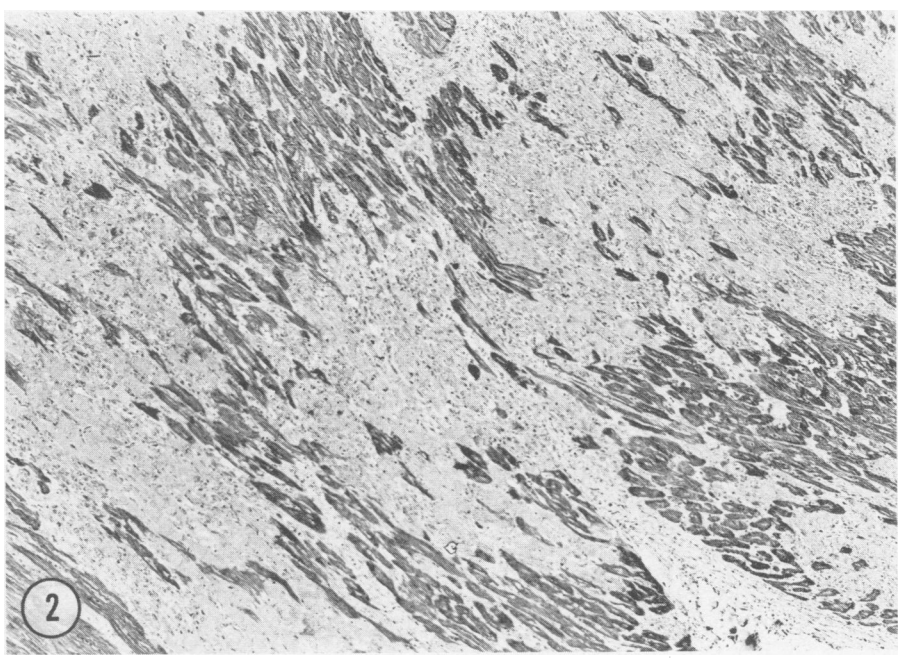

Fig. 2. Microscopical picture of the pale foci demonstrated in Fig. 1. The dark-stained fibers are normal whereas the faintly stained areas consist of fibers in different stages of degeneration. Masson's trichrome. X63.

CK AND LD ISOENZYME ACTIVITY

The detection limit for porcine CK-MM and human CK-MB was approximately 0.5 μkat/L after electrophoretic separation of the control samples.

The CK isoenzyme patterns after electrophoretic separation for heart muscular tissue in both man and pig are shown in Fig. 7b. It is evident that the proportion of CK-MB is higher (about 30%) in human than in porcine myocardium (about 5%). Furthermore only CK-MM activity was demonstrated in porcine skeletal muscular tissue (20). In porcine skeletal muscles (M. semitendinosus, biceps and longissimus dorsi) there is a predominance of LD-4 and LD-5 isoenzymes, whereas in the porcine heart muscular tissue the main activity is found in the LD-1 and LD-2 fractions (Fig. 7a).

The CK and LD activities gradually increased during the first day after stress, with the highest values after 24 hours. Therefore these are the only enzyme activities after the stress procedure presented in this report. The CK isoenzyme patterns in sera of the HN and HP pigs at rest and 24 hours after restraint stress are demonstrated in Table II. The total CK and CK-MM activities were significantly elevated, ($p < 0.001$), the CK-BB was slightly elevated, whereas the CK-MB activities remained low, not exceeding 1.0 μkat/L, 24 hours after stress in normal and stress-susceptible pigs.

In the present study higher total CK and CK-MM activities were demonstrated in HP than in HN pigs at rest and 24 hours after stress (Table II). This is described and have been commented upon also in earlier reports (19, 23).

There was a significant elevation in the total-LD and in all of the LD isoenzyme fractions in the HP pigs 24 hours after stress, compared with the values at rest, whereas only a slight significant difference in some of the LD frac-

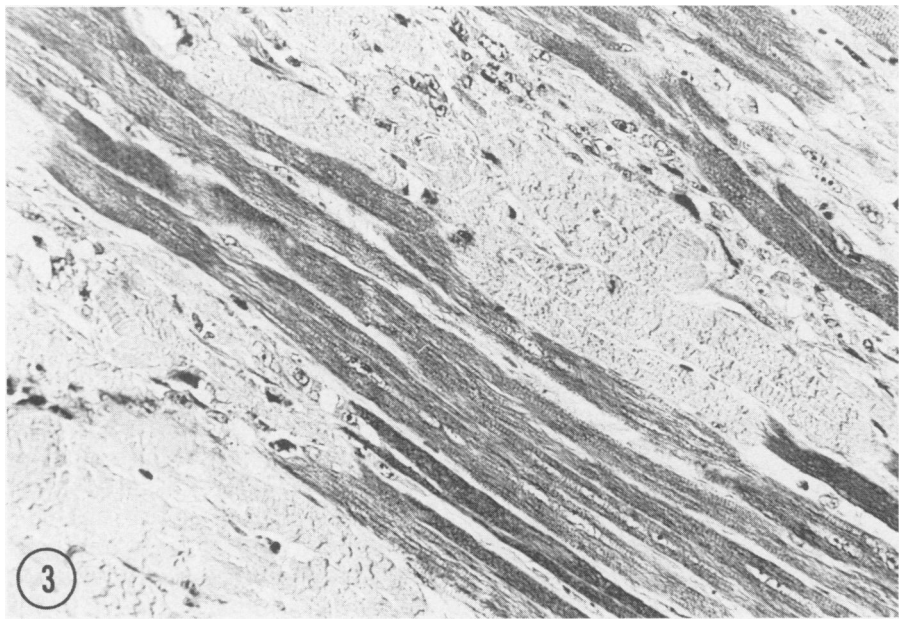

Fig. 3. Left ventricular myocardium of pig number 9. Necrotic tissue with contraction bands is well demarcated. There is some proliferation of macrophages and fibroblasts. Masson's trichrome. X320.

tions at rest and 24 hours after stress were demonstrated in the HN pigs (Table II). There was no significant difference in the LD activities between HP and HN pigs at rest, whereas pronounced differences were recorded following stress (Table II). Thus HP pigs had significantly higher total-LD, LD-4 and LD-5 activities than the normal pigs 24 hours after stress.

The correlation between the total CK and CK-MM activities and the severity of the skeletal muscular necroses (graded from zero to severe) was highly significant ($p < 0.001$) (Spearman rank coefficient, $r_s = +0.86$) whereas no significant correlation was obtained between the total-CK, CK-MM or CK-MB activities and the severity of myocardial necrosis (graded 0.5) when all pigs were concerned. Furthermore there was a highly significant correlation between total-LD and LD-5 activities and the severity of the skeletal muscle damage. No significant correlation was demonstrated between the severity of myocardial damage and the total LD or any LD-isoenzyme activities when all pigs were concerned (Table II).

In order to demonstrate the effect of myocardial damage on the CK and LD isoenzyme activities in serum, three HN pigs free from skeletal muscular necrosis were compared with three HN pigs with slight or moderate necrosis in different muscle groups 24 hours after stress (Table III). All pigs showed moderate to severe myocardial damage. Pigs with skeletal muscular necrosis had higher total CK and CK-MM activity values than pigs without skeletal muscular damage. Furthermore, significantly higher total-LD, LD-1, LD-3, LD-4 and LD-5 values were recorded after stress in pigs with skeletal muscular lesions, compared with pigs subjected to myocardial lesions only.

Furthermore, in pigs with myocardial damage only, the total-CK and CK-MM values were significantly elevated 24 hours after stress, compared with the levels at rest, and a significant elevation of the total-LD, LD-1 and LD-2 activities vis-à-vis the levels at rest was also demonstrated in these pigs. In animals subjected to skeletal muscular damage there was a significant elevation in all LD fractions 24 hours after stress, compared with the levels at rest (Table III)

In pigs where no skeletal muscular lesions were demonstrated, a highly significant correlation was obtained between the total-CK and CK-MM values as well as the total-LD, LD-1 and LD-2 values and the severity of the myocardial damage (graded 2-4) (Spearman rank coeff. = 1.0), (Table II).

In serum from human subjects collected during the course of acute myocardial infarction (AMI), distinct CK-MB isoenzyme fractions were obtained after electrophoretic separation and the CK-MB activity in these sera varied

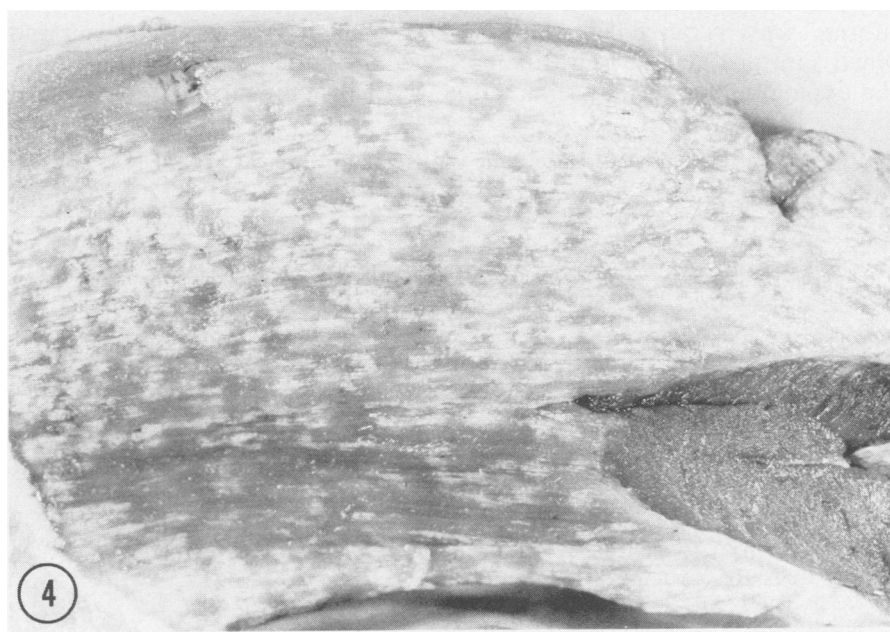

Fig. 4. Specimen from M. extensor carpi radialis of pig number 8. The muscular tissue shows widespread distribution of pale foci of necrosis.

Fig. 5. Microscopical picture of M. semimembranosus from pig number 4. Some fibers show granular disintegration and floccular changes. There is moderate proliferation of mononuclear cells. Two damaged fibers in the centre of the picture are mineralized. H&E. X135.

between 1.0 and 5.0 μkat/L (Fig. 8a). The isoenzyme pattern in serum from HN pigs at rest and in pigs subjected to severe myocardial necrosis (graded 4-5) is demonstrated in Fig. 8b. Only a slight, nonsignificant increase in the CK-MB activity was demonstrated in pigs with severe myocardial lesions.

DISCUSSION

In erythrocytes, a relatively high degree of LD activity is present, particularly in the anodic fractions (LD 1-3) (7). Haemolysis must therefore be avoided in blood samples assayed for LD activity. In CK analysis, haemolysis does not interfere however, and no detectable CK activity could be detected in porcine erythrocytes (22). With the catheter inserted into a main vein as described above, blood was drawn without risk of haemolysis. Furthermore, a permanent vein tubing was required to obtain blood samples at rest and during the experimental period so as not to excite the animal and to avoid risk of contamination of muscular tissue — factors which could cause falsely elevated CK activity in the blood plasma (22).

A number of procedures have been reported for the separation and quantitation of CK isoenzymes. In general these techniques involve electrophoretic separation or ion exchange chromatography followed by fluorimetric or spectrophotometric quantitation. Recently immunoinhibition methods using antibodies against CK-M or CK-B subunits as well as bioluminiscence assay for CK estimation have been introduced (5, 14). The high sensitivity of these methods makes it possible to measure accurately even minor increases in CK activity and diagnose even minor myocardial damage in man. However, as CK-BB is normally present in porcine serum (20, 21) (Fig. 8b), inhibition of CK-B by use of specific antibodies in order to calculate the CK-MB activity is thus not adequate in pigs.

Furthermore, there is no complete cross-reaction between porcine CK isoenzyme and antibodies against human CK-B and CK-M subunits (20). As little is known about the CK isoenzyme pattern in the normal and pathological sera of pigs, a screening method for separation of CK isoenzymes with high sensitivity was required and was preferred in terms of reliability (it provides immediate insight

TABLE II. Total Serum-CK, -LD and Related Isoenzyme Activities in Halothane-sensitive and Normal Pigs at Rest and 24 Hours After Restraint Stress. n = Number of Pigs Examined

Time	Halothane reactivity	n	Total CK	CK-1	CK-2	CK-3	Total LD	LD-1	LD-2	LD-3	LD-4	LD-5
Rest	HP	9	26 ± 12^{a}	2.8 ± 1.7^{b}	0.8 ± 0.5^{c}	24 ± 11^{d}	15 ± 6^{i}	3.4 ± 1.5^{k}	2.3 ± 0.6^{l}	2.7 ± 1.0^{m}	2.8 ± 1.5^{n}	2.7 ± 0^{o}
	HN	6	7.1 ± 2.1^{e}	1.4 ± 0.8^{f}	0.5 ± 0.2^{g}	4.5 ± 1.3^{h}	11 ± 2.7^{p}	2.0 ± 0.8^{q}	1.5 ± 0.5^{r}	1.8 ± 0.6^{s}	1.8 ± 0.4^{t}	2.1 ± 0.9^{u}
Statistics (rest):			p<0.02	ns	ns	p<0.05	ns	ns	ns	ns	ns	ns
24 h	HP	9	598 ± 160^{a}	7.3 ± 3.2^{b}	0.7 ± 0.3^{c}	580 ± 168^{d}	56 ± 17^{i}	11 ± 6.8^{k}	9.6 ± 4.7^{l}	7.5 ± 5.5^{m}	9.8 ± 4.4^{n}	14 ± 6^{o}
	HN	6	252 ± 107^{e}	3.7 ± 1.3^{f}	0.8 ± 0.2^{g}	250 ± 101^{h}	27 ± 12^{p}	7.8 ± 3.7^{q}	4.0 ± 1.8^{r}	4.2 ± 2.2^{s}	4.6 ± 3.8^{t}	7.2 ± 3.5^{u}
Statistics (24 h):			p<0.02	p<0.05	ns	p<0.02	p<0.02	ns	ns	ns	p<0.05	p<0.02

CK-1 = CK-BB	aa; p<0.001	ff; p<0.05	11; p<0.001	qq; p<0.05
CK-2 = CK-MB	bb; p<0.02	gg; ns	mm; p<0.02	rr; p<0.05
CK-3 = CK-MM	cc; ns	hh; p<0.001	nn; p<0.001	ss; ns
	dd; p<0.001	ii; p<0.001	oo; p<0.001	tt; ns
	ee; p<0.001	kk; p<0.02	pp; p<0.05	uu; p<0.05

Spearman rank correlation coefficient: extent of skeletal muscular necroses versus a+e and d+h; p<0.001, versus i+p and o+u p<0.001
Spearman rank correlation coefficient: extent of myocardial necroses versus a - h: ns, versus i - u: ns

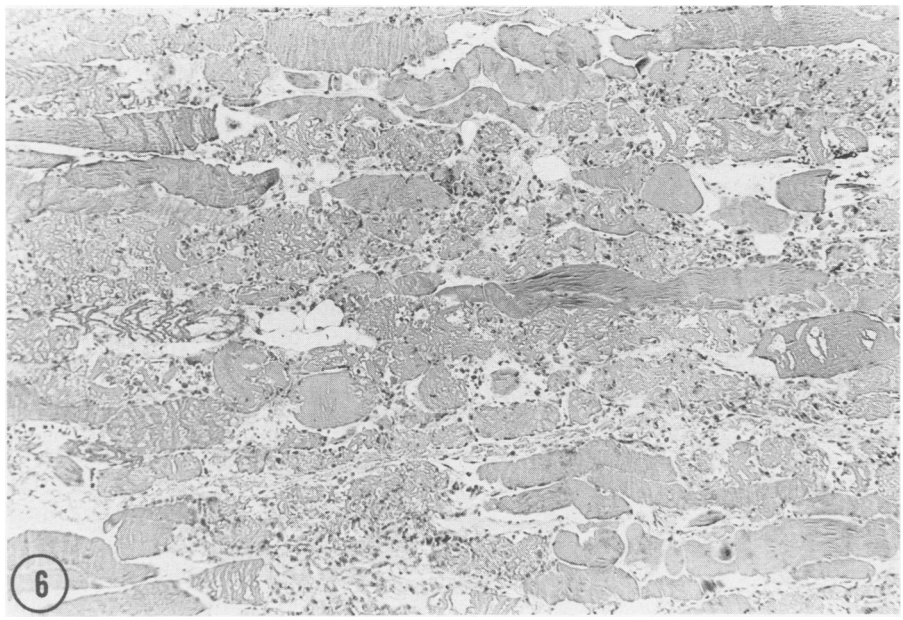

Fig. 6. Histopathological picture of the muscular tissue demonstrated in Fig. 4. Most muscle fibers are severely damaged. H&E. X80.

into the distribution of CK isoenzymes). Electrophoretic separation on prepared gel plates combined with fluorimetric development of the zymogram meets these criteria. However, when using this method, measurement of minor changes in the CK activity (less than 0.5 μkat/L) may occur without being accurately estimated.

In porcine myocardial tissue only about 4-5% CK-MB was detected (20), (Fig. 7b), whereas about 30% CK-MB was found in human heart tissue in addition to CK-MM (Fig. 7b). In other studies an isoenzyme composition of 10-20% MB in addition to CK-MM was found in porcine heart muscular tissue (2, 6). However, the colorimetric staining technique used by these authors is not comparable to the fluorimetric development of the zymograms used in the present study. (These methodological problems are further commented upon in an earlier report [20]). In human heart muscular tissue, various methods of distinguishing CK isoenzymes revealed an approximate composition of 20-30% CK-MB in addition to CK-MM (8, 15, 17, 24).

In serum from healthy adult human subjects normally only CK-MM is detected but in cases of acute necrotic lesions in the heart muscle (AMI), CK-MB activity appears within 24 hours after the myocardial damage (4, 12, 17). Furthermore, a positive relationship between the size of the infarction and the elevation of CK-MB in the plasma has been established in man (13, 18). In cases with only small areas of necrosis in the myocardial tissue the CK-MB activity in serum is only slightly raised, and a highly sensitive method such as column chromatography or immunoinhibition techniques, combined with bioluminescence assay, is required for the quantitation of CK-MB (3, 14). In cases of more severe myocardial lesions and a pronounced elevation of the CK-MB activity, however, electrophoretic separation does fulfil the criteria for necessary sensibility and reproductibility in order to detect CK-MB, as demonstrated in the present study.

It should be noted that the term AMI in man as used in this study is based upon a clinical diagnosis only, as no morphological exami-

TABLE III. Total CK, LD and Related Isoenzyme Activities in Serum of Pigs with Moderate to Severe Myocardial Damage but with Varying Degrees of Skeletal Muscular Lesions. n = Number of Pigs Examined

Time	Extent of skeletal muscle necrosis	Extent of myocardial necrosis (grade 0-5)	n	Total CK	CK-1	CK-2	CK-3	Total LD	LD-1	LD-2	LD-3	LD-4	LD-5
Rest	None	0	6	7.1 ± 2.1^{a}	1.4 ± 0.8^{b}	0.5 ± 0.2^{c}	4.5 ± 1.3^{d}	10.7 ± 2.7^{n}	2.1 ± 0.8^{j}	1.5 ± 0.5^{k}	1.8 ± 0.6^{l}	1.8 ± 0.4^{m}	2.1 ± 0.9^{n}
24 h	None	2-4	3	144 ± 105^{e}	3.3 ± 2.0^{f}	0.8 ± 0.2^{g}	140 ± 99^{h}	14 ± 2.9^{o}	5.3 ± 1.0^{p}	3.1 ± 1.0^{q}	2.6 ± 1.4^{r}	1.4 ± 0.6^{s}	2.8 ± 2.0^{t}
	Slight-moderate	1-4	3	360 ± 109	4.2 ± 1.6	0.7 ± 0.2	359 ± 107	40 ± 10^{u}	9.9 ± 2.3^{v}	4.4 ± 0.9^{x}	5.9 ± 1.6^{y}	7.9 ± 2.2^{z}	11.8 ± 3.3^{w}
Statistics (24 h)				$p<0.05$	ns	ns	$p<0.05$	$p<0.05$	$p<0.02$	ns	$p<0.05$	$p<0.05$	$p<0.02$
Student's t-test				a e; $p<0.02$ b f; $p<0.05$ d h; $p<0.02$				i o; $p<0.02$ j p; $p<0.02$ k q; $p<0.05$		l r; ns m s; ns n t; ns	i u; $p<0.001$ j v; $p<0.001$ k x; $p<0.001$ l y; $p<0.002$ m z; $p<0.001$ n w; $p<0.001$		

Spearman rank correlation coefficient; extent of myocardial necroses versus e and h; $p<0.001$, versus o, p and q; $p<0.001$

nation of the myocardial tissue was performed in connection with the analysis of CK isoenzymes.

Although extensive necroses were present in the myocardial tissue of several pigs (Table I, Fig. 1) no significant elevation of the CK-MB activity was registered in serum (Fig. 8b). Nevertheless, it cannot be ruled out that a slight elevation in the CK-MB activity might have occurred, which was not detectable with the technique used in this study. On the other hand a pronounced elevation of the CK-MM activity in serum was observed in those animals that had large necrotic lesions in the cardiac muscles. It therefore seems possible that the differences in CK isoenzyme composition in the heart tissue between man and swine may be responsible for the differences in the CK-MM and CK-MB isoenzyme patterns in serum in connection with acute myocardial necrosis/infarction in these species.

The significance of the necrosis in skeletal muscles in HP and HN pigs will not be further discussed here but it seems probable that these lesions contribute to a considerable extent the dramatic increase in serum CK and LD activities in the pigs following the restraint stress procedure. Particularly in the halothane-sensitive animals, very high CK-MM activity was observed (Table II) and it is obvious from the morphological examinations that severe skeletal muscle damage occurred in these pigs (Table I, Figs. 4-6).

Judging from those animals in which various types of skeletal muscles were examined, there is a close correlation between the severity of the lesions within M. semimembranosus and other muscular groups, Mm. longissimus, biceps and triceps. In some pigs where only M. semimembranosus was examined (Table I) it cannot be excluded that lesions might have been present in other muscles, but if such was the case it seems reasonable to assume that they would only have been minor. This is further supported by the fact that in the HN pigs without alterations in M. semimembranosus but with pronounced myocardial lesions a close correlation between the CK-MM activity and the grade of myocardial damage was registered, whereas no significant correlation in that respect was obtained when all pigs are considered (Tables II and III). This means that in cases of skeletal muscular necrosis, at least in moderate to severe cases, the release of CK-MM activity originating from the myocardial tissue will not be manifest in the statistical calculations.

Heart muscle tissue is characterized by having a predominance of LD-1 and LD-2 isoenzymes (Fig. 7a) and in pigs only small differences in isoenzymes composition exist between various parts of this organ (7). In skeletal muscles the cathodic migrating LD isoenzymes are the dominant fractions (LD 3-5), (Fig. 7a), (7).

The pronounced increase in the LD activity and particularly in the LD-5 fraction after stress in the

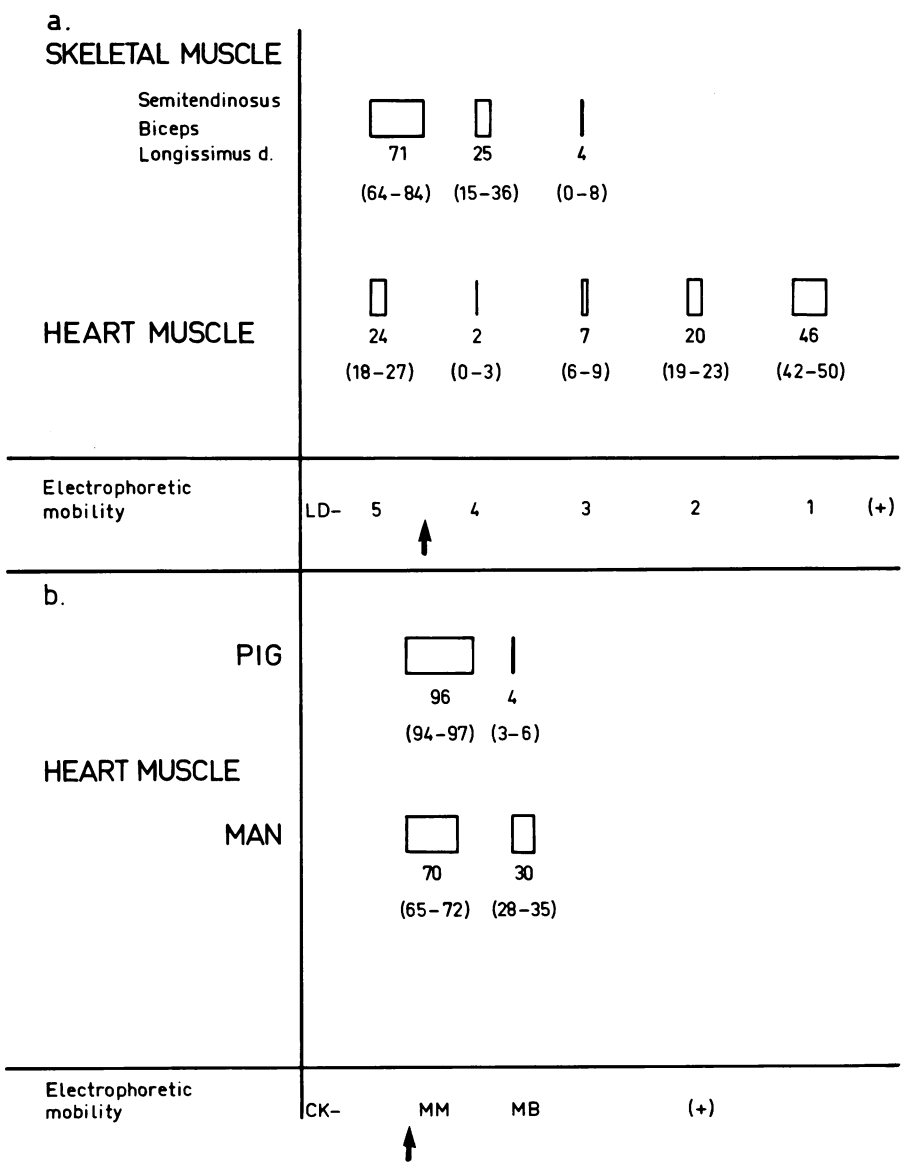

Fig. 7a. The relative composition of LD isoenzymes in percent of the total enzyme activity in homogenates from porcine skeletal and heart muscle. Each value represent ten different samples, mean and range are given. ↑ indicates application of samples.

Fig. 7b. The relative composition of CK isoenzymes in percent of the total enzyme activity in homogenates from porcine and human heart muscle. Each value represent ten different samples, mean and range are given. ↑ indicates application of samples.

HP pigs (Table III) is probably related to the skeletal muscular damage and it seems probable therefore that CK-MM isoenzyme is released from the skeletal muscles in these pigs. However, as there is a significant increase in the anodal LD fractions too (LD 1-2), it seems likely that necroses in the myocardial tissue also contribute to the release of CK-MM to a certain extent in these pigs. In the HN pigs the CK-MM activity was moderately elevated and the increase in LD activity was related mainly to the anodic isoenzyme fractions (LD 1-2) in those HN pigs in which no skeletal muscle lesions occurred (Table III), a fact which probably reflects the myocardial damage in these animals.

Creatine kinase is the most closely correlated serum enzyme with stress susceptibility, and halothane sensitive pigs show significantly higher total-CK, CK-MM and somewhat higher CK-BB activities under normal conditions at rest, than nonreacting littermates at different intervals during the fattening period, (19, 23). This was demonstrated for HP and HN pigs also in this study (Table II).

The reason for this elevation in the CK isoenzyme fractions seen under normal conditions in HP pigs is not fully understood. Enhanced metabolism in the skeletal muscular cells, pathological disorders such as disturbances in the membrane permeability of the muscular cells, as well as differences in the endocrinological system between stress susceptible and normal pigs have been suggested as basic mechanisms in the development of stress susceptibility in domestic pigs.

The differences in the CK and LD activities between HP and HN pigs which are more pronounced one day after stress as demonstrated in this study seems related to the severeness of muscular damage at this stage. However, it is still obscure why HP pigs are more prone to develop lesions and necroses in the striated muscular tissue at stress than HN pigs. This phenomenon will be subjected to further studies. Earlier investigations have shown great differences in the sympathetic response to various forms of stress in HN and HP pigs resulting in a significant higher catecholamine activity in the blood plasma in HP pigs (12) a fact which may indicate differences in the hormonal activity and/or a higher sensibility to the hormonal substances in the stress susceptible pig.

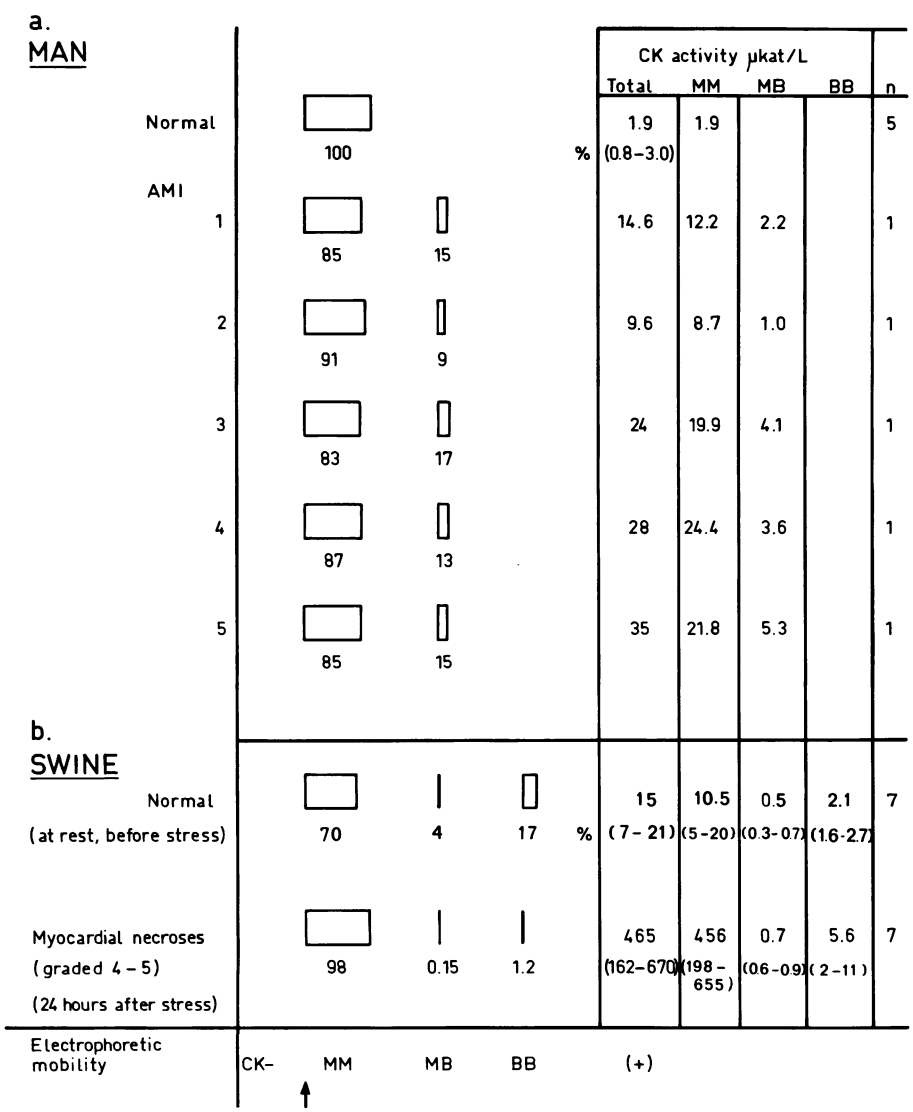

Fig. 8. Creatine kinase isoenzyme activity in serum from human subjects (a) and pigs (b) collected during the course of acute myocardial infarction/myocardial necrosis. The relative composition (in percent) and the absolute CK isoenzyme activities (μkat/L) are given. n = number of individuals examined. ↑ indicates application of samples.

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