Protein synthesis, myosin ATPase activity and myofibrillar protein composition in hearts from tumour-bearing rats and mice

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Growing rats and adult weight-stable mice bearing a transplantable methylcholanthrene-induced sarcoma were compared with animals with various states of malnutrition. Heart protein synthesis was measured *in vivo*. Myocardial RNA, myofibrillar protein composition and the Ca^{2+} -activated ATPase activity in heavy chains of native myosin were measured. 'Fingerprints' were made from myosin by trypsin treatment to evaluate possible structural changes in the protein. Cardiac protein-synthesis rate was decreased by 20 % in growing tumour-bearing rats, by 35 % in protein-malnourished (rats) and by 47 % in starved rats, compared with freely fed controls ($P < 0.05$). Adult tumour-bearing mice showed no significant decrease in myocardial protein synthesis. Pair-weighted control mice had significantly depressed heart protein synthesis. Protein translational efficiency was maintained in both tumour-bearing rats and mice, but was decreased in several groups of malnourished control animals. The Ca^{2+} -activated myosin ATPase activity was decreased in all groups of malnourished animals, including tumour-bearing mice and rats, without any evidence of a change in cardiac isomyosin composition. We conclude that loss of cardiac muscle mass in tumour disease is communicated by both depressed synthesis and increased degradation largely owing to anorexia and host malnutrition. Increased adrenergic sensitivity in hearts from tumour-bearing and malnourished animals is not communicated by increased Ca^{2+} -activated ATPase activity. This may be down-regulated in all groups with malnutrition, without any observable alterations in the isomyosin profile.

INTRODUCTION

Growth of malignant tumours is associated with a negative nitrogen balance, which leads to loss of cardiac contractile mass (Medigreceanu, 1910; Lundholm *et al.*, 1980; Karlberg *et al.*, 1981; Wood *et al.*, 1982; Drott *et al.*, 1986). Our previous investigations on ultrastructure in hearts from tumour-bearing mice have demonstrated a decreased cross-sectional area of myocytes and a decreased number of myofibrils and myosin filaments per myofibre (Sjöström *et al.*, 1987). Similar morphological changes were associated with an increased net release of phenylalanine in perfused hearts from tumour-bearing rats, demonstrating an increased net breakdown of heart proteins (Drott & Lundholm, 1989). Heart function, however, does not seem to deteriorate in parallel with the ultrastructural changes and the loss of contractile mass in cancer cachexia (Ko & Paradise, 1972; Heymsfield *et al.*, 1978; Nutter *et al.*, 1979; Drott *et al.*, 1986). This dissociation suggests an adaptation including functional protection (Ransnäs *et al.*, 1989; Drott *et al.*, 1987). In contrast with pure undernutrition, the tumour-bearing state is associated with increased whole-body energy expenditure (Lindmark *et al.*, 1983, 1984; Warnold *et al.*, 1978). This situation may theoretically impose a proportionately larger load on hearts in a tumour-bearing host compared with hearts subjected only to uncomplicated undernutrition (Drott *et al.*, 1986). The combination of starvation, stress and inflammation in tumour disease may therefore necessitate a functional adaptation which may include alterations in both structure and function of proteins.

The purpose of this study was to evaluate if our previous observations of increased adrenergic sensitivity in hearts from tumour-bearing animals (Drott *et al.*, 1986, 1987; Ransnäs *et al.*, 1989) are associated with a higher myosin ATPase activity and a different myofibrillar protein composition from that in undernourished reference animals without tumour disease.

MATERIALS AND METHODS

Chemicals and radiochemicals

L-[U- ^{14}C]Phenylalanine (525 mCi/mmol) was from New England Nuclear Chemicals, Dreieich, Germany. Electrophoresis-grade chemicals were obtained from Pharmacia, Uppsala, Sweden, and Bio-Rad, Richmond, CA, U.S.A. Myosin (M-1636), actin (A-4533), troponin (T-3515) and tropomyosin (T-3640) standards and all other chemicals and reagents were of analytical grade, from Sigma Chemical Co., St. Louis, MO, U.S.A.

Experimental animals and tumour model

Rats and mice with the same kind of tumour were used. Our previous experiments on cardiac metabolism were performed in perfused hearts from sarcoma-bearing rats. Tumour-bearing rats represent a model of growing animals. Undernutrition in such models generally represents retarded growth rather than true wasting. Therefore our main model of adult sarcoma-bearing mice was also used, in which undernutrition represents true wasting. Growing male Sprague-Dawley rats weighing 80–90 g were from Anticimex, Södertälje,

Abbreviations used: PCM, protein-energy-malnourished.

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Sweden. The 3-month-old weight-stable female C57Bl/6J mice were from Bomholtgård, Ry, Denmark. The methylcholanthrene-induced sarcomas were implanted subcutaneously in the flanks by using a trocar. These tumours do not metastasize. The tumours did not seem visibly to impair animal movements, although some effects on motor activity are likely to occur (Morrison *et al.*, 1984). No tumour ulceration occurred. Tumour-bearing and freely fed control animals had free access to standard chow diet. The amount and the time course of food intake have been described elsewhere for our freely fed tumour-bearing rats and mice (Lundholm *et al.*, 1980; Drott *et al.*, 1986).

Starved control rats were totally deprived of food for 96 h and mice for 48 h before experiments, to agree with our previous experiments (Drott *et al.*, 1986, 1987; Ransnäs *et al.*, 1989). Protein-energy malnutrition (PCM) in non-tumour-bearing controls was achieved by giving rats a protein-free diet *ad libitum* for 2 weeks before the experiments (Drott *et al.*, 1986). Pair-weighted control mice received a daily amount of food so that the rate, duration and magnitude of weight loss before death agreed with that of the carcass of freely fed sarcoma-bearing mice (Svaninger *et al.*, 1986). Pair-fed control rats and mice received the same amount of food as the spontaneous intake of tumour-bearing animals. Pair-fed mice are more food-restricted than pair-fed rats, since the anorexia is more pronounced in sarcoma-bearing mice (Lundholm *et al.*, 1980; Drott *et al.*, 1986). Pair-weighted control mice are more malnourished than pair-fed mice, since weight-pairing is done with the purpose of matching for both the anorexia and the alterations in body composition. Animals were kept in single cages in a room with constant temperature and humidity, and were maintained on a 12 h-light/12 h-dark cycle.

Sarcoma-bearing mice die spontaneously with cachexia 15–16 days after tumour implantation. All experiments on mice were performed on day 11, when the tumour comprised approx. 14% of body weight. Sarcoma-bearing rats die spontaneously 24–28 days after tumour implantation. Rats were killed for experiments 3 weeks after tumour implantation, when tumours comprised around 12% of body weight.

Animals were randomly allocated to groups either for determination of heart protein composition or for studies of heart protein synthesis. All animals were weighed before death. Rats were anaesthetized by intraperitoneal injection of pentobarbital (Nembutal, 60 mg/kg body wt.). Mice were killed by cervical dislocation. Blood was drawn from beating hearts after anaesthesia or cervical dislocation. The hearts were excised and cleared of blood with 0.9% NaCl. Atrial and extra-cardiac tissues were carefully removed before weighing and immersion in liquid N₂. Hearts and plasma were stored at -70 °C until analysed. The tumours were carefully dissected and weighed.

Cardiac myofibrillar composition

Tissue samples (50 mg) were homogenized in an Ultra-Turrax homogenizer (IKA, Labassco, Sweden) in 20 mM-potassium phosphate buffer (pH 7.0) and transferred quantitatively to a graduated tube with 20% (w/v) SDS. The mixture was heated in boiling water for 3 min and then centrifuged at 10000 g for 1 min at room temperature (Everett *et al.*, 1983). The supernatants were used for separation of myofibrillar protein by SDS/

polyacrylamide-gel electrophoresis overnight at 80 V in 25 mM-Tris/192 mM-glycine buffer containing 0.1% SDS in a LKB 2001 vertical unit, with a 5–20% gradient gel. Gels were stained with 0.1% Coomassie Brilliant Blue. Proteins were quantified by densitometry (Shimadzu, model CS 930).

Myosin ATPase activity and isomyosins

Ca²⁺-activated myosin ATPase activity was determined by precipitation of liberated phosphate as calcium phosphate in non-denaturing gels as described by Hoh *et al.* (1976, 1977). Briefly, myocardial tissue was minced with scissors and washed in phosphate-buffered saline (40 mM-NaCl, 3 mM-sodium phosphate, pH 7.0 at 2 °C). The washed muscle was homogenized in the same buffer for a total of 90 s, with periods of 30 s homogenization and 30 s interruption. The homogenate was centrifuged at 3640 g for 30 min at 1 °C. The pellet was washed and re-centrifuged. The pellet was immersed in the extraction buffer and re-centrifuged at 48000 g for 3 h at 1 °C. The supernatant was recovered and mixed with an equal volume of ice-cold glycerol and stored at -20 °C (Hoh *et al.*, 1976, 1977). This supernatant was also used for separation of isomyosins by pulsed-field electrophoresis.

Polyacrylamide slab gels (1.5 mm thick) were used in determinations of myosin ATPase activity. The electrophoresis buffer [20 mM-Na₂P₂O₇ and 10% (v/v) glycerol, pH 8; 2 °C] was recirculated during electrophoresis. A pre-run of 60 min was carried out at 70 V to remove ammonium persulphate. Equal concentrations of myofibrillar proteins in 50% glycerol were mixed with trace amounts of Bromophenol Blue and electrophoresed for 16 h at 170 V (gradient of approx. 14 V/cm). Pyrophosphate ions after protein separation were removed by electrophoresis during 2–4 h in the presence of 20 mM-Tris, 190 mM-glycine, 5 mM-ATP and 10% glycerol, pH 8.8 at 2 °C. The gels were supported by a glass plate and were transferred to 'Seal a Meal Bags' containing 100 ml of an ATPase incubation buffer (600 mM-KCl, 5 mM-ATP, 5 mM-β-mercaptoethanol, 15 mM-CaCl₂, 25 mM-Tris, 190 mM-glycine adjusted to pH 8.8 at 20 °C). The bags were maintained for 3 h at 37 °C in a water bath with gentle agitation. The precipitated calcium phosphate was quantified by densitometry at 550 nm after various times of incubation to monitor the ATPase reaction. After scanning for ATPase activity, the gels were stained for protein with Coomassie Brilliant Blue. Densitometer tracings were performed and the specific ATPase activity was calculated. To ascertain that contaminating proteins did not have the same mobility as native myosin during undenatured conditions, high-molecular-mass (669000–67000 Da) and low-molecular-mass (94000–14400 Da) standard proteins were electrophoresed under identical conditions. All of these proteins migrated out at the bottom of the gels.

Isomyosins were estimated by densitometry after pulsed-field-electrophoresis separation in 0.7% agarose gels and pyrophosphate buffer as described above. Pulse-field electrophoresis (2015 Pulsaphore System, LKB Pharmacia; pulse time N/S 90 s, E/W 90 s, stepping with hexagonal electrodes, 20 h, 125 V, 3 °C) gave better resolution of isomyosin distribution in our hands compared with either slab gels or rods.

Tryptic digestion of myosin

Crude myosin preparations were isolated as described

above before mixing with glycerol. Tryptic digestion was performed as described by Bhan & Malhotra (1976). Briefly, a saturated solution of $(\text{NH}_4)_2\text{SO}_4$ with 10 mM-EDTA was slowly added to the crude myosin preparation with gentle stirring on ice until 35% saturation with $(\text{NH}_4)_2\text{SO}_4$ was reached. After centrifugation at 25000 *g* for 15 min, the pellet was discarded and the supernatant was adjusted to 45% saturation with $(\text{NH}_4)_2\text{SO}_4$. After centrifugation at 25000 *g* for 15 min, the myosin pellet was dissolved in 0.5 M-KCl, pH 7.0, and dialysed against 0.3 M-KCl/1 mM-EDTA until it was free of sulphate ions. Protein concentration was determined on a sample (Lowry *et al.*, 1951). Samples were diluted to obtain equal concentrations of myosin. Trypsin (myosin/trypsin ratio 100:1, w/w) was added to the myosin sample, which was dissolved in 0.5 M-KCl, pH 7.0, and incubated for 5, 15, 20 and 30 min at room temperature. At the end of the incubation period, a 2.5-fold excess of soya-bean trypsin inhibitor over trypsin was added to the reaction mixture, which was rapidly chilled on ice. The samples were subjected to SDS/polyacrylamide-gel electrophoresis and densitometry.

Protein synthesis

Heart protein synthesis was measured by the flooding technique as described by Garlick *et al.* (1980) and Pain *et al.* (1984). A single intraperitoneal injection of L-[U- ^{14}C]phenylalanine (0.4 $\mu\text{Ci/g}$ body wt.) was given together with phenylalanine (150 $\mu\text{mol}/100$ g body wt.). Mice were injected intraperitoneally without anaesthesia, whereas rats were injected intravenously in a lateral tail vein after pentobarbital anaesthesia (Nembutal, 20 mg/kg body wt.). The animals were killed at 5 and 10 min after injection. Hearts were removed 10 min after phenylalanine injections. Plasma amino acids were extracted in methanol overnight. The specific radioactivity of soluble plasma phenylalanine was quantified by h.p.l.c., with a pre-column derivative-formation procedure (Hill *et al.*, 1979), on a $\mu\text{Bondapak C18}$ column (Waters Associates Chromatographic Liquid Systems, U.S.A.). The plasma specific radioactivity of phenylalanine at 5 min after injection was used as an estimate of the mean specific radioactivity in the precursor pool for heart protein synthesis (Garlick *et al.*, 1980). The time course of free amino acid labelling after injection agreed with previous reports (Garlick *et al.*, 1980).

Phenylalanine in heart proteins was isolated by

homogenizing heart tissue in distilled water at 4 °C. Proteins were precipitated with 30% trichloroacetic acid at 80 °C and isolated as described elsewhere (Lundholm *et al.*, 1979). Proteins were hydrolysed in 6 M-HCl for 24 h at 110 °C. The amino acids were bound to a strong cation-exchange resin (AG 50W-X8, 100–200 mesh; Bio-Rad) and eluted with 4 M- NH_3 . Ammonia was removed by evaporation to dryness, and the amino acids were resuspended in 3 ml of 0.5 M-sodium citrate, pH 6.3, for enzymic conversion of phenylalanine into β -phenethylamine with phenylalanine decarboxylase (EC 4.1.1.53) from *Streptococcus faecalis* (Garlick *et al.*, 1980). The enzyme was suspended in 0.5 mg of pyridoxal phosphate/ml. β -Phenethylamine was quantified in a Hitachi Perkin-Elmer MPF-2A fluorescence spectrophotometer (Suzuki & Yagi, 1976).

Measurement of heart RNA and protein content

RNA was extracted and quantified as described by Munro & Fleck (1966). Protein was determined as described by Lowry *et al.* (1951), with bovine albumin as the standard.

Statistics

Values are given as means $\pm$ S.E.M. Statistical testing among groups of animals were performed by one-factor ANOVA. Differences between specific groups of animals were tested by a multiple-range test. *P* values less than 0.05 were regarded as statistically significant.

RESULTS

The tumour comprised 12% of body weight in rats, and 14% in mice, at the time of experiments. The carcass weight of tumour-bearing mice (body weight minus tumour weight) was not significantly different from the body weight of pair-fed and pair-weighted mice, but was 11% lower than that of freely fed control mice ($P < 0.05$). Sarcoma-bearing rats had a 9% lower carcass weight compared with freely fed control rats, but a considerably higher carcass weight than protein-energy-malnourished (PCM) and starved rats ($P < 0.05$). The 12% and 16% difference in body weight between tumour-bearing mice and rats respectively compared with pair-fed controls is partly explained by a considerably higher water content in tumour tissue ($\sim 83\%$) than in normal tissues (70–75%) and a different

Table 1. Body and heart weights and heart protein content in sarcoma-bearing and control mice

Results are means $\pm$ S.E.M. for the numbers of animals in parentheses. Tumour weight was 3.15 ± 0.21 g. Mice: TB, tumour-bearing; PF, pair-fed; PW, pair-weighted; S, starved (48 h); C, freely fed controls. For the last column, $P < 0.05$ among the groups (ANOVA); $^aP < 0.05$ versus freely fed controls, and $^bP < 0.05$ versus tumour-bearing animals (both by multiple range test).

Animal group ...	TB (19)	PF (12)	PW (19)	S (6)	C (19)	<i>P</i>
Final body wt. (g)	$22.7 \pm 1.1^*$	20.0 ± 0.7^a	$19.1 \pm 0.5^{a,b}$	18.7 ± 0.3^a	22.0 ± 0.7	< 0.05
Heart wet wt. (mg)	79.4 ± 4.4^a	89.8 ± 6.4	83.3 ± 3.0	87.1 ± 3.2	93.5 ± 5.9	< 0.05
Heart protein concn. (mg/g)	198 ± 5.3	199 ± 5.4	200 ± 7.0	—	196 ± 6.8	
Heart protein (mg)	17.5 ± 0.4^a	18.7 ± 0.6	17.6 ± 0.4^a	—	20.6 ± 0.5	< 0.05

* Includes tumour weight.

Table 2. Body and heart weights and heart protein content in sarcoma-bearing and control rats

Results are means $\pm$ S.E.M. for the numbers of animals in parentheses. Tumour weight was 29 ± 4 g. Rats: TB, tumour-bearing; PF, pair-fed; PCM, protein-energy-malnourished; S, starved (96 h); C, freely fed controls. For the last column, $P < 0.05$ among the groups (ANOVA); $^aP < 0.05$ versus freely fed controls, and $^bP < 0.05$ versus tumour-bearing animals (both by multiple range test).

Animal group ...	TB (14)	PF (6)	PCM (14)	S (14)	C (20)	P
Final body wt. (g)	235 $\pm$ 5	197 $\pm$ 4 ^a	99 $\pm$ 1 ^{a,b}	158 $\pm$ 2 ^{a,b}	233 $\pm$ 3	< 0.05
Heart wet wt. (mg)	763 $\pm$ 29 ^a	690 $\pm$ 26 ^a	387 $\pm$ 12 ^b	561 $\pm$ 18 ^{a,b}	810 $\pm$ 18	< 0.05
Heart protein concn. (mg/g)	196 $\pm$ 4	203 $\pm$ 5	201 $\pm$ 4	198 $\pm$ 5	195 $\pm$ 4	
Heart protein content (mg)	145 $\pm$ 3 ^a	140 $\pm$ 5 ^a	78 $\pm$ 6 ^{a,b}	111 $\pm$ 8 ^{a,b}	158 $\pm$ 3	< 0.05

Table 3. Contractile proteins in hearts from tumour-bearing (TB) and control rats (C, PCM)

Results are means $\pm$ S.E.M. for four determinations on pooled samples from 10 animals in each group). Myofibrillar, soluble and collagen proteins are equally decreased in tumour-bearing animals (TB) compared with controls (C) (Drott *et al.*, 1986).

Animal group ...	Protein (% of myofibrillar proteins)		
	TB	PCM	C
Myosin	50.1 $\pm$ 3.6	51.5 $\pm$ 2.0	51.0 $\pm$ 2.4
Actin	24.3 $\pm$ 2.2	23.7 $\pm$ 2.0	24.7 $\pm$ 2.1
Tropomyosin	15.4 $\pm$ 0.8	15.5 $\pm$ 0.6	15.7 $\pm$ 0.9
Troponin	10.2 $\pm$ 0.6	9.3 $\pm$ 0.6	8.6 $\pm$ 0.8

accumulation of tumour tissue per food intake compared with average host tissues (Lundholm *et al.*, 1980).

Heart protein concentration did not differ between study and control animals in mice and rats. Heart weight and total heart protein content were significantly lower in tumour-bearing mice and rats than in freely fed controls but were not different from those of pair-fed and pair-weighted control animals (Tables 1 and 2). We have previously reported that contractile proteins are not selectively decreased in hearts from tumour-bearing mice (Sjöström *et al.*, 1987). This was also confirmed in rats (Table 3).

Heart protein synthesis

Growing rats (Table 4). The amount of cardiac protein synthesized per day was decreased by 20% in tumour-bearing rats and by around 65% in starved and PCM rats compared with freely fed control rats ($P < 0.05$). The RNA content was significantly decreased only in the most undernourished rats (PCM and starved). RNA activity (protein synthesis per unit of RNA) was significantly decreased only in starved rats.

Adult mice (Table 5). Tumour-bearing and pair-fed control mice had around 15% lower heart protein synthesis compared with freely fed controls, although this difference did not reach statistical significance. Protein synthesis in hearts from pair-weighted mice was significantly lower compared with freely fed control mice ($P < 0.05$).

The RNA content in hearts from sarcoma-bearing mice and pair-weighted controls was significantly lower compared with freely fed control mice. RNA activity was not decreased in tumour-bearing mice, but was in both pair-fed and pair-weighted controls.

ATPase activity and isomyosin

Figs. 1 and 2 show estimates of ATPase activity in purified myosin from rats and mice. The specific activity of Ca^{2+} -dependent myosin ATPase activities was decreased in tumour-bearing, protein-malnourished and starved animals compared with freely fed controls. The ATPase activity was measured as a mean value for the

Table 4. Heart protein synthesis in sarcoma-bearing and control rats

Flooding technique with [^{14}C]phenylalanine was used. For experimental details see the Materials and methods section. Results are means $\pm$ S.E.M. for the numbers of animals in parentheses. Rats: TB, tumour-bearing; PF, pair-fed; PCM, protein-energy-malnourished; S, starved (96 h); C, freely fed controls. For the last column, $P < 0.05$ among the groups (ANOVA); $^aP < 0.05$ versus freely fed controls, and $^bP < 0.05$ versus tumour-bearing animals (both by multiple range test).

Animal group ...	TB (7)	PF (6)	PCM (7)	S (6)	C (13) ^P	
Protein synthesis (%/day)	13.4 $\pm$ 1.9 ^a	15.1 $\pm$ 1.6	10.8 $\pm$ 2.3 ^a	8.8 $\pm$ 1.6 ^{a,b}	16.6 $\pm$ 0.8	< 0.05
Protein synthesis rate (mg/day)	20.1 $\pm$ 1.9 ^a	21.7 $\pm$ 2.2	8.4 $\pm$ 3.1 ^{a,b}	9.8 $\pm$ 2.0 ^{a,b}	25.3 $\pm$ 1.4	< 0.05
RNA content ($\mu\text{g}/\text{mg}$ of protein)	9.2 $\pm$ 1.1	10.3 $\pm$ 0.9	8.8 $\pm$ 0.3 ^a	8.2 $\pm$ 0.8 ^a	11.2 $\pm$ 0.8	< 0.05
RNA activity (mg of protein/day per mg of RNA)	15.1 $\pm$ 1.5	14.6 $\pm$ 1.3	12.3 $\pm$ 1.3	10.7 $\pm$ 1.1 ^a	14.2 $\pm$ 1.3	< 0.05

Table 5. Heart protein synthesis and RNA content in sarcoma-bearing and control mice

The flooding technique with [^{14}C]phenylalanine was used. For experimental details see the Materials and methods section. Results are means $\pm$ S.E.M. for the numbers of animals in parentheses. Mice: TB, tumour-bearing; PF, pair-fed; PW, pair-weighted; C, freely fed controls. For the last column, $P < 0.05$ among the groups (ANOVA); *0.05 versus freely fed controls, and $^b0.05$ versus tumour-bearing animals (multiple range test).

Animal group ...	TB (7)	PF (7)	PW (7)	C (7)	P
Protein synthesis (%/day)	8.1 $\pm$ 0.5	7.4 $\pm$ 0.5	6.8 $\pm$ 0.8	9.0 $\pm$ 0.8	
Protein synthesis (mg/day)	1.40 $\pm$ 0.12	1.38 $\pm$ 0.18	1.19 $\pm$ 0.13 ^a	1.65 $\pm$ 0.20	< 0.05
RNA content ($\mu\text{g}/\text{mg}$ of protein)	10.4 $\pm$ 0.5 ^a	11.4 $\pm$ 0.5	10.3 $\pm$ 0.6 ^a	11.7 $\pm$ 0.5	< 0.05
RNA activity (mg of protein/ day per mg of RNA)	9.8 $\pm$ 0.7	6.9 $\pm$ 0.5 ^{a,b}	7.2 $\pm$ 0.5 ^{a,b}	9.0 $\pm$ 0.5	< 0.05

Fig. 1. Myocardial ATPase activity in purified rat myosin

For details see the Materials and methods section. Rats: TB, tumour-bearing ($\blacklozenge$); S, starved ($\blacktriangle$); PCM, protein-energy malnourished ($\times$); C, freely fed control ($\blacksquare$). Pooled heart tissue from six animals in each group was used. Gels were scanned at 550 nm for both calcium phosphate and proteins. Values on ordinates are areas under the densitometric curves.

three isomyosin forms V_1 – V_3 . The V_1 form is known to have the severalfold highest specific activity (Lompré *et al.*, 1981).

There was no indication of significant myosin isoenzyme alterations among any animal groups. V_1 was the dominant form in adult animals (Fig. 3). Our estimates of isomyosin distribution in mixed heart tissue from mice and rats agreed with previous reports (Hoh *et al.*, 1977; Lompré *et al.*, 1981). 'Tryptic fingerprints' of purified mouse and rat ventricular myosin were also separated to evaluate possible structural differences among the animal groups (Schwartz *et al.*, 1982). Before this technique was applied to study and control animals, we checked that this approach was sensitive enough to detect possible differences by demonstrating the relative differences in peptide maps of fetal myosin as compared with myosin from adult mouse hearts (Fig. 4). In mouse fetal heart tissue V_3 is the dominant isomyosin (Lompré *et al.*, 1981; Schwartz *et al.*, 1982).

Trypsin treatment and tryptic fingerprints of native rat ventricular myosin are shown in Figs. 5(a) and 5(b).

Fig. 2. Myocardial ATPase activity in purified mouse myosin

Mice: TB, Tumour-bearing ($\blacklozenge$); S, starved ($\blacktriangle$); C, freely fed controls ($\blacksquare$). Two separate experiments, using 15 and 30 μg of protein respectively, were performed. Pooled heart tissue from six animals in each group was used.

All animal groups showed a thin actin band, which was due to retention of small amounts of actomyosin in the initial purification step. Densitometer tracings before tryptic digestion revealed that the relative amount of actin compared with the amount of myosin was different between the groups ($P < 0.05$, ANOVA) (tumour-bearing 0.40 ± 0.04 ; PCM, 0.32 ± 0.05 ; starved, 0.18 ± 0.03 ; control, 0.21 ± 0.05 ; five repeated analyses, six animals in each group). This may indicate a more tightly bound actomyosin complex in tumour-bearing and PCM rats compared with starved and freely fed controls. Further analysis of myosin before trypsin treatment showed that the amount of recovered myosin relative to the total protein content excluding actin in the purified fraction was 0.68 ± 0.07 in tumour-bearing, 0.60 ± 0.05 in PCM, 0.27 ± 0.04 in starved and 0.56 ± 0.06 in freely fed control rats ($P < 0.05$, based on four analyses and six animals in each group). This may be interpreted as a lower stability or yield of myosin from starved rats during the purification process. The appearance of 'fingerprints' 15 min after addition of trypsin to myosin from sarcoma-bearing, PCM, starved and freely fed control rats is shown in Fig. 5(b). The differences among the groups

Fig. 3. Separation of isomyosins (V_1 – V_3) by pulse-field electrophoresis as described in the Materials and methods section

Significant redistribution between V_1 to V_3 was not found within either mouse or rat animal groups. Abbreviations: rTB, tumour-bearing rats; rC, freely fed control rats; rS, 96 h-starved rats; rPCM, protein-energy-malnourished rats; mC, freely fed control mice; mTB, tumour-bearing mice; mS, 48 h-starved mice. The protein loads applied to the gels were 19 μ g and 28 μ g from rats and mice respectively.

were mainly of a quantitative nature. No intact myosin remained after 20 min trypsin treatment in samples from tumour-bearing, starved and freely fed control rats, whereas 16% of the initial amount of myosin was undigested after 15 min and 3% after 30 min trypsin treatment in the PCM group. Thus myosin from PCM rats may have been more resistant to trypsin treatment compared with the other groups.

DISCUSSION

This study has evaluated the consequences of cardiac muscle loss with regard to alterations in synthesis, composition and structure of heart proteins. Growing rats and adult non-growing mice with the same kind of malignant sarcoma were used. The reason for including both rats and mice was that our previous experiments in perfused hearts have been carried out on sarcoma-bearing rats (Drott *et al.*, 1986, 1987; Drott & Lundholm, 1989; Ransnäs *et al.*, 1989), but our major work on tumour-host metabolism has been carried out in sarcoma-bearing mice (Lundholm *et al.*, 1978, 1979, 1980; Karlberg *et al.*, 1981; Lindmark *et al.*, 1983; Svaninger *et al.*, 1983, 1986, 1987a,b). It is also possible that the distant effect of a malignant tumour may be different in growing and non-growing hosts. Tumour-bearing mice were compared with pair-fed, pair-weighted, starved and freely fed controls, whereas tumour-bearing rats were compared with pair-fed, PCM, starved and freely fed controls, to agree with our previous work.

Our results demonstrate that tumour-bearing animals with malnutrition suffered from a net loss of cardiac proteins, which did not seem to involve selectively structural proteins or subcomponents. This protein loss seemed to be dependent on nutritional imbalances and anorexia in tumour-bearing animals. To us it is likely that a normal composition of the various myofibrillar proteins should maintain physiological relationships in both moderate and severe undernutrition, although this

Fig. 4. Densitometer tracings from SDS/polyacrylamide-gel (5–15%) gradient electrophoresis of trypsin-treated fetal mouse myosin (mainly V_3 isomyosin) and trypsin-treated myosin from adult weight-stable freely fed mice (mainly V_1 isomyosin)

The area of small fragments (gradient 11–15%; 45000–13000 Da) is shown. Eight fetal hearts and four adult hearts were pooled.

is not consistently found (Clark & Wildenthal, 1986). Differences in a selective depression of protein synthesis with regard to one or a few contractile proteins should then be tightly counter-regulated by a correspondingly increased degradation of other contractile proteins if the heart function is not to be impaired. This view has support in our results of unchanged relative proportions of myofibrillar proteins among well-nourished and severely undernourished animals (Sjöström *et al.*, 1987; Drott *et al.*, 1986; and Table 3). In perfused hearts from undernourished rats we have found no evidence of impaired pumping performance (Drott *et al.*, 1986, 1987), and our results agree with previous findings of the influence of starvation and protein deprivation in cardiac mRNA levels (Zähringer *et al.*, 1985).

Theoretically the loss of cardiac proteins in tumour-bearing animals may be achieved by decreased protein synthesis, an increase in breakdown or a combination of both. There was, however, no consistent alteration in net protein synthesis in tumour-bearing and malnourished

Fig. 5. Electrophoretic separation of myosin before and after trypsin treatment

(a) SDS/polyacrylamide-gel electrophoresis of myocardial myosin from tumour-bearing (TB), starved (S), protein-energy-malnourished (PCM) and freely fed control (C) rats before and after 20 min of tryptic digestion.

animals, although one may conclude from our results that heart protein synthesis probably started to decline in animals suffering from malnutrition. Protein synthesis may then reach a statistically significant level of depression when malnutrition reached a certain degree. However, RNA activity was quite normal in both tumour-bearing rats and mice, demonstrating that the translational machinery was efficiently utilized. Consequently, the lower heart protein mass in growing tumour-bearing rats may be mediated by both depressed synthesis and increased breakdown, whereas in non-growing mice it is likely to be the result of an initially increased breakdown. In a more severe stage, when malnutrition has progressed, inhibition of protein synthesis may also be clear-cut in adult mice, as suggested by the decreased synthesis in pair-weighted controls.

Densitometric tracings of myofibrillar proteins in our previous and present experiments did not confirm any changes in contractile protein concentration among normal and severely malnourished animals. Such experiments may, however, not entirely exclude the possibility of more subtle alterations in myofibrillar function and structure. Differences have been reported in the helix content and amino acid composition of ventricular myosin from euthyroid and hyperthyroid rabbits. This indicates that structural differences in ventricular myosin heavy chain may occur after hormonal alterations (Everett *et al.*, 1984). Our previous studies have revealed that non-growing sarcoma-bearing mice suffer from increased corticosteroid production (Svaninger *et al.*, 1987a) and increased secretion of growth hormone (Svaninger *et al.*, 1987b), in combination with insulin resistance and low plasma levels of tri-iodothyronine (Svaninger *et al.*, 1986). Such hormonal alterations would thus provide possible mechanisms to induce either the expression of different heavy-chain myosin genes (Everett *et al.*, 1984) or altered isomyosins (Dillmann, 1985; Rovetto *et al.*, 1972), as reported for rats suffering from chronic partial starvation for 4 weeks (Dillmann, 1980; Dillmann *et al.*, 1983). Such rats also had depressed Ca^{2+} -activated ATPase activity. In our experiments, the Ca^{2+} -activated ATPase activity in purified myosin was also consistently decreased in all groups of malnourished animals, both rats and mice. The most simple explanation to this could be that myosin V_1 decreased and V_3 increased, as reported for both diabetic and hypothyroid animals (Hoh *et al.*, 1977; Garber & Neely, 1983; Garber *et al.*, 1983), since V_1 is known to have the highest specific ATPase activity. We were, however, unable to detect any evidence of altered proportions of isomyosins in tumour-bearing animals as an explanation of the functional adaptation, in spite of depressed ATPase activity and a lowered thyroid-hormone concentration (Fig. 3; Svaninger *et al.*, 1986). It is likely that isomyosin alterations would need a considerable time period, since myosin has a comparatively long half-life, around 6 days in rats (Martin *et al.*, 1977). It should then be necessary

Samples were pooled from six rats in each group. (b) Densitometer tracings from SDS/polyacrylamide-gel electrophoresis of trypsin-treated cardiac myosin from tumour-bearing (TB), starved (S), protein-energy-malnourished (PCM) and freely fed control rats (C). The abscissa represents 200 000 Da. Samples were pooled from six rats in each group; trypsin treatment was for 20 min.

for more than 12 days to pass before confirming a significantly altered myosin composition in tumour-bearing animals. This time period is considerably longer than that used in our acute starvation experiments. Tumour-bearing mice develop cachexia over 5–7 days, and tumour-bearing rats over 10–12 days, after the onset of tumour growth. Thus it is unlikely that the depressed ATPase activity, which was actually most pronounced in our starved rats, reflects an existing but undiscovered isomyosin composition. Therefore our results suggest that ATPase activity can be independently down-regulated without simultaneous alterations in the transcription of myosin genes. To confirm this hypothesis, measurements of transcription of myosin genes must be performed in combination with measurements of ATPase activity.

In conclusion, this study provides evidence that loss of cardiac muscle mass in tumour disease is communicated by both depressed synthesis and increased protein breakdown explained by anorexia and nutritional imbalance. Decreased RNA capacity seems to be an earlier change than loss of RNA activity in tumour-bearing animals. Increased adrenergic sensitivity and reactivity in hearts from a tumour-bearing host, as found in our previous studies (Drott *et al.*, 1986, 1987; Ransnäs *et al.*, 1989), are not associated with or communicated by increased myosin ATPase activity. On the contrary, depressed ATPase activity was found in all groups of animals with undernutrition. In addition, depressed ATPase activity appeared without any detectable change in the distribution of isomyosin in animals suffering from tumour disease, acute starvation or protein deprivation. The functional protection, particularly in hearts from tumour-bearing animals, is unclear, although membrane receptor changes seem to be involved (Ransnäs *et al.*, 1989).

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