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HEART FUNCTION AND METABOLISM IN MALNUTRITION

Investigations with special reference to cancer cachexia

by

Christer Drott

Göteborg 1987

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av

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1. Drott, C., Ekman, L., Holm, S., Waldenström, A. & Lundholm, K.: Effects of tumor-load on myocardial function in the isolated working rat heart. J Mol Cell Cardiol 18: 1165 - 1176, 1986.

2. Drott, C., Waldenström, A. & Lundholm, K.: Cardiac sensitivity and responsiveness to B-adrenergic stimulation in experimental cancer and undernutrition. J Mol Cell Cardiol, in press 1987.

3. Ransnäs, I., Drott, C., Lundholm, K., Hjalmarson, Å. & Jacobson, B.: Effects of malnutrition on rat myocardial B-adrenergic and muscarinic receptors. Accepted for publication in Circ Res 1987.

4, Drott, C. & Lundholm, K.: Glucose uptake and amino acid metabolism in perfused hearts from tumor-bearing rats. Submitted for publication 1987.

5. Drott, C., Lönnroth, C. & Lundholm, K.: Protein synthesis, myosin ATPase activity and myofibrillar protein composition in hearts from tumor-bearing rats and mice. Submitted for publication 1987.

6. Drott, C., Persson, H. & Lundholm, K.: Metabolic response to adrenaline infusion in malnourished patients with and without cancer. Submitted for publication 1987.

ABSTRACT

Drott, C. Heart function and metabolism in malnutrition. Department of Surgery, Institution I, University of Gothenburg, Sahlgrenska Hospital, Gothenburg, Sweden.

This study has investigated the functional effects of cardiac hypotrophy, alterations of cardiac energy and protein metabolism, myosin ATPase activity and adrenergic adaptation in conditions with malnutrition, particularly in tumor-induced malnutrition.

Growing rats and adult weight stable mice were used. Tumors (methylcholanthrene induced) were transplanted subcutaneously in syngenic animals. In addition to freely fed control animals, reference animals with pure malnutrition were used. Starvation (96 hrs) and feeding a protein free diet for 2 weeks (PCM) induced severe malnutrition and subsequent heart hypotrophy. The isolated working rat heart model was employed to assess heart pumping performance and reaction to catecholamine stimulation in vitro. Protein synthesis was measured in vivo by the injection of large doses of radioactive amino acids (flooding technique). Cardiac B-receptor affinities were investigated by radioligand binding experiments. Myosin concentration and pattern of myosin tryptic digests were determined by SDS poly acrylamide gel electrophoresis. Ca^{++} activated ATPase activities were assessed in purified native myosin. Finally, malnourished patients with and without cancer were infused with adrenaline in comparison to healthy control individuals.

Cardiac pumping performance was well maintained in hypotrophic hearts from both tumor-bearing rats and rats with benign conditions of malnutrition. The functional protection of the heart was associated with increased sensitivity and reactivity to catecholamines and increased B-receptor affinities. Cancer patients had elevated plasma adrenaline concentration. The metabolic response to adrenaline infusion was maintained and in some respect increased in malnourished patients. The wasting of cardiac muscle was rather dependent on increased protein breakdown in tumor-bearing animals compared to states of pure malnutrition where depressed synthesis dominated. No transcriptionally regulated myosin alterations could be detected although myosin ATPase activities were markedly depressed in all hypotrophic hearts. Tumor-bearing rats had a reduced cardiac glucose uptake and a consistently increased oxygen consumption compared to all other animal groups.

It is concluded that functional protection prevents heart insufficiency in cancer cachexia and probably also in benign severe malnutrition. Adaptation mechanisms, operating through adrenergic modulation, seem to be a response to general heart wasting rather than being tumor-specific. Hearts from tumor-bearing hosts exhibit increased preference of fat as energy substrate. Myocardial wasting in tumor disease is a composite of reduced protein synthesis and increased protein degradation rate. The increased oxygen uptake in hearts from tumor-bearing rats may indicate that the heart has been identified as an energy draining compartment in a tumor-bearing host.

Key words: Heart function, cancer, myosin ATPase, heart contractile proteins, heart protein synthesis, cardiac B-receptors, catecholamines, cardiac glucose metabolism, cardiac oxygen uptake.

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These papers are referred to by their Arabic numerals in the text.

Abbreviations employed

ATP, adenosine triphosphate

cAMP, cyclic adenosine monophosphate

DNA, deoxy ribo nucleic acid

dP/dt, first derivative of pressure development with time

EDTA, ethylene diamine tetra acetic acid disodium

GTP, guanosine triphosphate

Gpp(NH)p, 5'-guanylyl-imidodiphosphate

GDP, guanosine diphosphate

HPLC, high pressure liquid chromatography

ICYP, iodo cyano pindolol

kPa, kilo Pascal

N_s, stimulatory guanine nucleotide binding regulatory protein

PAGE, poly acrylamide gel electrophoresis

PCM, protein calorie malnutrition

PF, pair-fed control animals

PW, pair-weighed control animals

QNB, quinuclidinyl benzilate

RNA, ribo nucleic acid

SDS, sodium dodecyl sulphate

uCi, micro Curie

CONTENTS

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BACKGROUND

There has been a myth that the heart is in some way special and does not suffer adversely from the effects of malnutrition. Sparing of heart tissue was supposed to reflect "the wisdom of nature" to protect vital organs (Foster, 1895; Evans, 1945).This teleologic reasoning has, however, not proved to be entirely correct.The origin of this myth is a study performed by Carl Voit in 1866 (Voit, 1866). He starved one cat for 14 days and found a 33 percent loss of body weight but only a 2,6 percent loss of heart weight. Carl Voit himself did not put any emphasis on his finding but in his paper referred to Chossat who already 1843 performed detailed studies of organ weights in starved pigeons (Chossat, 1843). Chossat found 45 percent reduction of heart dry weight compared to 51 percent loss of skeletal muscle in starvation. Despite the careful studies of Chossat the unfortunate single cat experiment by Carl Voit had a prolonged impact on the general view of the heart in inanition (Vaquez, 1921; Evans, 1945). In 1920 Krieger published a review of the literature dealing with heart weights in various catabolic conditions (Krieger, 1920). Regardless of the cause of cachexia, all species of experimental animals and man showed a pronounced hypotrophy of the heart. The first comprehensive experiments with tumor-bearing rats and mice were performed in 1910 by Medigreceanu who found hypertrophy of the liver but hypotrophy of the heart (Medigreceanu, 1910).

The horrors of Nazi concentration camps led to pronounced emaciation of a vast number of people during World War II. Despite cardiac hypotrophy, impairment of heart function was not a prominent feature. Infections, primarily pulmonary were instead identified as the cause of death in the majority of patients dying from starvation (Dmochowski & Moore, 1975). Sudden death during refeeding was, however, observed and might in the light of more recent observations have been associated with cardiac arrhythmias.

The so called Minnesota experiments were performed on human volonteers who were subjected to semistarvation leading to severe cachexia (Keys et al, 1950). Semistarvation was found to cause bradycardia, hypotension, reduced cardiac output, reduced whole body energy expenditure and hypotrophy of the heart. Electrocardiographic changes included low voltage and prolonged systole. Despite all these alterations of heart function, no subject showed signs of circulatory insufficiency. The con-

clusions of these studies were that heart muscle mass and performance were adapted to the decreased whole body energy expenditure and the subsequent reduced circulatory demand.

Clinical studies of marasmus or kwashiorkor have shown that heart weight, heart rate, cardiac output and stroke volume decline in a linear fashion with reduction of body weight but clinical signs of heart failure are infrequent (Kerpel-Fronius & Varga, 1949; Smythe et al, 1962; Alleyne, 1966; Viart, 1977). Some investigators have found myocardial fibrosis, interstitial oedema and heart dilatation (Piza et al, 1971; Thomas et al, 1972). These clinical studies are, however, often confounded by known or unknown incidence of sepsis and vitamin or mineral deficiency which have specific adverse effects on the heart (Cohen et al, 1976; O'Connor et al, 1977). Uncomplicated starvation such as anorexia nervosa leads to reduction of heart mass but left ventricular function has been found unimpaired (Gottdiener et al, 1978). On the other hand, several studies have shown sudden death associated with refractory ventricular arrhythmias in patients undergoing extreme weight reduction regimens (Isner et al, 1979; Sours et al, 1981). Myocardial changes due to dietary factors might thus induce electrical instability of the heart. On the other hand it seems that the incidence of myocardial infarction in patients with cachexia is lower than expected (Wilens et al, 1967). Malnutrition is associated with increased morbidity and mortality in patients undergoing cardiac procedures (Blackburn et al, 1977). There are, however, no evidence that heart failure is responsible for this increased complication rate.

In summary, the functional significance of cardiac hypotrophy is not clear and clinical studies of the heart in malnutrition have not shown evidence of heart failure but rather an adaptation to the concomitant reduction of circulatory demand. The hemodynamic characteristics are as whole comparable with the well known changes seen in hypothyroidism (Morkin et al, 1983).

INTRODUCTION

Cachexia is a common manifestation of cancer and contributes to physical disability and mortality. The incidence of cancer is increasing and the number of tumors for which we have options of cure or long term remission are also increasing. Modern treatment with surgery, radiotherapy and chemotherapy often add to catabolism of the patient. Cancer cachexia

is thus an increasing clinical problem. Since Warren, 55 years ago, in a study of 500 autopsies on cancer patients, reported that cachexia was the principal cause of death (Warren, 1932) much research effort has been devoted to evaluate the mechanisms behind and the effects of progressive catabolism in malignant tumor disease. Only sparse information is, however, available on specific organ derangements in cancer patients. This is the first systematic study of the distant influence of malignant tumor growth on the heart.

The degree of cachexia in cancer seems often to exceed that which may reasonably be expected on the grounds of the extent of the neoplastic process. Thus it is acknowledged that cancer profoundly influences the host metabolism in a way which is completely separate from the physical effects of tissue organ invasion. It is therefore clear that the tumor can communicate its prescence to distant non-involved organs. Effects on the brain cause anorexia by some "uncoupling" of the physiological fine tuning of food intake which normally match the demands. Anorexia is consequently a prominent feature that can explain most of the tumor associated cachexia (DeWys, 1977; Lundholm et al 1980; Lundholm et al 1981;). It has been reported that overt cachexia can occur in patients with tumors which comprise less than 0.01 percent of the total body weight (Nathanson & Hall, 1974). The total tumor mass of the majority of terminal cancer patients, even without obstruction of vital organs, seldom exceeds 500 grams (Costa, 1977). Tumors therefore, are often lethal in spite of the fact that they only constitute a minor part of the host. The pregnant woman on the other hand is able to nourish the growing fetus to a final weight of around 3.5 kilograms without developing cachexia. The human body is thus able to compensate for and support a much larger and more rapidly growing "parasitic" mass of tissue than is the case in cancer. Apart from the increase of food intake pregnancy leads to adaptive increase of heart mass as a response to increased circulatory demands (Katz et al, 1978; Buttrick et al, 1987). In contrast malignant tumor growth is associated with inadequate nutrient intake despite increased energy expenditure (Warnold et al, 1978; Bastable et al, 1979; Costa, 1977; DeWys, 1977; Young, 1977; Walker & Gray, 1983; Lindmark et al, 1984).

In contrast to skeletal muscle the heart must function continually in order to maintain life and it is, of course, vital that the heart can respond

properly to the increased circulatory demands in tumor disease. Heart muscle wasting is,however, almost proportional to the general loss of lean body mass in cancer and not different from the cardiac hypotrophy in non-tumor states of cachexia where circulatory demands often are decreased (Smith,1928; Addis et al, 1936; Wood et al, 1982). The cardiac hypotrophy thus stands in conflict with increased circulatory demands in cancer disease but not necessarily in pure starvation.This may be further emphasized in conditions with complications and stressful treatment regimens. Despite hypotrophy of cardiac muscle, overt signs of heart failure is not a general clinical feature in cancer disease. Cardiodepressant circulatory factors have been reported in specific malignant diseases e.g. leukemia (Mir, 1981) and certain therapeutic regimens e.g. doxorubicin have direct cardiotoxic side effects (Bristow et al, 1981). There are, however, few reports addressing the impact of tumor disease itself on heart function.

Electrocardiographic findings in starvation includes low voltage, bradycardia, prolonged QRS complex and increased Q-T interval (Simonson et al, 1948; Burch et al, 1968). In contrast, clinical cancer is often associated with increased heart rate even in the abscence of sepsis or other complications (Karlberg et al, 1981; Lindmark et al, 1984). A large epidemiological study has shown that increased heart rate might constitute an independant risk factor for cancer mortality in men (Persky et al, 1981). Feldman et al found that patients with a QRS complex shorter than 0,08 seconds had a significantly higher incidence of neoplasia compared to patients with longer QRS complex (Feldman et al, 1982). The increased heart rate in cancer disease is consistent with the increased whole body energy expenditure and may be secondary to increased circulatory demand mediated by hormonal and neural changes. Alterations of circulating substrate levels and changes of myocardial metabolism in malignant disease compared to non-malignant causes of heart cachexia might also play a roll. The heart constitutes less than one percent of total body weight but utilizes about ten percent of total basal oxygen and energy consumed (Brachfeld, 1978). This makes the heart the metabolically most active organ of the body. Consequently, alterations of heart metabolism can considerably affect whole body oxygen consumption. The heart might thus be involved as an energy draining organ in progressive cancer cachexia.

AIMS OF THE STUDY

It has previously been shown that the heart undergoes hypotrophy in cancer cachexia. If heart function should deteriorate in parallel with the loss of myocardium one would expect symptoms of cardiac dysfunction. Clinical empirism, however, has not given support to this expectation. This is not necessarily evidence of a nonexistent problem but could also indicate that the issue have not received appropriate attention and investigation. If protection of function occurs in the hypotrophic heart, this may include alterations in protein composition, energy metabolism and hormonal regulation. Knowledge of these processes are of prime interest in the understanding of adaptive mechanisms and may provide a basis for therapeutical approach. Since one of the main features of cancer cachexia is the associated anorexia several different reference states of malnutrition are almost mandatory in addition to freely-fed control animals in studying the distant effects of tumors.The overall strategy of this study was to explore the distant influence of malignant tumor growth on the heart in an animal model and thereafter to assess whether some of its implications were valid also in malnourished patients with and without cancer.

The specific aims of this study were:

1. To evaluate the effects of tumor associated cardiac hypotrophy on heart pumping performance and oxygen consumption.
2. To investigate whether altered adrenergic regulation of the heart is involved in the functional adaptation to cardiac hypotrophy in tumor disease.
3. To evaluate whether the presence of a malignant tumor affects myocardial energy metabolism.
4. To evaluate heart protein metabolism and composition of cardiac contractile elements in tumor disease.
5. To investigate whether malnourished cancer patients have adaptive changes in their whole body metabolism in response to alterations in the adrenergic state.

METHODOLOGICAL CONSIDERATIONS

ANIMAL STUDIES

Tumor and animal model

The tumor-bearing animal models used in this study have previously been extensively used in our laboratory and are well characterized (Lundholm 1975; Ekman 1980; Lindmark 1985a; Moldawer 1986; Ternell 1986; Svaninger 1987b). Growing rats were used in heart perfusion experiments. The rationale for this choice was that rat hearts are big enough to permit technically easy perfusions and that transplantable tumors grow poorly in adult rats. For in vivo experiments on heart protein synthesis, adult non-growing mice were used in addition to rats. In using both growing rats and non-growing mice it must be born in mind that host response to tumor growth may not be the same in young growing and weight stable adult animals due to inherent differences in hormone levels.

Male, growing Sprague-Dawley rats were obtained from Anticimex, Södertälje, Sweden. All animals in each series of experiments were born on the same day. On arrival the rats were randomly allocated to tumor inoculation or the various control groups. Tumor inoculation was performed during light ether anaesthesia in 3 - 4 weeks old animals (body weight 80 - 100 grams). The tumor (methylcholanthrene induced sarcoma) was transplanted subcutaneously in the flanks under aseptic conditions using a trocar. After 10 - 14 days the tumors become palpable and after 25 - 30 days the rats die spontaneously with cachexia. Experiments were performed three weeks after tumor implantation. At this time the rats appeared healthy apart from their tumor lumps and reduction of subcutaneous fat. The tumor did not impair their movements and no signs of metastasis were seen. A few rats with tumor ulceration of the skin or intraabdominal tumor growth (from inadvertent intraabdominal puncture at the time of inoculation) were discarded. At the time of sacrifize tumor weight comprised 13 - 20 percent of body weight. Female adult weight stable mice were used for in vivo studies of protein synthesis (C57/BL 6J, Bomholtgård, Ry, Denmark). A methylcholanthrene induced sarcoma was transplanted as described above for rats. The mouse tumor has the same biological behaviour as the previously described rat tumor apart from a faster relative growth rate. The mice die with cachexia 15 - 17 days following tumor implantation. Experiments were performed 11 - 12 days after tumor transplantation when the tumor comprised 10 - 15 percent of body weight.

Caution is necessary when extrapolating data from animal models to the clinical situation. The main differences are the very rapid growth and great tumor mass in experimental tumors compared to clinical cancer. Thus, experimentally induced tumors in animals may constitute 20 - 30 percent of body weight whereas the tumor burden in human cancer rarely exceeds 5 percent of the body weight (Costa, 1977). It has, however, previously been shown that many host reactions are similar in tumor-bearing animals and cancer-bearing man (Lundholm et al, 1978a).

Reference and control animals

An important aspect in all research concerning cancer is selection of appropriate controls. Much, and perhaps even the main part of the distant effects of malignant tumor-growth may be related to the concomitant anorexia (DeWys, 1977; Lundholm et al, 1980; Lundholm et al, 1981). The decreased food intake is of course dependent on the tumor but alterations of metabolism, such as acute phase response and increased energy expenditure may add to the catabolic effects of anorexia (Bastable et al, 1979; Lindmark et al, 1983; Lindmark et al, 1984). To strictly define tumor-specific effects would thus require controls not only matched for food intake but also with an inflammatory reaction of the same magnitude as that of tumor-bearing animals. It is, however, in practice impossible to create conditions that exactly mimic the pattern of spontaneous food intake of tumor-bearing animals. A sterile inflammatory reaction can be induced by e.g. injection of turpentine but it is impossible to obtain a graded response. In these studies we therefore mainly chose reference conditions of very severe malnutrition in order to exaggerate the nutritional effects on the heart, thereby focusing more on qualitative alterations for comparison to the tumor-bearing state.

Apart from non-tumor-bearing freely-fed control animals, two groups of nutritionally deprived rats were used as additional references. Starvation and protein calorie malnutrition were prolonged to obtain very pronounced cardiac hypotrophy in order to fully explore functional and metabolic alterations. Starvation for four days resulted in a final body weight around 65 percent of freely-fed control animals. Protein-calorie malnutrition represented a more prolonged way to induce cachexia. This was accomplished by giving rats a protein free diet for two weeks resulting in a final body weight around 40 percent of freely-fed control rats. The composition of protein free diet is shown in Paper 1. This chow also led

to a drastically reduced caloric intake thus yielding rats with both protein and calorie malnutrition. The time course of food intake and body weight for all groups is shown in Paper 1. In an attempt to distinguish between effects of the tumor per se from the effects of anorexia on heart protein synthesis, pair-weighed and pair-fed animals were used. The rationale to use these reference groups in the mouse experiments is the fact that the mouse tumor model is associated with early and a more pronounced anorectic effect than the rat tumor model. Thus sarcoma-bearing in rats caused a 12 percent decrease in cumulative food intake during the period from tumor transplantation to the time of experiment, whereas sarcoma-bearing mice showed a 30 percent reduction. Pair-fed animals were given the same amount of food as the spontaneously eating tumor-bearing animals on a daily basis. Pair-weighed animals were offered a daily amount of food in order to obtain a loss of body weight corresponding to the carcass weight loss of tumor-bearing animals. Food was given twice daily. Pair-fed animals can control for depressed food intake to some extent by adaptation of energy and nitrogen metabolism. Pair-weighed animals are more calorie restricted than pair-fed. Thus pair-weighed mice received 56 percent of the spontaneous food intake of freely-fed control mice. However, both models of calorie and nitrogen restriction are hampered with the problems of meal feeding and altered diurnal eatings compared to freely-fed animals. All animals had free access to tap water and were kept in a room with constant temperature and humidity on a 12 hour light-dark schedule. In rats general anaesthesia was induced by intraperitoneal injection of pentobarbital (Nembutal^{R} 60 mg/kg b w) before heart exstirpation. Mice were killed by cervical dislocation.

Heart composition and tumor growth

The tumors consisted of two well defined lumps that could easily be dissected free for weighing. Heart dissection and lipid extraction are described in detail in Paper 1. Care was taken to dissect the chambers free from atrial and extracardiac tissues. Hearts were dried to constant weight at +80^{o} C and no difference of cardiac water content was found between groups after perfusions or in unperfused hearts.

The isolated perfused, working rat heart model

Langendorff introduced the method of isolated hearts perfused in a retrograde fashion in 1895 (Langendorff, 1895). The retrogradely perfused heart is often referred to as "non-working" which correctly speaking is

misleading since the heart indeed contracts rythmically and produces a ventricular pressure although minimal volume work is performed. In the antegradely perfused heart, "working" preparation, both the aorta and the left atrium are cannulated which enables the heart to do external work and maintain its physiological role as a pump in vitro (Neely et al, 1967). In comparison with other in vitro muscle preparations the isolated heart has many advantages. Oxygen, substrates and hormones are provided in a physiological way through the capillary network and changes in uptake and release are easily measured. Vast amounts of investigations have used this model in studies on heart physiology and metabolism in hypertension, diabetes, ischemia, and nutritional manipulation. The usefulness of this model in investigations of heart physiology and metabolism has recently been reviewed (Williamsson & Kobayashi, 1984). When comparing the isolated perfused heart to the in vivo situation it must be emphasized that the heart is influenced in vivo by factors such as lipolysis leading to increased circulating free fatty acids as well as other variations of substrate and hormone levels and possible interactions between these factors. It must also be pointed out that the isolated heart is denervated thus excluding the effects of systemic nervous reflexes. However, as the initial purpose of this study was to delineate intrinsic changes of the heart induced by tumor growth it was advantageous to eliminate direct humoral and neural influences. Thus, the isolated working heart preparation was considered useful for the present experiments.

Rat heart perfusions

The rats were heparinized (1000 IU/ kg b w given i.p.) 30 minutes prior to heart exstirpation. After anaesthesia, the chest was opened by lateral incisions at both sides of the midline so that the whole anterior part of the thorax could be lifted upwards to expose the heart. The heart was carefully picked up and the large vessels were severed with one single cut with a pair of scissors. The heart was immediately immersed in ice-chilled saline. When the heart had stopped beating, the aorta was attached to the perfusion cannula by a ligature (Fig 1). Retrograde preperfusion was immediately started from a reservoir 70 cm above the heart. Preperfusion was carried out for 5 - 10 minutes and the perfusate discarded after one passage through the heart. During preperfusion the left atrium was cannulated through a pulmonary vein. Care was taken to ligate all other pulmonary veins to prevent leakage from the left atrium. The pulmonary artery was cannulated by a fine polyethylene catheter (Fig 1).

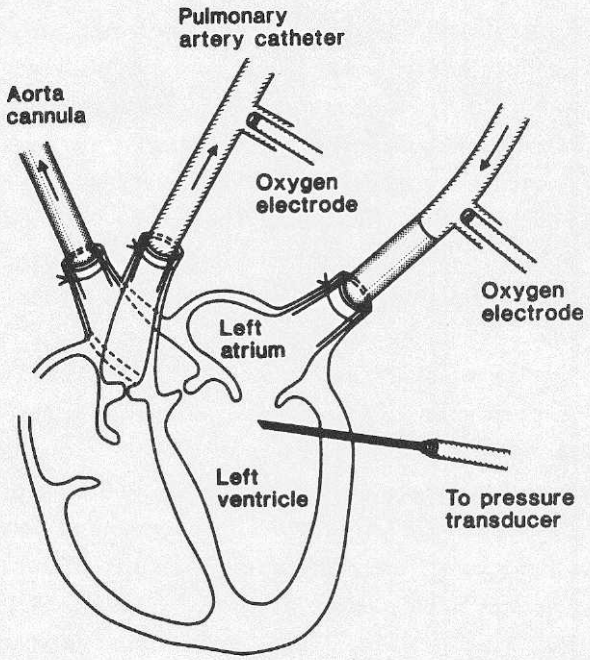

Figure 1. Preparation of the heart in the isolated, working, perfused heart model. The aorta was first attached to a steel cannula by a ligature. This route was used for retrograde preperfusion during which the left atrium was connected to a steel cannula and the pulmonary artery to a polyethylene catheter.Oxygen electrodes were placed in contact with the perfusate just prior to entry into the heart and after passage through the myocardium thus enabling measurement of myocardial oxygen uptake when the preparation was switched to antegrade perfusion. A cannula connected to a pressure transducer was inserted in the left ventricle. Pacing was performed via the aorta and left ventricular cannulas.

Antegrade perfusion with recirculating buffer (in most experiments 100 ml) was started by clamping the tube from the preperfusion reservoir and unclamping the tube from the atrial bubble trap (Fig 2). Left atrial filling pressure was altered between 5 and 20 cm water by changing the vertical distance from the overflow outlet of the bubble trap to the left atrium. The left ventricle pumped the perfusate via a pressure chamber with 2,5 ml air in order to provide elasticity. Aortic output was measured at the top of the oxygenating chamber 80 cm above the heart. The coronary flow was measured as the sum of perfusate ejected through the pulmonary artery cannula and fluid dropping from the heart. Flow was quantified by collection in a graded cylinder. In some experiments maximal afterload was

accomplished by clamping the aortic tube. Left ventricular pressure, maximum positive dP/dt and heart rate were recorded with a Statham p 23Db transducer connected to a 18 gauge cannula inserted through the left ventricular wall (Fig 1). Recordings were obtained on a Grass polygraph model 7D (Grass Instruments Co, Mass, USA). In some experiments the hearts were atrially paced at 350 beats/minute by means of a pacemaker (Elema-Schönander, Sweden). The energy of the pace signal was set to two times the measured threshold value necessary for eliciting a contraction in each individual heart. Hearts were discarded if there were signs of air embolism, leakage from the left atrium, marked arrythmia or an aortic pressure less than 80 cm water. To ensure the stability of the experimental set up a few perfusions were carried out during four hours after which the hearts were still vigorously working. Krebs-Henseleits buffer was used through all experiments (Krebs & Henseleit, 1932). Disodium EDTA (0,5 mM) was included to chelate trace quantities of heavy metals in the perfusate. The final concentration of the buffer expressed as mM was: glucose 14; NaCl 118; KCl 4,7; $CaCl_2$ 2,5; $MgSO_4$ 1,2; KH_2PO_4 1,2; $NaHCO_3$ 25. All perfusions were carried out at $+37^{o}C$.

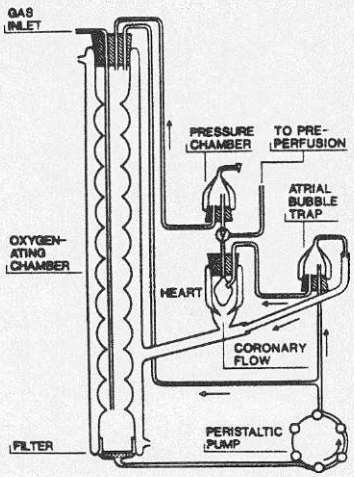

Figure 2. Perfusion apparatus used for in vitro rat heart perfusions. A peristaltic pump circulates the perfusate from the oxygenating chamber to a bubble trap used to avoid air embolism to the heart. The height of the bubble trap can be varied to obtain different hydrostatic pressure of the inflow to the left atrium, thereby altering preload. A pressure chamber filled with air is located on the outflow side of the system thus providing elasticity as the tubes are stiff. The left ventricle pumped the perfusate to the top of the oxygenating chamber 80 cm above the heart.

Mechanical heart performance

Evaluation of cardiac performance has long been a problem that has plagued investigators. This problem is derived from the fact that performance of the heart as a pump may be altered by multiple factors, the major of which include: 1) the initial myofibre lenght often indirectly assessed by the end diastolic ventricular volume or the left atrial filling pressure (preload); 2) the resistance to ventricular emptying, as determined in a complex manner by the aortic pressure (afterload); 3) alterations in the contractile properties of the muscle itself (contractility) (Brutsaert & Sonnenblick, 1973). The distinction between changes in cardiac performance due to the Frank-Starling mechanism and those due to changes in contractility are crucial. The Frank-Starling mechanism is the phenomenon that the mechanical response increases progressively as the length at which the muscle fibre is held is increased. An altered response of developed force, fibre shortening or velocity of shortening which is independent of muscle fibre length means a change in contractility. By definition the study of hearts in various cachectic states means that heart size in study and control groups differ. Differences in heart volume and wall thickness means that a given left atrial filling pressure does not yield the same end diastolic left ventricular wall tension in various hearts according to the law of Laplace. Thus, the wall stress is proportional to intraventricular pressure and radius and inversely proportional to wall thickness (Hopkins et al, 1973). The translation of left atrial filling pressure to end diastolic fibre length is further complicated by possible differences of left ventricular compliance and geometry of the heart. In theory the heart shape may vary from a thin walled spherical to a thick walled ellipsoid form. These differences occur between various hearts and within the same heart during contraction. Furthermore, the orientation of muscle fibers within the heart is extremely complex and not parallel but oblique. In conclusion, there does not appear to be a variable which is a well validated index of myocardial contractility in the intact heart (Noble, 1972; Brutsaert & Sonnenblick, 1973). Differences in cardiac output, left ventricular peak systolic pressure and pressure development during systole (dP/dt) between hearts are thus net reflections of a variety of possible heart changes including the theoretical concept of contractility. The methods used in this thesis in order to assess the mechanical activity of the hearts included the measurement of coronary and aortic flow, the left ventricular pressure development during systole and the peak left ventricular systolic pressure. By sliding the atrial bubble trap up and down

(Fig 2) the left ventricular filling pressure was varied between 5 and 20 cm water. For simplicity we used the expression preload for left ventricular filling pressure despite the theoretical objections mentioned above. The reason to use pacing was to normalize diastolic filling time and to avoid the so called Treppe phenomenon which means that increased heart rate is associated with increased contractility.

Oxygen uptake and adequacy of oxygenation

Hemoglobin free oxygen transport requires high flow rates to provide adequate tissue oxygenation from the physically dissolved oxygen. Thus, coronary flow was high but the coronary vascular bed was not maximally dilated as it was able to adjust to increasing work loads. Oxygen tension of the affluent perfusate was continuously measured just before entering the left atrium and the effluent was measured just after leaving the heart by the pulmonary artery (Fig 1). The electrodes and calibration procedure are described in detail in Paper 1. Oxygen tension of the affluent perfusate was adjusted to around 70 kPa. No arterio-venous shunting occurs in the isolated perfused rat heart (Huhman et al, 1967) and the coronary flow is uniform throughout the heart (Shipp et al, 1964). The fact that coronary effluent oxygen tension was always above 10 kPa thus indicates a sufficient oxygen supply to the myocardium. Moreover, perfusion with blood instead of oxygenated buffer does not change the oxygen uptake even at high pressure work (Gamble et al, 1970). In a recent review Opie came to the conclusion that oxygenation of the isolated, working perfused rat heart is adequate (Opie, 1984).

Perfusions in the presence of catecholamines

To avoid oxidation, noradrenaline and isoproterenol solutions were made fresh each morning and kept in darkness, at $+4^{o}C$ in the presence of an antioxidant (ascorbic acid). The catecholamines were added to the perfusate in the oxygenating chamber to allow dilution before entering the heart. The amount of catecholamines necessary to elicit a barely detectable response was defined in pilot studies and that concentration was used at the start. Addition of catecholamines in excess of the highest concentrations used in the experiments gave no further increased response. In order to avoid differences in desensitization the hearts were perfused for exactly three minutes on each level of catecholamine concentration.

Cardiac glucose uptake in vitro

Glucose uptake can be measured as the disappearance of glucose from the perfusate but the use of radioactively labeled glucose has been reported to be a more sensitive method (Cheung et al. 1978). (2-^{3}H) glucose was evaporated to dryness in order to eliminate any ^{3}-H_2O before addition to the perfusate of the heart preparation. The initial five minutes of antegrade perfusion was used for stabilization of the hearts and equilibration between perfusate and tissue pools. Thereafter, the perfusions were carried out during one hour at steady state conditions with a preload of 10 cm water and pacing. Samples of the perfusate were removed 5, 35 and 65 minutes following the start of antegrade perfusion. The tritium incorporated into water was quantified in a scintillation counter after isolation of pure water by vacuum destillation at low temperature. The hydrogen in the 2-position is the first to be transferred after glucose entry into the cell. It takes place by the isomerization reaction of glucose-6-phosphate to fructose-6-phosphate and thus reflects the total uptake of glucose and is not a direct measure of oxidation of glucose (Katz, 1976). The conversion of carbon radiolabel from glucose into carbon dioxide provides a mean to quantify the oxidation rate but this method does not account for glucose conversion into lactate, amino acids or glycogen. The rationale to use tritium labelled glucose to obtain total cellular uptake ratios was the fact that hormonal and other regulatory mechanisms of glucose metabolism mainly operate by modifying transmembrane transport (Opie, 1968; Brachfeld, 1978).

Cardiac amino acid metabolism

Perfusate samples obtained simultaneously with those for glucose uptake determination as described above were analyzed with respect to amino acid content by the use of high pressure liquid chromatography (HPLC). After a precolumn derivatization procedure (Hill, 1979), the amino acids were separated in a gradient system on a reverse phase uBondapac-C18 column (Waters associates liquid chromatographic system). The amino acids were detected fluorometrically and finally quantitated with a data module system (Waters, model 730) for automatic integration of the peak areas using standard amino acids. As the aim was to study protein metabolism rather than total protein breakdown, protein synthesis inhibitors were not used. Thus, the measured release of aminoacids reflects the net effects of protein breakdown, protein synthesis, amino acid oxidation and de novo synthesis of amino acids. Since amino acids such as phenylalanine and

tyrosine are not metabolized in the heart they represent a mean to quantify net protein balance.

Plasma and heart tissue catecholamines

The recovery of catecholamines from biological samples are very sensitive to factors in the work-up procedure and the nature of stabilizing agents. Therefore, heart tissue was immediately immersed in liquid nitrogen after removal from the rats. The frozen tissue was cut into small pieces put into a tissue grinder and weighed. One millilitre of perchloric acid (0.1 mol/l) containing EDTA (2.7 mmol/l) and the internal standard dihydroxybenzylamine (DHBA) (0.2 umol/l) was added per 0.1 g of tissue and the homogenization was performed in an ice-bath. The homogenate was then centrifuged in a refrigerated centrifuge at $4^{o}C$ for 10 minutes at 25.000 g. The supernatant was transferred to a new tube and frozen ($-70^{o}C$) until analysis. The blood samples were collected into chilled tubes containing 20 ul/ml blood of a solution of the anticoagulant ethyleneglycol-bis-(B-aminoethylether) N',N'-tetraacetic acid (EGTA) (0.2 mol/l) and the antioxidant reduced glutathione (GSH) (0.2 mol/l). After centrifugation (1000 g for 5 min) at $4^{o}C$ the plasma phase was separated and stored at $-70^{o}C$ until analysis. The catecholamines were separated from the sample by adsorption to alumina and then desorbed by elution with perchloric acid. The assay was performed using liquid chromtography and electrochemical detection. The method has previously been described in detail and validated against the radioenzymatic method, showing good agreement (Eriksson and Persson, 1982).

Cardiac protein synthesis in vivo

Protein accounts for some 80 percent of heart dry weight in rats (Munro, 1970). The renewal rate of heart proteins is a key regulating process of heart composition and subsequent function. In steady-state, synthesis may be estimated from degradation of labelled proteins, but in non-steady state incorporation of labelled amino acids into newly synthesized proteins is a more valid approach in estimating synthesis (Schreiber, 1982). This can be accomplished by a variety of analytical techniques, each with its own advantages but none without disadvantages and assumptions to make them valid (Waterlow et al, 1978). The main difficulties is to achieve a correct measurement of the immediate precursor pool for protein synthesis. Constant infusion of radio-labelled amino acids relies on the assumption of isotopic steady state and that radioactivity in the acid soluble

fractions are true reflections of the specific radioactivity of the free amino acids at the site of protein synthesis. The validity of this assumption has been questioned as intracellular compartmentalization may cause the specific activity of amino-acyl-tRNA to be different from that of the total free intracellular pool (Martin et al, 1977; McKee et al, 1978). Furthermore, additional errors might arise from recycling of tracer during longer infusion periods. The constant infusion technique has subsequently received considerable criticism (Waterlow et al, 1978; McNurlan et al, 1979; McNurlan et al, 1982;). The flooding technique relies on the assumption that a massive administration of amino acids will saturate all of the free amino acid pools,thus reducing compartmentalization and thereby minimizing the problem of defining the correct precursor pool (Garlick et al, 1980). This method, using the plasma specific activity as a measure of the precursor pool is convenient and the short time span of the experiment lowers the risk of recycling of radiolabel. In direct comparison, the flooding dose methodology has been reported to give significantly higher estimates of protein synthesis compared to the continuous infusion method (Emery et al, 1984; Pomposelli et al, 1985). We have recently validated the flooding technique against the "pulse-labelling" tracer technique which represents a reliable method to quantify protein synthesis rate (Garlick, 1980). The rates of protein synthesis obtained with a flooding dose of either leucine or phenylalanine were significantly higher than those obtained with pulse labeling technique (Ternell, 1986). The major disadvantage of the pulse labelling method is that it requires a large number of animals. A further possible disadvantage is compartmentalisation of aminoacyl tRNA which may occur within the cell (Martin et al, 1977). There are thus still controversies regarding methodology in measuring the true protein synthetic rate in vivo. However, in the relative comparisons between groups of animals the flooding technique may represent a valid and convenient method why we chose this technique. The isotopes were given in a lateral tail vein in rats. After intravenous injection, there is a fast mixing of the substance in blood (Waterlow et al, 1978). In mice intravenous injection is technically difficult why we chose intraperitoneal administration as it has been shown that watersoluble substances like amino acids are rapidly absorbed into the circulation from the peritoneal cavity (Henshaw et al, 1971). A suitable radioactive aminoacid for investigation of the rate of protein synthesis should have no possibility of conversion to other amino acids. In the heart ^{14}C phenylalanine fulfils this criterium, whereas leucin may be less appropriate. We have, how-

ever, determined that more than 95 percent of the radioactivity incorporated into tissue protein was as leucine 30 minutes following an injection of L-(U-^{14}C)leucine (unpublished observations). The animals were given a single injection of a large dose of phenylalanine (150 umol "cold" phe/100 g body weight and 0.4 uCi L-(U-^{14}C)-phe/g bw) or leucine (100 umol "cold" lucine/100 g b w and 0.4 uCi L-(1-^{14}C) leucine/g b w) which give linear kinetics in plasma and tissue pools (Garlick et al, 1980). Plasma proteins were extracted in absolute methanol overnight. The specific radioactivity of phenylalanine or leucine was quantified by means of high pressure liquid chromatography with a precolumn derivatization procedure (Hill et al, 1979). Plasma specific radioactivity was determined during 10 minutes for phenylalanine and during 30 minutes for leucine in a large number of animals. Plasma specific radioactivity of phenylalanine was almost stable during 10 minutes whereas that of leucine showed a linear decrease leaving 20 percent of initial activity at 30 minutes. The disappearance curve for the specific radioactivity thus followed zero order kinetics which simplified calculations of protein synthesis rates. This permitted the calculation of the average plasma specific radioactivities from single measurements obtained at sacrifize at 10 and 30 minutes for animals injected with phenylalanine and leucine respectively. These average plasma values were regarded as the precursor pool for protein synthesis. Amino acids in heart proteins were quantified by conversion of phenylalanine into B-phenetylamine (Garlick et al, 1980) and subsequent spectrophotometric quantification (Suzuki & Yagi, 1976) or by suspension in water for HPLC separation. The leucine content was estimated to be 8 percent and phenylalanine 3 percent of total heart protein (Waterlow et al, 1978). Protein concentration was determined according to Lowry (Lowry et al, 1951). The fractional synthesis rate (k_s) was determined using the equation (Garlick et al, 1980):

$k_s = S_b / S_p \times t$

where S_b is the specific radioactivity of heart protein, S_p is the average specific activity of the free plasma amino acid pool and t is the incorporation time. Myocardial RNA was extracted and quantified as described by Munro and Fleck (1966).

Heart protein composition

In all studies of the heart, but especially in compositional studies one must bear in mind that heart muscle is a mixture of cell types (myocytes, fibroblasts, neural and endothelial cells). Thus 75 percent of myocardial

DNA is associated with connective tissue and endothelial cells (Munro, 1970). Myocytes are, however, so much larger than other cell types that they constitute at least 75 percent of the heart by volume (Wildenthal, 1980). Thus, cardiac protein content may be a better reflection of myocyte mass than the amount of DNA. The heart contains an abundance of mitochondria which constitute around 46 percent of total cardiac protein (Wildenthal, 1980). This fact necessitates isolation of the protein compartments involved in the contractile process when studying functionally important myofibrillar alterations in pathological conditions. We therefore concentrated on determination of contractile proteins. Heart tissue was homogenized in 20 mM potassium phosphate buffer. Total SDS (sodium dodecyl sulphate) lysates were used as this method has been reported to give a higher recovery of myosin than ordinary high salt concentration extractions (Everett et al, 1983). During extraction and purification there is a risk of myosin degradation. Everett has, however, shown that addition of protease inhibitors did not increase the yield of myosin in these preparations (Everett et al, 1983). After denaturation and centrifugation, the supernatants were subjected to SDS polyacrylamid gel electrophoresis. Following electrophoresis, the gels were dried, stained with Coomassie brilliant blue and destained with ethanol, acetic acid and distilled water. Identification of the bands were obtained by comigration of standards of myosin, actin, troponin and tropomyosin. The relative concentration in each band was quantified by densitometer scanning and integrating the area under the curve.

Tryptic digestion of myosin

Functionally important alterations of myocardial myosin composition can be obtained by changes in the expression of specific genetic information of the cell. Thus, three distinct ventricular isomyosines V_1, V_2 and V_3 have been identified by pyrophosphate gel electrophoresis of native molecules (Hoh et al, 1977). The relative isomyosin composition is dependent on species and age (Lompre et al, 1981) and can be influenced by various physiological and pathological conditions (Dillmann, 1985; Mercadier et al, 1981). In our initial experiments we found no difference of electrophoretic mobility of myosin between groups. Separation of the classical three isomyosines by electrophoresis of native molecules might, however, not be sensitive enough to detect minor shifts in their relative distribution. Furthermore, post-transscriptional modification of the myosin molecules can occur and is not necessarily reflected by altered mobility of the

intact molecule in pyrophosphate gel electrophoresis (Scheuer & Bhan, 1979). The point at which myosin is cleaved by trypsin into the meromyosins represents a flexible region of the tail because the susceptibility to cleavage by proteolytic enzymes is greater where the coil conformation is less rigid (Katz, 1977). The peptides thus obtained constitutes a "finger print" of the molecule and may be more sensitive than traditional methods to detect subtle differences in myosin structure. Myosin was extracted by a previously described method (Hoh et al, 1976; Hoh et al, 1977). Ventricular tissue was minced in an extraction buffer with the following composition: 100 mM $Na_4P_2O_7$, 5mM EGTA, 2 mM 2-mercaptoethanol, pH 8.8 at +2C. The homogenate was centrifuged for 3h at 48.000 g. The pellet was discarded and the supernatant thoroughly mixed with an equal volume of ice-cold glycerol. Trypsination was then performed according to Bhan and Malhotra at high ionic strength (0.5 M KCl) to avoid aggregation of myosin which may occur at low ionic strength (Bhan & Malhotra, 1976). Aggregation of myosin might render some of the susceptible bonds unaccessible to trypsin, thus yielding incomplete digestion. The cleavage products were separated in SDS polyacrylamide gel elecrophoresis and quantitated by densitometer scanning.

Cardiac myosin ATPase activity

The splitting of ATP provides energy for the cyclic interaction of myosin and actin, the molecular basis for muscular contraction. It is therefore tempting to postulate that the activity of myofibrillar ATPase is an important control factor in the whole complex chain of events known as excitation - contraction coupling. The main problem is, however, to define which ATPase to measure and during which conditions to measure it in order to obtain an optimal correlation to in vivo contractile properties of the intact myocyte. Thus crude myofibrillar preparations retain the physiologic interrelations of actin and myosin but also contain mitochondria, sarcoplasmatic reticulum and sarcolemma, all of which have their own ATPase activities. At the other end of the purity scale of preparations one can study ATPase activity of proteolytic subfragments of myosin where the enzymatically inactive portion of the molecule has been eliminated (Bhan & Malhotra, 1976). Proteolysis of myosin might, however, lead to degradation of subunits creating artifacts in the determination of ATPase activity (Scheuer & Bhan, 1979). By using pure myosin preparations the problems of contaminating ATPase and preparation artifacts may be avoided. On the other hand, purified intact myosin is removed from actin

and the regulatory proteins tropomyosin and troponin which might alter the enzymatic activity. However, good correlations have generally been found between purified myosin ATPase activity and physiological function (Rovetto et al, 1972; Schwartz et al, 1981; Garber & Neely, 1983; Alpert & Mulieri, 1986). Myosin was isolated as described above. As myosin is insoluble in low ionic strength buffers normally used for electrophoretic studies, a high ionic strength pyrophosphate buffer where myosin is quite soluble was used (Hoh et al, 1976). After electrophoresis, the gels were incubated in ATPase reagent. Determination of ATPase activity was performed according to Hoh et al with spectrophotometric quantification of calcium phosphate precipitate (Hoh et al, 1977). This scanning process was repeated four times during the course of incubation. After scanning for ATPase activity, the gels were stained for protein with Coomassie brilliant blue and scanned again at 550 nm using the same absorbance scale and scan rate as before. The ratio of the area under the calcium phosphate peaks to the area under the protein peaks (the absorbance ratio) was plotted against incubation time. The ATPase activity found in freely-fed control rats of seven weeks age in this study was approximately three times higher than previously found in adult rats (Hoh et al, 1977).

Cardiac B-adrenergic and muscarinic receptor affinities

Radioligand binding techniques in isolated cardiomyocyte membrane preparations were used to determine adrenergic and muscarinic receptor affinities. Preparation of cell membranes is crucial. In earlier studies on avian and amphibian erythrocytes, membranes could be prepared with ease and high purity. Myocytes require a more extensive procedure to give pure membrane preparations. In order to avoid excessive non-specific agonist binding a high degree of purity is necessary. An important step is to remove contractile proteins which is accomplished by washing with 0,6 M potassium chloride. Excessive washing and prolonged centrifugation at high force may, however, damage membrane proteins and cause depletion of guanosine triphosphate which is necessary for adenylate cyclase activation (Pecker & Hanoune, 1977). Therefore, only five minutes exposure of the membranes to KCL followed by centrifugation at 25.000 x g for ten minutes was used. Receptor analysis depends on the use of radiolabelled tracers. Binding affinities of receptors coupled to the adenylate cyclase system are different for agonists and antagonists. Antagonist binding displays only one affinity while agonists exhibit variable binding affini-

ties. Thus, the tracer used should be an antagonist. The antagonist tracers used were (^{3}H)-quinuclidinyl benzilate (QNB) and (^{125}I)-iodocyanopindolol (ICYP) for muscarinic and B-adrenergic receptors respectively. ICYP was chosen because it has lower non-specific binding and a high specificity to B-adrenergic receptors compared to previously used B-adrenergic ligands (Brodde et al, 1981). Carbachol and oxotremorine were used as muscarinic agonists and isoproterenol as B-adrenergic agonist. Binding assays were carried out in phosphate-buffered saline (composition see Paper 3). Receptor agonist kinetics was studied by incubating membranes with either 0.4 nM tritiated QNB or 0.05nM iodinated CYP and various concentrations of carbachol or isoproterenol, respectively. The reactions were carried out at + 26 C and terminated after one hour by ice-cold phosphate-buffered saline. The samples were poured over a Whatman glass filter (GF/F 25 mm), followed by a wash with the same buffer. Radioactivity was then quantified in a Packard scintillation spectrometer. By using equilibrium binding studies and plotting the data according to Scatchard (bound/free ligand against bound ligand concentrations) the eqilibrium dissociation constant and the binding site concentration can be determined. Scatchard plots were utilized in order to ensure that observed agonist affinity changes were not due to altered antagonist binding. Agonist-receptor affinity is influenced by receptor binding to guanine nucleotide binding proteins, which are essential for adenylate cyclase effects. Thus, free receptors bind agonists with a lower affinity than the complex between receptors and guanine nucleotide binding proteins. Guanosine triphosphate (GTP) induces a shift in affinity leaving all the receptors in the free, low affinity form. Antagonist displacement was studied in both the absence and presence of a non-hydrolyzable GTP analogue, 5'-guanylyl-imidodiphosphate (Gpp(NH)p). Non-specific binding was assessed by a 100 fold excess of either scopolamine or alprenolol and never exceeded 5 percent. Data analysis was carried out using computer assisted both linear (Minneman et al, 1979) and non-linear (Feldman, 1972) regression analysis of saturation and competition binding curves.
To determine if the data were fit significantly ($p<0.001$) better by the multi-site model, the residual sums of squares of the respective fits were compared using an F-test (DeLean et al, 1982). Finally, binding parameters were corrected according to Cheng and Prusoff (Cheng & Prusoff, 1973).

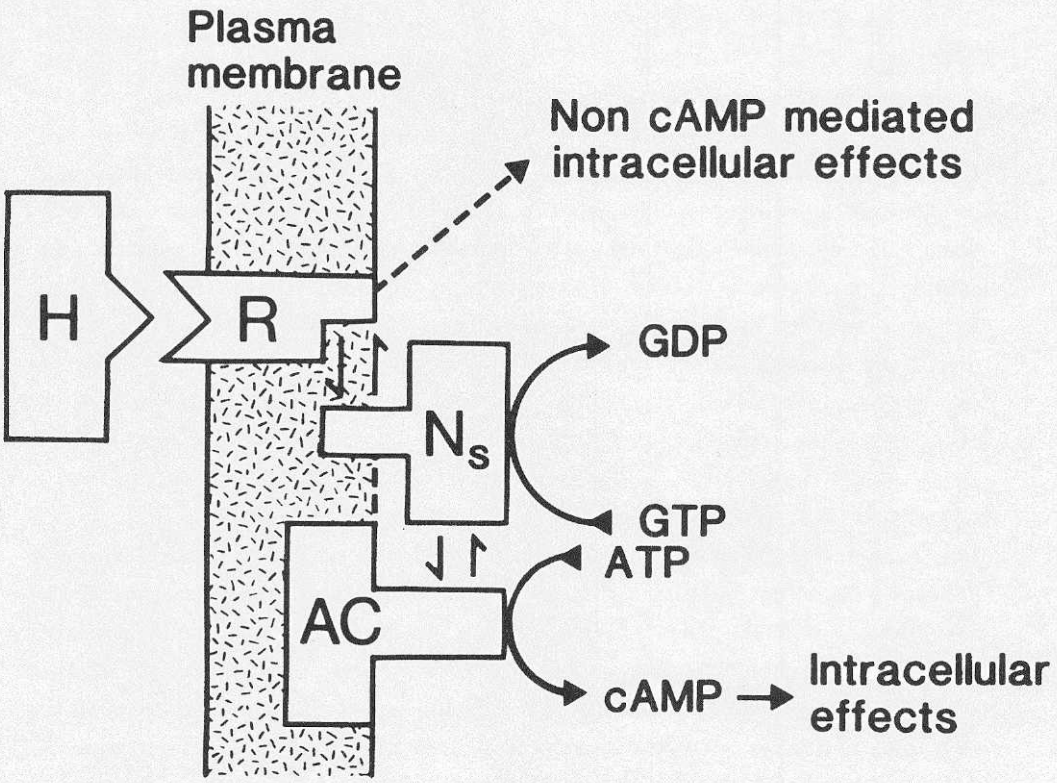

Figure 3.
The mechanism of B-receptor mediated adenylate cyclase stimulation. H = hormone; R = receptor; N_s = stimulatory nucleotide regulatory protein; AC = adenylate cyclase. In the resting state R, N_s and AC do not interact with each other. B-adrenergic agonists (but not antagonists) promote the functional coupling between R and Ns. This favours the release of GDP from N_s and its exhange by other guanine nucleotides such as GTP which in turn destabilizes the R-N_s complex so that it dissociates into R-H and N_s-GTP. The free N_s-GTP complex is then able to stimulate AC. Hydrolysis of GTP into GDP terminates the AC stimulation. When GTP is replaced by a non-hydrolysable analogue such as Gpp(NH)p AC stimulation persists and all B-receptors are left in the low affinity form (R is free and not bound to NH_s).

Adenylate cyclase activity

Adenylate cyclase activity was determined in cell according to the method described by Hanoune et al (1977). The method is described in detail in Paper 3. Adenylate cyclase activity was assayed by increasing concentrations of adrenaline from 10^{-8} to 10^{-4} M. Inhibition of adenylate cyclase activity mediated via muscarinic receptors was monitored in the prescence of 10 uM adrenaline/0.1 mM Gpp (NH)p and increasing concentrations of the muscarinic agonist oxotremorine-M (10^{-10} - 10^{-4} M).

CLINICAL STUDY

Patients

Consecutive malnurished surgical patients with and without cancer disease and well nourished control patients were investigated. The diagnoses of cancer patients were all histologically proven. Nutritional status and body composition were assessed by history of weight loss, body weight, anthropometric measurements, total body potassium content (^{40}K whole body counter) and serum albumin concentration. To account for differences in age and length, body weight index and total body potassium index were calculated from tables derived from a local reference population (Bengtsson et al, 1981) and relationships between ideal body weight and total body potassium (Boddy et al, 1972).

Adrenaline infusion

The experiments were carried out in the morning after an overnight fast. Following baseline measurements, constant adrenaline infusion was started at a rate of 0.005 ug/kg b w/min during 40 minutes resulting in a slight but detectable rise of serum adrenaline conc. After discontinuation of the infusion, the plasma adrenaline concentration promptly returned to preinfusion levels during 40 minutes of rest. The subsequent infusion rate of 0.02 ug/kg/min during 40 minutes gave plasma concentration in the range seen in moderate trauma (Davies et al, 1984). Higher infusion rates were judged to be hazarduous in these elderly and malnourished patients. As adrenaline is sensitive to oxidative degradation, care was taken to protect the infusion lines and bottles from light. All blood samples were obtained from an indwelling arterial cannula which was also used for measurements of blood pressure and heart rate.

Indirect calorimetry

Indirect calorimetry was performed before the infusions started and during the latter half of each of the 40 minutes periods. The system used has previously been described in detail (Lindmark et al, 1985b). The patients head was placed in a ventilated hood where a soft face mask was placed 1/2 cm above the mouth and nose. This allowed the patient to breathe without restrain or discomfort. The air from the hood was drawn by a membrane pump and the flow measured by a flow meter with a precision of 2 %. The air sample for gas analysis was dried in a column. Oxygen was measured with a paramagnetic Servomex 1100 (Taylor, Servomex) and carbondioxide with an LB-2 infrared analyzer (Beckman Instru-

ments). The output signals from each instrument were led to a portable desk computer (Epson HX 20) via an analogue/digital coupler (KEBO Computer AB, Stockholm, Sweden). The computer collected values every 10 seconds from the instruments and processed the data to give oxygen consumption, carbondioxide production and respiratory quotient after a complete run. The integrated system has a coefficient of variance of 3 - 4 percent when used repeatedly on the same subjects over several days (Lindmark et al, 1985b).

STATISTICS

Values are expressed as mean $\pm$ SEM. For comparison of means the non-parametric Mann-Whitney U-test (Siegel, 1956) was used when the numbers of observations were small to avoid assumptions of normal distribution and the parametric t-test (Colton, 1974) was used when larger numbers of observations were computed. When analysis of variance (Afifi & Azen, 1979) was used, possible individual differences between groups were analyzed by a multiple range test (Woolf, 1968). Least square regression analysis was used in calculations of correlation. P values less than 0.05 were considered as statistically significant.

RESULTS

Body and heart weights

Table 1 shows body weight, heart dry weight and ratio of heart weight to body weight in tumor-bearing and the various control groups of rats (pooled data from all the studies). The tumor comprised approximately 15 percent of body weight leaving a carcass (tumor-free body weight) weight in tumor-bearing rats of around 82 percent of the body weight of freely-fed control rats.

Table 1. Body weight, heart dry weight and the ratio of heart dry weight to body weight in tumor-bearing (TB), starved (S) and protein-calorie malnourished (PCM) rats expressed as percent of freely fed control (C) rats (pooled data from all the studies). Mean ± SEM.

	Body weight (%)	Heart dry weight (%)	Heart dry weight/ body weight (%)
TB	97±3	88±2[a]	89±1[a]
S	65±4[a]	69±4[a]	107±1[a]
PCM	38±2[a]	45±3[a]	116±1[a]
C	100±2	100±2	100±0.5

a)$p<0.01$ vs C.

Cardiac mechanical performance and oxygen uptake

Cardiac output, left ventricular peak systolic pressure and contractility were well maintained in the hypotrophic hearts from both sarcoma-bearing, starved and PCM rats during various left atrial filling pressures (paper 1) as well as during catecholamine stimulation (Paper 1 and 2). The pumping ability expressed per gram myocardial mass was thus improved when depicted as Frank-Starling relationships (Fig. 4). Oxygen uptake was consistently increased in tumor-bearing rats compared to freely-fed control rats regardless if it was normalized to the entire heart, heart dry weight or left ventricular work (Fig.5; Paper 1 and 2). Starvation and PCM were on the other hand associated with decreased oxygen consumption compared to freely-fed controls.

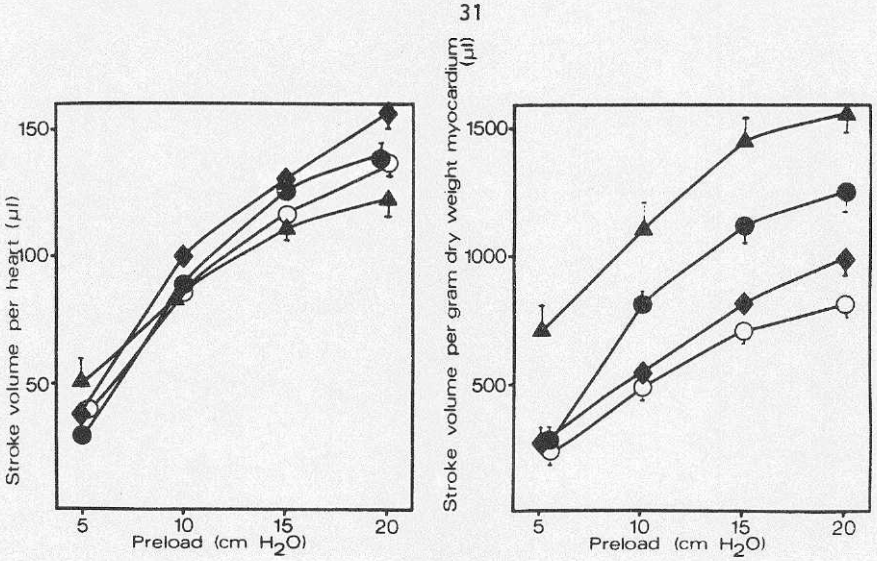

Figure 4.
Frank-Starling relationships when hearts were paced at 350 beats/min ◆ Freely-fed tumor-bearing animals (n = 20); ● Starved rats (n = 20); ▲ PCM rats (n = 10); ○ Freely-fed controls (n = 20).

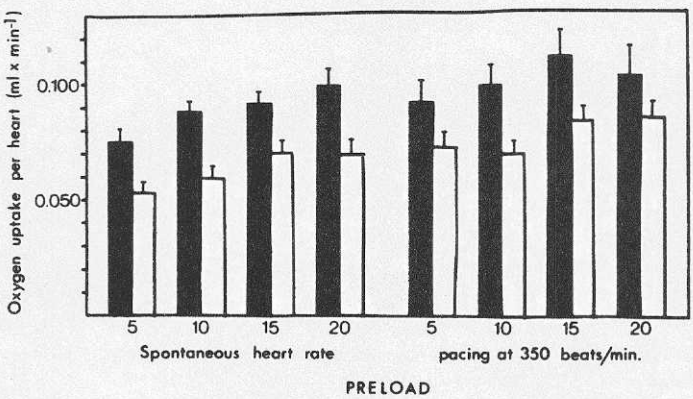

Figure 5.
Oxygen uptake in perfused hearts from tumor-bearing rats compared with freely-fed controls. Tumor-bearing rats had statistically significantly higher oxygen uptake at all examined conditions ($p<0.01$). The difference are even more pronounced when oxygen uptake was normalized to heart dry weight ■ tumor-bearing rats (n = 20); □ freely-fed controls (n = 20).

Cardiac sensitivity and responsiveness to catecholamines

The reaction of heart rate, left ventricular peak systolic pressure and contractility in the graded exposure to isoproterenol and noradrenaline revealed an increased sensitivity (the slope of the relative increase) and responsiveness (maximum relative response) in hearts from tumor-bearing, starved and PCM rats compared to freely-fed controls (Fig 6; Paper 1 and 2).

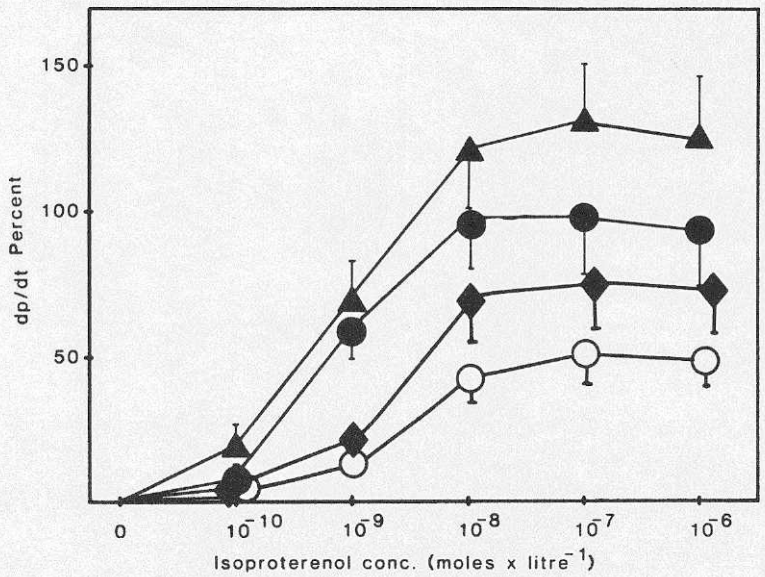

Figure 6.
The relative increase in contractility at increasing Isoproterenol concentrations. Mean ± SEM. ◆ Freely-fed tumor-bearing rats (n = 19); ● Starved rats (96 hrs) (n = 10); ▲ PCM rats (n = 10); ○ Freely-fed control rats (n = 16).
Baseline levels (mm Hg/s) (T) 6027 ± 504; (S) 6511 ± 596; (PCM) 6018 ± 591; (C) 5200 ± 252. The responsiveness is defined as the maximum relative response and the sensitivity as the regression coefficient of the slope at Isoproterenol concentrations between 10^{-10} and 10^{-8}.
Responsiveness: T vs S n.s; T vs PCM $p<0.0001$; T vs C $p<0.05$. Sensitivity: T vs S $p<0.005$; T vs PCM $p<0.001$; T vs C $p<0.001$.

Cardiac B-adrenergic and muscarinic receptor characteristics

Neither B-adrenergic nor muscarinic receptor numbers were different in groups of tumor-bearing, starved, PCM and freely-fed control rats. Agonist affinities of B-adrenergic and muscarinic receptors were, however, markedly increased in myocardial cell membranes derived from all groups of malnourished rats compared to freely-fed controls (Fig. 7). Adrenaline stimulation and muscarin agonist inhibition of adenylate cyclase activity showed no differences between the various rat groups (Paper 3).

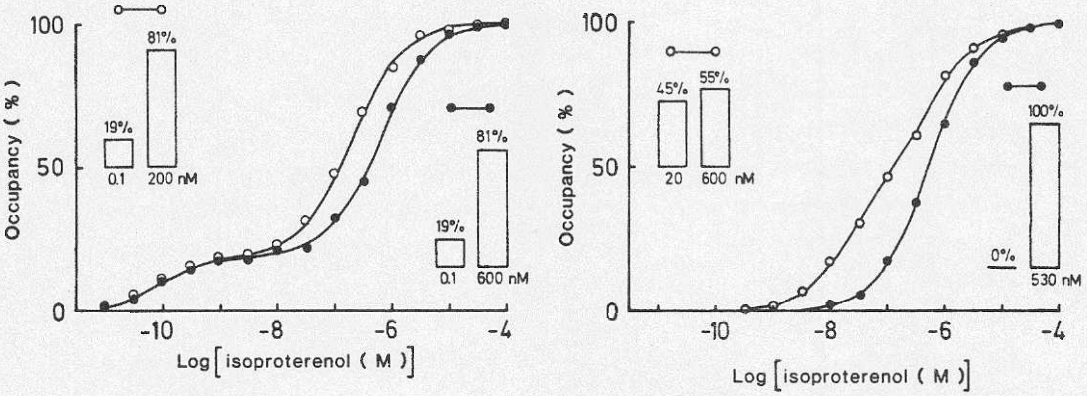

Fig 7. (^{125}I)-ICYP/isoproterenol competition binding in the abscence (o-o) or presence (●-●) of Gpp(NH)p on myocardial membranes from tumor-bearing (left panel) and freely-fed control rats (right panel). The insets demonstrate the affinity state distribution.

Cardiac energy metabolism

Tumor-bearing rats had a decreased cardiac glucose uptake and a lower release rate of lactate compared to both starved and freely-fed control rats during in vitro perfusion. This indicated a greater dependence on endogenous lipids as energy substrate in the cardiac metabolism of tumor-bearing rats (Table 2; Paper 4).

Table 2. Glucose uptake per heart, per gram dry weight myocardium and per left ventricular work in perfused hearts from tumor-bearing (TB), starved (S) and freely-fed control rats (C).

	Per heart (umol/h)	Per g d w (umol/h x g)	Per l v w (umol 10^{-6}/ mm Hg x ml)
TB	30±5	206±33[b,c]	82±15[b]
S	33±3[a]	298±18	143±36
C	47±4	293±25	139±14

a $p<0.01$ vs. control ; b $p< 0.05$ vs control; c $p<0.01$ vs starved rats.

Cardiac protein metabolism

Protein synthesis, assessed in vivo, was decreased by 19 percent in growing sarcoma-bearing rats compared to freely-fed controls. This depression of synthesis was, however, slight compared to starved and PCM rats whose cardiac protein synthetic rates were reduced by almost 50 percent. In adult non-growing sarcoma-bearing mice the capacity for protein synthesis (RNA content) was decreased, whereas the efficiency of protein synthesis (protein synthesis/RNA) and total cardiac fractional synthesis rate were maintained (Table 3). Malnourished reference mice (pair-fed and pair-weighed) had a depressed efficiency of protein synthesis and a tendency towards decreased whole heart fractional synthesis rate (not statistically significant) (Paper 5). The net release rates of amino acids during in vitro perfusions of hearts were different between sarcoma-bearing, starved and freely-fed control rats. Thus, sarcoma-bearing rats showed a higher net release rate of the non-metabolized amino acids (tyrosine, methionine and phenylalanine) whereas the main gluconeogenetic amino acids (alanine and glutamine) were released at lower rates compared to freely-fed control rats. These altered release rates were not evident in hearts from starved rats.

Table 3. Heart protein synthesis in tumor-bearing (TB), pair-fed (PF), pair-weighed (PW) and freely fed control (C) mice.

	TB	PF	PW	C
Fractional synthesis rate (%/day)	5.4±0.4	4.9±0.4	4.5±0.8	5.5±0.8

Cardiac contractile proteins

The concentrations of contractile cardiac proteins were not different between hearts from tumor-bearing, PCM and freely-fed control rats. Figure 8 shows SDS polyacrylamid gel electrophoresis of myosin and tryptic digests of myosin. There were signs of more tightly bound actomyosin complexes in the tumor-bearing and PCM groups compared to the starved and freely fed control groups. This conclusion is drawn from the fact that the area under the actin peak to the area under the myosin peak was higher in tumor-bearing (0.40) and PCM (0.32) than in starved (0.18) and control (0.21) rats. Tryptic digestion of fetal mouse myosin

(mainly V_3 isomyosin) showed a clearly different peptide pattern compared to adult control mouse myosin (mainly V_1 isomyosin). This method is thus able to detect transscriptionally regulated major isomyosin shifts. When myosin from tumor-bearing, starved and PCM animals were subjected to trypsination, the pattern of fragments showed essentially quantitative differences compared to freely-fed control animals. Myosin from PCM rats seemed more resistant to tryptic digestion compared to the other groups. Thus all myosin was lysed in the TB, S and C groups within 15 min whereas 16 percent of the initial amount of myosin remained intact in PCM rats after 15 min and 3 percent afer 30 min trypsination.

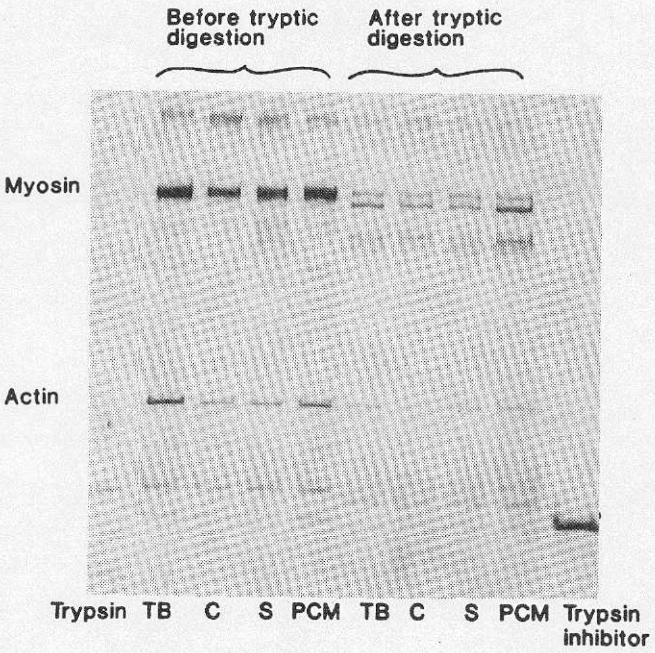

Fig.8. SDS polyacrylamide gel electrophoresis of myocardial myosin from tumor-bearing (TB), starved (S), protein-calorie malnourished (PCM) and freely-fed control (C) rats before and after tryptic digestion.

Myosin ATPase activity

ATPase activity of purified myosin from growing rats was lowest in hearts from starved animals, intermediate in hearts from tumor-bearing and PCM animals and highest in hearts from freely-fed controls (Fig.9). Non-growing adult mice showed the same pattern as that of rats when ATPase activity was assessed in hearts from tumor-bearing, starved and freely-fed control animals (Paper 5).

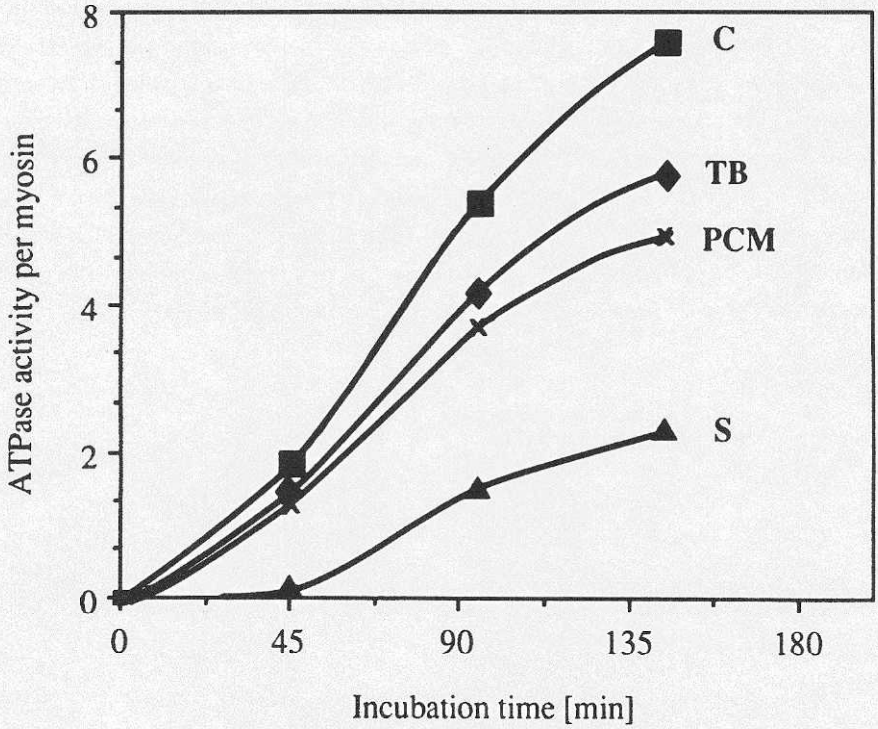

Fig 9. ATPase activities in purified myosin from tumor-bearing (TB), starved (S), protein malnourished (PCM) and freely-fed control (C) rats.

Metabolic response to adrenaline infusion in malnourished patients

Malnourished cancer patients had increased plasma concentrations of adrenaline compared to well nourished control patients. Whole body oxygen uptake, carbon dioxide production increased and mean arterial pressure decreased significantly in response to adrenaline infusion in all patients. Plasma concentration of glucose and insulin did not change during adrenaline infusion, while FFA concentration increased in all patients. The regression plots of whole body oxygen uptake, carbondioxide production and plasma FFA concentrations versus plasma adrenaline concentrations were statistically significantly steeper in malnourished patients compared to well-nourished patients when values from all subjects were treated as pooled observations (Paper 6).

DISCUSSION

Although much research effort has been spent all over the world on evaluating the mechanisms and effects of cancer cachexia, very little interest has been focused on the heart despite its virtue as a vital organ in the real sense of the word. This thesis consists of various experiments utilizing methods ranging from studies of isolated protein fragments to in vitro heart perfusions, in vivo studies in animals and clinical research in patients with the aim to evaluate the distant influence of malignant tumor disease on the circulatory system. It is a logical continuation of the previous investigations, dealing with the effects of tumor growth on host tissues performed in our laboratory during the past years.

Anorexia is recognized as a prominent feature in cancer disease (DeWys, 1977; Lundholm et al, 1980; Lundholm et al 1981) but many observations support the view that some metabolic changes arising in cancer patients are distinct from those associated with simple starvation or benign disease (Warnold et al, 1978; Shapot, 1979; Lindmark et al, 1984). It has also been shown that cancer can affect the host early and well in advance of nutritional stress (Wood et al, 1982). Therefore, in order to discriminate qualitatively between the effects of pure malnutrition and tumor specific metabolic and functional cardiac alterations groups of non-tumor animals with malnutrition were used in addition to freely fed controls.

A common denominator of malignant tumor disease and conditions of pure starvation or undernutrition is negative energy and protein balance necessitating mobilization of endogenous tissues. The regulation of the subsequently altered metabolism and the adaptation of individual organs are not completly understood. In figure 10 our present view of the influence of malignant tumor growth on the heart is illustrated.

Skeletal muscle constitutes the main, readily exchangeable pool of body protein. It is well recognized that net breakdown of skeletal muscle proteins represents an essential adaptation in situations of inadequate nutrient intake. Previous studies have also indicated tumor-specific acceleration of skeletal protein net degradation, independant of food intake (Stein et al, 1976; Goodlad & Clark, 1980; Norton et al, 1981; Kawamura et al, 1982). This might translate into abnormal depletion of body composition including a greater loss of body cell mass, in cancer patients

compared to states of pure undernutrition such as anorexia nervosa (Moley et al, 1987). The present study confirms previous work in refuting that the heart is spared from this general wasting of muscle tissue (Keys et al, 1950; Smythe et al, 1962; Wilens et al, 1967; Viart, 1977; Heymsfield et al, 1978; Nutter et al, 1979). Heart catabolism was, however, not tumor-specific as the heart weights were reduced in proportion to the loss of carcass (tumor-free body weight) in both tumor-bearing and benign reference states of malnutrition.

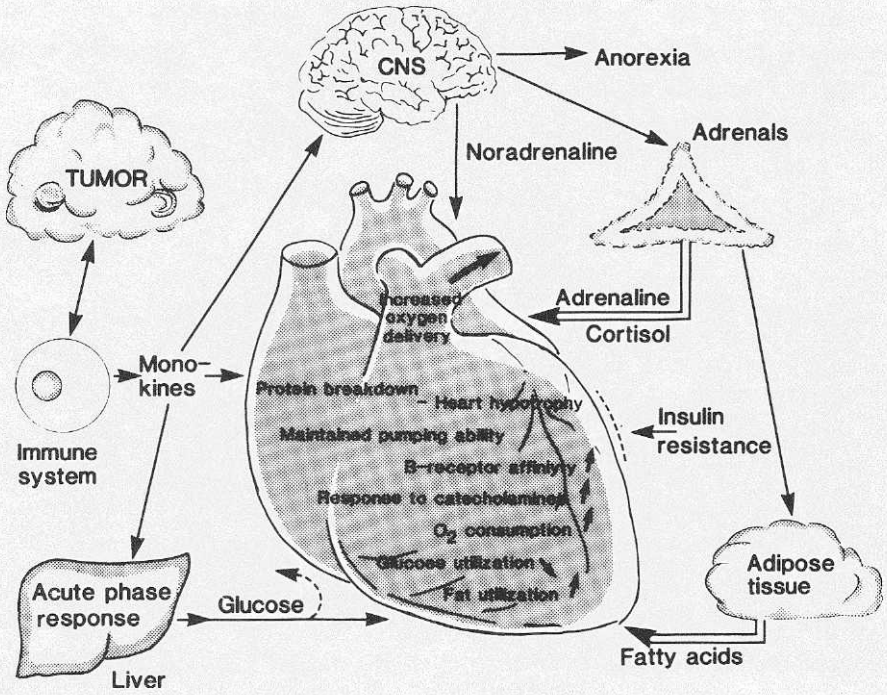

Figure 10. Interrelationship between the malignant tumor and the heart. The tumor elicits an immunological response with subsequent release of immune proteins (monokines and related substances). These proteins may act as mediators to produce anorexia, acute phase response of the liver and hormone alterations. Anorexia and the acute phase response contribute to protein breakdown in the heart with resulting cardiac hypotrophy. The heart, however, retains pumping ability to meet increased demands of oxygen supply. This may be achieved by increased catecholamine stimulation in combination with increased B-receptor affinity. Insulin resistance and increased adrenergic tone leads to decreased glucose and increased fat utilization as myocardial energy substrate. Increased myocardial oxygen consumption may contribute significantly to the elevated whole body energy expenditure in cancer.

A well known effect of skeletal muscle mass reduction is the feeling of weakness in both inanition and cancer disease. Studies on individual muscles, including electrically induced involuntary twitches, have confirmed the decreased function in hypotrophic skeletal muscle (Jeejeebhoy, 1986). If cardiac function should deteriorate in parallel with myocardial wasting it is not necessarily catastrophic provided that the circulatory demand of the host is diminished to the same extent. This might be the case in unstressed patients with undernutrition where energy expenditure is decreased (Keys et al, 1950) and, as shown in this study, the ratio of heart weight to body weight acually is increased. The situation in the tumor-bearing rats was, however, quite different as myocardial mass was reduced in parallel with the loss of carcass weight. The circulatory requirements of the tumor in combination with the acute phase response of the host consequently lead to increased demands on the heart as a pump. The fact that the tumor "consumes and replaces" lean tissues was revealed by the decreased ratio of heart muscle to body weight implying that "cardiac reserves" were quantitatively decreased in tumor disease.

Fortunately the heart does not lose its functional capacity in proportion to its diminished mass. On the contrary, this study shows that cardiac function is well maintained in hypotrophy and even improved if normalized to heart weight. This effect was not specific for tumor induced cachexia but occurred in hearts from starved and protein-calorie malnourished rats as well. Our findings are in concert with reports of normal or enhanced left ventricular function in cachectic patients (Heymsfield et al, 1978; Gottdiener et al, 1978) and in vitro heart preparations in protein-calorie undernourished rats (Nutter et al, 1979; Penpargkul et al, 1980). Interestingly, it has recently been reported an improvement of left ventricular function and cardiac performance during caloric restriction in obesity (Caviezel et al, 1986). In contrast, the comprehensive studies of Abel et al showed that left ventricular pressure and dp/dt were depressed in severely malnourished dogs (Abel et al, 1979). These hypotrophic canine hearts had, however, an increased peak developed force and as intraventricular baloons were used, cardiac output could not be measured thus making conclusions of net cardiac pumping performance hazardous. Others have suggested adverse cardiac effects of malnutrition. Thus, Kyger et al found decreased pumping ability in hearts from protein-calorie malnourished rats (Kyger et al, 1978). The malnourished rats had however a lower heart rate not accounted for by pacing and an unphysiological

high preload was used which caused several atria to rupture. Freund and Holroyde used a non-working Langendorff model and found only indirect suggestions of impaired cardiac pumping ability in malnourished rats (Freund & Holroyde, 1986). In these studies which argue for a detrimental effect of malnutrition on heart function, no account is taken for the quantitative loss of myocardium. In conclusion, previous reports do in no way provide definite contradictions to our findings but might reflect differences in methodology.

The ability of the heart to pump blood is of course the crucial variable in assessing cardiac adaptation in pathological conditions. However, to provide understanding of the underlying mechanisms and form a basis for therapeutical intervention detailed knowledge of myofibre function is essential. Due to the complexity of cardiac geometry the well maintained pumping ability of hearts from malnourished rats as found in this study does not necessarily mean that improved myofibre function accounts for all of the augmented performance per unit tissue. Thus, conformational and wall thickness changes of the hearts and subsequently altered compliance and wall stress might prevent the translation of a given left atrial filling pressure into an equal myofibre end diastolic length in hearts with variable size making the interpretation of the Frank-Starling relationships hazardous. In fact, the impossibility to accurately assess fibre length in intact muscles, especially in a syncytium like the myocardium has led to questioning of the whole concept of Starling's law of the heart (Altschule, 1986).

However, when maximal afterload was induced by clamping the aortic outflow in combination with high preload this study showed that the contractility and intraventricular peak systolic pressure were well maintained in all hypotrophic hearts sustaining the view that individual myofibre function was improved. In support of this conclusion are experiments performed on isolated strips of myocardium where the problems of differences in heart size are reduced. Such experiments have shown increased developed tension in atrial strips derived from animals after prolonged starvation (Ko & Paradise, 1972) and augmentation of tension development in left ventricular trabecular muscles derived from rats subjected to prolonged undernutrition (Cohen et al, 1976; Nutter et al, 1979).

Understanding of the mechanisms behind enhanced myofibre performance in hypotrophic hearts requires knowledge of cellular and subcellular adaptation, the protein metabolism being of prime interest. Net loss of lean tissue in weight stable organisms or the retarded growth in growing individuals result from an imbalance between protein synthesis and breakdown. A number of combinations of changes in these processes may occur. Skeletal muscle depends mainly on depressed protein synthesis in the regulation of wasting in chronic malnutrition, whereas increased degradation seem to play a role in acute trauma and sepsis (Millward et al, 1976; Rennie, 1985). The contribution of changes in protein synthesis and degradation to wasting of muscle have received less attention in myocardium compared to skeletal muscle. Thus, in cancer disease no studies are available on protein metabolism in the heart but in pure undernutrition it has been shown that protein synthesis is reduced during both acute starvation and more prolonged nutrient deprivation (Garlick et al, 1975; Preedy et al, 1984). This study confirmes that cardiac protein synthesis is markedly depressed in conditions of acute starvation and severe protein-calorie malnutrition, whereas tumor-bearing led to a moderate reduction of synthesis in growing rats. The synthesis of protein is regulated by gene transscription and translational modulation permitting fine tuning of the synthetic rate of each individual protein. Such unique mechanisms permitting selective modulation of degradative processes of individual proteins may not exist. Thus, alteration of synthesis rate is presumably the general key regulator in organ adaptation to altered demands.

Our knowledge of the regulation and mechanisms of protein synthesis is far more advanced than our understanding of intracellular protein breakdown largely because of technical problems involved in the study of degradative processes (Wildenthal, 1980). We could not determine whether increased protein degradation was involved in the myocardial wasting in growing tumor-bearing rats as no measurements of the time course changes of heart protein content during tumor growth were performed. However, indirect evidence of increased protein degradation could be derived from the experiments on tumor-bearing adult weight-stable mice. Thus, protein synthetic efficiency was not impaired and fractional protein synthesis rate was not depressed in tumor-bearing mice implying that increased degradation must have contributed to the net loss of heart tissue. This may be in keeping with increased activity of lysosomal enzymes (e.g. Cathepsin D)

in hearts from tumor-bearing mice (Lundholm et al 1978 a). A plausible explanation for the finding that the net loss of heart tissue in tumor-bearing mice is more dependent on increased protein breakdown, than in uncomplicated undernutrition where depressed synthesis is the main regulator, might be the net result of the combination of anorexia and acute phase response. Thus the nutritional deprivation would strive to depress synthesis, whereas the increased work load imposed on the heart by the circulatory demand of the tumor-bearing host could be a stimulus for increased protein synthesis. Increased heart work is a powerful and well described stimulator of protein synthesis (Isaksson, 1972; Moalic et al, 1984; Rappaport et al, 1986) but leaves degradation unchanged (Smith & Sugden, 1983). Degradation in tumor-disease might on the other hand be increased by the acute phase response which is known to have similarities with situations of trauma and sepsis where protein breakdown is elevated (Bastable et al, 1979; Jeevanandam et al, 1984). The suggestion that cardiac protein degradation is increased in tumor disease stands in contrast to findings in skeletal muscle of both mice (Svaninger et al, 1983) and man (Lundholm et al, 1982). One explanation to this might be the decreased demands on skeletal muscle in comparison with the increased work load of the heart in progressive tumor growth. In line with this hypothesis is a recent report of a protective effect of increased muscle work load on skeletal muscle wasting in rats (Norton et al, 1979).

Protein synthesis was in this study as in most other studies measured over a very short period of time. This can of course just give a momentary glimpse of the metabolic events that are subject to diurnal variation and are part of a dynamic and progressive disease state. It is thus possible that the metabolic adaptation in the early stages of tumor growth is different from that of more advanced disease. Such variation during the time course of a pathological condition can be seen in starvation where protein synthesis is promptly decreased at the onset of food deprivation whereas protein degradation has been reported initially decreased (Crie et al, 1980; Smith & Sugden, 1986) followed by a final increase as some sort of last resort emergency when the animal becomes desperately depleted (Millward et al, 1976; Waterlow et al, 1978).

The information obtained from measurements of total protein synthesis is limited as the heart is a mixture of cell types and each cell contains hundreds of proteins, each with its own rate of synthesis and degrada-

tion. Heart proteins have half lives ranging from one hour to several days (Waterlow et al, 1978). In the response to a departure from the normal state ,the synthesis of some proteins may be increased and others decreased. This can be exemplified by the reaction of the liver to inflammation or tumor-growth where the protein synthetic rate of some proteins is reduced whereas others e.g. acute phase proteins are synthesized at markedly increased rates (Lundholm et al, 1979). During in vitro perfusion, hearts from tumor-bearing rats showed a decreased release of the sum of all amino acids which was partly explained by a lower efflux of glutamine and alanine whereas the non-metabolizable amino acids were released at increased rates. This is in keeping with a different turnover of individual cardiac proteins as suggested by others (Smith & Sugden, 1986).

Despite the possibility of altered protein turnover the relative distribution of soluble, myofibrillar and collagen types of protein has been found unaltered in hearts from tumor-bearing mice indicating that the variuos gross protein compartments are equally reduced in the catabolic process (Sjöström et al, 1987). However, each of the major protein compartments contain an abundance of individual proteins making further and more detailed characterization necessary in order to explore adaptive changes. The functionally most important cardiac protein compartment is the myofibrillar which is composed of the contractile proteins actin and myosin and the regulatory proteins troponin and tropomyosin. These individual proteins show heterogenous synthetic rates and newly synthesized molecules constitue a precursor pool for incorporation into myofibrils (Zak et al, 1976). The half life of the contractile proteins is shortest for myosin heavy chain (5 days), intermediate for troponin, tropomyosin, myosin light chain and longest for actin (8 days) (Wildenthal, 1980). This heterogeneity of the rate of synthesis of the various contractile proteins has been found maintained during overload and subsequently increased overall protein synthesis (Moalic et al, 1984), whereas starvation has been reported to induce discoordinate reduction of myofibrillar protein synthesis with a more profound reduction of actin synthesis compared to myosin synthesis (Clark & Wildenthal, 1986). In this study the relative distribution of these major subgroups of cardiac contractile proteins was found unaffected by tumor-bearing, starvation and protein-calorie malnutrition compared to freely eating controls. In concert with our finding is the report of Zähringer et al who found the relative content of specific mRNA

coding for the major myofibrillar cardiac proteins unchanged in starvation and protein deprivation (Zähringer et al, 1985).

There is, however, the possibility of qualitative alterations of individul myofibrillar proteins with subsequent influence on their functional characteristics. Myosin occupies a central position in the transduction of chemical to mechanical energy and in the mammalian heart polymorphism of cardiac myosins seems to be a general feature. Native ventricular myosin can be separated in non-denaturing pyrophosphate gels into three isozymes V_1, V_2 and V_3 in order of decreasing electrophoretic mobility and ATPase activity. The isomyosin distribution is determined by changes in transscription of specific genes coding for different forms of myosin heavy chains. The expression of the isomyosin genes appears to be under multifactorial control and varies with animal species (Lompré et al, 1981). The pattern of isomyosin composition is also altered by age and is influenced by various pathological conditions such as volume overload, pressure overload, diabetes, thyroid and growth hormone aberrations (Hoh et al, 1977; Morkin et al, 1983; Dillmann et al, 1984; Dillman et al, 1985). Several authors have found correlations between cardiac function and isomyosin composition (Schwartz et al, 1981; Alpert & Mulieri, 1986). We found no difference in electrophoretic mobility of native myosin among the animal groups. In order to detect possible differences in contractile protein structure, myosin molecules were subjected to trypsin digestion and the peptides, separated electrophoretically, thus creating a "fingerprint". The results indicated merely minor quantitative differences between the various animal groups. Some indications of altered actomyosin properties were, however, found in SDS polyacrylamide gel electrophoresis as actin seemed more tightly bound to myosin in hearts from tumor-bearing and PCM rats compared to starved and freely-fed control rats. The functional significance of this finding is, however, not clear.

Despite the fact that we were unable to detect any major shifts of the V_1 -V_3 isomyosin distribution or tryptic cleavage pattern between groups, the ATPase activities in purified myosin preparations were depressed in hearts from all groups of cachectic animals which is in agreement with previous reports from studies of various states of undernutrition (Garber & Neely, 1983; Dillman et al, 1985). Discordant patterns of total myosin ATPase activity and isomyosin distribution has previously been reported (Winegrad et al, 1987). The decreased ATPase activities might partly be

mediated through hormonal mechanisms. Thyroid hormones affect myosin synthesis through binding to nuclear receptors which alters transcription of specific genes, low hormone levels yielding isoforms of low ATPase activity (Garber et al, 1983; Everett et al, 1984). Low insulin levels are similarly associated with decreased ATPase activities (Garber et al, 1983; Dillman et al, 1985). Consequently, the low T_3 (Burman et al, 1980; Persson et al, 1985; Svaninger et al, 1986) and insulin (Lundholm et al, 1978b; Svaninger et al, 1987a) levels in cachexia might play a regulatory role for the decreased ATPase activities observed in tumor-bearing and protein-calorie malnourished animals. Transscriptional regulation of myosin can, however, hardly explain the decreased enzyme activity found in 96 h starved rats in this study and by others (Garber & Neely, 1983) as the turnover of myosin is slow. An alternative mechanism might be post-translational modification of myosin (Morkin et al, 1983). Such conformational myosin modulation with influence on ATPase activity may include alterations of sulfhydryl residues near the active sites on the globular head of the heavy chains (Scheuer & Bhan, 1979).

Our finding of decreased myosin ATPase activity and increased function in hypotrophic hearts seem contradictory but it has previously been reported a dissociation of myosin Ca^{++} ATPase activity and contractile function in rat myocardium (Effron et al, 1983; Bhatnagar et al, 1985). The relevance and physiological significance of these apparantly conflicting findings are unclear. It is, however, unlikely that ATPase activity of purified myosin alone can provide information that is definitive regarding the contractile process in vivo. ATPase activity can be measured in a number of different ways apart from Ca^{++} activated purified myosin. However, the physiological importance of various in vitro ATPase activity measurements is far from clear. The ATP splitting of myosin is activated in vivo by making contact with actin and using magnesium as cofactor. Thus the most important cation for physiological activity of actin regulated myosin ATPase is magnesium, yet magnesium inhibits pure myosin ATPase activity and therefore has not been used in purified myosin preparations (Scheuer & Bhan, 1979). Heart contractile function has also been reported dissociated from magnesium stimulated myofibrillar ATPase activity (Dowell, 1984) and discoordinate results of ATPase activity during different conditions of assay has been shown in hypothyroidism where Ca^{++} ATPase activity is reduced whereas K^{+}-EDTA ATPase is unaffected (Rovetto et al, 1972; Garber & Neely, 1983). In addition to

myosin, the loci of control that might be important in alterations of contractile function include the ionic movements and electrical currents controlled by the sarcolemma, the energy dependent uptake and release of calcium by the sarcoplasmic reticulum and the sensitivity of the troponin-tropomyosin system to alterations in calcium concentration in the cell (Philipson et al, 1980). Such control of the activity of contractile proteins might be regulated by the phosphorylation of myosin light chains and troponin (Kopp et al, 1979; Stull et al, 1980; Morano et al, 1986). Another major structural component of the sarcomere is actin of which exist isoforms with functional significance (Schwartz et al, 1986).

Interestingly Ca^{++} activated myosin ATPase activity has been considered as an index of the rate of myosin cross-bridge cycling, high enzyme activity leading to increased velocity of shortening whereas low activity is associated with prolongation of the muscular twitch i.e. the muscle shortens and develops force more slowly throughout a longer period and consequently contracts more economically (Alpert, 1986; Lecarpentier et al, 1987). Hence, lowering of myosin ATPase activity in malnutrition may be an adaptive physiological response that results in the more efficient conversion of chemical energy into cardiac contraction.

Hypothetically the low circulating levels of thyroid hormones detected in both man (Persson et al, 1985) and experimental animals (Svaninger et al, 1986) with cancer cachexia and other types of malnutrition might be part of a logic down regulation of metabolism in an effort to optimize survival in situations of inadequate nutrient intake. As mentioned above, the effects on the heart may be a decreased ATPase activity and subsequently diminished oxygen consumption. A consistent finding in this study was the decreased oxygen uptake in hearts from starved and protein-calorie malnourished rats sustaining this hypothesis. However, in conditions with increased circulatory demand, the heart must be able to respond by pumping more vigorously. This requires a regulatory system to relay the message of increased circulatory needs to the heart. The adrenergic system constitutes the most powerful stimulator of cardiac performance by influencing both chronotropic and inotropic mechanisms making it a possible candidate for such regulatory function (Opie, 1969). This study clearly shows experimental evidence that the physiological response to adrenergic stimulation is increased in malnutrition, regardless of the cause of cachexia. Thus both sensitivity and reactivity was augmented to

graded isoproterenol and noradrenaline exposure in malnourished rats. The regressions plots of several parameters versus plasma adrenaline concentration were statistically significantly increased in malnourished patients compared to well nourished controls during adrenaline infusion. The variables that showed this increased response were associated with heart function i.e. mean arterial pressure and heart rate apart from whole body energy metabolism. An increased response to adrenergic stimulation in malnutrition has previously been shown in adipocytes (Dax et al, 1981; Wolfe et al, 1987). Although this study was not designed to evaluate lipolysis, the increased plasma glycerol levels in malnourished patients sustains these previous investigations. The increased physiological response to catecholamines was evident in all the various states of malnutrition. Our results are in agreement with previous clinical (Jayarajan et al, 1985) and experimental (Herlihy et al, 1984) reports. An intriguing possibility is that modulation of adrenergic stimulation may be one of several common adaptations in response to the loss of cardiac contractile muscle elements. Thus intrinsic myocardial dysfunction in acute sepsis (Smith et al, 1986) and in cardiomyopathic hamsters (Rossner & Coudrai, 1986) were associated with increased sensitivity to isoproterenol.

Possible pathways to obtain increased adrenergic drive on the heart include increased exposure to catecholamines, increased receptor numbers, increased receptor affinity or a combination of these. Pure undernutrition is generally associated with signs of decreased adrenergic activity such as bradycardia, hypotension and decreased energy expenditure as well as decreased noradrenaline turnover (Young & Landsberg, 1977; Avakian, 1982; Rappaport et al, 1982). Plasma levels of catecholamines and urine output of catecholamine metabolites are also decreased in uncomplicated starvation (Jung et al, 1979; DeHaven et al, 1980; Gross et al, 1979). Clinical undernutrition is, however, seldom uncomplicated and pure but is often associated with stress from disease and treatment. Thus, cancer cachexia is associated with increased circulating levels of catecholamines as shown in this study and by others (Russel et al, 1984). Furthermore, we have recently shown that urine excretion of catecholamines is significantly higher in cancer patients compared to control patients matched for malnutrition and inflammation (Drott et al, 1987). The increased physiological response to catecholamines can thus hardly be explained by receptor upregulation due to decreased exposure to adrenaline and noradrenaline. Regulation of the adrenergic response to prolonged alterations of catecho-

lamine exposure can operate via modulation of receptor density. We could, however, not detect any impact of pure malnutrition or tumor-growth on B-receptor numbers, confirming previous reports from studies on calorie restricted animals (Crandall et al, 1983).

The increased physiologic response to catecholamines was instead associated with altered affinity of the B-adrenergic receptors. Assessed in isolated cardiomyocyte membranes, the affinity was at least ten-fold increased in all the malnourished groups of rats compared to freely-fed controls. Previous ligand binding studies of B-adrenergic receptors have suggested the ternary complex model in which the receptors show high affinity towards agonists if coupled to the stimulatory guanine nucleotide binding protein (N_s) (DeLean et al, 1980). Guanine nucleotides convert all the high affinity receptors to the low affinity state which is believed to mirror the dissociation of the receptor-N_s-complex. The extent to which an agonist may stimulate the activity of adenylate cyclase correlates with the percentage of adrenergic receptors in the high affinity state,i.e. equivalent to the number of receptors shifted in agonist affinity by guanine nucleotides. Several features of isoproterenol binding to B-receptors in hearts from malnourished rats were, however, not compatible with the ternary complex model. Thus, despite increased affinity of the high affinity state the percentage of receptors in this state was diminished and addition of a GTP analogue shifted the affinity of the low affinity state but not the high affinity state as expected in hearts from PCM and tumor-bearing rats. The reaction to a GTP analogue in cardiomyocyte membranes from starved rats was similar to that of control rats but attenuated. The difference between receptor characteristics in acute and more prolonged undernutrition might indicate that the adaptation requires some time to develop. In general, catecholamines stimulate adenylate cyclase with an order of potency that parallels their inotropic potency. We were, however, not able to detect any increase of adenylate cyclase activity despite the intriguing combination of increased receptor affinity and increased physiological response. This does, however, not exclude the possibility that cAMP relays the message in vivo as the concentration range of adrenaline used for in vitro stimulation (umol levels) is unphysiologically high as compared to the in vivo situation (nM levels) but other possible pathways for transmembrane signaling do also exist. Thus, catecholamine induction of increased contractility has been reported without cyclic nucleotide formation (Opie, 1969; Benfey et al, 1974). Such B-receptor

mediated influence on heart contractility by mechanisms other than adenylate cyclase stimulation might operate through regulation of magnesium ion transport (Maguire, 1984). Furthermore, phosphorylation of myosin has functional effects and myosin light chain kinase is not dependent on cyclic nucleotides for activity but requires the calcium binding protein calmodulin and calcium for activity (Adelstein, 1980). It has also been reported that agents coupled to the inhibitory nucleotide binding protein can influence glucose transport in isolated adipocytes through a non-cyclic AMP associated mechanism (Lönnroth et al, 1987).

Glucocorticoid production is increased in cancer disease (Schaur et al, 1979; Svaninger et al, 1987c). These hormones have a permissive effect in the adrenergic regulation of cardiac contractility. This effect is obtained by modification of calcium influx through the sarcolemmal membrane and is not mediated by adrenergic receptors. Thus, corticosteroids can influence the process of contraction by potentiating adrenergic stimulation by a non-cAMP dependent mechanism (Seleznev & Martynov, 1982). An alternative explanation to the increased receptor affinities and the lack of increase in cAMP production might be a receptor population that has a high agonist affinity but does not couple to adenylate cyclase (Dixon et al, 1987). The adrenergic hypersensitivity could not be explained by an attenuated muscarinic inhibition of adenylate cyclase activity, as muscarinic receptor numbers were unaltered and their affinities increased in the various states of malnutrition.

In contrast to conditions of starvation and undernutrition where energy requirements are decreased, the tumor-bearing host exhibits increased energy expenditure (Warnold et al, 1978; Lindmark et al, 1983; Lindmark et al, 1984). This has been found associated with increased heart rate (Karlberg et al, 1981). The tissues and organs mainly responsible for the elevated oxygen consumption in cancer disease has hitherto not been clearly identified. This study indicates that the heart might be a significant contributor to the increased oxygen demand in the tumor situation as oxygen uptake was constantly increased during all experimental conditions in hearts from tumor-bearing rats, whereas starvation and protein-calorie malnutrition led to decreased oxygen uptake compared to freely fed controls. Increased cardiac oxygen consumption might be secondary to elevated circulatory demands due to metabolic alterations in peripheral tissues including the tumor itself. It has recently been suggested that the presen-

ce of the tumor directly causes an increase in the rate of fuel oxidation in brown adipose tissue and that the wasting of other tissues is then caused by the demand of brown adipose tissues for metabolic substrates (Brooks et al, 1981; Edström et al, 1986; Shellock et al, 1986). However, this cannot explain the increased oxygen consumption per work load that was evident in hearts from tumor-bearing rats. An increased utilization of fat as energy fuel might contribute to the increased oxygen consumption as more oxygen is required to metabolize equicaloric amounts of fat compared to glucose (Siess et al, 1985). Thus, the heart itself might be involved as an energy draining organ in cancer disease. Further indications of an altered energy metabolism is evident from the experiments on starved and tumor-bearing rats.Hearts from tumor-bearing rats had a significantly lower glucose uptake compared to both starved and freely-fed control rats. We did not measure fat oxidation directly but it can be deduced from the increased oxygen consumption and decreased glucose uptake that hearts from tumor-bearing rats must rely more on fat substrate than hearts from control rats. Endogenous myocardial fat is available and can account for an increased triglyceride oxidation rate during the comparatively short in vitro experiment (Olson & Hoeschen, 1967; Crass et al, 1972; Morgan et al, 1984). In vivo this might translate into increased utilization of circulating fat substrate as shown in patients with cancer (Hansell et al, 1986). In support of this hypothesis are elevated plasma glycerol levels in cancer patients (Edén et al, 1985; Paper 6). The increased plasma concentrations of catecholamines in combination with increased catecholamine sensitivity might mediate the increased fat oxidation in tumor disease. Thus, adrenergic stimulation has been shown to enhance respiration of fat and decrease glucose uptake (Opie, 1969).

The low thyroid state might cause a prolonged systole and subsequent prolonged Q-T time which has been shown clinically in malnourished patients. The prolonged Q-T time is also associated with an increased risk for life threatening ventricular arrhythmias (Moss, 1986). Sudden, unexpected death has been reported in patients who have lost considerable weight on the very low energy liquid protein diets (Isner et al, 1979) and in patients with weight loss complicating anorexia nervosa (Isner et al, 1985). One hypothetical explanation for these fatal arrhythmias might be that situations of stress induce elevated adrenergic activity which in combination with increased B-adrenergic sensitivity could induce electrical instability in the heart of the malnourished individual (Opie et al 1979).

We have recently addressed the question of altered electrical properties in hearts from malnourished rats both in vitro and in vivo. Despite the known difficulties to elicit ventricular arrhythmia in rat hearts preliminary data show that starvation and tumor-bearing are associated with cardioelectrical abberations including a significant delay of the vulnerable period to the end of the QT interval and a tendency towards increased timespan where ventricular fibrillation could be induced.

SUMMARY AND CONCLUSIONS

In summary, this study shows that cardiac hypotrophy in tumor disease involves alterations of energy and protein metabolism as well as adrenergic modulation.The resulting functional adaptation of the hypotrophic heart was, however, not limited to tumor-bearing rats but was also evident in starved and protein malnourished rats. Teleologically, the increased sensitivity and reactivity to adrenergic stimulation and concomitantly increased B-adrenoceptor affinities may be general means to minimize deterioration of pumping performance during malnutrition rather than being specific to tumor disease. Maintained and perhaps even increased sensitivity to adrenaline was confirmed in malnourished patients. Ca^{++} activated purified myosin ATPase activities were depressed in hearts from all groups of malnourished animals although only minor alterations of myosin structure could be detected.

Some observations in tumor-bearing hosts might indicate specific effects of the presence of the tumor in addition to the effects of malnutrition. Thus, cancer patients had elevated plasma levels of adrenaline and in common with tumor-bearing rats showed signs of increased fat utilization as energy substrate. Hearts from tumor-bearing rats had an increased oxygen uptake, even after normalization to left ventricular work, which might indicate that the heart contributes to the increased whole body energy expenditure in cancer disease. Although wasting of cardiac muscle was evident in all hypotrophic hearts, tumor-bearing animals showed evidence of an increased protein breakdown compared with animals with pure undernutrition. In starved and protein malnourished controls, cardiac hypotrophy was explained by a decreased protein synthesis. It is still unclear how the "functional reorganization" of the heart in cancer disease and malnutrition is coordinated and the fine details of how each change contributes to specific as well as overall performance remains to be clarified. One important challange for the future is to elucidate whether electrical instability might impose a risk for arrythmia.

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