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A generally nonlethal Salmonella typhimurium infection in weanling rats produced bacterial myocarditis and myocardial hyperplasia. Myocardial lesions were characterized by focal infiltrates of inflammatory cells (predominantly mononuclear), segmental myocyte necrosis, and incipient fibrosis. Although bacterial infections are infrequently associated with myocarditis, the S. typhimurium infection in young rats produced a new experimental model of diffuse myocardial inflammatory foci. Biochemical changes in the myocardium (over)

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included great increases in total myocardial contents of protein (23%), RNA (39%) and DNA (43%) and several lipid fractions (35-55%) as well as in tissue activities of acid hydrolases, such as cathepsin D (124%) and β-glucuronidase (135%), all of which contrasted with the relatively limited areas of histologic involvement (1.5%). To study the effects of additional stress in this model infection, some rats were exercised by forced running in wheels for 2 hours and others were fasted for 24 hours before samples were obtained. The short period of forced exercise in this infection caused an additional increase of myocardial protein content (47%) but with no additional change in histology. The expected fasting-induced degradation of protein as well as an infection-associated increase in myocardial lipids were each prevented when rats were fasted during ongoing acute infection. Protein degradation, as reflected by heightened acid hydrolase activities, seemed to occur at a similar rate regardless of other stresses, whereas the rate of myocardial protein synthesis appeared to be alterable.

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Running Title: Exercise and fasting in bacterial myocarditis

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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ABSTRACT

A generally nonlethal Salmonella typhimurium infection in weanling rats produced bacterial myocarditis and myocardial hyperplasia. Myocardial lesions were characterized by focal infiltrates of inflammatory cells (predominantly mononuclear), segmental myocyte necrosis, and incipient fibrosis. Although bacterial infections are infrequently associated with myocarditis, the S. typhimurium infection in young rats produced a new experimental model of diffuse myocardial inflammatory foci. Biochemical changes in the myocardium included great increases in total myocardial contents of protein (23%), RNA (39%) and DNA (43%) and several lipid fractions (35-55%) as well as in tissue activities of acid hydrolases, such as cathepsin D (124%) and β-glucuronidase (135%), all of which contrasted with the relatively limited areas of histologic involvement (1.5%). To study the effects of additional stress in this model infection, some rats were exercised by forced running in wheels for 2 hours and others were fasted for 24 hours before samples were obtained. The short period of forced exercise in this infection caused an additional increase of myocardial protein content (47%) but with no additional change in histology. The expected fasting-induced degradation of protein as well as an infection-associated increase in myocardial lipids were each prevented when rats were fasted during ongoing acute infection. Protein degradation, as reflected by heightened acid hydrolase activities, seemed to occur at a similar rate regardless of other stresses, whereas the rate of myocardial protein synthesis appeared to be alterable.

INTRODUCTION

Myocardial involvement in infectious diseases and the possible detrimental effects of exercise during infection are matters of great clinical importance. Although the pathogenesis of myocarditis is not fully understood, certain microorganisms, such as Coxsackie viruses or Trypanosoma cruzi, are highly myocardiotropic and may be used experimentally to initiate degeneration or necrosis and invasion of inflammatory cells in the myocardium of mice or monkeys (10, 20, 21). These lesions are associated with replication of microorganisms within the myocardium and with increased myocardial weight. Weight increases are attributable to edema and the inflammatory infiltrate, as well as to myocardial hypertrophy or hyperplasia (10, 31). Myocardial involvement may become more pronounced if Coxsackie virus-infected mice are forced to exercise (21, 31) or are subjected to undernutrition (37). Conversely, bacterial invasion of the myocardium is rare (2) and there seems to be no experimental studies of bacterial myocarditis nor any previously described animal model.

Increased protein synthesis in muscle is a normal response to physical training (24), whereas in various infections, protein degradation takes place in striated muscle to provide fuel for gluconeogenesis and substrate for de novo protein synthesis in the liver (3, 34). Recent studies of tularemia in the rat, an infection not associated with myocarditis, showed that forced exercise during the acute phase of infection could counteract the myocardial protein degradation associated with the infection (12, 17).

The objectives of the present study were: 1) to investigate the effects of a standardized bacterial infection due to S. typhimurium on protein and

lipid metabolism (Study 1) and histopathology (Study 2) in the myocardium of rats fed a restricted, well-balanced starch diet, and 2) to study interactions of forced exercise and fasting. Energy shortage is integral in most infections due to the accompanying anorexia (3, 34). Determinations of RNA and lysosomal acid hydrolase activity were included in the biochemical analyses in order to evaluate rates of protein synthesis and degradation; growing rats were used to take advantage of their higher metabolic turnover rate.

MATERIAL AND METHODS

Animals. In a first study (Study 1), weanling 21 day old male Sprague-Dawley rats (Charles River Breeding Labs, Wilmington, Mass.) were randomly assigned (n = 7 in group 1 and n = 8 in groups 2-6) into 6 groups: 1) noninfected fed controls (C); 2) noninfected fasted controls (F); 3) infected, fasted (IF); 4) infected, fed (I); 5) infected, fed and exercised IEx); and 6) exercised, fed controls (Ex). In a second study (Study 2) additional rats were randomized in similar groups (n = 7 per group) for histopathological examination of tissue samples.

The rats were housed individually in an air-conditioned, isolated room with a temperature of 24 $\pm$ 1^{o} and fluorescent lighting from 07:30-19:30 hours. Infected and control rats were studied simultaneously. Individual food consumption and weight gains were recorded daily. For the 14-day-trials, all rats were fed a nutritionally balanced standard corn oil diet (30) at 80% of the ad libitum intake, with corn meal replacing sucrose/fructose as in previously described diets (Ilback et al, in prep 1982). This restriction was performed in order to standardize the energy intake in the face of anorexia

present in this relatively mild infection. Fresh water was available ad libitum. Care and management were performed between 08:00-09:30 hours each day; thereafter the room was locked against further entry.

Infection. After 7 days acclimatization and food adaptation, the rats were inoculated (day 7) intraperitoneally (IP) with 0.2 ml of 10^7 colony forming units/ml (CFU) of a nonwashed, nutrient agar grown, virulent S. typhimurium culture prepared from a lyophilized culture previously standardized in our laboratory. In preliminary experiments, this dose and route of administration were shown to cause a clinically recognizable illness that produced a cumulative lethality of less than 5% at 9 days of disease in nonexercised rats. In both studies, body temperatures were recorded daily for 5 days, starting on the day of inoculation, using a thermocouple adapted to the neck of the rat. After 7 days of the infection, tissue and plasma were obtained (day 14).

Exercise. Rats in appropriate groups were forced to exercise on the seventh day after inoculation (day 14); exercise consisted of running in one of 16 interconnected exercise wheels for 120 minutes at 10 m/min immediately prior to tissue and plasma sampling. This exercise caused considerable exhaustion of the rats.

Fasting. For 24 hours before tissue sampling, beginning on day 13, food was withdrawn from rats in the fasted groups, but fresh water was available ad libitum.

Sampling and tissue preparation. Following rat decapitation on day 14, the heart was immediately taken out while still beating; atria, vessels and blood quickly removed; and in Study 1, the remaining ventricular muscle frozen

in dry ice/acetone. The samples were then stored at -70° C until analyzed (within 2 weeks). After thawing, the prepared muscle sample was rapidly weighed, cut into small pieces with scissors and homogenized in 20% (wt/vol) ice-cold distilled water in all-glass homogenizers operated manually. The homogenates were divided for different assays; those for protein, RNA, DNA and acid hydrolase determinations were further diluted in ice cold buffer (150 mM KCl, 50 mM $KHCO_3$, 6 mM EDTA, pH 7.4) to 3% (wt/vol). The entire procedure was performed at 0-4°C. The homogenates for acid hydrolase assays were made 0.1% with respect to Triton X-100 concentration.

In Study 2, the heart was placed in buffered 10% formalin for routine histological processing and preparation of H & E slides.

Assays of protein, RNA, DNA, lysosomal enzyme activities and lipids (Study 1). The protein concentration was determined after incubating 0.1 ml homogenate with 0.1 ml 20% KOH at 80C for 60 min according to Lowry (23). RNA and DNA were measured according to Wannemacher (33). The activities of cathepsin D (Cat. D.: E.C. 3.4.23.5) (7) and β-glucuronidase (GUase: E.C. 3.2.1.31) (1) were determined and considered to reflect the protein degradation rate and lysosomal capacity in the heart muscle. Heart lipids were analyzed in 6 fractions; triglycerides (TG), diglycerides (DG), monoglycerides (MG), free fatty acids (FFA), cholesterol (Chol) and cholesterol esters (Chol.E) by thin layer chromatography (5). All assays were performed on homogenates prepared from the entire ventricular muscle and calculated per whole organ and per gram "wet" muscle.

Pathology (Study 2). The myocardium was embedded in paraffin and cut in sections that contained ventricles and septum. Myocardial sections were

stained with hematoxylin-eosin, coded to blind the group source of the slide, and studied in detail by one of the investigators (A.J.J.) before the code was disclosed. Areas of myocardial necrosis, cellular infiltration, and/or fibrosis were recorded and quantitated morphometrically according to their size. The size of these lesions was compared to the total area of each myocardial tissue section and was expressed as a percential quantitation of the inflammatory tissue. The percentage was obtained from a summation of measures taken from 3 serial 6: μm sections of myocardium per rat. The rat-group code was not disclosed until all microscopic analyses had been completed.

Statistics. The effects of infection, exercise and fasting were calculated by means of two-way analysis of variance. Correlations were performed and tested for significance by the methods of least-squares and the t-test, respectively.

RESULTS

Effects of infection. All infected rats, in both studies, responded with fever which appeared 48 hours postinoculation and lasted for 48 hours. Practically all supplied food was consumed throughout the experiments; this feeding pattern was compatible with an infection of low intensity. The infection produced consistent splenic enlargement and histologic signs of myocarditis in all bacteremic rats, with a concomitant increase of heart weight (Table I) that correlated to spleen weight (Fig. 2A). An increased heart to body weight ratio was recorded (Fig. 1A). The myocarditis was characterized by focal infiltration of histiocytes and lymphocytes as well as segmental necrosis of myocytes, but only a few neutrophils were seen. The

septum was more involved than either the right or left ventricular walls which had a similar density of lesions. The myocarditis was designated subacute because of the predominance of mononuclear cells. Fibrosis, appearing as widened bands separated from the myocytes, was minimal when present. Connective tissue separation and a stranded appearance of the myocytes were interpreted to be effects of localized tissue edema.

The infection increased the content of total protein, RNA and DNA, as well as the activities of cathepsin D and β-glucuronidase in the ventricular myocardium (Table II). The myocardial content of these enzymes and of RNA were all significantly correlated to the heart weight. For β-glucuronidase, this correlation persisted even when the activity was expressed per gram of tissue (Fig. 2B, C). The average increase in β-glucuronidase activity was 5 times that of the heart weight (Table II). Similarly, DNA increased its concentration in infection and the rate of DNA formation was higher than that of protein, as reflected in a decreased protein versus DNA ratio (Fig. 1A). The infection caused the total amount of myocardial lipids to increase due to an increase of measured lipid fractions, except for the diglycerides and triglycerides which showed no significant change (Fig. 3).

Effects of exercise. A 2-hour period of exercise in noninfected rats did not cause myocardial lesions, alterations in the heart weight (Table I), or a change in any measured indicator of myocardial protein turnover (Table II). Similar exercise in the infected rats caused no increase of heart weight in addition to that attributable to the infection per se, but the amount of protein in the heart rose further (Table II) resulting in an increased protein concentration (Figs. 1A and B). This increased protein

content in exercised infected rats was not associated with any difference in the histological picture as compared to that found in infected sedentary rats (Table I). Similarly, the activities of cathepsin D and β-glucuronidase were uninfluenced by the superimposition of exercise on the infection. The myocardial content of triglycerides was redued by exercise in healthy, but not in infected rats (Fig. 3). All other lipid fractions were uninfluenced by exercise.

Effects of fasting. Fasting for 24 hours before the study of noninfected rats reduced the heart weight, the protein content, RNA and all the lipid fractions in the heart. However, the DNA content and the activities of cathepsin D and β-glucuronidase were uninfluenced (Table II, Figs. 1 and 2) and the histology was normal. In infected rats, fasting produced a similar pattern of biochemical changes except that protein was spared. This resulted in a higher protein concentration than that seen in infection alone or in fasting alone (Figs. 1A and B). Fasting during infection also induced a higher heart versus body weight ration, since body weight was lost more rapidly than heart weight (Fig. 1A).

In two of the fasted infected rats, the myocardial histology was almost normal, showing an area of inflammatory tissue corresponding to only 0.1-0.2% of the total tissue area. Although the average inflammatory response for the fasted infected group was not significantly altered by the fasting, the somewhat lower myocardial inflammatory response (Study 2) was in keeping with decreased spleen weight and a loss of correlation between spleen and heart weight (Study 1) recorded in fasted infected rats (Fig. 2A). The RNA versus DNA ratio was decreased only with fasting (Fig. 1A) and the correlation

between RNA content and heart weight existed in all groups except the fasted control group (Fig. 2B). Fasted infected rats showed a decreased myocardial content of free fatty acids, triglycerides and total lipids when compared to fed infected rats and all lipid fractions (except for triglycerides) were in the same ranges as those for fed noninfected control rats (Fig. 3).

DISCUSSION

The present relatively mild S. typhimurium infection in the young rat provided a suitable experimental model, apparently not described before, for the study of myocarditis in bacterial infection. Thus, this infection evoked classical histologic signs of myocarditis. Further, the heart weight and the heart versus body weight ratio increased as did total protein, RNA, DNA and lipid content of the myocardium. The cardiac hyperplasia correlated with the severity of the infection. Based on our indirect determination of myocardial protein turnover, the synthesis rate during ongoing acute infection was apparently higher than the degradation rate, as evidenced by both myocardial growth and protein formation. A single 2 hour period of forced exercise during the infection increased the myocardial protein content further, without significantly altering the extent of histological lesions, lipid content or heart weight. A 24 hour period of fasting during the infection reduced the heart weight, reversed the infection-induced myocardial lipid-accumulation, but allowed the protein content to increase. The cell mass subjected to our biochemical studies was almost exclusively composed of myocardial cells rather than inflammatory cells, since the latter averaged only 1.0 to 1.4% of the tissue area in serial histopathological sections. We concluded that the increase in cardiac weight, including protein formation and the hyperplasia

response, in this S. typhimurium-associated myocarditis could not be explained only by the presence of an inflammatory infiltrate, edema or fibrosis. Rather, it was predominantly a result of increased protein and DNA synthesis in myocytes and/or nonmuscular cells.

Effects of infection. Spleen size in tularemic rats provides a valid estimate of disease intensity (25). This observation is apparently also true in this S. typhimurium infection, since myocardial enlargement correlated positively with spleen weight. As shown in experimental viral myocarditis, cardiac alterations include myofiber necrosis and infiltration of inflammatory cells (37) as well as an acute increase in heart weight. As in this bacterial myocarditis, the increase was not attributable solely to edema and the inflammatory infiltrate (21, 31). Although not confirmed biochemically, the heart weight increase in viral myocarditis is commonly interpreted as a hypertrophy caused by a thickening and dilatation of the ventricular wall (2).

In infection of rats with S. typhimurium (34), as with several other bacteria (3), the flux and movement of plasma amino acids from skeletal muscle to the liver increases. This redistribution process is accompanied by altered concentration of individual amino acids in plasma (11) which could in turn possibly affect myocardial fractional extraction and utilization rates of circulating free amino acids. However, there are no turnover studies to show whether or not a similar flux of amino acids occurs from the cardiac muscle to the liver or how the anabolic and catabolic rates of the myocardium are affected by this infection. The protein synthetic capacity of myocardium is normally dependent on the RNA concentration, energy availabililty and amino acid supply (36); in vitro, an increase in the concentration of plasma amino

acids can induce an increased synthesis of whole heart protein and myosin (26). The present findings of increased RNA concentration (Fig. 1B) and its positive correlation with protein formation (Table 2, Fig. 3) support the concept that net synthesis of cardiac protein occured in this infection despite the activation of cathepsin C and β-glucuronidase which, in turn, suggests that the degradation rate for protein was increased also (6). The magnitude of the increase in protein content was similar to that of the heart weight (23 and 21%, respectively). Thus, the protein concentration was relatively unchanged but DNA accumulated (43%).

During an inflammatory response, phagocytizing cells containing protein, RNA, DNA, and lysosomal enzymes, such as cathepsin D, migrate into the involved tissue (19, 39). Some of the increase in biochemical variables in the present myocardial study may thus be attributable to the cellular infiltrate. However, our histologic data show that this proportion should be minor, since the foci of inflammatory or fibrotic cells occupied less than 1.5% of the area of each histopathological section. Similarly, myocardial enlargement in excess of that caused by an inflammatory exudate was found in coxsackie virus myocarditis (10, 21, 31). Edema can also be ruled out as a significant cause for the increased heart weight in this infection, since the biochemical results clearly indicate a net synthesis of DNA and protein (i.e., a hyperplasia response) as distinguished from one of only a net synthesis of protein only (i.e., hypertrophy). By comparison, in nonmyocarditic model infections in mice, such as tularemia and influenza, a net protein degradation occurs in the myocardium (12, 17) and in Newcastele virus disease of chicks, even DNA is degraded (29).

The normal growth of skeletal and cardiac muscle in rats of a similar young age as those of our rats is chiefly attributable to hypertrophy rather than to hyperplasia (18). However, increased metabolic demands in noninfected, growing, normal animals can necessitate increased nuclear DNA or ploidy in some enlarged myocytes (24). Accordingly, extracts from myocardial cytoplasma from young rats can stimulate DNA synthesis in adult myocardial nuclei (22). Furthermore, mitochondrial DNA can increase in small amounts in hypertrophying myocardium although such a source probably does not contribute more than 10% to the total cellular DNA (27). The magnitude of the present hyperplasia response was similar to that recorded after experimentally induced aortic constriction, which produced gross hypertrophy (44%) and an increase in total ventricular DNA content by 54% (16). In another S. typhimurium model infection of a higher intensity but of a shorter duration in adult rats of the same strain, no myocardial hyperplasia was seen (unpublished results). Similarly, the age of the rats (20) and the duration and intensity of the disase process (39) may all influence the susceptibility and risk of developing myocarditis and heart enlargement in experimental coxsackie virus infections. Thus, the present infection appeared to initiate a process of myocardial DNA synthesis and possibly cell division, although the factors mediating this response are not known. Possibly, some cellular or humoral constituents of the inflammatory exudate or the presence of microorganisms in the tissues may be a crucial stimulator of myocardial hyperplasia in S. typhimurium myocarditis.

The release of leukocytic endogenous mediators (LEM/Interlukin-1) from activated phagocytic cells occurs early in bacterial infections (35). These

hormone-like mediators of fever also induce an enhanced production of neutrophils, an accelerated hepatic synthesis of "acute phase" serum proteins, and a stimulation of phagocyte and lymphocyte activity. Analogous mediators have now been shown to induce skeletal muscle proteolysis as well, through a process secondarily mediated by prostaglandin synthesis or deactivation of naturally occurring cathepsin inhibitors (4). Thus, even myocardial metabolism might be influenced by endogenously produced mediators in infection.

Acid hydrolases including β-glucuronidase and cathepsin D are activated in states in which a catabolic component exists(12), 13, 17). These include muscle ischaemia (9) and necrosis (32) followed by repair, or more generally in states of increased metabolic turnover, including infections (12, 13). Acid hydrolase activation of a magnitude similar to that of the present study has also been recorded in the myocardium in nonmyocarditic infections, such as tularemia, where degradative processes predominate and result in catabolic loses of myocardial tissue and protein content (12, 17). Macrophages also contain and release acid hydrolases (19) but his source was probably of a minor importance in the present infection because of the small local inflammatory involvement (1.4% of tissue area is equivalent to $1/\sqrt{2}$%, or 0.71% of tissue volume). The fact that the concentration of myocardial lipids was unaffected in infection, although the total content increased, ruled out fat metamorphosis as a significant cause for the myocardial enlargement.

Effects of exercise. There is an increased protein synthesis in striated muscle when the functional demand increases (24, 36). Similarly, physiological myocardial hypertrophy can occur due to various exercise

regimens (7). Nor unexpectedly, the single 2 hour period of exercise immediately before tissue sampling in the healthy control rats was not enough to cause any measurable myocardial alterations. In the infected rats, on the other hand, this exercise caused a considerable increase of the protein content over and above that associated with the infection alone. An increase in "trainabililty" of infected versus healthy rats has been observed in tularemia (12). However, since there was a considerable increase in protein content (47%) although the exercise lasted only for 2 hours in the present study, it remains possible that when exercise was superimposed on the myocarditis, a further increase of blood flow and capillary permeability led to a localized accumulation of various plasma proteins. The histological signs of myocardtis were not significantly different in exercising or infected rats. This present finding contrasts to the exercise-induced deterioration observed in coxsackie virus myocarditis (21, 31, 39), although the latter may be explained by a more prolonged exercise program or by an enhanced virus multiplication within myocytes.

Effects of fasting. In fasting, the synthesis rate of skeletal muscle protein falls. Increased demands for amino acids needed for gluconeogenesis and nonmuscle protein synthesis result in a decreased muscle mass (3, 15). Cathepsin D is one of the enzymes capable of degrading myofibrillar proteins (6) and its activity increases during starvation (8). However, we observed no activation of cathepsin D in noninfected fasted rats. Rather, an abrupt fall in protein and RNA and a lost correlation between RNA content and heart weight indicated a reduction of myocardial protein synthesis. On the other hand, fasted infected rats showed an unchanged myocardial protein content despite

activation of cathepsin D. The preserved correlation of RNA versus heart weight also suggested an increased metabolic turnover rate. These observations may further support a concept that an infection-induced mediator is capable of stimulating anabolic as well as catabolic processes, depending on the tissue under study (4).

The total content of myocardial lipids decreased due to fasting, regardless of infection, and alterations in the different fractions were similar to those caused by exercise. In fasting as in exercise this accords with their contribution to energy metabolism with emphasis of triglycerides and free fatty acids. The 14% lower heart weight in fasted as compared to fed infected rats cannot be accounted for solely by a different content of lipids (Fig. 3) or glycogen (114), since these constituents can account for only a few percent of the myocardial weight. A possible explanation for part of the lost heart weight with fasting might be a reduced inflammatory reaction and a diminished fluid content of the myocardium. Smilarly, a great loss of spleen weight (35%) occurred when infected rats were fasted.

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TABLE I Experimental design and effects of infection, exercise and fasting on tissue weights (study I) and on the development of histologic signs of myocarditis (inflammatory lesions in percent of tissue area in ventricular sections) (study 2) in S. typhimurium infected rats. All values are means ± S.D. Asterisks denote statistically significant differences (*P<0.05, **P<0.01, ***P<0.001) compared to results in fed, sedentary noninfected controls (group C) and letters (a P<0.05, b P<0.01, c P<0.001) are interposed between those values that differ significantly from each other.

Study group		Variable: Infected 7 days	Exercised 2 hrs	Fasted 24 hrs	Body weight g	Heart weight mg	Spleen weight mg	Inflammatory tissue %
Fed Controls	(C)	No	No	No	124 ± 5	441 ± 25	519 ± 158	+
Fasted Controls	(F)	No	No	Yes	95 ± 5***	361 ± 27 ** b	306 ± 121 c	+
Infected, Fasted	(IF)	Yes	No	Yes	92 ± 6*** c	458 ± 58 a	1352 ± 215*** c	1.0 ± 0.7
Infected, Fed	(I)	Yes	No	No	118 ± 12	533 ± 62***	2094 ± 652***	1.4 ± 0.6
Infected, Fed, Exercised	(IEx)	Yes	Yes	No	123 ± 7	550 ± 101*** c	2331 ± 477*** c	1.1 ± 0.7
Exercised, Fed, Controls	(Ex)	No	Yes	No	118 ± 5	422 ± 31	438 ± 173	+

+ essentially normal tissue

TABLE II Biochemical response of the ventricular myocardium to infection, exercise and fasting in S. typhimurium infected rats (study 1).

All values are means ± S.D. of total myocardial contents. Asterisks denote statistically significant differences (*P<0.05, **P<0.01, ***P<0.001) compared to fed, sedentary noninfected controls (group C) and letters (a P<0.05, b P<0.01, c P<0.001) are interposed between those values that differ significantly from each other.

Study group		Protein mg	RNA mg x 10^{-2}	DNA mg x 10^{-3}	Cat.D µg Alb/min	GUase nmol/min
Fed Controls	(C)	47.8 ± 3.9	132 ± 12	836 ± 58	191 ± 34	29.6 ± 3.2
Fasted Controls	(F)	37.4 ± 4.7 * c	98 ± 13 * b	832 ± 96 b	189 ± 44 b	24.2 ± 3.4 c
Infected, Fasted	(IF)	62.0 ± 11.6 **	146 ± 34 a	1058 ± 191 *	353 ± 90 **	62.9 ± 21.5 **
Infected, Fed	(I)	58.8 ± 8.3 * c	184 ± 48 **	1197 ± 207***	428 ± 160***	69.5 ± 28.0***
Infected, Fed, Exercised	(IEx)	86.4 ± 16.0*** c	189 ± 46*** c	1223 ± 245*** c	458 ± 173*** c	76.4 ± 30.3*** c
Exercised, Fed, Controls	(Ex)	43.5 ± 3.6	125 ± 9	807 ± 45	193 ± 35	28.3 ± 3.4

FIGURE LEGENDS

Fig. 1. Myocardial parameters (means ± S.E.) related to the turnover of myocardial protein in rats expressed in % deviation from values given in parenthesis in fed, sedentary noninfected control rats. Biochemical parameters are given as concentrations in DNA units (fig. A) or in "wet muscle" units (fig. B).

A) Heart/body weight (3.59 ± 0.12 mg x g body weight^{-1}) RNA (1.58 ± 0.03 mg x mg DNA^{-1}), protein (57.3 ± 1.6 mg x mg DNA^{-1}) and cathepsin D (228 ± 12 µg albumin x min^{-1} x mg DNA^{-1}).

B) DNA (1.90 ± 0.04 mg x g muscle^{-1}), RNA (2.99 ± 0.07 mg x g muscle^{-1}), protein (108 ± 2 mg x g muscle^{-1}) and cathepsin D (431 ± 21 µg albumin x min^{-1} x g muscle^{-1}).

Groups are as follows: fasted controls (□) infected fasted (▨) infected fed (■), infected, fed, exercised (▩) and exercised, fed, controls (▧). Asterisks denote statistically significant differences.

(*$P<0.05$, **$P<0.01$ and ***$P<0.001$) when compared to results in fed, sedentary, noninfected, controls and letters (a) $P<0.05$, b) $P<0.01$, c) $P<0.001$) are interposed between bars that show a statistically significant difference from each other.

Fig. 2. Correlation of heart weight (mg) to

A) spleen weight (mg)

B) total myocardial RNA content (mg)

C) myocardial β-glucuronidase activity (nmol x min x g muscle^{-1}). Correlation coefficients varied between 0.834-0.988 and were all significant except that (0.694) of the fasted noninfected group (group F).

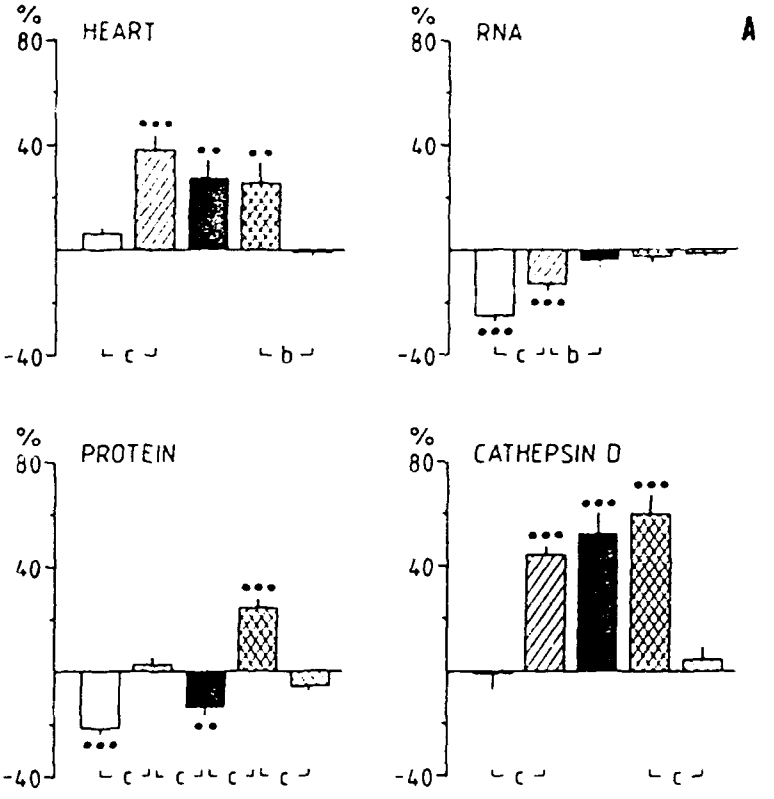
Groups are as follows: fed controls (C), fasted controls (F), infected, fasted (IF), infected, fed (I),infected, fed, exercised (IEx) and exercised, fed, controls (Ex).

r = correlation coefficients, the statistical probability values of which are given when significant.

Fig. 3. Total myocardial contents (means ± S.E.) of lipids (mg x 10^{-3}) in infected, exercised and fasted rats expressed in % deviation from values (given in parenthesis) in fed, sedentary noninfected control rats.

Lipid fractions are as follows: triglycerides (TG: 330 ± 22), diglycerides (DG: 440 ± 18), monoglycerides (MG: 194 ± 6), free fatty acids, (FFA: 1227 ± 54), cholesterol (CHOL: 953 ± 6), cholesterol esters (CHOL.E: 125 ± 11) and total lipids (TOT.LIPIDS: 3268 ± 97). Groups and statistical symbols are the same as in Fig. 1.

Fig. 1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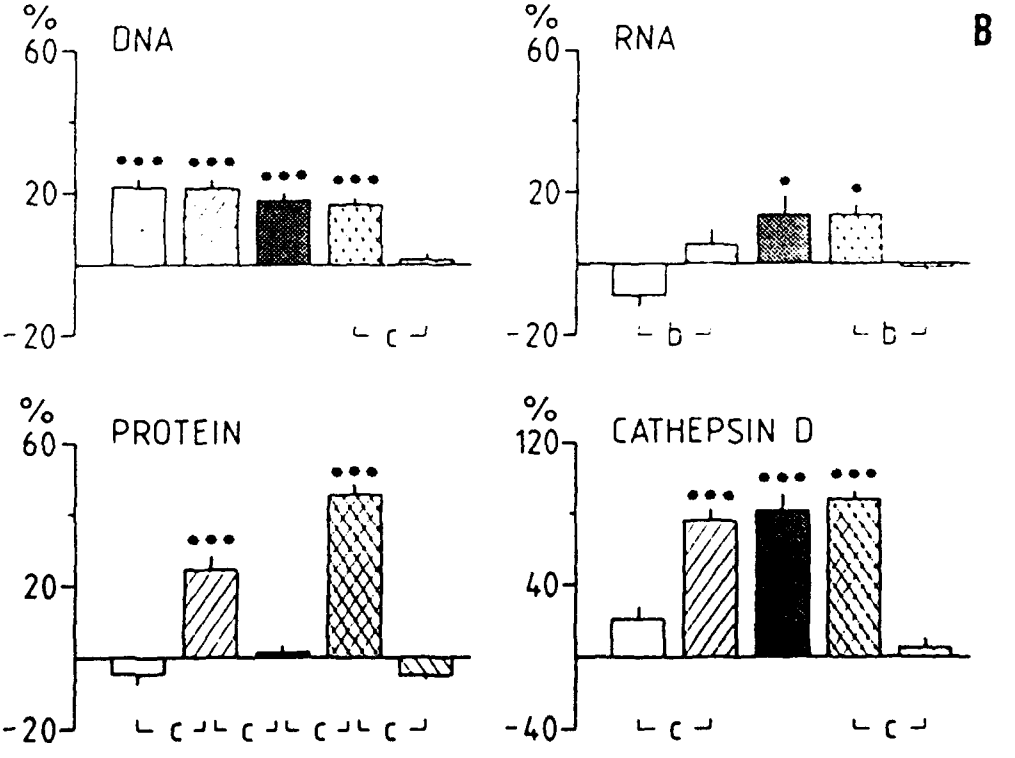
HEART
RNA
A
PROTEIN
CATHEPSIN D
c
b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DNA
RNA
B
PROTEIN
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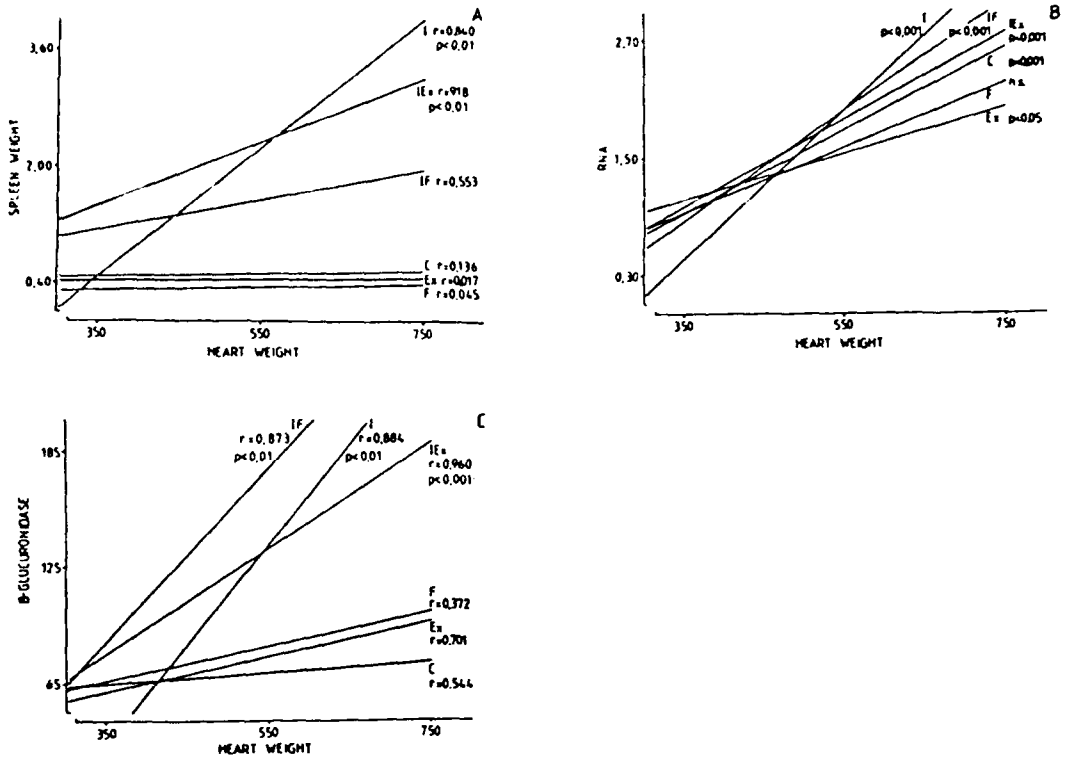
40
-40
CATHEPSIN D
c
b

Fig. 2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C r=0136
Es r=0017
F r=0,045
350
550
750
HEART WEIGHT
B
RNA
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I p<0,001
IF p<0,001
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C p<0,001
n.s.
F
Es p<0,05
350
550
750
HEART WEIGHT
C
B-GLUCURONIDASE
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125
65
IF r=0,873 p<0,01
I r=0,884 p<0,01
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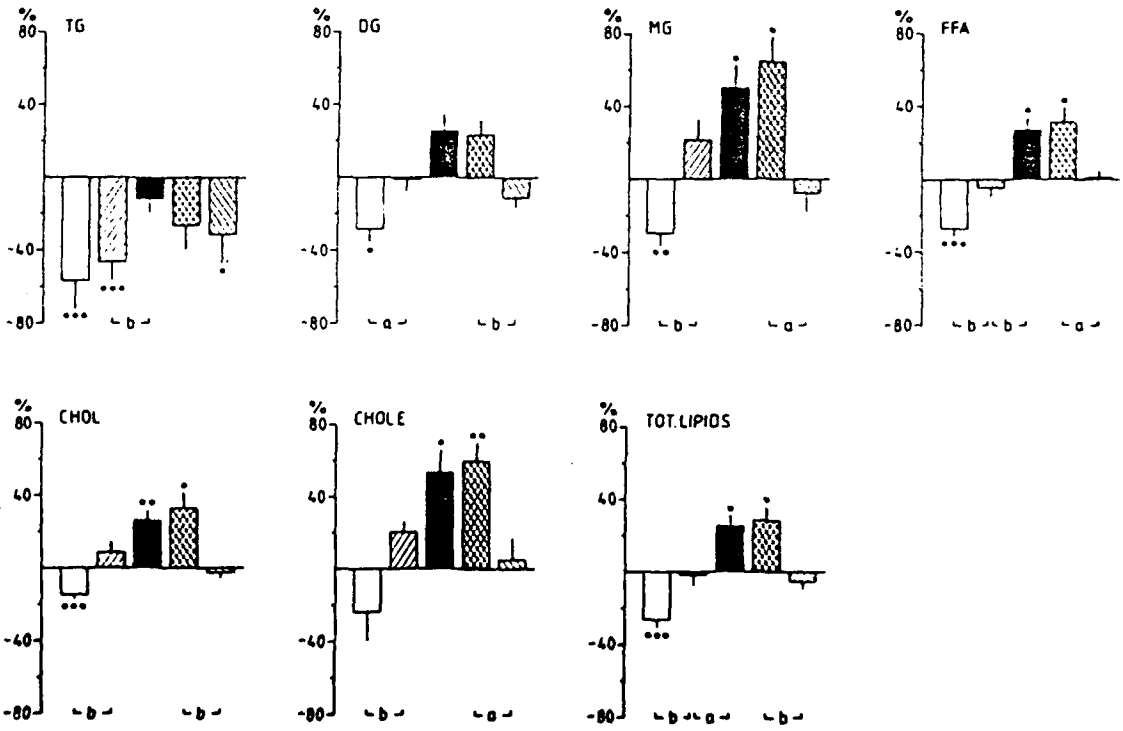
550
750
HEART WEIGHT

Fig. 3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DG
MG
FFA
CHOL
CHOLE
TOT.LIPIDS
a
b