SUGGESTED SHORT TITLE

Effects of diet and exercise on nitrogen status of the body.

IMMEDIATE AND DELAYED EFFECT OF EXERCISE ON VARIOUS NITROGENOUS COMPONENTS OF BLOOD, MUSCLE AND SKIN OF YOUNG ADULT RATS CONSUMING ADEQUATE VERSUS INADEQUATE LEVELS OF PROTEIN

by

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I INTRODUCTION

One of the most important consequences of the technical revolution that has marked our century is the replacement of man- and animal-power by mechanical energy and the resulting trend towards sedentarism in man. This change in the way-of-living of a large proportion of the world population has affected the human species in such a way that it has led a well-known scientist (Passmore, 1964) to talk of the development of a "new type of man and woman, homo sedentarius". In spite of this general tendency, there are still individuals whose level of activity can be considered as heavy. In this category are sportsmen, members of the armed forces undergoing heavy drill and certain types of industrial and agricultural workers.

The influence of physical exercise on nutritional requirements remains therefore a subject of interest, especially to investigators in the field of human nutrition. The relationship between the level of activity and energy requirements is quite well established, but the question of protein requirement for exercise is still a matter of controversy. Yet protein is not only an essential nutrient for all, but it has been one of the first dietary components to be recognized as such. In a recent publication, a leading group of experts on protein requirements (FAO/WHO Joint Expert Group, 1965) had to admit that "scientific evidence of a need for more protein.....in heavy workers is inconclusive" and recommended further research on the subject. One of the main difficulties encountered in studying this problem, as well as that of protein requirements in general, is the inadequacy of the criteria available for measuring slight

deficiencies and small changes in the need for protein. The evaluation of labile protein reserves constitutes a means of detecting small variations in the state of protein nutrition that might result from a change in the level of activity or from any other factor. It is only in recent years, however, that simple and sensitive methods have been proposed for the measurement of protein reserves (Allison et al., 1962).

This approach was used by Christensen (1963a) to study the influence of physical exercise on the protein status of rats fed different levels of protein. The results of this study not only indicated that the labile protein of some of the body compartments decreased when activity was increased, but they revealed important changes following exercise, in the nitrogenous composition of certain muscles involved in the imposed exercise. Another interesting observation from the same report is the significant difference found in their response to exercise between animals that had been submitted to intermittent periods of training before the experiment and rats that had not been trained prior to the experimental period. These findings gave weight to the hypothesis that exercise increases protein requirements, but left many pointsto be clarified. They opened the way to further research in relation to the effect of the level of physical activity on labile and even on so-called stable body nitrogen. This report also demonstrated the importance of rest periods between periods of exercise, especially with respect to changes occurring in muscle. These questions are among several that need to be answered before any conclusion can be reached regarding the influence of the level of physical activity on the need for dietary protein.

The present study was undertaken to obtain some information on the sequence of appearance of changes observed in rats as a consequence of intermittent exercise, on the reversibility of such changes and on the influence of the level of dietary protein on the production and permanence of these changes. More specifically, this experiment was aimed to evaluate the immediate and delayed effects of a prolonged period of exercise and of dietary protein level on the nitrogenous composition of some of the tissues of the rat that were shown to be susceptible to this influence, namely: blood serum, skeletal muscle and skin.

II REVIEW OF LITERATURE

A - Body nitrogen

1 - Body nitrogen under normal conditions.

a - Importance and functions of nitrogenous substances in the body.

The metabolizing mass of the living body may be thought of as being essentially a mixture of water and protein. Protein accounts for approximately 20% of the fat-free composition of the body of mammals, and for about three-quarters of the dry, fat-free weight of their carcass. Proteins have a structural and a functional role in the body: they are an essential part of the structure of cells and, as constituents of enzymes and hormones, they permit and regulate the metabolic reactions by which life is maintained. If the body is invaded by foreign bodies, proteins play a defensive role either as constituents of antibodies or as detoxifying agents.

b - Types of nitrogenous substances in the body.

In the cell, body nitrogen exists as free amino acids, as structured proteins and as conjugated proteins such as nucleoproteins and lipoproteins. The nucleus contains desoxyribonucleic acid (DNA), one of the constituents of chromosomes and a carrier of genetic information. Ribonucleic acid (RNA), another nucleoprotein, acts as a template for protein synthesis and is found mostly in the ribosomes of the endoplasmic reticulum and in the cell sap. Conjugated with lipids, proteins are essential features of internal and external cellular membranes.

Non-protein nitrogen is found predominantly in intra- and extra-cellular body fluids. It includes: 1) free amino acids forming the amino acid pool upon which the cells can draw for the synthesis of their individual characteristic proteins; 2) important energy reservoirs such as creatine and creatinine; 3) end-products of protein metabolism such as urea, uric acid and ammonia.

The type of protein found in the protoplasm of any particular cell of the body appears to be specific for tissue and for species. It is the chemical structure of the protein constituents of a tissue which ascribes a special function to this tissue. Blood proteins include haemoglobin and plasma proteins. The latter are referred to as albumins, globulins and fibrinogen, which are general terms including a large number of simple and conjugated proteins as well as traces of enzymes, hormones and antibodies. Serum proteins can be separated electrophoretically into albumin and at least 4 globulin fractions termed: α_1, α_2, β and γ globulins, each component differing from the other in the size of its molecule. The quantitative importance of each fraction varies with the species. While serum albumin is synthesized mainly in the liver, gamma globulin originates mostly in non-hepatic tissues (Wannemacher et al., 1963).

Muscle is composed of formed elements or myofibrils and of sarcoplasm in which the myofibrils are immersed. The myofibrils contain three types of proteins: myosin which

incorporates L- and H- meromyosins, actin that can be present either in the G- or F-form, the two being interconvertible, and tropomyosin. Actin and myosin can unite to form actomyosin. These three components have different molecular weights, physical and chemical properties and play a leading role in muscle contraction. Sarcoplasm contains globulin X, myoglobin and other nitrogenous components, largely enzymes and nucleoproteins. Extracellular proteins of muscle tissue include collagen, elastin and reticulin.

While the epidermis or outer layer of the skin is composed mainly of keratin, a highly insoluble protein of the albuminoid type, the dermis or inner layer contains other albuminoids such as collagen, elastin and reticulin. The skin interstitial fluid contains the same proteins as those normally found in the plasma (Humphrey et al., 1957).

c - Dynamic state of body nitrogen.

As a consequence of the work of Schoenheimer (1942), Whipple (1948) and others, it has been recognized that there is a constant turnover of nitrogenous components in the body. Proteins are continually being broken down into amino acids which are pooled and reused for synthetic purposes. The turnover rate varies with the type of protein, some being renewed at a slow pace, while others are rebuilt more rapidly. In the event of a deficiency, the former will be maintained at the expense of the latter.

These observations led Whipple (1948) to distinguish two types of body proteins: stable proteins, the constant turnover of which causes no change in the amount in which it exists in the body or in each cell, and labile proteins, which are that part of tissue protein that can be easily depleted and repleted by varying the intake of protein in the diet. Stable proteins are associated mainly with structural parts of cells such as the nucleus, the membrane and with essential tissues such as nervous tissue. Cellular DNA and serum globulins are examples of stable nitrogenous components (Allison et al., 1963). Labile proteins are found in greater concentrations in the cytoplasm and in more dispensable organs such as liver and viscera. This type of proteins include, among other components, cellular RNA and serum albumin (Allison et al., 1963).

Allison and coworkers (1963) make a further differentiation between "dynamic protein" and "labile protein reserves". "Labile protein reserves" would account for not more than 5% of the body nitrogen and would be more closely associated with liver, intestinal and pancreatic tissues. Munro (1964) considers this fraction as a mere extension of the free amino acid pool of the body. "Dynamic protein" is a more general term which would include all the dispensable protein stores, represent 20% or more of body nitrogen and be distributed among cells of numerous tissues including skeletal and cardiac muscles and skin. According to Shapiro and Fisher (1962), protein reserves could be maintained through metabolic shifts between dynamic and labile proteins. These authors believe that reserve proteins are made up partially

of cellular and extracellular non-protein nitrogen, and contain substantial amounts of non-essential amino acids. But according to Munro (1964), it is more likely that most of the labile nitrogen is in the form of protein.

d - Importance of protein reserves.

The level at which protein reserves should be maintained in order to assure optimum health and efficiency is still a much debated question. If one defines protein reserves as "a moiety beyond current needs which may be called upon to meet situations of privation or stress" (Holt et al., 1962), it can be assumed that protein stores should be built up only as long as they are beneficial to meet a stress situation. It has proved difficult however, to demonstrate any advantage to the accumulation of large amounts of protein by animals submitted subsequently to different stresses. Grossman and others (1954) could not establish any relationship between the level of dietary protein and wound tensile strength in rats. Furthermore, Halac (1961) and Halac (1962) found that in the same species growth, endurance to forced exercise, survival to starvation and to withdrawal of protein from the diet were better when the animals were on a normal-protein than when they were on a high-protein diet. Resistance to X-ray exposure was the same on both levels of protein (Halac, 1961). Vaughan et al., (1962), who experimented with pigs, believe that a high-protein diet makes these animals less able to cope with the stress of a sudden protein deprivation. And according to Ross (1959), protein restriction prolongs life by retarding growth: an increase in life span would result, at least

in part, from the decrease in respiratory ailments and in age-associated diseases observed in growth-retarded animals.

On the other hand, beneficial effects of a high-protein diet have been reported in piglets by Bauer and Filer (Anonymous, 1960) under conditions of both starvation and thirst, in rats, by Halac (1961) in relation to water starvation, and in chicks by Fisher et al., (1964) in response to amino acid imbalance or contamination with a virus. Differences in the actual levels of protein used in these various experiments would explain the discrepancies in the results (Fisher et al., 1964).

e - Protein composition of the body of the rat.

Body composition of rats, like that of other mammals, varies mainly in its fat and water contents. The amount of water decreases with physiological age, but beyond the point of chemical maturity, there appears to be little change in the gross chemical composition of the dry, fat-free carcass (Bailey et al., 1960). Table I summarizes different values reported in the literature on the composition of the body of normal adult rats. It will be noticed that the nitrogen or protein content of the fat-free body is fairly constant. According to Bender and Miller (1953), the protein composition of the fat-free carcass is highly correlated with its water content in animals of the same age and strain.

Blood proteins

Total blood proteins, serum proteins and serum protein fractions were studied in the rat by several workers. Representative values for these components are shown in table II. Results are expressed in relation to either whole blood or blood serum.

TABLE I

Body composition of normal adult rats *

Authors	Body weight and sex (g.)	Whole carcass				Dry carcass				Fat-free wet tissue			Dry, fat-free carcass	
		H2O	Fat	N	Protein	Fat	Non-fat N	Protein		H_2O	N	Protein	N	Protein
Widdowson and McCance (1956)	315 M	59	17	-	23									
	232 F	56	21	-	19									
Stanier (1957)	300 M	63	13	3	-					73	3	-		
Elkinton and Widdowson (1959)	? M	63	13	-	-					71	3	-	11	-
	250 M	65-69	-	-	-	27-39	60-73	8-10	-				13-14	-
Pratt and Putney (1959)	300 M	64-67	-	-	-	27-36	63-73	9-10	-				13-14	-
Michelsen and Anderson (1959)	319 F	58	21	-	18									
Lee and Lucia (1961)	200 M	-	6	-	-					72	-	19		
	? M	-	16	-	-					68	-	21		

* expressed in percentage to the nearest whole percent.

TABLE II

Blood proteins of adult rats

Authors	in whole blood			in blood serum					
	Total proteins	Albumin	Globulins α_1 α_2 β γ	Total proteins	Albumin	Globulins α_1	α_2	β	γ	A/G
	%	%	%	g.%	g.%	g.%				
Jeffay and Winzler (1958a)	7 (% of whole blood)	55	5 10 10 20 (% of blood proteins)							
Weimer et al. (1959a)				5.8	2.0	1.5	0.7	1.1	0.5	
						3.8				0.5
Christensen (1963a)				6.6	2.6	3.9				0.7
Leathem (1964)				6.1	3.3	0.6	0.6	0.9	0.7	
						2.7				
				5.9	2.2	0.8	1.0	1.1	0.9	
						3.9				

The turnover rate of rat γ globulin is lower than that of other serum proteins. Its half-life was found to be of 5 days by Jeffay and Winzler (1958a) and of 4.7 days by Freeman and Gordon (1964). These two groups of workers estimated respectively at 3.7 and 2.7 days the half-life of serum albumin.

Muscle proteins

Skeletal muscle of the adult rat does not vary to any large extent in its protein content even if one compares different muscles of the body. Table III shows that authors agree well in estimating the amount of protein in skeletal muscle. There is a wide divergence of opinion, however, in the estimation of DNA in skeletal muscle of rats. This variety of views might be due to differences in the age of the animals or to dissimilarities in the techniques chosen for its determination.

Age affects the DNA content of tissues to a considerable extent. After maturity is attained, however, the DNA content of any diploid nucleus of any tissue within a species is considered constant (Davidson, 1954). Most adult rat tissues contain about 0.65 picogram of DNA.P per nucleus (approximately 8.0 picograms DNA, assuming 8.1% P in DNA), except liver nuclei which would yield values approximating 0.913 picogram DNA.P per nucleus (11 picograms DNA), due to the presence of tetraploid cells (Davidson, 1954). In this case, the amount of DNA per cell would depend on the number of nuclei in each cell and since the quantity of polyploid cells increases with age, the DNA value per cell would depend upon the age and the body weight of animals (Fukuda and Sibatani, 1953), or else upon its ideal weight (Waterlow and

TABLE III

Protein and DNA composition of skeletal muscle of adult rats

Authors	Muscle analyzed	Protein (1)			DNA (1)
		g./100 g. wet muscle	g./100 g.fat-free wet muscle	g./100 g.fat-free dry muscle	m./100 g. wet tissue
Mandel et al. (1949)	hind leg	18 *			39 - 50
Stanier (1957)	"skeletal"	21 *			
Hagan and Scow (1957)	left thigh	20			
Mendes and Waterlow (1958)					85 **
Elkinton and Widdowson (1959)	"skeletal"		21 *	84 *	
Kao and McGavack (1959)	lower leg	20			
Christensen (1963b)	gastrocnemius	21 *		94 *	124

(1) figures rounded to the nearest whole number.
* computed from the published data, using 6.25 as the factor of conversion of N to protein.
** computed from a figure given as mg. of DNA.P, assuming 8.1% P in DNA.

Weisz, 1956). The multiplication of polynuclear cells with aging in tissues other than liver in the rat is questionable. Because of its relative stability, DNA is often used as a reference standard in metabolic experiments involving changes in other constituents of the cell (Thomson et al., 1953; Gray and de Luca, 1956; Allison and Fitzpatrick, 1960).

In a study on the influence of age on the composition of connective tissue in rats, Kao and McGavack (1959) observed no consistent influence of age on insoluble collagen of the upper leg muscle, but an increase with age in the amount of insoluble collagen in the lower leg and abdominal muscles. The amount of soluble protein, including soluble collagen, per 100 grams of tissue in 8-month-old female rats was found to be 19.9 ± 1.4 grams in the lower leg muscle, and that of insoluble collagen in the same muscle, 1.4 ± 0.3 grams.

Skin proteins

On a percentage basis, rat's fresh skin contains slightly less protein than fresh muscle but much more collagen (cf. table IV). Total collagen increases with age but soluble collagen decreases, according to Kao and McGavack (1959). Houck and Jacob (1958) observed a decrease with age in the concentration of hydroxyproline, and hence of soluble collagen in the skin, but only in rats weighing more than 280 grams. In smaller rats, the amount of hydroxyproline in skin was found to increase with age.

TABLE IV

Protein, collagen and DNA composition of skin of adult rats

Authors	Protein (1)			Collagen (1)		DNA (1)
	g./100 g. wet skin	g./100 g. fat-free wet skin	g./100 g. fat-free dry skin	Total g./100 g. wet skin	Insoluble g./100 g. wet skin	mg./100 g. fresh skin
Stanier (1957)	15 *					
Rodesh and Mandel (1958)						dermis: 72** epidermis: 110**
Elkinton and Widdowson (1959)		27 *	77 *			
Kao and McGavack (1959)	13 (soluble protein only)			21	15	
Christensen (1963b)	19 *		86 *			110

(1) figures rounded to the nearest whole number.
* computed from the published data using 5.38 as the factor of conversion of N to protein.
** computed from a figure given as DNA.P, assuming 8.1% P in DNA.

2 - Body nitrogen as affected by stress

Dietary restriction or imbalance, severe physical exercise, extreme variations in environmental temperature, as well as several other factors can be grouped under the heading of stressor agents. When reviewing the effect of such factors, and particularly of the first two, on body nitrogen, one finds that there is a group of effects that are common to all of these factors and that reflect the effort of the body to adapt itself to a sudden change in environment.

a - General effects of stress on body nitrogen.

Systemic stress, especially during the "alarm" stage, causes a rapid breakdown of cytoplasm and even of entire cells (Selye, 1950), resulting in the liberation of proteins and protein catabolites which can either be excreted, causing a negative nitrogen balance, or be reused for the synthesis of tissues. The former situation usually predominates, as shown by an elevated urinary excretion of urea, creatinine, uric acid, sulphur and amino acids (Leathem, 1964), and by the occasional occurrence of proteinuria (Selye, 1950). Plasma proteins, especially albumin, fall, while non-protein nitrogen in the blood tends to rise, at least in the initial stage of the stress state (Selye, 1950). The tissues lost more readily are those from the thymus and other lymphatic organs, large parenchymatous glands such as the liver and pancreas, connective tissue and musculature. Quantitatively, because they are large organs, the carcass and the skin represent important sources of the protein degradation

products. The more abundant the protein reserves, the more severe are the nitrogen losses resulting from stress. The immediate result of such a stimulation of catabolism is a more rapid turnover of tissue proteins, but soon the regeneration capacity of tissues wears off, and the protein reserves of the animal become exhausted.

b - Role of hormones in the response of body nitrogen to stress.

1) Adrenal hormones

Adrenal hormones play a definite role in the changes in protein metabolism induced by stress, as indicated by the fact that the adrenal cortex is the only organ that tends to gain weight under stressful conditions (Selye, 1950). An acute stress brings about an increased secretion of corticosterone which, through gluconeogenesis, furnishes the organism with the fuel that it urgently requires. Engel (Boulouard, 1963) believes that an increased need for carbohydrate would be, in fact, the first general effect of stress. In order to maintain the level of blood sugar, adrenal corticoids enhance catabolism of peripheral tissue proteins. This in turn creates an accumulation of amino acids in the blood and the liver. The amino acids that are not transformed into glucose are resynthesized into liver proteins (Korner, 1960) and, under certain conditions, into blood proteins such as γ globulin (Trémolières et al., 1954). Corticoids can therefore be anabolic or catabolic depending on the tissue involved. Korner (1960) found that adrenal

corticoids increase the proportion of large-sized ribosome particles containing RNA in the liver and believes that this fact is linked with the increased ability of the liver to synthesize protein under the influence of these hormones. On the other hand, adrenal steroids reduce the rate of amino acid incorporation into muscle, into diaphragm, into mouse kidney and into rat skin (Leathem, 1964).

2) Other hormones.

Several hormones other than the adrenal corticoids are involved in protein metabolism and protein synthesis. Stress may, in some cases, disturb hormonal balance and affect body nitrogen through influencing quantitatively and/or qualitatively the endocrine secretion of the pancreas, of the pituitary, of the gonads and of the thyroid. A review of the role of hormones in protein metabolism is, however, outside the scope of this section. The present review is therefore limited to general and important studies published on this subject in recent years.

It must be remembered that the fundamental mechanisms of hormonal action at the level of cellular physiology are still poorly understood (Querido, 1962; Randle, 1963). Protein anabolic hormones such as the pituitary growth hormone, androgens and insulin were shown to accelerate amino acid incorporation into peptides (Krahl, 1961; Korner, 1962; Leathem, 1964). This effect would be specific of the organ affected rather than specific of the hormone (Kassenaar et al., 1962), with the result

that the same hormone will cause an increase in protein synthesis in some tissues, and an increase in protein breakdown in other tissues. In the case of androgens, Aschkenasy (1959) believes that their main effect is not nitrogen retention but a specific redistribution of proteins among organs and tissues. The over-all effect of hormones on nitrogen balance depends on the importance of the target organs affected by the hormone concerned. While growth hormone and androgens cause a positive nitrogen balance because they induce nitrogen retention in large organs such as skeletal muscle, adrenal corticoids and thyroid hormones increase protein synthesis in the liver and perhaps in other tissues, but have a general catabolic effect because they increase the breakdown of peripheral muscles. Estrogens produce an increase in the size and protein content of sexual tissues only (Leathem, 1964). Insulin is protein anabolic and is essential for an optimal action of growth hormone and androgens (Leathem, 1964). When the hormonal action results in a positive nitrogen balance, it is usually due to hypertrophy, but the work of Kassenaar et al. (1962) indicates that androgens induce hyperplasia in the seminal vesicles of the rat. The composition of a muscle that increased in size as a result of the administration of growth hormone was found to remain unchanged (Leathem, 1964).

3 - Body nitrogen as affected by diet.

In addition to general effects of stress, a dietary restriction, whether of calories or of protein produces

specific changes in body nitrogen.

a - Effect of a caloric restriction:

1) On the whole body or carcass composition.

The results of a caloric restriction on the protein composition of the body of animals are somewhat different depending on whether the animal is completely starved or is chronically underfed. In both cases, there is a loss of weight corresponding to a decrease in the percentage of both fat and fat-free solids, but in adult animals, the proportion of fat lost is greater during chronic undernutrition than during fasting (Widdowson and McCance, 1956).

Widdowson and McCance (1956) reported that the amount of protein in the carcasses of male rats, either underfed for 5 weeks or starved for 6 days, was 80% and 84% respectively of that of control animals, while comparable percentages in female animals were 91% and 92%; the body weights of the same animals were 84%, 85%, 85% and 84% of those of the controls in the same 4 groups. The proportion of nitrogen in the bodies of the rats that were restricted in their caloric intake was therefore either the same or higher than in the control animals. This finding was confirmed by Stanier (1957) and by Sobel et al., (1959). This last group of workers found that, if expressed as a percentage, the loss in carcass nitrogen was inferior to the loss in body weight until the latter reached 30%, at which point the loss of nitrogen equalized that of body

weight. The absolute amount of nitrogen in the carcass is also reduced in caloric restriction (Garcia and Roderuck, 1964b).

Widdowson and McCance (1956) observed no difference in the total amount of water in the bodies of control and food-deprived animals. However, Stanier (1957) and Elkinton and Widdowson (1959) found a higher percentage of water in the whole carcass, but a reduced amount of water in the lean body mass of rats that had been underfed for periods varying from 6 days to 13 weeks, compared to normally-fed animals.

Longer experiments on food restriction failed to demonstrate any marked change in the body composition of animals. Fisher and Griminger (1963) observed no difference in either nitrogen, lipids or moisture contents of the bodies of chicks fed limited amounts of food for 3 years. In a study in which they compared the body composition of rats maintained, by means of food restriction, at a weight of 200 grams for periods up to 26 weeks with that of normal rats of the same weight, Lee and Lucia (1961) found no significant difference in most body components, including the proportion of body weight represented by protein. But they observed that, although body weight remained constant, feed intake gradually decreased for the first 44 days, at which time it settled at a level 35% below the initial intake. The authors believe that this decrease in the caloric requirement

is the result of adjustments involving several mechanisms: improvement in the efficiency of feed absorption, decreased metabolic rate, slowing of the activity and changes in the utilization of food, particularly in carbohydrate metabolism. Although this group of workers did not notice any change in the voluntary activity of their underfed animals, Cabak et al., (1963a) observed a reduction in the activity of a group of young rats fed restricted amounts of a high-protein diet.

2) On organs and tissues.

The first organs to suffer from a caloric restriction are the liver, the pancreas and the intestine. Ju and Nasset (1959), report that in adult rats these three organs lose about one half of their nitrogen in a 192-hour fast. A decrease in the quantity of digestive enzymes would account for a large part of this decrease in nitrogen.

Effect on blood nitrogen.

Inanition, in rats from which food is withheld until they lose 25% of their initial weight, causes a decrease in serum albumin, α_1, α_2, and β globulins, according to Weimer et al., (1959a), but increases the concentration of γ globulin. Total blood nitrogen rose steadily in adult rats fasted for 192 hours (Ju and Nasset, 1959). Such an increase, as well as that of γ globulin, could simply reflect a decrease in blood volume, but the published data does not allow

one to conclude on the validity of this assumption. On the other hand, Cabak and others (1963a) found no appreciable change in the albumin fraction under conditions of caloric restriction in young rats but a decrease in the α and β globulins, as well as a fall in total plasma proteins.

Effect on muscle nitrogen.

Muscle, and skeletal muscle in particular, is greatly affected by a caloric insufficiency. During fast periods of 3 to 7 days, the thigh muscles of young rats lost weight in proportion to the body weight loss, and of the calories represented by this weight loss, 13% to 27% were derived from protein (Hagan and Scow, 1957). There was no appreciable change in the H_2O/protein ratio nor in the nitrogen content of the non-protein nitrogen or of the stroma fractions. Myosin values were lower than for controls in rats that were starved for the longest period (7 days) only, but sarcoplasmic proteins ("water soluble") suffered a considerable loss (21% of initial weight) in all the starved animals.

The picture is slightly different for chronic undernutrition, in which case most workers report an increase in the amount of water per unit weight of muscle (Elkinton and Widdowson, 1959; Dickerson and McCance, 1960; Widdowson et al., 1960). Over-hydration was found to be due to an increase in

extracellular water, while intracellular fluid decreased (Cabak et al., 1963a). These changes were accompanied by an increase in non-protein nitrogen and in extracellular protein, and by a decrease in sarcoplasmic and fibrillar protein. Similar results were obtained in chicks by Dickerson and McCance (1960) and in young pigs by Widdowson and collaborators (1960). Caloric deficiency affects the total amount of certains enzymes of muscle, but does not alter their unit activity (Wainio et al., 1959).

Effect on skin nitrogen.

The skin of calorie-restricted animals does not retain greater amounts of water than that of normal animals according to Elkinton and Widdowson (1959) working with rats, and to Widdowson et al.,(1960), with adult pigs. It could even contain less water and more nitrogen per kilogram of fat-free tissue, at least in young rats (Cabak et al., 1963a). No difference in the collagen/nitrogen ratio of skin was found by this group of workers nor by Fisher and Griminger (1963) in the chick. In pigs, Widdowson et al., (1960) noted an increase in such a ratio only when underfeeding was started at a very early age.

b - Effect of a protein restriction.

Through its effect on labile protein fractions, protein intake affects the nitrogen content of the body as a whole and its distribution in the different body compartments. The picture of protein depletion varies depending on the stage of the depletion and the severity of the dietary restriction. The effects of protein depletion on body composition are mediated through the influence of such a restriction on:

- food intake and body weight;
- nitrogen utilization and excretion;
- nitrogen balance and protein synthesis.

1) Effect of dietary protein level on food intake and body weight.

A voluntary restriction of food intake is not specific of a protein deficiency but is characteristic of all imbalanced diets. On a protein-free diet, animals rapidly lose their appetite (Williams, 1961) and consequently, lose weight. Adult rats will lose up to approximately 50% of their initial weight at the end of 60 to 80 days of deprivation (Jacob et al., 1951; Williams, 1961).

Usually, animals eat to satisfy their energy requirements (Wagle et al., 1962; Andik et al., 1963). Once a depletion state is established, the need for energy of the protein-depleted animals is reduced because of a lower protoplasmic mass and consequently, of a lower basal metabolism (Black, 1939; Aschkenasy,

1960). On a "per cell" basis, however, Gray and Deluca (1956) could not observe any difference in the metabolic activity of tissues of rats fed either a low- or a high-protein ration. In young animals, the limitation of growth imposed by protein restriction also reduces the need for food energy. Up to a certain limit, adult animals try to compensate a deficiency of protein by an increase in food consumption (Garcia and Roderuck, 1964a). But according to Meyer and Hargus (1959), animals would be limited in their ability to increase their food intake in order to obtain more protein by their capacity to store or dissipate energy. Andik et al., (1963) confirmed this assumption by finding that the food intake and the survival rate of young rats given a low-protein diet were higher if the animals were maintained in a cold temperature than if they remained at room temperature.

2) Effect of dietary protein level on nitrogen utilization.

The level of protein in the diet affects both nitrogen digestibility and nitrogen retention. The proportion of nitrogen consumed that is absorbed increases with an increase in the percentage of dietary protein, mostly because the proportion of fecal nitrogen represented by metabolic fecal nitrogen varies inversely with the level of protein intake (Rutherford, 1955;Hartsook and Hershberger, 1963). In addition, certain types of proteins are poorly digested in protein-depleted animals, probably due to a lack of digestive

enzymes or other substances present in the digestive tract (Allison, 1957).

The rate of general turnover of nitrogen in the body is also proportional to the level of nitrogen intake, so that increased intakes will result in increased outputs in the urine (Hartsook and Hershberger, 1963). When the protein intake is less than the requirement, Allison (Allison and Fitzpatrick, 1960) showed that the excretion of urinary nitrogen is a function of the magnitude of protein stores. The biological value of the protein and the caloric adequacy of the diet also influence the amount of nitrogen excreted. Urinary nitrogen excreted by an animal with well-supplied protein stores is both of "endogenous" and "exogenous" origin i.e. it comes both from tissue protein, including the body amino acid pool, and from food protein. The first type is represented by urinary creatinine, the amount of which does not vary with protein intake, but is correlated with the protoplasmic mass or better, with the functional nuclei of muscle mass (Allison, 1956). Urea and uric acid, for their part, are mostly the breakdown products of exogenous protein and constitute almost 100% of the fraction of urinary nitrogen that varies with protein intake.

3) Effect of dietary protein level on nitrogen balance and protein synthesis.

Through its influence on nitrogen excretion, the level of dietary protein affects the sign and the value of nitrogen balance in the body but its effect is not necessarily in the same direction or of the same magnitude for the whole organism and for its different compartments. During protein depletion, two types of changes take place which are closely related one to the other (Garrow, 1959): 1) a breaking-down of body proteins affecting first the more labile proteins such as plasma albumin, proteins of the liver, of the intestine, of the pancreas; 2) a change in the distribution of protein synthesis in favour of the essential organs. The over-all result is a general slowing down of protein metabolism (Jeffay and Winzler 1958b; Yuile et al., 1959b). As the level of dietary protein is increased, the turnover rate of tissue proteins is increased, reaching a maximum, in weanling rats, when casein is the only source of protein, at the level of 25% dietary protein for intestinal and liver proteins, and at levels up to 40% dietary protein for other proteins (Muramatsu et al., (1963).

The magnitude of the over-all nitrogen balance in the organism or in a tissue depends not only on the level of protein fed, but also on the supply and balance of amino acids and on the supply of energy, both of which being limiting factors on the rate of synthesis (Munro et al.,

1953; Munro, 1954). The influence of the caloric intake on nitrogen retention varies with the protein intake. With a protein-free diet, it is evident that addition of energy can do little to increase the rate of protein synthesis (Allison and Fitzpatrick, 1960). But when dietary protein is adequate, an increase in calories will cause a linear improvement in nitrogen balance (Munro et al., 1962a). It has been observed (Allison, 1956; Munro et al., 1962a).that a restriction in the caloric consumption does not alter utilization of dietary protein unless it falls below 50% of the requirement; at that point, dietary protein is used for energetic purposes and is no longer available for the synthesis of tissues or to replenish body protein reserves. With very low caloric intakes, the magnitude of nitrogen balance would be a function of the importance of protein stores (Allison, 1956).

The effect of protein intake on protein synthesis is reflected in the amount of RNA in the cell. RNA participates in protein synthesis in two ways (Simkin, 1959): 1) soluble RNA, found mostly in the cytoplasm, is involved in the transport of activated amino acid residues to the site of protein synthesis; 2) ribonucleoprotein RNA, concentrated in the microsome of the cell, receives the soluble RNA-amino acid complex giving the amino acids their sequence to form a characteristic protein. It is believed that a deficiency in protein does not affect the

total amount of RNA synthesized, at least in the liver, but that it speeds up its breakdown, this effect being confined to the microsomal fraction (Munro and Clark, 1960). An increase in the quantity of ribonuclease, the enzyme that catabolizes RNA, in tissues of protein-deprived animals confirms the relationships between RNA, protein anabolism and the level of dietary protein (Zigman and Allison, 1959).

4) Effect of a protein deficiency on the nitrogen composition of the body.

The combined effects of a dietary protein restriction on tissue degradation and on protein synthesis can be measured in the profound modifications undergone by the body under the influence of such a deficiency. These changes can be observed at 3 levels: that of the whole organism, that of tissues and organs and that of the cell.

Effect of a nitrogen deficiency on the nitrogenous components of the whole body.

Studying the effects of a protein-free diet on the total carcass composition of adult rats, Stanier (1957) concluded that these effects were not much different from those of a caloric-deficient high-protein diet. There was overhydration of the body, less fat (8%) than in control animals (13.2%) but more than in animals restricted in energy (2.5%), and practically the same proportion of nitrogen (3.15, 3.25 and 3.20 grams nitrogen per 100 grams)

in the fat-free carcass of protein-depleted, control and underfed animals. The percentage loss of nitrogen was lower than that of body weight so that the nitrogen concentration of the body was increased.

In weanling rats made protein-deficient, the proportion of non-collagen nitrogen per kilogram of fat-free tissue is decreased (Cabak et al., 1963a). Similarly to what happens in caloric restriction, collagen would not become degraded unless deprivation is prolonged. No loss of collagen was observed in chicks that were either starved or protein-depleted until they lost one third of their initial body weight (Summers and Fisher, 1960). But if the same birds were repleted and starved again for 6 days, a decrease of 20% in the collagen was registeredin both calorie-restricted and protein-restricted groups. In mice, Harkness et al. (1955) estimated at 13% the decrease in total collagen that followed a 17-day period on a protein-free diet, while residual protein nitrogen was lowered by 37%.

Effect of a nitrogen deficiency on the nitrogenous components of organs and tissues.

As mentioned earlier, nitrogen losses during protein depletion are confined to the labile or, in severe cases, to the dynamic fraction of body nitrogen. Moreover, protein deprivation results in

shifts in tissue synthesis in favour of the essential organs. Consequently, different organs and tissues lose and gain nitrogen at different rates when protein intake is below the requirement. In early protein depletion, protein is lost from readily available pools such as that of serum and liver (Garrow, 1959). Extravascular protein would contribute twice as much nitrogen as the circulating plasma during this phase (Yuile et al., 1959a). Later during depletion, proteins of slower turnover are used to furnish essential amino acids to the essential organs. Of other tissues which could contribute amino acids to an over-all metabolic pool during stress, muscle would be one of the most important (Munro et al., 1953; Yuile et al., 1959b; Waterlow, 1963; Allison et al., 1963).

Skin, because of its considerable mass, accounts for the largest percentage of the nitrogen of the body after muscle and skeleton and for this reason, it has been suggested as a possible reservoir of readily available nitrogen (Sobel et al., 1959). The observation that, during rehabilitation from protein or caloric undernutrition, one-third of the nitrogen retained by young rats was deposited in the skin (Cabak et al., 1963b), supports this opinion.

Effect of a protein deficiency on blood nitrogen.

Long-term protein deprivation in the rat results

in decreased blood volume, plasma volume, packed cell volume and circulating haemoglobin (Ambegaokar and Chandran, 1959). A change in blood volume can mask variations in blood components making it difficult to compare results obtained by different workers. In general, total circulating proteins and total albumin, as well as their concentration in the plasma or the serum, are substantially decreased during severe protein depletion. This is true in the rat (Lippman, 1948; Weimer et al., 1959a, 1959b; Ambegaonkar and Chandran, 1959; Mulgaonkar et al., 1959), in the dog (Kawashima, 1956; Wannemacher et al., 1963), in the cat (Coles, 1960), and in the human (Kulkarni et al., 1960). In long-term protein malnutrition in humans, serum proteins and serum albumin may appear normal up to the final stages of deficiency (Picou and Waterlow, 1962) presumably because the decrease in synthesis of these substances is compensated by a reduction in their catabolism.

The effect of protein depletion on the globulin fractions of the serum is more inconstant. There is usually a decrease in the relative proportionsof α_1, α_2, and β globulins (Kawashima, 1956; Weimer et al., 1959a, 1959b; Mulgaonkar et al., 1959; Wannemacher et al., 1963) but γ globulin either stays unchanged (Wannemacher et al., 1963) or

increases (Weimer et al., 1959a, 1959b). This last observation may be the result of hemoconcentration. The effect of protein depletion on serum proteins appears thus to be restricted to those proteins such as serum albumin which are synthesized in the liver (Wannemacher et al., 1963). Because of the relative stability of γ globulin, which is the principal component of the globulins, serum albumin/globulin or A/G ratio is usually considered a good index of protein depletion. At least in early stages of depletion, the A/G ratio of protein-deprived rats is lower than normal (Weimer et al., 1959a, 1959b).

Among other blood proteinsaffected by protein deprivation are glycoproteins (Weimer et al., 1959c) and certain enzymes such as aldolase and pseudo-cholinesterase (Barrows and Chow, 1959; Allison and Fitzpatrick, 1960). Plasma esterase, plasma lipase and serum amylase would be reduced in the protein deficient human (Waterlow, 1959).

Effect of a protein deficiency on muscle nitrogen.

During protein depletion, muscle weight and muscle protein are reduced, the latter to even a greater extent than the former. Hind leg muscles of adult rats lost 55% to 65% of their protein after 61 to 69 days of a protein-free regime, while they lost only 50% to 60% of their weight

(Mandel et al., 1949). In spite of the depletion, extracellular proteins particularly collagen, continue to grow, and therefore to increase in concentration and in absolute amounts (Mendes and Waterlow, 1958; Cabak et al., 1963a). But the amount of sarcoplasmic and fibrillar protein decreases (Cabak et al., 1963a), as well as that of RNA (Mandel et al., 1949). The concentration of DNA is reported to have increased during prolonged protein deprivation but its absolute amount per muscle was unaffected (Mandel et al., 1949). In similar conditions, Mendes and Waterlow (1958) observed a decrease in the quantity of DNA per muscle and believe that this decrease reflected a diminution in the number of cells.

During protein depletion, muscle non-protein nitrogen and particularly free amino acids, tend to decrease for the first 2 to 3 weeks (Thomson et al., 1950) and then to rise (Cabak et al., 1963a). This last observation, according to Allison et al., (1963) reinforces the assumption that muscle supplies overall metabolic pools with amino acids during stress. There is even a tendency for depleted animals to maintain higher levels of free amino acids in the muscles than in the liver (Allison et al., 1963).

Part of the nitrogen lost by skeletal muscle in protein deprivation is accounted for by a reduction

in the quantity of enzymes. Wainio et al., (1959), studying cytochrome oxidase, succinate-cytochrome reductase, DPN.H-cytochrome C reductase and aldolase in the gastrocnemius muscle of the rat, observed a decrease of 10% to 20% in unit activities and of 50% to 60% in the total activities of these enzymes. Muscle lactic dehydrogenase, on the contrary, is not influenced by protein level and succinoxidase is increased under conditions of deficiency (Anonymous, 1959).

Effect of a protein deficiency on skin nitrogen.

The results of subnormal protein intakes on the skin of animals such as the rat are in line with those shown in muscle but they are less pronounced. Rodesh and Mandel (1958), giving a protein-free diet to adult rats during 30 to 60 days, noted a decrease in RNA of 45% in the epidermis and of 40% in the dermis, the results being given per unit surface area. When expressed the same way, DNA showed no change, but its concentration per 100 grams of fresh skin was increased. The amount of nitrogen per 100 grams of fresh skin remained unchanged which presumably means, assuming a loss in other constituents, that the concentration of nitrogen in the skin was decreased. Total collagen was found to be markedly decreased in the skin of mice submitted to a

protein-free diet for 20 days (Harkness et al., 1958). In an experiment involving young rats fed a low-protein diet (Cabak et al., 1963a), the concentration of collagen, as well as that of skin nitrogen was found to increase, due to dehydration, but this was ascribed to a shortage of calories, rather than to one of protein.

Effect of a protein deficiency on the nitrogen composition of other organs and tissues.

As mentioned earlier, liver and pancreas are among the first organs to be affected by a protein deficiency. Within a few days, the liver loses as much as 40% of its protein (Waterlow, 1956). As it might be expected, a large proportion of the lost nitrogen is in the form of enzymes (Albanese, 1959 ; Allison and Fitzpatrick, 1960). There is also a substantial loss of RNA (Weill et al., 1956; Allison et al., 1963). Pancreas responds quickly to a deficiency of protein by a loss of nitrogen, RNA and even, according to Mandel et al. (1954), by an absolute loss of DNA. Histological changes such as a gradual disappearance of zymogen granules and atrophy of the acini (Wachstein and Meizel, 1959) would explain the decrease in pancreatic enzymes observed in the protein deficient rat (Snook and Meyer, 1964) and human (Waterlow, 1954).

Protein and RNA are also lost from the spleen (Jacob et al., 1951), the kidney (Addis et al., 1940;

Allison et al., 1962) and the adrenals (Munro et al., 1962b) as a result of a protein deficiency. The first two of these organs, as well as the heart, suffer a loss of enzymes (Wainio et al., 1959). The brain appears very resistant to protein depletion (Mandel et al., 1950; Wainio et al., 1959; Allison et al., 1962).

Effect of a nitrogen deficiency on the nitrogenous components of the cell.

The impact of protein nutrition on the cell has been studied mostly in the liver, first because this organ contains a high proportion of labile nitrogen, and second, because of the ease with which it can be isolated and separated into its different components.

While Thomson et al. (1952) and Munro (1954) believe that the number of liver cells is not altered by protein depletion, Williams (1961) reported a decrease in the number of cells per liver both in rats that were protein deficient and in their pair-fed controls indicating that this result was due to inanition. But if one considers the number of cells per unit weight of liver, then the figure gradually goes up as protein depletion progresses (Williams, 1961).

Total nitrogen per cell falls gradually in the course of protein depletion, partly due to inanition. After 80 days of a protein-free diet, the nitrogen concentration in the liver cell of depleted rats

was found to be only 68% to 75% of that of pair-fed controls (Williams, 1961). The nucleus nitrogen does not suffer at all from this loss (Muntwyler et al., 1950; Moulé, 1959) which would be shared uniformly by all cytoplasmic fractions (Munro, 1954). Analyzing each fraction more precisely, however, led Muntwyler et al.(1950) to think that the depletion is most pronounced in the microsomes (47.4% of total), then in the residual material (30.9% of the loss) and least in the mitochondria (20% of the loss). Similar conclusions were reached by Wirramanayake et al.(1953).

Following protein deprivation, there would be a reduction in the amino acid pool of the liver cell (Allison et al., 1963) and a reduction in cell RNA (Thomson et al., 1952; Munro, 1954; Moulé, 1959). The loss of RNA in the microsomal fraction of hepatic cells accounts for practically all the decrease of this component in the liver (Wirramanayake et al., 1953; Munro, 1954).

Contrarily to RNA, DNA is usually considered a stable component of the liver cell. That the amount of DNA per nucleus is not affected by dietary means is recognized by Campbell and Kosterlitz (1952), Thomson et al. (1952) and by Davidson (1954). Disagreement with this finding was enunciated by Ely and Ross (1951), by Lecompte and de Smul (1952)

and by Umana (1965). All three groups of workers observed higher than normal values for DNA in liver cell nuclei of young rats previously submitted to protein depletion. The inanition consecutive to protein deficiency would cause, according to Ely and Ross (1951), severe pycnosis in the cell resulting in the disappearance of nuclei without a corresponding decrease in the amount of DNA. Umana (1965) believes that protein deficiency increases the polyploid population of the liver, and not the DNA content of each nucleus.

4 - Body nitrogen as affected by exercise.

a - General effects of exercise on the body.

Physical activity is the cause of metabolic, physiological and physical changes appearing in this order in the organism. The increased demand for energy accelerates the metabolism, particularly in muscle. This in turn, increases the need for energy-yielding nutrients and for oxygen. The first of these requirements is met through an augmented consumption of food, and the second, through physiological adjustments involving the respiratory and circulatory systems. Further physiological adaptations are needed for the removal of excess waste products and heat, in order for the body to maintain homeostasis; increased blood flow, sweating are the most important of these changes. Finally, prolonged exercise will cause adaptative changes in the size and composition of tissues, organs and of the whole body in order that work be performed with maximum efficiency. All of these physical and chemical changes occuring in the working organism are controlled through sensitive nervous and hormonal mechanisms. The existence, magnitude and permanence of such changes depend, to a large extent, on 3 factors:

- the duration of the physical activity;
- the intensity of the physical activity;
- the repetition of the same type of activity (training).

1) Influence of the duration of physical activity.

The length of time during which a certain activity

is performed and the moment at which the physiological or metabolic response of the body is measured greatly influence the type and size of this response. First, the total amount of work performed, and consequently the energy spent, are proportional to the length of the exercise period. Moreover, the effect of long sustained effort on the body is quite different, in some instances, from that of exercise periods of more intensity interrupted with frequent rests. For example, the first type of activity would not cause any hypertrophy of skeletal muscle, contrarily to the second type (Steinhaus et al., 1931b). Finally, within one period of work of any duration, the metabolic effects of activity vary with the phase during which the measurements are taken. Muscular exercise is usually considered as including 3 steps (Bugard et al., 1961): 1 - a period of muscular effort corresponding to a period of oxygen debt and of shifts of water and electrolytes between intra-and extra-cellular phases; 2 - a period of fatigue during which certain metabolites such as lactic acid accumulate in the blood; 3 - a period of recovery in which more stable conditions are gradually reestablished. An increase in the secretion of protein-catabolizing corticoids would accompany the first phase, while more of the protein-anabolizing androgens would be produced in the resting phase. During the latter, oxygen consumption would only gradually decrease to the pre-exercise level, and

Passmore and Johnson (1960) observed that oxygen consumption was sustained for 7 hours above basal level after human subjects had completed a 10-mile walk at moderate speed.

2) Influence of the intensity of the physical activity.

Duration and intensity of work are the two factors which determine the total amount of work produced, and therefore, the energy expenditure. Young et al. (1959) found that caloric expenditure was highly correlated with the grade of inclination in treadmill running in dogs. Oxygen debt would also be related to the intensity of work, according to the same workers. The latter consider body temperature as the best criterion of physical exhaustion. Many of the changes arising in the body as a consequence of exercise are functions, not so much of the total work produced, but of the work load or work performed per unit of time. This is true of the increase in muscle size consecutive to speed running in rats (Steinhaus, 1933), of the increase in muscle strength resulting from training (Hettinger, 1961) as well as of the nitrogen loss induced by muscular activity in man (Gontzea et al.,1962a). There are indications (Fowler et al., 1962) that the level of certain serum enzymes rises as the intensity of work increases in humans during treadmill activity.

3) Influence of training.

It is widely recognized that the same work load does not require the same amount of energy from comparable subjects, or even from the same subject during different trials. This difference is due to training. Frequent and regular repetition of a certain activity results in important changes that tend to facilitate the performance of this activity, to retard the development of fatigue, and to improve the efficiency of the body in doing this exercise. As examples of some of these changes, Morehouse and Miller (1953) mention: a greater ability for the muscles to absorb and utilize oxygen, an improved circulation due to an increase in the number of capillaries and/or to a dilatation of existing vessels in the heart, muscle, and cerebral cortex, a larger stoke volume but a slightly slower heart rate, and adjustments in the nervous system resulting in better skill.

Severe training may bring about an enlargement of the heart and sometimes a change in the size of other organs and tissues. In this respect, Kimeldorf and Baum (1954) who studied the effect of exhaustive exercise on organ growth in rats, make a distinction between the effects of stress, which are non-specific, and those which are structural adaptations of the body to facilitate the performance of exercise. In the first category they place adrenal enlargement, thymic involution and reduced body weight, and in the second category, heart hypertrophy

and a relative increase in the size of the muscle or muscles participating in the exercise. That the effect of training varies with the length and intensity of the exercise periods is illustrated by the fact that endurance sports enlarge the right side of the heart while short, intense repeted movements produce similar changes in the left side of the heart (Morehouse and Miller, 1953). Since trainability of muscle is greater in the male than in the female, it is thought that it has a direct relationship with the availability of male sex hormones (Hettinger, 1961). The hypertrophy of muscle and the increase in its components caused by physical activity and by male sex hormone were found to be similar in all respects (Hettinger, 1961).

b - Effects of exercise on body nitrogen.

Similarly to protein depletion, physical exercise exerts its effects on body nitrogen through several channels. For the convenience of comparison, these effects of exercise will be examined and grouped under the same headings as when the effects of the level of dietary protein were reviewed.

1)- Effect of exercise on food intake and body weight.

While moderate exercise does not appear to interfere with normal growth or weight gain of rats (Steinhaus, 1933; Mayer et al., 1954), strenuous exercise has a definite depressing effect on the rate of weight gain of both young (Guerrant et al., 1939) and adult rats (Kimeldorf and Baum, 1954; Hearn and Wainio, 1956;

Gould et al., 1959; Gollnick and Hearn, 1961). Growth retardation due to severe exercise would not affect the final weight attained by the animal, but only delay its achievement (Guerrant et al., 1939). The difference observed between the influence of moderate and of strenuous exercise tends to confirm the opinion of Kimeldorf and Baum (1954) that the failure to gain weight under conditions of hard work is a reflection of a depressed desire to eat that follows any stressful situation.

That an increase in energy output is not always compensated by an increase in energy intake is testified by several authors, among which Guerrant et al. (1939), Kuncova and Vinaricky (1958), Gollnick and Hearn (1961). When rats are moderately exercised, according to Mayer and associates (1954), the food intake increases in direct proportion to the length of the exercise period. This does not hold true if the animals are exercised for less than 1 hour per day, or for more than 6 hours per day. This last point would represent a threshold above which exercise could be considered "stressful". Thomas and Miller (1958) report a similar trend: up to a level of approximately 1 mile a day, the food intake of exercised rats remained below that of control animals, but above this level, it equaled or exceeded that of the controls. Moreover, on rest days, the food deficit was largely compensated by a food intake greater than

that of non-exercised animals. Thomas and Miller (1958) further observed that this increase in food consumption persisted after termination of exercise and that the rate of gain of rats that had been subjected to exercise was considerably elevated for fairly long periods of time. Another interesting observation of these same workers is that on exercise days and, to a lesser extent, on resting days, the spontaneous activity of the exercised animals was greatly depressed.

2) --Effect of exercise on nitrogen utilization and excretion.

No consistant effect on the digestibility of protein food can be attributed to physical exercise. Exercise has been reported in turn to increase the efficiency of food utilization (Guerrant et al., 1939), to have no influence on nitrogen utilization (Konishi and McCoy, 1960), and to increase fecal nitrogen in horses (Harvey et al., 1939) and in humans (Gontzea et al., 1959).

Physical activity modifies the metabolism of nitrogen in the body in the same way, if not to the same extent, that it affects the metabolism of other energy-yielding nutrients. Terroine has shown (Terroine and Sorg-Matter, 1927) that the expenditure of "endogenous" nitrogen, or of this portion of the catabolized nitrogen that is not of immediate dietary origin, is proportional to basal metabolism. On the

other hand, basal metabolism would increase as a consequence of physical activity (Black, 1939; Rougier and Osouf, 1960; Brozek, 1961). One may then agree with Gontzea et al. (1959) that the increase in energy metabolism that accompanies physical exercise intensifies endogenous nitrogen consumption. Munro (1964), however, disagrees with this opinion, and claims that any increase in the nitrogen output resulting from exercise is related to the level of protein in the diet and not to an increase in endogenous nitrogen metabolism. In other words, the effect of physical exercise on protein metabolism would be mostly on "exogenous" nitrogen, or that portion of catablized nitrogen that varies directly with the dietary intake of nitrogen. This view is supported by reports showing a considerable elevation in blood urea and in nitrogen excretion following physical activity.

Blood urea and to a smaller extent, blood uric acid and creatinine are significantly elevated during exercise (Chailley-Bert et al., 1961). The explanation proposed by Chailley-Bert and his collaborators (1961) is that there would be in the blood a degraded nitrogen fraction that would neutralize the excess of acids liberated in the process of muscular contraction. After the activity has ceased, this fraction would become useless and be excreted.

Physical activity accentuates nitrogen excretion

both in urine and, in the case of man, in sweat. Exercise proteinuria is a well known phenomenon that has been observed in several species, including man, and mostly in sportsmen. Immediately following exercise and for some time after, the urine of individuals performing strenuous exercise contains the same protein fractions as those found in blood serum at the same time (Nedbal and Seliger, 1958). The albumin/globulin ratio of blood and urine of young men running at top speed was found to be 1.59 compared to 0.57 during rest periods. Urinary excretion of mucoproteins was also found to be elevated during exercise (Dukes-Dobos et al., 1963). Exercise proteinuria has been attributed to the effect of an increased blood acidity on the glomerular membrane (Javitt and Miller, 1952) or to a decreased renal blood flow (Nedbal and Seliger, 1958; Taylor, 1960). These changes in the kidney would be secondary to an increased secretion of noradrenaline (Cantone and Cerretelli, 1960b) and therefore, would be indicative of over-all strain rather than be a specific effect of exercise. The fact that exercise proteinuria decreases with training (Cantone and Cerretelli, 1960b) and is not present in intermittent moderate exercise (Gontzea et al.,1962a) confirms this opinion.

Even in the absence of proteinuria, urinary excretion of nitrogen increases with an increase in activity. While total urinary nitrogen was found to

increase by 14% to 17% during the 4 days that men performed on a bicycle ergometer an amount of exercise corresponding to 1250 Calories, the excretion of urea during the same period was estimated 13% to 21% higher than in the preceeding days although the nitrogen intake was kept constant (Gontzea et al., 1962a). This is to say that it is mostly the intensification of the process of urogenesis that is responsible for the rise in nitrogen excretion through the kidney. Urea represents 70% to 90% of the nitrogen excreted according to the same author, and ammonia 3% to 6%. No appreciable modification in the excretion of ammonia was found to follow an exercise period, except when work was performed at high temperatures. Nitrogenuria stayed elevated for 1 to 3 days after exercise periods had ceased (Gontzea et al., 1959). The increase in nitrogen output associated with exercise is more pronounced after a meal than in the post-absorptive state (Munro, 1964).

Until recently, nitrogen excretion in sweat had not been given much attention and most studies on the effect of physical activity on nitrogen metabolism and nitrogen balance failed to consider this factor. More recent work, however, has attracted the attention of scientists on the important role of perspiration in the excretion of some products of nitrogen metabolism, especially in conditions where sweating is abundant.

In a study involving 63 men aged 14 to 56, Gontzea et al. (1959) from Rumania collected, by means of nylon sleeves, and analyzed 449 samples of sweat from three areas of the body: chest, arm and thigh, while the subjects were doing exercise on a bicycle ergometer. They found that in 75% of the individuals, nitrogen concentration of sweat varied between 0.30 and 0.55 gram per liter, with a mean value of 0.43 gram per liter. The higher the volume of sweat, the lower was its nitrogen concentration, but the decrease became marked only when the output exceeded 400 ml/hour: for an output of 200 to 300 ml per hour, the mean nitrogen concentration was 0.45 gram per liter and when the output was 400 to 500 ml per hour, the mean nitrogen content fell to 0.36 gram per liter. However, when the volume exceeded 600 ml/hour, the nitrogen concentration stopped decreasing. The nitrogen concentration of the sweat collected from the arm or thigh was slighly higher than that of samples taken on the chest. If one calculates from these figures, how much nitrogen was excreted per hour during work, average values of 112, 162 and 173 mg. are obtained for volumes of sweat of 250 ml, 450 ml, and 600 ml per hour. Gontzea and his collaborators (1959) estimated the rate of secretion of sweat at 250 ml per hour when room temperature was 20° C (68° F) and at 450 ml when it was 35° C (95° F).

Another group of workers: Consolazio et al. (1963) reported much higher concentrations of nitrogen in the perspiration of men doing exercise on a bicycle ergometer. They estimated at 149, 189 and 241 mg.per hour the amount of nitrogen excreted in sweat during work at 70°, 85° and 100° F. Although acclimatization to heat was found to lower these figures, the values reported by Consolazio et al. (1963) are still higher than the ones obtained by the Rumanian group of workers. Excretion of nitrogen in sweat is considerably lower during sleep. Under minimal sweating conditions, the average output is 15 mg. nitrogen per hour (Consolazio et al., 1963). An increase in the quantity of sweat nitrogen because of exercise is not compensated by a decrease in nitrogen excretion through urine or feces, according to Consolazio and coworkers (1963). Urea would account for the largest proportion of the total nitrogen excreted through sweating, ammonia, for about 8% and creatinine and uric acid, for about 1% (Consolazio et al., 1963).

The acceleration in protein metabolism that occurs as a result of exercise is not proportional to the increase in caloric expenditure, at least not in all phases and periods of exercise. Gontzea et al. (1962a, 1962b) showed that an increase in energy expenditure of 50% produces an increase of 14% to 17% in nitrogen excretion and that training reduces the excretion of

nitrogen to a greater extent than it diminishes the energy expenditure necessary to perform the same amount of work. They suggest that the catabolizing effect of adreno-cortical hormones might decrease with time. Moreover, in trained fed animals, the percentage of Calories expended that are derived from protein decreases with the length of time spent since a meal was consumed (Young et al., 1962) and decreases as the amount of work is increased (Young and Price, 1961). Young et al. (1962) believe that in the fed animal, protein oxidation provides a maximum of 7% of the total energy spent during work. The same group of workers figures that in the post-absorption state, approximately 60% of the variation in nitrogen excretion is due to energy expenditure per se. They do not make any suggestion as to what factor or factors might be responsible for the other 40% in variation but the literature on this subject would suggest stress as an important factor, at least in untrained individuals. The observation mentioned earlier (Gontzea et al., 1962a) that nitrogen losses were more closely related to the energy expenditure per unit of time than to the total amount of work performed, supports this concept.

3) Effect of exercise on nitrogen balance and protein synthesis.

The increased nitrogen excretion that follows physical exercise results in a negative

nitrogen balance for as long as excretion remains high, even if the intake was sufficient for equilibrium before exercise was started. With a diet supplying 1 gram of protein per kilogram of body weight, Kraut et al.(1958) observed a negative nitrogen balance in 2 men performing hard work for 23 weeks, when only 13% of the protein was of animal origin. But when this percentage was increased, the balance was positive. On the same amount of protein, but with about 1/3 being animal protein, 8 out of the 9 subjects observed by Gontzea et al. (1959) lost body protein while they were doing heavy physical exercise bringing their energy requirement up to a level of 3300 to 4100 Calories per day. Always supplying the same quantity of protein (1g/kg/day), Gontzea et al. (1962a) repeated this study with 18 men submitted for 4 consecutive days to a level of physical activity equivalent to 1250 Calories per day. Nitrogen balance became negative in 17 of the cases and remained negative during the following 4 days in 10 of the cases, even if energy requirements were all the time satisfied. The average loss was evaluated at 1.60 grams nitrogen or 10.0 grams of protein per day. When the protein intake was increased by 50%, nitrogen balance, under the same exercise conditions, was positive or, in the case of 2 individuals, only slightly negative. When the exercise period was prolonged to 3 weeks,

a negative nitrogen balance was established in the first 2 days in 6 out of 8 cases; it was accentuated during the following two days, but became gradually less negative in the rest of the 3 weeks. At the end of this time, nitrogen losses had decreased by 75% to 85% compared to the first 2 days, while energy expenditure had decreased by 20%. Nitrogen losses averaged 1.27 grams per day in the first 2 days to end up at 0.19 gram per day after 3 weeks. Giving the same intake of protein (1g./kg/day), Yoshimura (1960) observed a positive nitrogen balance in his subjects during heavy muscular exercise but noted the appearance of anemia and hypoproteinemia after 10 days of exercise. These symptoms disappeared after 2 to 3 weeks, the activity being maintained at the same level, and failed to appear when the intake of protein was raised to 2 grams per kilogram per day.

On an intake of 1.2 grams of protein per kilogram of body weight per day, young men showed a fall in nitrogen retention when physical training was started, but a rise above control values as the exercise period was prolonged (Watkin et al., 1963). On the contrary, when the diet contained only 0.4 gram of protein per kilogram of body weight, nitrogen losses increased progressively during each of the work phases. A deficient intake (4 to 8 grams nitrogen per day) led to considerable nitrogen losses

(51 grams nitrogen) in individuals participating in a 3-day bicycle contest (Chailley-Bert et al., 1961).

There is little experimental evidence to support the concept that protein synthesis is increased in certain tissues during exercise. In vitro experiments on working muscles indicate that during the rest period that immediately follows activity, synthesis of muscle protein occurs when the supply of protein is adequate (Popova, 1951). Further data is needed to confirm this report and to determine if this synthesis represents an increase over the normal rate of protein synthesis in muscle and in other tissues of the living animal.

4) Effect of exercise on the nitrogen composition of the body.

Two opposite influences tend to modify the nitrogen composition of the body as a result of physical exercise: on one hand, severe exercise delays growth in the young and, in the older animal, produces a temporary negative nitrogen balance; on the other hand, exercise stimulates protein metabolism and perhaps protein synthesis, at least in certain body compartments such as muscle. The result of these two tendencies is reflected in changes of variable duration in the blood, muscle, skin, and other organs and tissues, but appears to have no measurable effect on total body nitrogen.

Effect on total body composition.

If total body nitrogen is not affected by exercise, the proportion of body weight represented by fat is considerably decreased in exercised animals (Steinhaus et al., 1931b). The observation that the body of guinea pigs that had been exercised for 8 months had a higher specific gravity than that of control animals (Brozek, 1961) corroborates this finding. After three weeks' strenuous training, the body water of soldiers was found to have increased, and body density to have increased slightly, according to the same author (Brozek, 1961).

Effect of exercise on blood nitrogen.

In 1963, Christensen reported that long-term moderate exercise significantly reduced total serum proteins, serum albumin, and, to a slighter degree, serum globulins, in adult rats, resulting in a lower serum A/G ration in the exercised group (cf. table V). Exercise consisted of running on a revolving drum for 3½ hours a day, 6 days a week for 28 days. This report came rather unexpected for only short-lasting effects of exercise had been previously demonstrated on serum proteins. In humans performing exercise for 30 minutes on a bicycle ergometer, De Lanne et al. (1959) showed that total plasma proteins were slightly increased during submaximal and maximal work and in the period that immediately followed the exercise.

TABLE V

Nitrogen composition of tissues of rats submitted to long-term moderate exercise

(Christensen, 1963a)

Group	Blood serum				Muscle		Skin	
	proteins g.%	albumin g.%	globulins g.%	A/G ratio	DNA mg./muscle	N mg./muscle	DNA mg./sample	N mg./sample
Non-exercised	6.6	2.6	3.9	0.7	4.0	111	10.8	362
Exercised	6.0	2.3	3.7	0.6	4.5	124	10.8	377

The increase in the concentration of total proteins and of albumins were not thought to be due entirely to hemoconcentration (De Lanne et al., 1958). Beta-and gamma-globulins, contrarily to albumins and to α_1 -and α_2 -globulins, tended to drop during submaximal work; but these levels, as well as those of other fractions gradually returned to normal values, always reached after 60 minutes of recovery. The albumin/globulin ratio was increased during the first minutes of submaximal work, remained steady thereafter during submaximal work, decreased markedly 5 minutes after maximal work and returned to normal within 60 minutes after the end of exercise. De Lanne and collaborators (1958) believe that all plasma protein fractions except β globulins can be rapidly replaced from intercellular resources. According to Johnson and Wong (1961) there are exchanges of proteins of low molecular weight, such as albumin and certain globulins, between the plasma and the lymph, and these exchanges are made possible by alterations of capillary pores. Climatic conditions could modify the response of plasma proteins to physical exercise (De Lanne et al., 1958).

Important changes in serum enzymes have been observed during and following physical exercise. A significant elevation of glutamic-oxaloacetic transaminase (SGOT), of glutamic-pyruvic transaminase

(SGPT), of lactic dehydrogenase (SLD) and of aldolase have been reported in rats (Altland and Highman, 1961) and in humans (Fowler et al., 1962) following treadmill activity. The study of Altland and Highman (1961) involved an exceptionally long exercise period of 16 hours: in this case the value of SLD was found to return to normal within 24 hours, that of SGPT, within 72 hours, and those of SGOT and of serum aldolase, to remain slightly above normal even after 144 hours of rest. Fowler et al. (1962) did not evaluate the time necessary for enzyme levels to return to their initial values after exercise, but Cantone and Cerretelli (1960a), in an experiment where human individuals performed exercise for 30 minutes on a treadmill, estimated at 75 minutes the time needed for aldolase activity to return to normal. Fowler et al. (1962) suggest that there is a relationship between the magnitude and duration of exercise and serum enzyme levels and that the increase in enzymes would be considerably less when the activity is more intense. The elevation in blood enzyme levels would be caused by an increase in the permeability of cells and particularly of the mitochondria or of the cellular membrane (Altland and Highman, 1961 ; Fowler et al., 1962) or to an acceleration in muscle enzyme synthesis and turnover with diffusion of larger quantities of the enzymes out of the cells (Fowler et al., 1962).

Effect of exercise on muscle nitrogen.

When considering the effect of exercise on muscle, the severity and type of exercise and the amount of training are of great importance. In an extensive review on the chronic effects of moderate exercise published in 1933, Steinhaus reports that during training by treadmill running, dogs' muscles gain in size, strength and endurance, and that the increase in size is due exclusively to an increase in sarcoplasm, and not to changes in the length of fibers, in the number of nuclei nor in the number or size of fibrilli in muscle cells. This hypertrophy of muscle would be a function of the work performed per unit of time. Helander (1961) also found that calf muscles of guinea pigs exercised for 4 months had a slight tendency to increase in size with a rise in the degree of activity. The total nitrogen content of wet muscle tended to be higher. In contradiction with Steinhaus (1933), this author is of the opinion that the increase in total nitrogen reflects an increase in myofilamental nitrogen, as he observed no change in either sarcoplasmic protein, stroma protein nor NPN. Helander (1961) concludes that one could expect an increase in the contractile strength of muscle following this elevation in the concentration of myofilamental nitrogen.

This hypothesis is confirmed by Hettinger (1961) who related muscle strength to muscle cross section and

found that training not only increased the size of muscle fibers, but also the absolute number of nuclei in the muscles and their protein content. Christensen (1963a) found a significant increase in the fresh weight of gastrocnemii muscles of rats that had been submitted to prolonged intermittent exercise and then exercised again for 28 days, compared with those that had received the preliminary training but had remained idle during the last period of the experiment. Furthermore, Christensen (1963a) observed increased values for total DNA, total nitrogen and total RNA per muscle in exercised rats that had received previous training, in comparison with trained rats that served as controls (cf. table V). Differences in muscle N/DNA and RNA/DNA ratios were, however, not significant.

Contrarily to moderate exercise, forced swimming results in a decrease in the absolute size of muscles of rats, whether practiced at the rate of ½ hour per day for a minimum of 35 days (Hearn and Wainio, 1956; Gollnick and Hearn, 1961) or to exhaustion, for 30 days (Kimeldorf and Baum, 1954). Hearn and Wainio (1956) found the wet weights of gastrocnemii muscles of adult rats to be 1.861 and 2.005 grams respectively for the exercised and control groups, while in Gollnick and Hearn's experiment (1961), comparable weights were 2.377 and 2.433 grams for corresponding groups. In another experiment involving exhaustive

exercise in rats, Kimeldorf and Baum (1954) reported that the gluteus maximus muscle was significantly smaller in the exercised group, but represented a larger part of body weight.

The nitrogenous composition of muscle is not believed to vary much as a result of severe, short-term physical activity. Gollnick and Hearn (1961) estimated the protein content of skeletal muscle, as represented by the gastrocnemius, to be 217.17mg. per gram of wet weight in the case of exercised rats and 218.72mg. for the control group: this difference was not significant. No change was observed, following exercise, in the activity of muscle succinic dehydrogenase (Hearn and Wainio, 1956), malic dehydrogenase and phosphorylase (Gould and Rawlinson, 1959), and lactic dehydrogenase (Gould and Rawlinson, 1959; Gollnick and Hearn, 1961).

Effect of exercise on skin nitrogen.

Exercise does not influence the DNA content of skin, nor does it affect significantly the N/DNA or RNA/DNA ratios in this tissue, according to Christensen (1963a). However, in rats that had been submitted to prolonged intermittent exercise, these ratios were slightly elevated in the exercised group compared to the idle group, and were much lower in the preconditioned rats, including both exercised and non-exercised groups, than in the rats that had not

been trained before the experiment. Long-term intermittent exercise would then result in lower N/DNA and RNA/DNA ratios with a predisposition to high ratios during a subsequent exercise period.

Effect of exercise on other organs and tissues.

The size of many organs and glands is affected by exercise, but the way each one is affected and the intensity of this effect depend again on whether the exercise is one that requires strength, speed and a short but exhaustive effort such as swimming or speed running, or one of endurance such as treadmill walking. Table VI illustrates the effect of both types of exercise on some important organs and glands. Except for the heart and adrenals, the data reported in this table does not warrant definite conclusions and more rigidly controlled exercise is needed to support valuable statements on the effect of exercise on liver, kidneys, spleen. Christensen (1963a) found no effect of exercise in adult rats on the DNA content of liver. However, in exercised animals, the fresh liver weight was slightly decreased, and the N/DNA ratio was increased but not significantly.

Whether the exercise is one of endurance such as running, or one that requires speed or intense effort, such as swimming, there is a marked enlargement of the heart, especially of the two ventricules (cf. tables VI and VII). Steinhaus et al. (1931a) remarks that

TABLE VI

Effect of exercise on fresh weights of organs and glands

Authors	Species of animal	Type of exercise	Length of exercise period	Change in fresh weight *				
				Liver	Heart	Adrenals	Kidney	Spleen
Hatai (1915)	rat	endurance	3-6 months	+18%	+23%	-	+19%	-24%
Steinhaus (1931b)	dog	short speed	?	+16%	+13%	+28%	+27%	-20%
Steinhaus (1931b)	dog	endurance	?	no diff.	+5%	-21%	+3%	+6%
Kimeldorf and Baum (1954)	rat	short speed	3-30 days	-	incr.	+21%	decr.	incr.
Hearn and Wainio (1956)	rat	short speed	½-hour/day 5 - 8 weeks	-	+5%	+21%	-	-
Gollnick and Hearn (1961)	rat	short speed	½-hour/day for 35 days	-	+2% (ventricle)	+32%	-	-

* expressed in percentage to the nearest whole percent.

TABLE VII

Adrenal and heart weights of exercised (swimming) and non-exercised, pair-fed adult rats

Authors	Initial weight of rats (grams)	Length of study	Weight gain/day (grams)		Adrenal weight (milligrams)		Heart weight (milligrams)	
			Exercised	Controls	Exercised	Controls	Exercised	Controls
Hearn and Wainio (1956)	250	8 weeks	1.4	2.0	23	19	1861	2005
Gollnick and Hearn (1961)	340	35 days	1.1	1.8	37	28	1014	991

this increase in size is not paralleled by a corresponding gain in body weight, so that the ratio of heart weight to body weight is higher in exercised animals. There are indications that heart size regresses when exercise is discontinued (Steinhaus et al., 1931a). Cardiac enlargement would represent an adaptive change to exercise (Kimeldorf and Baum, 1954). Protein content (Gollnick and Hearn, 1961) and unit activity of lactic dehydrogenase (Gollnick and Hearn, 1961) and of succinic dehydrogenase (Hearn and Wainio, 1956) of the heart were not found to be modified by exercise. But the total activity of lactic dehydrogenase was significantly increased (Gollnick and Hearn, 1961).

Another fairly constant feature of exercise is a marked enlargement of the adrenal glands: this would be a non-specific response of the organism to stress (Kimeldorf and Baum, 1954; Hearn and Wainio, 1956).

B - Dietary protein requirements for exercise.

Dietary protein is essentially needed by the body for two main purposes:

1) to make up for obligatory losses of nitrogen through urine, feces and skin;

2) to supply the amino acids necessary for growth of new tissues as well as for the chemical maturation of existing tissues.

Protein requirements of rats under maintenance conditions and during growth are given in the appendix (table 2-A).

During exercise, both of these basic needs are increased, for it has been shown that exercise:

- increases the loss of nitrogen in the urine, at least at the beginning of a training period (Gontzea et al., 1959; Gontzea et al., 1962a);
- increases the loss of nitrogen through the skin (Gontzea et al., 1959; Consolazio et al., 1963);
- promotes growth of certain muscles participating in the exercise (Steinhaus, 1933; Helander, 1961; Hettinger, 1961; Christensen, 1963a).

Moreover, there is considerable evidence (Yoshida et al., 1957; Abraham et al., 1961; Wagle et al., 1962; Crampton, 1964) that optimum growth and nitrogen utilization are closely related to the balance between the protein and the caloric content of the diet, which would suggest that an increase in the requirement for energy because of activity should be accompanied by a proportional increase in the protein intake. Information on this subject is still, however, far from complete,

and most authorities in the field of human nutrition reserve their opinion on this matter until further study is completed. Widely used dietary standards (U.S. Food and Nutrition Board, 1964; Canadian Council on Nutrition, 1964) assume that physical activity does not increase protein requirements. This opinion is based on earlier nitrogen balance studies that showed no increase in nitrogen retention as a consequence of physical activity.

The validity of nitrogen balance as a criterion to assess protein requirements has been questioned (Christensen, 1963a; Crampton, 1964) on the basis that nitrogen equilibrium may be established over a wide range of nitrogen intakes, and that an over-all equilibrium does not necessarily implicate that protein is available in sufficient amounts in all body compartments. Using various criteria to determine the adequacy of the diet, a few investigators have recently attempted to estimate quantitatively the need for protein during exercise. Yoshimura (1960) evaluated at 2.0 grams per kilogram of body weight per day the amount of mixed protein needed to prevent symptoms of protein deficiency in men doing heavy muscular exercise. Gontzea and co-workers (1960) for their part, recommend that the diet of young men performing work and necessitating 3300 to 4100 Calories per day, should provide 12% to 14% of the Calories in the form of protein when about 1/3 of the protein is of animal origin. According to Watkin et al. (1963), 0.8 to 1.0 gram of protein of good quality per kilogram of body weight per day is needed to maintain nitrogen equilibrium in physically active adult individuals. A more rigorous

approach was used by Crampton (1964) to estimate at 19 grams of digestible protein per 1000 digestible Calories the amount of dietary protein needed for work. This proportion is the same as that needed for maintenance and applies to any species of mammals, whatever their size. As far as the FAO/WHO Joint Expert Group (1965) they do not make a specific recommendation for an extra allowance of protein for work because of "inconclusive evidence". But they strongly recommend that research be continued on this subject.

III BASIS AND NATURE OF THE PRESENT STUDY

The literature shows that exercise has a stimulating effect on protein metabolism as demonstrated by an increase in nitrogen excretion and a negative nitrogen balance, at least for some time following the beginning of the training period. The elevation in the level of certain nitrogenous fractions and enzymes in the blood during exercise is also indicative of a slowing of the catabolism and/or of an increase in the anabolism of these compounds as a result of exercise. Part of these changes could, however, be attributed to the stress of meeting a new situation, since they tend to recede with time. As training advances, other tendencies appear which could be more validly related to exercise "per se": transfer of nitrogen from labile stores such as serum albumin, liver proteins, to other body compartments including muscle and perhaps, skin.

Christensen (1963a) has shown that these changes affect not only labile but also so-called "stable" nitrogenous compounds such as DNA. In some cases, however, the appearance of such changes is linked with the presence of several successive periods of exercise and rest. This raises the questions of the time of appearance and of the reversibility of these effects. Are these modifications instigated during an exercise period and maintained or perhaps enhanced during the rest period that immediately follows, or are they delayed effects of exercise, showing only after the imposed activity has been stopped for some time? How long do these results last and how long does it take for all the nitrogenous components of tissues to return to pre-exercise levels, supposing that these changes are reversible? As yet no answer can be given

to these questions.

Further work is then needed to clarify the following points:
1) to find out whether changes in the nitrogenous components of blood serum, muscle and skin following prolonged intermittent exercise are a continuous process or are realized according to a pattern presenting peaks and pits of varying heights;
2) to determine the length of time that it takes for these components to return to levels comparable with those obtained in controls;
3) to evaluate the influence of the dietary protein level on the changes observed as a consequence of exercise in the nitrogenous components of these same tissues.

The present study aims to furnish some of the basic data necessary to answer these questions. It is postulated that certain of the changes observed by Christensen (1963a) in the nitrogen status of rats submitted to several intermittent periods of exercise are delayed effects of exercise, showing only after the animals have been allowed to rest for a certain length of time. This hypothesis does not exclude the possibility that such changes could be intensified or reversed by subsequently imposing another period of exercise to the animals. But the present study is limited to the observation of the immediate and delayed effects of only one period of exercise on the protein status of rats. Blood serum, skeletal muscle and skin were analyzed with respect to some of their most important nitrogenous components, namely: total nitrogen in blood serum, muscle and skin; albumin and globulin in blood serum; DNA in muscle and skin, and collagen in skin only. It is assumed that albumin/globulin and N/DNA ratios are reliable criteria of protein

nutrition when comparing groups in which the "stable" components of the ratios i.e. globulin or DNA, are not different from one group to another. In cases where there is a treatment effect on globulin or on DNA, such a ratio could not validly be used for evaluating protein reserves in that particular tissue.

Three levels of dietary protein were used in this experiment: one corresponding to the level generally considered adequate for maintenance in adult rats, one which was equivalent to twice the maintenance level and one which was only half of the maintenance level. The use of these three diets enabled one to observe any possible interaction between dietary protein and exercise and between dietary protein and the length of the rest period allowed after exercise was ceased. It was assumed that, if protein needs were not increased in proportion to caloric needs during exercise periods, the augmented feed and caloric intake resulting from an increase in activity would raise the protein consumption of rats consuming sub-maintenance level of protein to the maintenance level; similarly, such an increase in protein intake would raise the intake of animals already receiving amounts of protein sufficient for maintenance to super-maintenance level. In such cases, this improvement in the protein status would be reflected in the protein reserves. If, on the contrary, the supplement of protein consumed by active rats was needed to meet the increased demand of exercise, the levels of labile protein reserves should be similar in the active and in the idle rats receiving the same level of dietary protein.

IV SPECIFIC OBJECTIVES OF THE RESEARCH

The purpose of this experiment was to observe the effect, on rats consuming super-maintenance, maintenance or sub-maintenance levels of protein, of a muscular exercise period of 8 weeks, followed by rest periods of zero, two or four weeks, as measured by:

1) the rate of weight gain and the feed efficiency ratio;
2) serum proteins and the serum albumin/globulin ratio;
3) muscle nitrogen, muscle DNA and the N/DNA ratio in muscle;
4) skin nitrogen, skin DNA, skin collagen as well as the N/DNA and the non-collagen N/DNA ratios in the skin.

V EXPERIMENTAL PROCEDURE

A - General plan of the study

The study included 2 replicates with 36 animals per replicate. The allotment plan (table VIII) gives the distribution of animals into the different treatment groups in each replicate.

The design of this experiment is a 3 x 3 x 2 x 2 factorial arrangement. The "E_1" and "E_0" main groups ("exercise" and "control"), included 18 animals each. These were subdivided into 3 sub-groups according to the length of the "recovery" period (0, 2 or 4 weeks) that followed the initial period of 8 weeks of exercise. During the whole test (exercise and recovery periods) two rats of each sub-group were maintained on either one of 3 diets:

- diet 1 (D_1) in which the level of protein was twice that which is considered adequate for maintenance*;
- diet 2 (D_2) in which the level of protein was equal to that which is adequate for maintenance*;
- diet 3 (D_3) in which the level of protein was only one half of the maintenance requirement*.

B - Animals

Eighteen-week-old female albino rats were used in this study. The animals were housed in individual 6" x 8" screen-bottomed cages. Food and water were supplied ad libitum except during the 3½-hour daily period during which exercised rats were confined to the exercise apparatus cages where no food was available. During this time, food was also withdrawn from cage-idle animals. The rats used in replicate II were of the same strain as those used

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* "maintenance requirement" in this thesis, designates that level of dietary protein considered sufficient for maintenance by the U.S. Committee on Animal Nutrition (cf. Appendix table 2-A).

TABLE VIII

Allotment plan (for each of 2 replicates)

Level of exercise	Length of the rest period after initial 8 weeks of exercise	Diet		
		Diet 1 (D_1)	Diet 2 (D_2)	Diet 3 (D_3)
Exercise (group E_1)	0 week (R_0)	Group E_1 D_1 R_0 2	Group E_1 D_2 R_0 2	Group E_1 D_3 R_0 2
	2 weeks (R_2)	Group E_1 D_1 R_2 2	Group E_1 D_2 R_2 2	Group E_1 D_3 R_2 2
	4 weeks (R_4)	Group E_1 D_1 R_4 2	Group E_1 D_2 R_4 2	Group E_1 D_3 R_4 2
No exercise (group E_0)	0 week (R_2)	Group E_0 D_1 R_0 2	Group E_0 D_2 R_0 2	Group E_0 D_3 R_0 2
	2 weeks (R_2)	Group E_0 D_1 R_2 2	Group E_0 D_2 R_2 2	Group E_0 D_3 R_2 2
	4 weeks (R_4)	Group E_0 D_1 R_4 2	Group E_0 D_2 R_4 2	Group E_0 D_3 R_4 2

in the first replicate, but did not originate from the same breeding farm and were smaller in size than rats in replicate I; their average weight was 258 grams, and that of rats in replicate I, 289 grams.

C - Diets

Diets were purified rations containing the following ingredients (given in percentages):

	Diet I (13.6% protein)	Diet 2 (6.8% protein)	Diet 3 (3.4% protein)
Vitamin-free casein	15.0	7.5	3.75
Corn oil	14.0	14.0	14.0
Corn starch	61.0	68.5	72.25
Alphacel	5.0	5.0	5.0
Salt mix (U.S.P. XIV)	4.0	4.0	4.0
Vitamin mix	1.0	1.0	1.0
	100.0	100.0	100.0

The composition of the vitamin mix is given in the Appendix (table 1-A).

Samples of each of the three diets used in every one of the two replicates were analyzed for energy, dry matter, crude protein and ether extract. The results of the analyses are shown in table IX. The low values obtained for ether extract and for energy in the case of the diets used in replicate I were thought to be due to an uneven blending of the oil through the mixture because of the inadequacy of the equipment used for mixing the diets. As the discrepancies were about the same for all three diets, however, it was assumed that this irregularity did not appreciably alter the results. If expressed as milligrams of protein per Calorie,

TABLE IX

Proximate composition of diets

Diet	Replicate	Dry matter %	Crude protein (on air-dry sample) %	Ether extract (on dry matter) %	Calories per 100 grams diet
1 * (15% casein)	I	90	14	8 (1)	450 (1)
2 * (7.5% casein)	I	91	7	8 (1)	440 (1)
3 * (3.75% casein)	I	90	4	8 (1)	430 (1)
1 ** (15% casein)	II	93	14	15	470
2 ** (7.5% casein)	II	92	8	15	460
3 ** (3.75% casein)	II	92	4	15	450

* mixed in several batches in small mixer.

** mixed in single batch in large mixer.

(1) sampling difficulties in the case of replicate I are believed to be responsible for the low values obtained for ether extract and for energy.

the protein content of diets 1, 2 and 3, is respectively of 30, 15 and 7.5 mg. Since the minimum level of dietary protein considered adequate for adult rats is 10 mg. per Calorie (cf. Appendix table 2-A), it is evident that the first two diets fulfill this requirement while diet 3 does not.

D - Exercise regimen

The rats in groups "E_1" were exercised 6 days a week for 8 weeks. The full daily work schedule consisted of 7 cycles of 25 minutes of work and 5 minutes of rest. Rats were gradually conditioned to exercise starting with only 5 minutes of exercise on the first day and increasing the length of the exercise periods every day until the 11th day at which time they ran the entire 3½-hour cycle.

The exercise apparatus was composed essentially of a motor-driven revolving drum on which the rats were forced to walk at a speed of approximately 48 feet per minute. Water was supplied ad libitum during exercise periods.

E - Records

The following data were collected on each individual animal during the trial:

1 - weekly feed consumption and body weight;

2 - fresh weight of the heart;

3 - fresh and dry, fat-free weights of the liver, of muscle (pair of gastrocnemii muscles) and of a skin sample shaved ante mortem;

4 - chemical determinations:

a) on blood serum: - total nitrogen

- albumin and globulins

- non-protein nitrogen

b) on muscle (gastrocnemii):

- total nitrogen

- DNA

c) on skin: - total nitrogen

- DNA

- hydroxyproline (collagen)

Hydroxyproline was also determined on muscle, but due to difficulty in reducing the fibrous material of muscle into a fine powder, and therefore in obtaining perfectly homogeneous samples, the results of these determinations were too variable to be utilized and had to be discarded.

F - Tissue collection and storage

Rats were killed by decapitation under light anesthesia. Exercised rats sacrificed immediately after the 8-week exercise period (group E_1R_0) were killed approximately 2 hours after the end of the exercise period. Tissues were collected and submitted to the treatment hereby described. Whenever weighing and homogenizing were necessary, these were done within 25 minutes to minimize effects of dehydration and autolysis.

1 - Blood.

Blood was recovered in a test tube, set aside for cooling, centrifuged at 2000 rpm for 10 minutes, and the serum collected by decantation. In certain cases mentioned in section G,

paragraph 1, the serum was analyzed immediately for albumin and globulins. In all other cases, it was kept at -5°C until needed for analyses.

2 - Muscle.

Gastrocnemii (2) muscles were dissected out, weighed and homogenized at high speed for 2 minutes in 95% ethanol using a Vir-Tis model 45 macro homogenizer. The homogenate was extracted 3 times for 24 hours each time, first with 95% ethanol, second, with a 50-50 mixture of 95% ethanol and ethyl ether and finally with ethyl ether. Each time, the tissue was recoved by filtration. After the last filtration, the tissue was allowed to dry at room temperature for 36 hours and weighed. It was then ground with a mortar and pestle and kept in a powdered form at -5°C until used for analyses.

3 - Liver and heart.

The liver was removed, weighed, homogenized, given the same treatment and kept in the same conditions as the gastrocnemii muscles. The heart was removed and weighed after excess blood had been wiped off with a paper towel.

4 - Skin.

The back of the rat was clipped to remove long hair just before the animal was decapitated. After bleeding, a section of the skin measuring approximately 8 x 5 centimeters was cut from the lumbar region. This sample, which included both epidermal and dermal layers of the skin was freed manually from lose subcutaneous fat. It was weighed and homogenized in 95% ethanol in a Waring blendor. After being extracted with

95% ethanol and ether and air-dried in the same fashion as muscle and liver, it was then ground in a micromill and kept at -5°C.

G - Chemical and physical methods.

1 - Albumin and globulins (serum)

The distribution of these serum fractions in total serum protein was determined by paper electrophoresis using a Spinco model R system composed of a Durrum cell, and using 0.075 ionic Veronal buffer at pH 8.6. A description of the method used can be found in the Spinco Technical Bulletin 6095A. Electric current was run for 17 hours at 3 milliamps per cell. The filter paper strips used were Schleicher and Schuell 2043A. The strips were stained with amidoblack dye, and the stained strips were scanned with a recording densitometer (model R Analytrol). This analysis was performed on fresh serum in the case of replicate II and the last third (Group R_4) of replicate 1, and on frozen serum in the case of the first two-thirds (groups R_0 and R_2) of replicate 1.

2 - Nitrogen (serum, muscle, skin)

Total nitrogen was determined by the micro Kjeldahl method as outlined in the A.O.A.C. manual (1960).

3 - Non-protein nitrogen (serum)

Non-protein nitrogen was determined by the Folin and Wu method, as described by Hawk, Oser and Summerson (1954), on pooled samples from the serum of the 2 rats receiving exactly the same treatment in each replicate, whenever enough serum was available for this determination. Since a gross

examination of these data revealed no difference between the results obtained in groups R_0, R_2 and R_4 (killed immediately after the 8-week exercise period or after 2 or 4 weeks of rest), the results were averaged for these three groups and the sera of rats of the three groups were pooled to estimate NPN in the cases where the quantity of serum from 2 rats only had not been sufficient to do this determination. The appropriate figure was then used to correct the total serum nitrogen values to protein nitrogen.

4 - Desoxyribonucleic acid (DNA) (muscle and skin)

The nucleic acids were extracted and determined according to the method outlined by Ceriotti (1952 and 1955). The extraction consisted of suspending the tissue powder in 10% (v/v) perchloric acid heated at 70°C, stirring constantly for 20 minutes. The supernatant was collected after centrifuging for 20 minutes at 1800 rpm at 5°C. The procedure was repeated twice and the extracts combined and made up to volume. DNA was determined on the extract by means of a colorimetric method based on the Dische reaction of DNA with indole (Ceriotti, 1952). A calibration curve was prepared using a purified DNA preparation (1) obtained from calf thymus.

5 - Hydroxyproline (collagen) (skin)

Protein from the skin was hydrolyzed by autoclaving the tissue with 6N HCl at 120°C in sealed tubes for 3 hours. The hydrochloric acid was then neutralized with NaOH, the amino acid mixture was filtered to remove humin, and the extract diluted to 100 mls. Hydroxyproline was then determined

(1) kindly supplied by Dr. R.H. Common, Director of the Chemistry Department, Macdonald College.

colorimetrically according to the Leach modification (Leach, 1960) of the Neuman and Logan method (Neuman and Logan, 1950). The percentage of collagen in the tissue was calculated using 7.46 as the factor of conversion of hydroxyproline to collagen. When calculating the percentage of collagen nitrogen in the tissue, it was assumed that collagen contained 18.6% nitrogen.

H - Statistical Analysis.

The data were analyzed by the variance method. The variation was distributed among the different sources of variation in the following manner:

Sources of variation		Degrees of freedom
Between all animals		71
Between groups		35
Replicate	1	
Level of exercise	1	
Length of rest period	2	
Level of dietary protein	2	
Interactions	29	
1st order:		
Replicate x level of exercise	1	
Replicate x length of rest period	2	
Replicate x level of dietary protein	2	
Exercise x length of rest period	2	
Exercise x level of dietary protein	2	
Length of rest period x level of dietary protein	4	
2nd and 3rd order interactions	16	
Remainder		36

In the analysis of variance, the second and third order interactions were added to the remainder, bringing the total number of degrees of freedom for error to 52.

In the cases where a significant "F" value was found for the length of rest period or the level of dietary protein, the least significant range was calculated in order to determine which ones of the three means involved were significantly different one from the other. The method used was Duncan's new multiple-range test as described by Steel and Torrie (1960).

During the course of laboratory manipulations, one of the blood samples was lost (from sub-group $E_0D_3R_2$). Since the variability of the results of the determinations on blood serum was fairly low, especially in the control group of animals, it was decided (1) that, in the statistical treatment of the data, the value obtained for the other animal receiving the same treatment would be used to replace the missing value, and 1 degree of freedom would be subtracted from the total variation and from the variation within pairs of animals receiving the same treatment combination.

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(1) after consultation with Dr. H.A. Steppler, Professor of Statistics, Macdonald College.

VI RESULTS

A - Format used in the presentation of results.

The results presented in this section are in the form of tables and of diagrams. In addition, full results are given in the Appendix tables 3-A to 17-A inclusively. Diagrams (fig. 1 to 9) illustrate various trends throughout the experiment or compare the results obtained on different tissues or during different phases of the experiment. Tables, which include tables of means (cf. table M) and tables of interactions (cf. table N) are numbered XV to XL and show separately and in combination the effects of exercise, length of the rest period after exercise, level of dietary protein, and replicate, on the variables measured.

Besides means, tables of means (cf. table M) give the standard error of each mean, differences between comparable means and indicate whether these differences are statistically significant or not. Tables of interactions (cf. table N) list the statistically significant first order interactions observed in each determination. In every case, means or other statistics are given first for the whole experiment and then, for each experimental period separately, the three experimental periods corresponding to the different lengths of time that the animals werekept alive, but idle after the end of the initial 8-week period of exercise. In the cases where experimental period and groups of rats are not confounded in the experiment, such as in the measurement of weight gains, feed or nutrient intakes, results are also given according to the groups of rats

TABLE M (Fac-simile of a table of means)

Mean.....
according to the level of exercise, replicate, level of dietary protein, or length of rest period.

Variable measured or experimental period	Level of exercise		Replicate		Level of dietary protein			Length of rest period		
	E_1	E_0	I	II	D_1	D_2	D_3	R_0	R_2	R_4
..... (n =72)	846±90	804±70	815±80	834±80	853±50	871±70	751±70	825±90	826±70	824±90
	42 *		19		18	120 **		1	2	
					102 **			1		

* significant at the 5% level ($p<0.05$)
** significant at the 1% level ($p<0.01$)

included in the experiment during each of the experimental periods.

In table M, each mean is followed by its standard error. Example: 846 ± 90. The difference between means are placed underneath and between the two means from which they were computed. When comparisons are made between three means, such as with levels of dietary protein or length of rest periods, the difference between the first and the second levels, and that between the second and third levels are placed immediately underneath the means, and the difference between the first and third values is placed on the following line in the center of the column where it belongs. Example:

Means (with s)	825±90(A)	826±70(B)	824±90(C)
Differences between A and B, B and C	1	2	
Difference between A and C		1	

Whenever they occur, significant differences are indicated by one (*) or two (**) asterisks, one * corresponding to a probability level of 5% ($p<0.05$) and two **, to a probability level of 1% ($p<0.01$).

The total number of animals included for the determination concerned is indicated in the column on the extreme left under the name of the variable measured (n =).

The following expressions and/or abbreviations are used in both tables of means and Appendix tables 3-A to 17-A:

- Levels of exercise:

  E_1 : exercised

  E_0 : controls - not exercised

- Levels of dietary protein:

  diet 1 (D_1) : 15% casein diet

  diet 2 (D_2) : 7.5% casein diet

  diet 3 (D_3) : 3.75% casein diet

- Experimental periods:

  period I : first 8 weeks of trial, during which the rats in the E_1 group (36) were exercised and those in the E_0 group (36) were cage-idle.

  period II : following 2 weeks of trial (weeks 9 and 10) during which all remaining rats (48) were cage-idle.

  period III: last 2 weeks of trial (weeks 11 and 12), during which all remaining rats (24) were cage-idle.

  group 0 (R_0) : group of rats (24) that lived only through period I and were killed immediately after the 8-week exercise period.

  group 2 (R_2) : group of rats (24) that lived through periods I and II and were killed after 10 weeks of experiment (8-week exercise period plus 2-week rest period).

  group 4 (R_4) : group of rats (24) that lived through periods I, II and III of the experiment and were killed after 12 weeks of trial (8-week exercise period plus 4-week rest period).

In table N (table of interactions), statistically significant interactions are indicated by one (*) or two (**) asterisks, depending whether they are significant at the 5% ($p<0.05$) or at the 1% ($p<0.01$) level. When the variable measured was such as A, which was the case for weight gains, feed and nutrient intakes, the results were considered according to experimental period and

TABLE N (Fac-simile of a table of interactions)

First order interactions observed on.....

Variable measured	Groups of rats and/or experimental period	E x Rep	E x D	E x R	Rep x D	Rep x R	D x R
A	Period I						
	Period II				**	**	
	Period III						
	All per. & groups						
	Groups 2, 4			**		**	
	Group 4			*			
B	All groups	*					
	Group O (R_0)						
	Group 2 (R_2)						
	Group 4 (R_4)	**	*				

* significant at the 5% level ($p<0.05$)
** significant at the 1% level ($p<0.01$)

according to groups. In all other cases, since experimental periods were confounded with groups, results were only divided according to groups.

The following abbreviations are used to designate the first order interactions:

E x Rep.	:	level of exercise x replicate
E x D	:	level of exercise x level of dietary protein
E x R	:	level of exercise x length of the rest period after the initial 8 weeks of exercise
Rep. x D	:	replicate x level of dietary protein
Rep. x R	:	replicate x length of the rest period after the initial 8 weeks of exercise
D x R	:	level of dietary protein x length of the rest period after the initial 8 weeks of exercise.

The term "muscle" used in connection with the present project means the gastrocnemius muscle, and the word "skin", that portion of the shaved skin that was removed for analysis, as described in section V under "Experimental procedure".

B - Feed intake and body weights.

Results on feed intake, nitrogen intake, body weight gains and body weights attained after 8, 10 or 12 weeks of trial are summarized in Figures 1, 2, 3 and 4. While Figure 1 shows the effect of exercise and of dietary protein level on feed consumption for each week of the experiment, Figures 2 and 3 illustrate the influence of these factors on nitrogen intakes (Fig. 2) and on weight gains (Fig. 3). One notices that, until the 4th or 5th experimental week, among the rats on maintenance (diet 2) or super-maintenance (diet 1) levels of protein, food consumption and weight gains of exercised rats were much below those of cage-idle rats. After this length of time, however, the appetite of exercised rats began to improve and the gains of weight of active rats exceeded those of the control animals. This acceleration in weight gain in exercised rats was intensified during the first rest period (period II) and, in the case of the rats on diet 1, during the second rest period (period III). Although a reduction in the rate of weight loss was noted in the rats on diet 3 after 4 or 5 weeks of trial and a slight gain was registered during period II, the weight curve showed an almost constant decline in both exercised and idle rats during the whole 12 weeks of experiment. Figure 4 summarizes the effects of diet and of exercise on feed intakes, nitrogen intakes and on weight gains during each of the three experimental periods.

1 - Feed intakes.

Average feed intakes per rat per week are given in

FIGURE 1

Weekly Feed Intakes

grams per rat per week

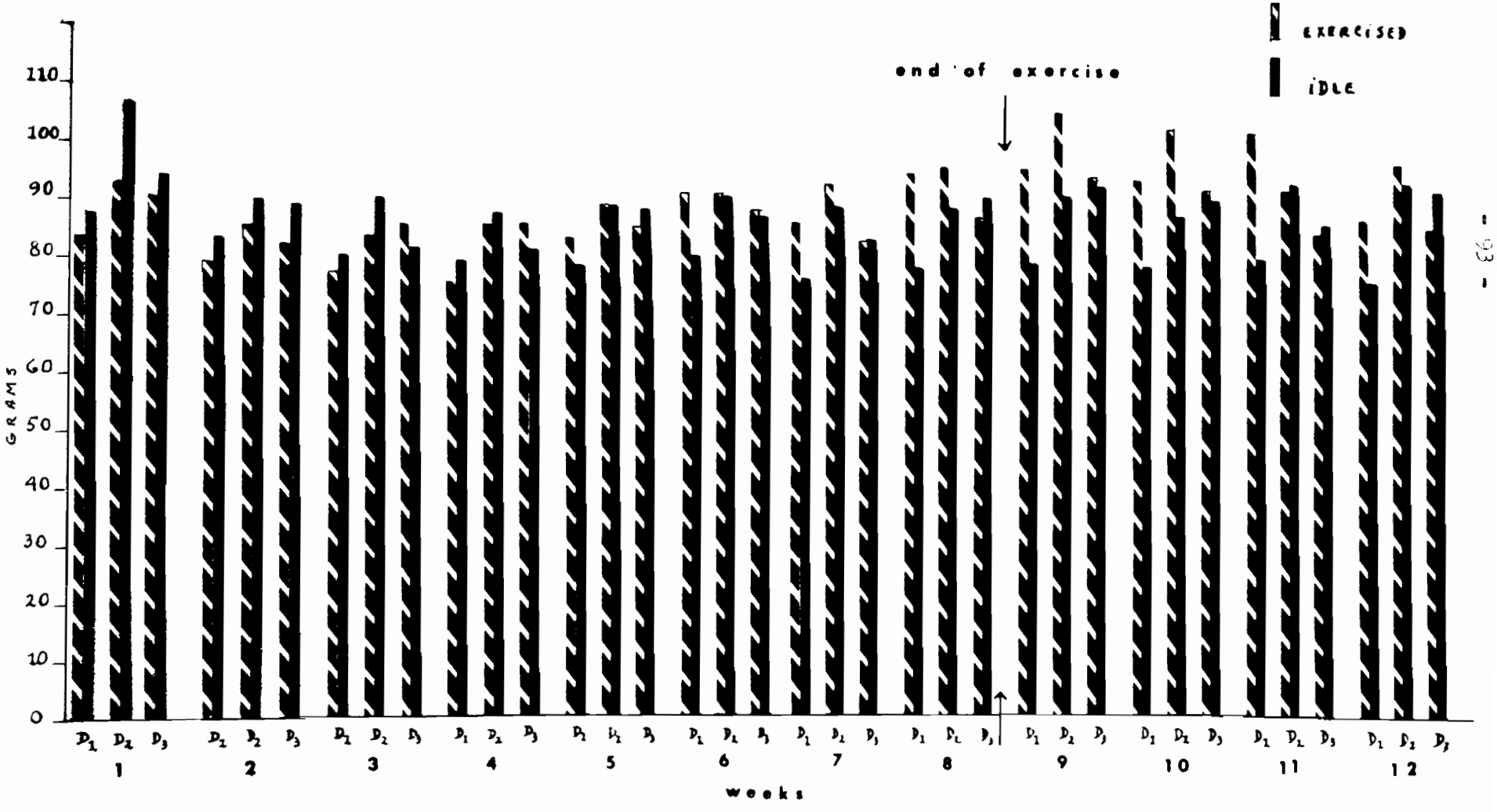

FIGURE 2
Weekly Nitrogen Intakes

grams per rat per week

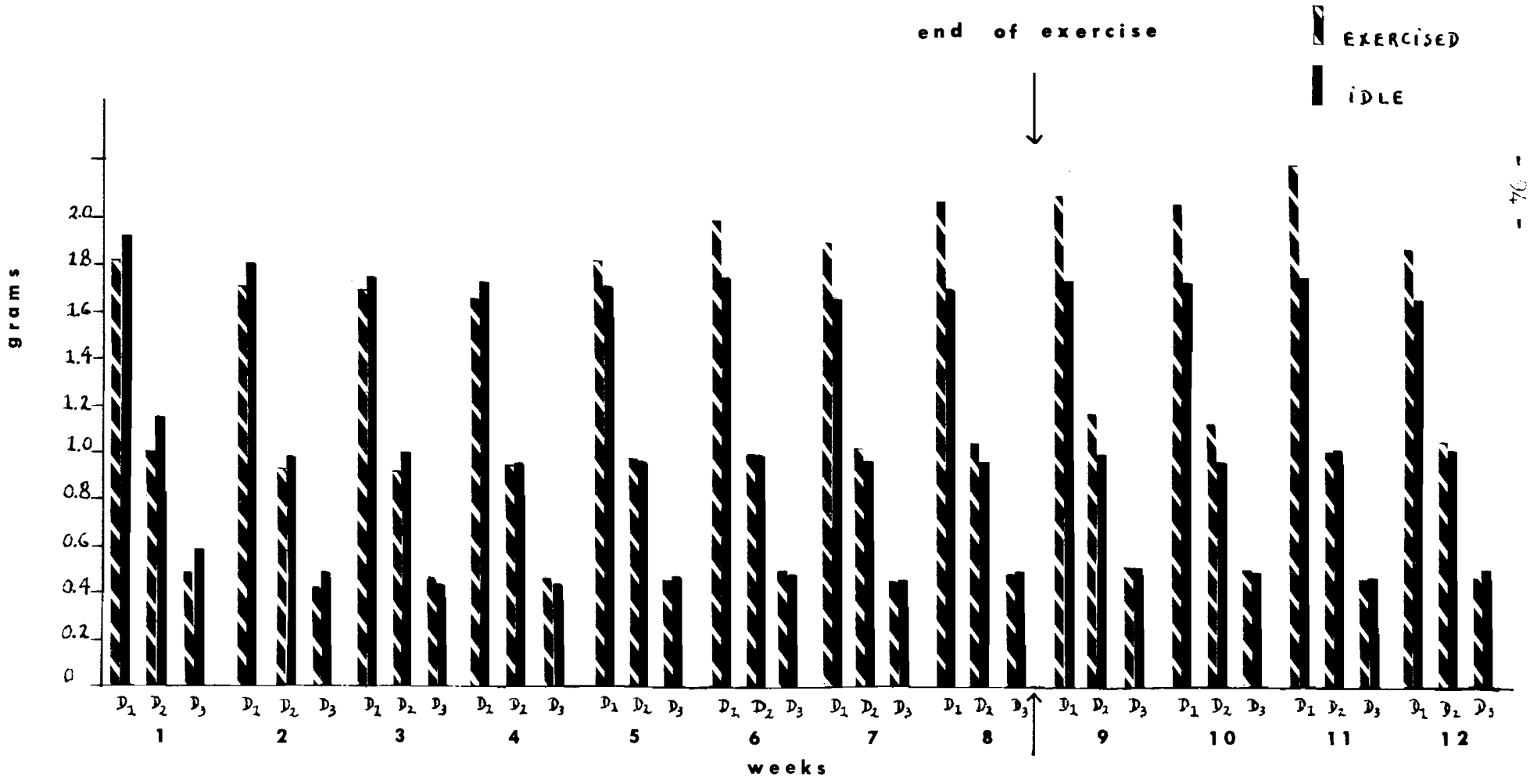

FIGURE 3

Weekly Weight Gains

grams per rat per week

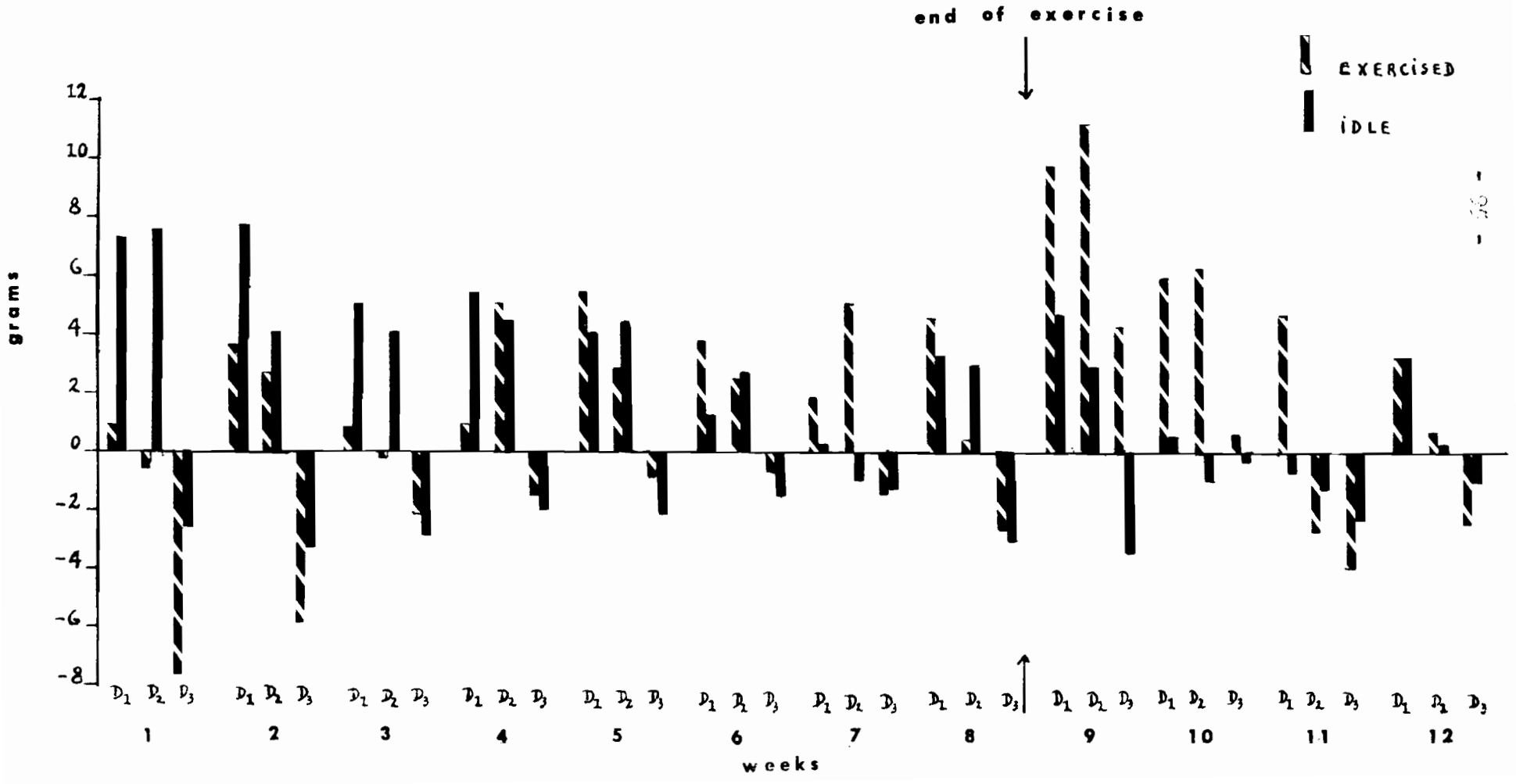

FIGURE 4

Weight Gains, N Intakes, and Feed Intakes

as affected by exercise, rest, or dietary protein

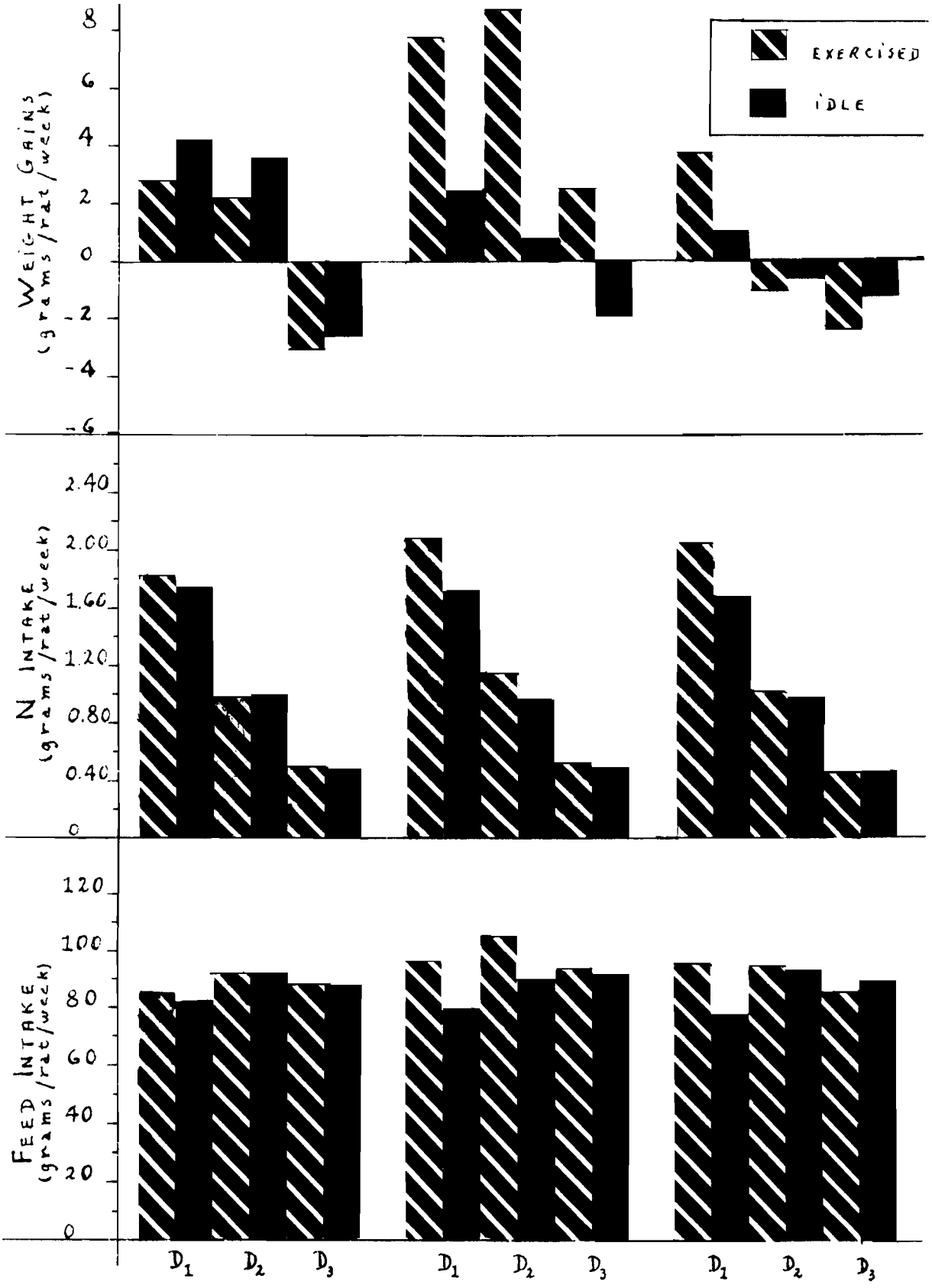

Table X. Although no tests of significance were done on these data, the following tendencies can be observed from this table:

a) feed consumption of exercised rats was higher during period II than in period I and, for replicate I, higher in period III than in period II, even if there was no imposed exercise during any of the last two periods;

b) during period I, the feed intake of exercised rats was approximately equal or only slightly superior to that of idle rats; in 2 cases, it was even slightly inferior;

c) during all periods and both in exercised and cage-idle animals, highest feed intakes were practically always registered in rats on diet 2;

d) animals in replicate I, in general, ate more than those in replicate II.

2 ª Energy intakes.

Since the chemical analysis of the diets showed slight differences between the energy contents of diets 1, 2 and 3 in the same replicate, and between the diets of similar composition in the two replicates (cf. table IX), average caloric intakes of the animals were computed and are summarized in table XI. The tendencies shown in the feed intakes are maintained, with the exception of the differences between replicates which are smaller, and, in a few instances, contrary to those observed in the feed intakes. The animals in replicate I ate more than those in replicate II, but since theenergy concentration of their

TABLE X

Average FEED INTAKE * according to level of exercise, replicate, experimental period and level dietary protein.

(grams of food per rat per week)

Level of exercise	Replicate	Period I (n = 72)			Period II (n = 48)			Period III (n = 24)		
		D_1	D_2	D_3	D_1	D_2	D_3	D_1	D_2	D_3
E_1 (exercised)	I	87	92	87	96	110	107	105	113	96
	II	83	89	87	96	102	82	86	78	76
E_0 (idle)	I	79	90	87	80	92	88	83	100	90
	II	83	94	87	79	88	97	74	88	90

* calculated to the nearest whole figure

TABLE XI

Average CALORIC INTAKE * according to level of exercise, replicate, experimental period and level of dietary protein.

(kilo-calories per rat per week)

Level of exercise	Replicate	Period I (n = 72)			Period II (n = 48)			Period III (n = 48)		
		D_1	D_2	D_3	D_1	D_2	D_3	D_1	D_2	D_3
E_1 (exercised)	I	389	404	372	431	481	455	473	494	409
	II	393	408	391	456	469	370	407	360	342
E_0 (idle)	I	357	394	373	360	404	373	373	439	382
	II	392	433	395	376	403	437	350	405	407

* calculated to the nearest whole figure

TABLE XII

Average NITROGEN INTAKE according to level of exercise, replicate, experimental period and level of dietary protein.

(grams nitrogen per rat per week)

Level of exercise	Replicate	Period I (n = 72)			Period II (n = 48)			Period III (n = 24)		
		D_1	D_2	D_3	D_1	D_2	D_3	D_1	D_2	D_3
E_1 (exercised)	I	1.9	1.0	0.5	2.1	1.2	0.6	2.3	1.2	0.5
	II	1.8	1.0	0.5	2.1	1.1	0.5	1.9	0.9	0.4
E_0 (idle)	I	1.7	1.0	0.5	1.7	1.0	0.5	1.8	1.1	0.5
	II	1.8	1.0	0.5	1.7	1.0	0.5	1.6	1.0	0.5

diets was lower, the energy intake was usually comparable in rats given otherwise the same treatment combination.

3 - Nitrogen intakes.

Average nitrogen intakes are summarized in table XII, assuming 16% nitrogen in the source of protein used in the diets (casein). The larger consumption of food of the rats on diet 2 tended to compensate for the lower protein content of their diet when compared with diet 1, and the nitrogen intake of these rats was in most cases more than half of the nitrogen consumption of rats on diet 1.

4 - Initial and final body weights.

Initial weights and final weights after 8, 10 or 12 weeks of trial are shown in tables 3-A and 4-A in the Appendix. Mean initial and final body weights as affected by the treatments are given in table XIII. Besides replicate, which influenced even initial weights, the level of dietary protein is the only factor that affected the final weights of the animals, the final weight of rats on diet 3 being significantly lower than that of rats on diet 2 and from that of rats on diet 1.

5 - Weight gains.

Average weight gains in each experimental period are reported in table XIIIa. The statistical analysis of weight gains revealed significant interactions between the level of exercise and the length of the rest period after exercise, and between replicate and the length of the rest period after exercise (cf. table XX). The effect of these combinations

TABLE XIII

Mean INITIAL and FINAL BODY WEIGHTS (1)
according to the level of exercise, replicate, level of dietary protein or length of rest period.
(grams per rat)

	Level of exercise		Replicate		Level of dietary protein			Length of rest period		
	E_1	E_0	I	II	D_1	D_2	D_3	R_0	R_2	R_4
Initial weights (n = 72)	275 ± 20	272 ± 19	289 ± 14	258 ± 7	272 ± 18	273 ± 18	276 ± 21	275 ± 15	273 ± 16	272 ± 23
	3		31**		1	3		2	1	
					4			3		
Final weights (n = 72)	290 ± 34	287 ± 35	302 ± 32	276 ± 31	310 ± 20	303 ± 26	253 ± 24	287 ± 31	294 ± 35	285 ± 37
	3		26**		7	50**		7	9	
					57**			2		

(1) calculated to the nearest whole figure.

TABLE XIIIa

Average WEIGHT GAINS according to level of exercise, replicate, experimental period and level of dietary protein.

(grams per rat per week)

Level of exercise	Replicate	Period I (n = 72)			Period II (n = 48)			Period III (n = 24)		
		D_1	D_2	D_3	D_1	D_2	D_3	D_1	D_2	D_3
E_1 (exercised)	I	2.8	1.8	-3.0	5.9	4.1	3.4	3.5	0.2	-4.5
	II	3.0	0.5	-2.8	10.0	13.6	1.6	4.5	-1.3	-2.0
E_0 (idle)	I	4.2	3.6	-2.7	-0.5	-1.1	-3.0	-0.5	-1.2	-2.8
	II	4.4	3.8	-2.1	5.8	3.0	-0.9	3.0	0.8	-0.5

of factors can be seen in table XIV. During period I, weight gains were smaller in the exercised than in the idle animals, but the difference was not statistically significant. In period II, the difference between weight gains of exercised and control rats was highly significant, the gains of the first group being about 12 times those of the second group. During the last experimental period, both exercised and idle animals lost weight and the loss was not significantly different in the two groups. Weight gains were similar for rats on diet 1 and on diet 2 for the first 2 periods of the trial, but those of animals on the low-protein diet were significantly smaller than those of the two other groups. During period III, both diet 2 and diet 3 caused a loss of weight in the animals, while a gain was registered in animals on diet 1.

Table XV shows the influence of the length of rest period on the weight gain of rats of a same group. As it can be observed from this table, the weight gains of rats in group 4 (R_4) were slightly inferior to those of groups 2 (R_2) and 4 (R_4) or 0 (R_0), 2 (R_2) and 4 (R_4) combined, and this, for the same period. But the same trend, i.e. an acceleration of the weight gain during the first rest period, can be observed in both group 4 (R_4) alone, and groups 2 (R_2) and and 4 (R_4) combined.

6 - Efficiency ratios.

a - Weight gain/energy intake

Since the different diets were not exactly isocaloric,

TABLE XIV

Mean WEIGHT GAINS in each experimental period,
according to the level of exercise, replicate or level of dietary protein.
(grams per rat per week)

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Period I (weeks 1 to 9) (n = 72)	0.8 ± 3.2	1.9 ± 3.6	1.4 ± 3.8	1.2 ± 3.1	3.6 ± 1.5	3.0 ± 2.5	-2.6 ± 1.8
	1.1		0.2		0.6		5.6**
					6.2**		
Period II (weeks 9, 10) (n = 48)	6.4 ± 5.5	0.5 ± 4.3	1.5 ± 4.8	5.5 ± 5.4	5.3 ± 5.4	4.9 ± 6.2	0.3 ± 4.4
	5.9**		4.0**		0.4		4.6**
					5.0**		
Period III (weeks 11,12) (n = 24)	-0.1 ± 3.9	-0.3 ± 2.1	-1.0 ± 3.2	0.7 ± 2.8	2.6 ± 2.1	-0.5 ± 2.4	-1.6 ± 2.2
	0.2		1.7		3.1**		1.1
					4.2**		

TABLE XV

Mean WEIGHT GAINS of groups of rats in experiment during each of the three experimental periods

(grams per rat per week)

	Experimental period		
	Period I	Period II	Period III
Groups 0, 2, 4 (n = 72)	1.3 ± 3.5	-	-
Groups 2, 4 (n = 48)	1.2 ± 3.6	3.5 ± 5.7	-
	2.3**		
Group 4 (n = 24)	0.9 ± 3.5	2.7 ± 5.2	0.2 ± 3.0
	1.8	2.5*	
		0.7	

(cf. table IX), the efficiency of the diets in producing a gain of weight was calculated as weight gained per 100 Calories consumed. In order to eliminate negative values, the weight gain figures were coded by adding 10 to weekly weight gains. The ratio was therefore estimated according to the following formula:

$$\frac{\text{weekly gain of weight in grams} + 10}{\text{Calories consumed per week}} \times 100$$

The mean ratios are presented according to treatment and period of experiment in table XVI, and according to groups of rats and length of the rest period in table XVII. Statistically significant interactions between main treatments are reported in table XX. The effect of treatments and of experimental periods on the caloric efficiency ratios was similar to that observed on weight gains: increased efficiency of exercised rats during period II and increasingly higher efficiencies with increasing levels of protein in all periods. A difference between replicates was also apparent during periods II and III.

b - Nitrogen intake/weight gain

This ratio was calculated according to the following formula:

$$\frac{\text{grams of nitrogen consumed per rat per week}}{\text{grams of weight gained per rat per week} + 10}$$

The value of 10 was added to weight gains for the same reason as when computing the weight gain/100

TABLE XVI

Mean WEIGHT GAINED PER 100 CALORIES CONSUMED, for each experimental period and according to the level of exercise, replicate or level of dietary protein (1)

100 (grams of weight gained per week + 10/calories consumed per week)

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Period I (n = 72)	2.7 ± 0.7	3.0 ± 0.9	3.0 ± 0.9	2.8 ± 0.7	3.6 ± 0.4	3.2 ± 0.5	1.9 ± 0.4
	0.3		0.2		0.4 *	1.3 **	
					1.7 **		
Period II (n = 48)	3.7 ± 1.1	2.7 ± 1.1	2.7 ± 0.9	3.7 ± 1.2	3.7 ± 1.0	3.6 ± 1.2	2.5 ± 1.0
	1.0		1.0		0.1	1.1	
					1.2		
Period III (n = 24)	2.4 ± 0.9	2.5 ± 0.7	2.1 ± 0.7	2.7 ± 0.8	3.2 ± 0.6	2.2 ± 0.6	2.0 ± 0.6
	0.1		0.6 **		1.0 **	0.2	
					1.2 **		

(1) in computing this ratio, a value of 10 was added to every mean weekly weight gain in order to eliminate negative values.

TABLE XVII

Mean WEIGHT GAINED PER 100 CALORIES CONSUMED
for groups of rats in experiment during each of the three experimental periods (1)

100 (grams of weight gained per week + 10/calories consumed per week)

	Experimental period		
	Period I	Period II	Period III
Groups 0, 2, 4 (n = 72)	2.9 ± 0.8		
Groups 2, 4 (n = 48)	2.9 ± 0.8	3.2 ± 1.2	
	0.3*		
Group 4 (N = 24)	2.8 ± 0.9	3.0 ± 1.1	2.4 ± 0.8
	0.2	0.6**	
		0.4*	

(1) in computing this ratio, a value of 10 was added to each mean weekly weight gain in order to eliminate negative values.

TABLE XVIII

Mean NITROGEN PER UNIT OF WEIGHT GAINED for each experimental period according to the level of exercise, replicate or level of dietary protein.(1)

(grams nitrogen consumed per week/gram of weight gained per week + 10)

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Period I (n = 72)	0.10 ± .03	0.09 ± .03	0.09 ± .03	0.09 ± .03	0.13 ± .01	0.08 ± .01	0.07 ± .02
	0.01		0		0.05**	0.01	
					0.06**		
Period II (n = 48)	0.08 ± .04	0.11 ± .06	0.11 ± .06	0.08 ± .03	0.14 ± .05	0.08 ± .03	0.06 ± .04
	0.03*		0.03**		0.06**	0.02	
					0.08**		
Period III (n = 24)	0.12 ± .05	0.11 ± .05	0.14 ± .05	0.09 ± .03	0.15 ± .03	0.12 ± .03	0.07 ± .03
	0.01		0.05**		0.03*	0.05*	
					0.08**		

(1) in computing this ratio, a value of 10 was added to every mean weekly weight gain in order to eliminate negative values.

TABLE XIX

Mean NITROGEN INTAKE PER UNIT OF WEIGHT GAINED for groups of rats in experiment during each of the three experimental periods. (1)

(grams nitrogen consumed per week/grams of weight gained per week + 10)

	Experimental period			
	Period I	Period II	Period III	
Groups 0, 2, 4 (n = 72)	0.09 ± .03			
Groups 2, 4 (n = 48)	0.09 ± .03	0.09 ± .02		
	0			
Group 4 (n = 24)	0.10 ± .03	0.10 ± .05	0.11 ± .05	
	0	0.01		
		0.01		

(1) in computing this ratio, a value of 10 was added to mean weekly weight gains in order to eliminate negative values.

TABLE XX

First order interactions observed on weight gains, weight gains/100 calories consumed or nitrogen intake/unit weight gained.

	Experimental period or groups of rats	E x rep	E x D	E x R	rep x D	rep x R	D x R
Weight gains (g.)	Period I						
	Period II				**	**	
	Period III						
	All per. & grps.						
	Groups 2, 4			**		**	
	Groups 4			*			
Weight gained/ 100 calories consumed	Period I						
	Period II					*	
	Period III						
	All per. & grps.						
	Groups 2, 4			**		**	
	Group 4			**			
N intake/unit weight gained	Period I						
	Period II						
	Period III						
	All per. & grps.						
	Groups 2, 4			**		**	
	Group 4			**			

Calorie ratio. Mean ratios are shown in tables XVIII and XIX and first order interactions, in table XX. Generally speaking, nitrogen efficiency ratios followed a pattern similar to that of energy efficiency ratios.

C - Weights of organs and tissues

Figure 5 illustrates the differences observed in the fresh weight of the heart and the dry, fat-free weights of liver and muscle with different diets, replicates and in each of the three groups of animals (groups 0, 2 and 4). For the purpose of comparison, final body weights of the animals are also included in this diagram. While liver and muscle weights are grossly proportional to final body weights, heart weight is related more closely to the level of exercise than to body weight. The influence of replicate, dietary protein and length of rest period varied with the tissue and will be discussed for each organ or tissue separately.

1 - Heart weight

Heart weights are given in the Appendix (table 5A). The levels of exercise and of dietary protein are the two factors which significantly affected the mean fresh weight of the heart as shown in table XXI. No statistically significant interaction was found in relation to heart weight (cf. table XXIV). The heart was significantly larger in the exercised animals on all three diets and during the 3 periods of the experiment. No regression in the mean heart weight of

FIGURE 5

Body and Organ Weights

as affected by exercise, rest, dietary protein, or replicate

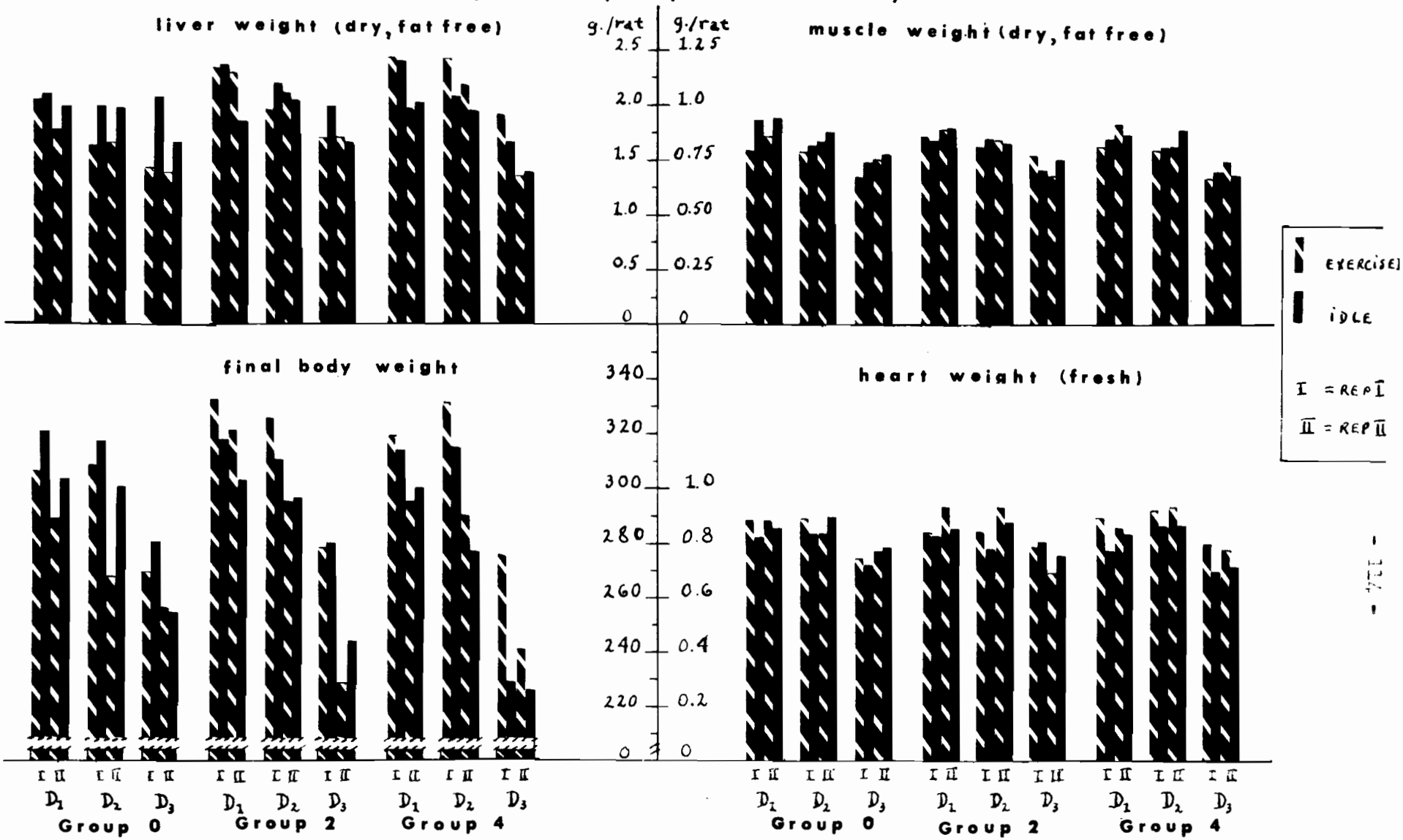

TABLE XXI

Mean HEART, LIVER AND MUSCLE WEIGHTS according to the level of exercise, replicate, level of dietary protein or length of the rest period.

(milligrams per rat)

	Level of exercise		Replicate		Level of dietary protein			Length of rest period		
	E_1	E_0	I	II	D_1	D_2	D_3	R_0	R_2	R_4
Heart weight (fresh) (n = 72)	846 ± 90	804 ± 70	815 ± 80	834 ± 80	853 ± 50	871 ± 70	751 ± 70	825 ± 90	826 ± 70	824 ± 90
	42**		19		18	120**		1	2	
					102**			1		
Liver weight (dry, fat-free) (n = 72)	1892 ± 400	1944 ± 290	2026 ± 370	1809 ± 300	2113 ± 290	1998 ± 330	1643 ± 250	1786 ± 290	1997 ± 295	1970 ± 430
	52		217**		115	355**		211**	27	
					470**			184**		
Muscle weight (dry, fat-free) (n = 72)	794 ± 80	814 ± 80	786 ± 80	821 ± 80	869 ± 60	824 ± 50	718 ± 50	811 ± 90	809 ± 80	792 ± 90
	20		35**		45*	106**		2	17	
					151**			19		

exercised rats was observed in the 4 weeks of rest included in this experiment. The heart did not show an increase in weight proportional to the increase in body weight during the 2 periods of rest, as indicated by the similarity of the heart weights of rats in group 0 (R_0), group 2 (R_2), and group 4 (R_4) (table XXI). No statistically different values were obtained for animals on diet 1 and those on diet 2. The hearts of rats in replicate II weighed slightly more than those of rats in the first replicate, although the final body weights of the former group of animals were lower than those of the latter group, but this difference was not significant.

2 - Liver weight

The difference between the weight of fresh liver and that of dry, fat-free liver was fairly constant for all the animals, and the percentage of weight loss resulting from dehydration and fat-extraction varied between 75.4% and 79.2%. The tendency was for the livers of rats on diet 3 to lose more weight during the fat extraction and drying procedure than those of animals on diets 1 or 2. It is presumed that this was due to the presence of a larger proportion of fat in the livers of the former. This finding coincides with the observation that in at least 10 out of 24 cases, the liver of rats on diet 3 was markedly paler in colour than that of other rats and appeared marbled

with yellow granules which were presumed to be fat. No microscopic examination or chemical analysis was done, however, to confirm this hypothesis.

The mean dry, fat-free weight of the liver was significantly affected by replicate, level of dietary protein and length of rest period, as shown in table XXI. Full data on liver weight are given in table 6A in the Appendix. A significant interaction between exercise and length of rest period was observed and can be seen more clearly in table XXII which gives mean liver weights for groups of animals rested for periods of various lengths after the initial 8 weeks of exercise. Liver dry, fat-free weight was significantly lower in exercised rats that had not been allowed to rest after the 8-week exercise period (group R_0) than in corresponding control animals, but this difference disappeared in rats that were allowed a 2-week or a 4-week rest period (groups R_2 and R_4). No difference in liver dry, fat-free weight was found between animals maintained on diet 1 and those consuming diet 2. In the case of group R_0, the difference in the value obtained for rats on diet 3 and that for rats on diet 2 was not statistically significant.

3 - Muscle weight

The percentage of fat and humidity lost by the muscles as a result of the ether and alcohol extraction and of drying was practically the same in all groups

TABLE XXII

Mean LIVER WEIGHT of groups of rats rested for 0, 2 or 4 weeks after an 8-week exercise period, according to the level of exercise, replicate or level of dietary protein.

(milligrams dry, fat-free liver per rat)

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Group 0 (n = 24)	1637 ± 250	1935 ± 260	1860 ± 340	1712 ± 230	1956 ± 180	1793 ± 300	1608 ± 300
	298**		148		163	185	
					348**		
Group 2 (n = 24)	1997 ± 340	1997 ± 270	2075 ± 310	1919 ± 270	2193 ± 290	2052 ± 220	1746 ± 190
	0		156		141	306*	
					447**		
Group 4 (n = 24)	2040 ± 490	1900 ± 360	2143 ± 440	1798 ± 360	2190 ± 350	2147 ± 380	1574 ± 250
	140		345		43	573*	
					616*		

of animals, and amounted to approximately 77%. The influence of the various treatments on the mean dry, fat-free weight of muscle is shown in table XXI and in table XXVII. Full results on muscle weight are given in the Appendix (table 7-A). As seen in table XXIII, exercise was associated with significantly lower muscle weight when considering group 0 (R_0) alone, but the difference was not significant when estimating the three groups together (table XXI). The lag in muscle growth ascribable to exercise was made up during the first rest period (period II). Muscle weight remained the same in exercised and cage-idle rats during period III.

Differences between replicates were in some respects similar to those found in heart size. Rats in replicate II had larger gastrocnemii muscles, especially during period I (significant difference in group 0), than the rats in replicate I, although their body weights were smaller. But this difference was not significant in groups 2 and 4. Animals maintained on a high level of protein (diet 1) had larger muscles than those on a maintenance level of protein (diet 2) if they were allowed no rest after the 8-week initial exercise period, but this difference diminished during the two rest periods and became non-significant. Muscles of rats on diet 3 were always smaller than those of rats on diets 1 or 2.

TABLE XXIII

Mean MUSCLE WEIGHT of groups of rats rested for 0, 2 or 4 weeks after an 8-week exercise period, according to level of exercise, replicate or level of dietary protein.

(milligrams dry, fat-free muscle per rat)

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Group 0 (n = 24)	781 ± 80	841 ± 80	785 ± 90	837 ± 70	879 ± 80	823 ± 60	732 ± 50
	60**		52*		56*	91**	
					147**		
Group 2 (n = 24)	809 ± 90	809 ± 70	806 ± 70	812 ± 80	869 ± 50	830 ± 30	727 ± 60
	0		6		39	103**	
					142**		
Group 4 (n = 24)	791 ± 90	792 ± 90	769 ± 80	815 ± 90	860 ± 70	819 ± 50	696 ± 50
	1		46		41	123**	
					164**		

TABLE XXIV

First order interactions observed on heart weights (fresh), liver weights (dry, fat-free) and muscle weights (dry, fat-free).

	Groups of rats	E x rep	E x D	E x R	rep x D	rep x R	D x R
Heart weight (fresh) (mg.)	All groups Group 0 (R_0) Group 2 (R_2) Group 4 (R_4)						
Liver weight (dry, fat-free) (mg.)	All groups Group 0 (R_0) Group 2 (R_2) Group 4 (R_4)			**			
Muscle weight (dry, fat-free) (mg.)	All groups Group 0 (R_0) Group 2 (R_2) Group 4 (R_4)						

4 - Skin weight.

As only a section of the skin was removed for analysis and skin samples were not identical in size, comparisons between the weights of the skin samples were of no meaningful value.

D - Total nitrogen composition of blood serum, muscle and skin

A comparison of the nitrogen composition of blood serum, muscle and skin can be made from table XXV and from figure 6. Detailed figures giving the nitrogen content of these tissues appear in tables 8-A, 10-A and 13-A in the Appendix. Replicate, level of dietary protein and, in the case of muscle, length of the rest period significantly affected the percentage of nitrogen in these tissues but exercise "per se" did not. In muscle, there was a significant interaction between exercise and the level of protein in the diet; this effect will be discussed with the results on muscle (paragraph F of the present section).

In the serum, the percentage of nitrogen was higher in rats of replicate I when compared with those of replicate II, but in muscle and skin, the opposite occurred. The level of protein in the diet was reflected directly in serum nitrogen, but it appeared to have but little influence on the percentage of nitrogen in muscle and skin. In muscle, the highest concentration of nitrogen was found in rats fed diet 2 and the lowest in those consuming diet 3, while the contrary was true for skin. In both serum and

FIGURE 6

Nitrogen in Serum, Muscle, and Skin

as affected by exercise, rest, dietary protein, or replicate

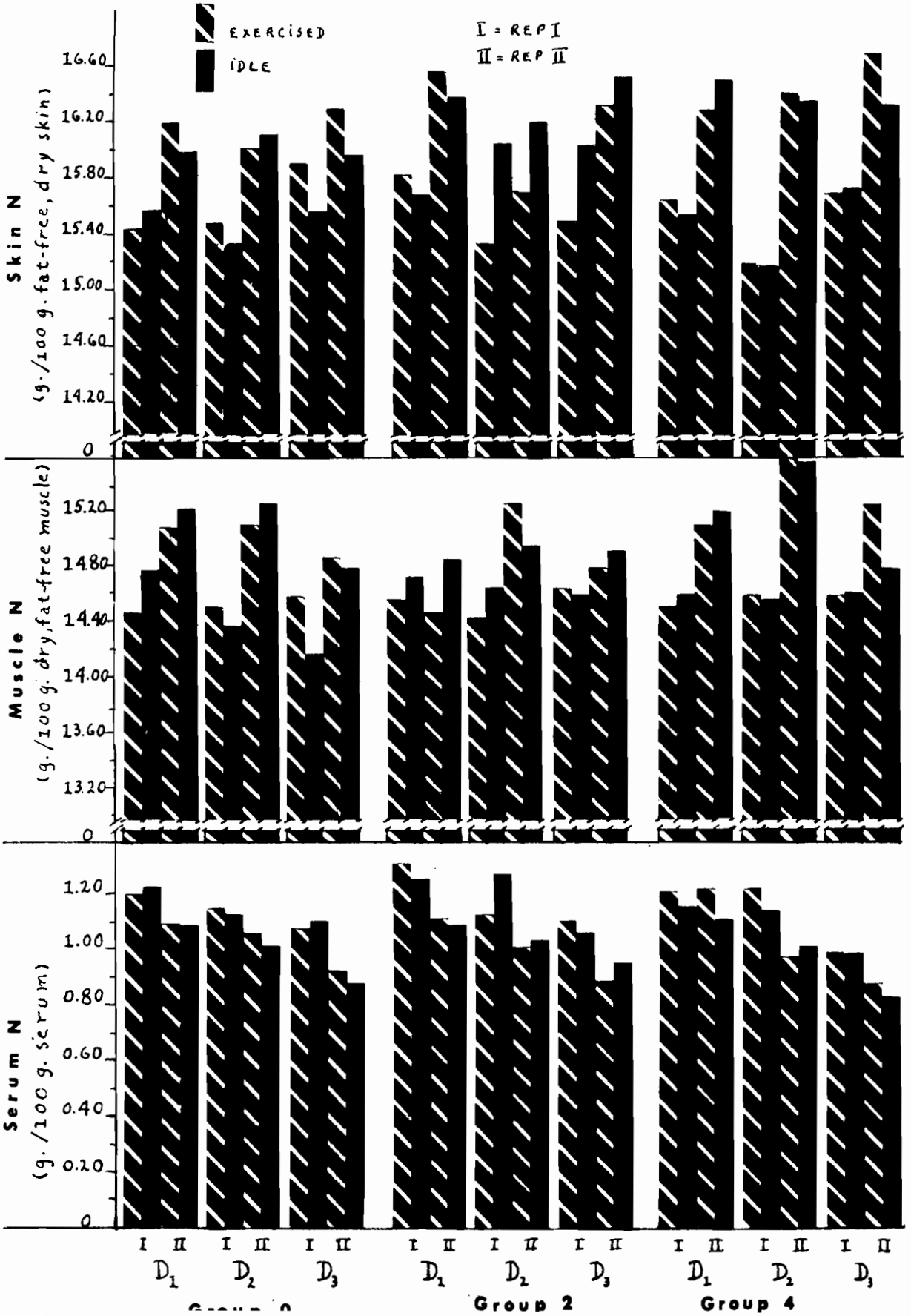

TABLE XXV

Mean BLOOD SERUM, MUSCLE AND SKIN NITROGEN according to the level of exercise, replicate, level of dietary protein or length of rest period.

(grams %)

	Level of exercise		Replicate		Level of dietary protein			Length of rest period		
	E_1	E_0	I	II	D_1	D_2	D_3	R_0	R_2	R_4
Serum N # (n=71) (1)	1.08±.13	1.08±.13	1.15±.11	1.01±.11	1.18±.08	1.09±.11	0.97±.11	1.08±.11	1.11±.14	1.06±.14
	0		0.14**		0.09**	0.12**		0.03	0.05*	
					0.21**			0.02		
Muscle N (n=72)	14.79±.38	14.80±37	14.54±.19	15.04±.34	14.78±.33	14.89±.46	14.70±.31	14.75±.37	14.72±.29	14.90±.43
	0.01		0.50**		0.11	0.19**		0.03	0.18**	
					0.08			0.15*		
Skin N (n=72)	15.90±.49	15.93±.48	15.57±.35	16.25±.32	15.95±.48	15.76±.51	16.03±.43	15.80±39	16.00±.46	15.94±.56
	0.03		0.68**		0.19*	0.27**		0.20	0.06	
					0.08			0.14		

(1) one sample missing from replicate II, group E_0, D_3, R_2.
\# corrected for non-protein nitrogen.

skin, there was a slight increase (not significant) in the percentage of nitrogen after 2 weeks of rest (group R_2 versus group R_0) and a decline after 4 weeks of rest (group R_4). In muscle, the concentration of nitrogen was sensibly the same after no rest and after 2 weeks of rest (groups R_0 and R_2) but significantly higher after 4 weeks of rest (group 4).

E - Nitrogenous components of blood serum

Mean serum proteins and albumin/globulin ratios are presented in table XXVI and detailed data on A/G ratio in table 9-A in the Appendix. Interactions found in these determinations are listed in table XXIX. The concentration of serum proteins was significantly higher in rats of replicate I than in rats of replicate II. This elevated value was the result not only of a higher albumin, but also of a higher globulin fraction (cf. figure 7). While the serum proteins and the A/G ratio fell with a decrease in dietary nitrogen, the percentage of globulins and of γ globulin in the serum was remarkably constant on all three levels of protein intake (cf. figure 7). Animals consuming diets 1 and 2 exhibited similar values for serum proteins in group 0 (R_0) only, and for the A/G ratio, in group 4 (R_4) only; otherwise, all 3 diets gave significantly different results in each of the three experimental periods.

Both serum proteins and serum globulins tended to increase after 2 weeks of rest (period II) and to decrease after 4 weeks of rest (period III), but these

TABLE XXVI

Mean SERUM PROTEINS (grams %) and SERUM ALBUMIN/GLOBULIN RATIO
according to the level of exercise, replicate, level of dietary protein or length of rest period.

	Level of exercise		Replicate		Level of dietary protein			Length of rest period		
	E_1	E_0	I	II	D_1	D_2	D_3	R_0	R_2	R_4
Serum Proteins (n=71) (1)	6.7±0.8	6.6±0.8	7.1±0.7	6.2±0.7	7.2±0.5	6.7±0.7	6.0±0.7	6.6±0.7	6.8±0.8	6.5±0.9
	0.1		0.9**		0.5**		0.7**	0.2		0.3
					1.2**			0.1		
Serum A/G Ratio (n=71) (1)	0.57±0.14	0.61±0.17	0.58±0.17	0.61±0.14	0.71±0.12	0.60±0.14	0.46±0.08	0.56±0.12	0.56±0.13	0.66±0.19
	0.04		0.03		0.11*		0.14**	0		0.10*
					0.25**			0.10*		

(1) one sample missing from replicate II, group E_0, D_3, R_2.

FIGURE 7

Distribution of Serum Protein Fractions

according to level of exercise, replicate, level of dietary protein, or length of rest period

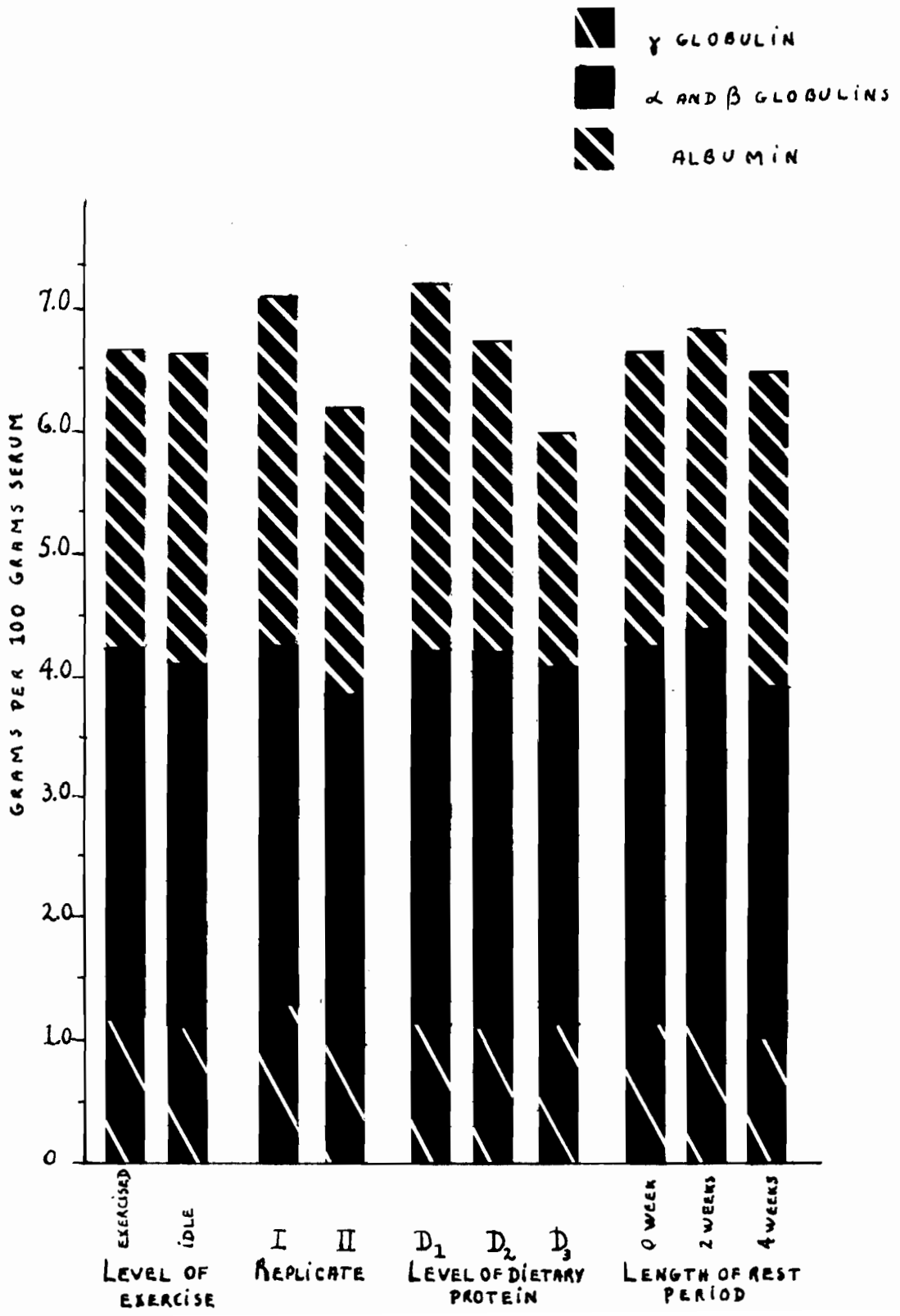

TABLE XXVII

Mean SERUM PROTEINS of groups of rats rested for 0, 2 or 4 weeks after an 8-week exercise period, according to the level of exercise, replicate or level of dietary protein.

(grams protein/100 grams serum)

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Group 0 (n = 24)	6.7 ± 0.7	6.6 ± 0.8	7.0 ± 0.5	6.2 ± 0.5	7.1 ± 0.5	6.7 ± 0.5	6.1 ± 0.7
	0.1		0.8**		0.4	0.6**	
					1.0**		
Group 2 (n = 23) (1)	6.7 ± 0.9	6.9 ± 0.8	7.3 ± 0.7	6.3 ± 0.5	7.3 ± 0.7	6.8 ± 0.8	6.2 ± 0.7
	0.2		1.0**		0.5*	0.6*	
					1.1**		
Group 4 (n = 24)	6.6 ± 1.0	6.4 ± 0.8	6.9 ± 0.7	6.1 ± 0.9	7.2 ± 0.4	6.6 ± 0.8	5.7 ± 0.6
	0.2		0.8**		0.6**	0.9**	
					1.5**		

(1) one sample missing from replicate II, group E_0, D_3.

TABLE XXVIII

Mean SERUM ALBUMIN/GLOBULIN RATIOS
of groups of rats rested for 0, 2 or 4 weeks after an 8-week exercise period,
according to the level of exercise, replicate or level of dietary protein.

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Group 0 (n = 24)	0.56 ± .14	0.56 ± .10	0.54 ± .09	0.58 ± .14	0.66 ± .12	0.55 ± .06	0.48 ± .09
	0		0.04		0.11**	0.07*	
					0.18**		
Group 2 (1) (n = 23)	0.54 ± .12	0.59 ± .15	0.55 ± .11	0.58 ± .16	0.69 ± .08	0.56 ± .09	0.43 ± .06
	0.05		0.03		0.13**	0.13**	
					0.26**		
Group 4 (n = 24)	0.62 ± .17	0.69 ± .22	0.65 ± .24	0.66 ± .12	0.80 ± .12	0.70 ± .18	0.47 ± .09
	0.07		0.01		0.10	0.23**	
					0.33**		

(1) one sample missing from replicate II, group E_0, D_3.

TABLE XXIX

First order interactions observed on blood serum proteins and albumin/globulin ratios.

	Groups of rats	E x rep	E x D	E x R	rep x D	rep x R	D x R
Serum proteins (g.%)	All groups Group 0 (R_0) Group 2 (R_2) Group 4 (R_4)				*		
Serum A/G ratio	All groups Group 0 (R_0) Group 2 (R_2) Group 4 (R_4)	 ** *			*		

changes were not statistically significant. However, a statistically significant increase occurred in the A/G ratio during period III, but this increase was observed in the cage-idle rats as well as in the exercised animals.

The effects of the length of the rest period on the nitrogen components of the serum can be estimated from tables XXVII and XXVIII where mean serum proteins and A/G ratios are reported according to the experimental period. Exercise had no significant effect on serum proteins nor on A/G ratio in either of the three periods, but there was a significant replicate-exercise interaction on the A/G ratio during the first 2 periods (cf. table XXIX). Exercised rats in replicate I had a lower A/G ratio than idle rats during period I, while in replicate II opposite tendencies were found. During period II, the contrary occurred. In the course of period III, animals in both replicates showed lower A/G ratios when exercised than when not exercised and this was true for animals on either level of protein.

F - Nitrogenous components of muscle

The mean concentration of nitrogen in muscle according to treatment, is given in table XXV, and that of DNA, in fig.8 and table XXX. This last table also shows the mean quantity of DNA per muscle as well as the mean N/DNA ratio in muscle as affected by treatments. The figures from which these means were calculated are found in tables 10-A, 11-A and 12-A in the Appendix. The level of dietary

protein is the only significant factor incluencing the percentage of DNA in muscle and the N/DNA ratio. The amount of DNA per muscle was affected by the level of protein in the diet and by replicate. While the quantity of DNA per 100 grams dry, fat-free muscle was higher, the quantity of DNA per muscle and the N/DNA ratio were lower in rats on diet 3 than in animals on either one of the other two diets. The quantity of DNA per muscle was also significantly higher in rats on diet 1 than in those on diet 2 because of the significantly different sizes of the muscles of these two groups of rats (cf. table XXI). For the same reason, the amount of DNA per muscle was greater in replicate II than in replicate I.

A significant interaction was found between replicate and the level of exercise on the amount of DNA per 100 grams muscle and on the N/DNA ratio (cf. table XXXIV). This was caused by the results obtained with group 4 (R_4): while in the exercised rats the percentage of muscle DNA was lower in replicate I than that in the controls, the situation was reversed in replicate II. As for the N/DNA ratio, its value was higher for exercised rats than for cage-idle animals in replicate I, and lower in replicate II.

The influence of the length of rest period on muscle components is clearly seen in tables XXXI, XXXII and XXXIII in which mean percentages of nitrogen, DNA and mean N/DNA ratios are given for each level of exercise, replicate

FIGURE 8

DNA in Muscle, and in Skin

as affected by exercise, rest, dietary protein, or replicate

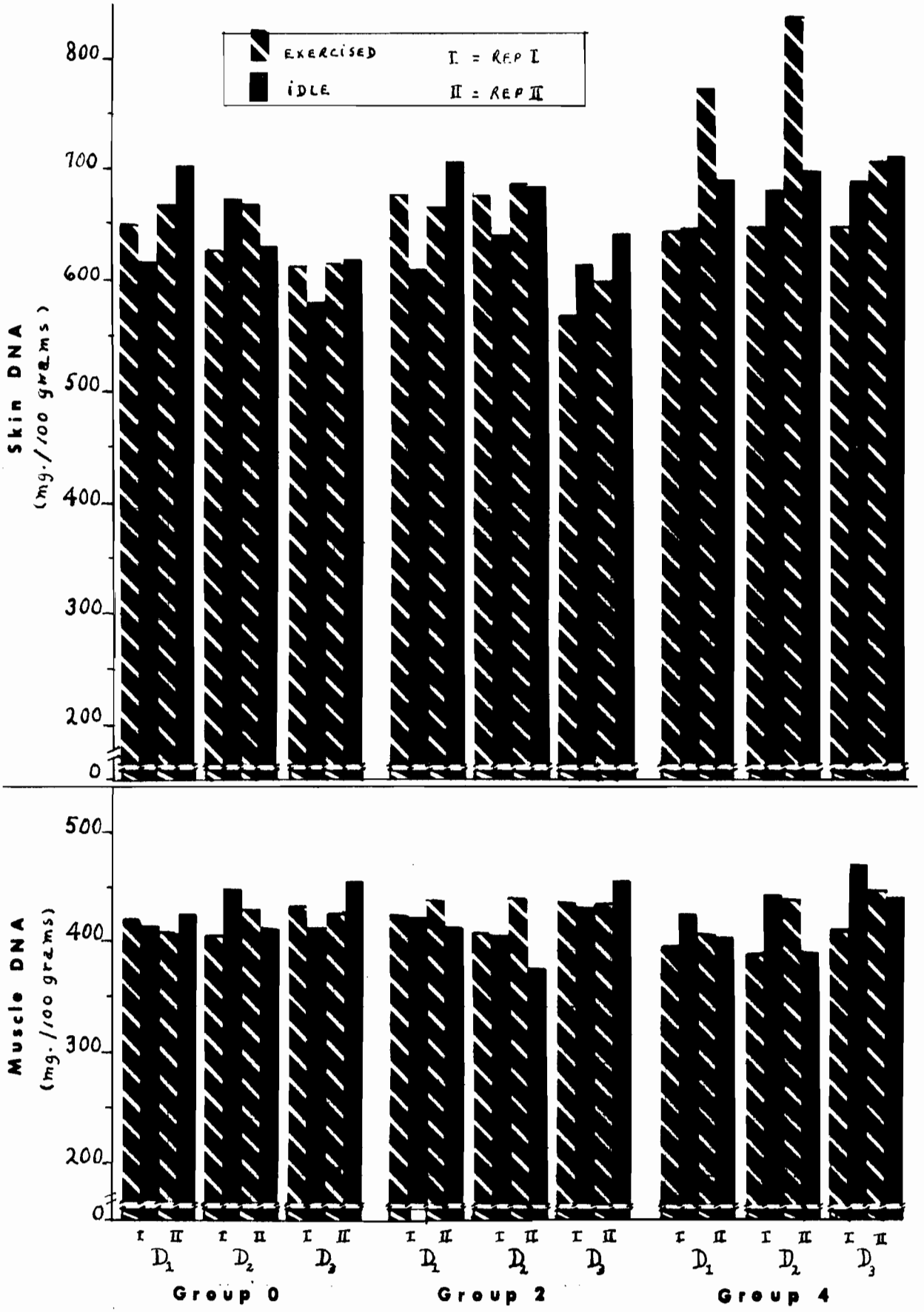

TABLE XXX

Mean MUSCLE DNA (mg./100 grams muscle [#] and mg./muscle [##]) and MUSCLE N/DNA RATIOS, according to the level of exercise, replicate, level of dietary protein or length of rest period.

	Level of exercise		Replicate		Level of dietary protein			Length of rest period		
	E_1	E_0	I	II	D_1	D_2	D_3	R_0	R_2	R_4
DNA (mg./100 grams dry, fat-free muscle) (n = 72)	423 ± 3	427 ± 3	424 ± 3	426 ± 2	418 ± 2	417 ± 2	439 ± 2	425 ± 3	426 ± 3	423 ± 3
	4		2		1	22**		1	3	
					21**			2		
DNA (mg./muscle) (n = 72)	3.4 ± .4	3.5 ± .3	3.3 ± .4	3.5 ± .3	3.6 ± .3	3.4 ± .3	3.2 ± .3	3.4 ± .4	3.4 ± .3	3.3 ± .3
	0.1		0.2*		0.2*	0.3**		0	0.1	
					0.5**			0.1		
N/DNA (n = 72)	35.1±2.3	34.8 ± 2.8	34.5±2.7	35.4±2.3	35.4±1.8	35.9±3.2	33.5±1.8	34.9±2.7	34.7 ± 2.4	35.3±2.6
	0.3		0.9		0.5	2.4**		0.2	0.6	
					1.9**			0.4		

\# dry, fat-free muscle

\## pair of gastrocnemii muscles.

TABLE XXXI

Mean MUSCLE NITROGEN in groups of rats rested for 0, 2 or 4 weeks after an 8-week exercise period, according to the level of exercise, replicate or level of dietary protein.

(grams N/100 grams dry, fat-free muscle)

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Group 0 (n = 24)	14.76 ± .31	14.74 ± .44	14.46 ± .23	15.04 ± .24	14.87 ± .35	14.79 ± .44	14.59 ± .30
	0.02		0.58**		0.08	0.20	
					0.28*		
Group 2 (n = 24)	14.67 ± .35	14.77 ± .22	14.59 ± .16	14.85 ± .33	14.64 ± .26	14.81 ± .34	14.72 ± .26
	0.10		0.26*		0.17	0.09	
					0.08		
Group 4 (n = 24)	14.92 ± .46	14.87 ± .42	14.57 ± .16	15.23 ± .36	14.84 ± .36	15.06 ± .56	14.80 ± .35
	0.05		0.34**		0.22	0.26	
					0.04		

TABLE XXXII

Mean MUSCLE DNA in groups of rats rested for 0, 2 or 4 weeks after an 8-week exercise period, according to the level of exercise, replicate or level of dietary protein.

(milligrams DNA/100 grams dry, fat-free muscle)

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Group 0 (n =24)	420 ± 30	430 ± 30	423 ± 40	427 ± 20	418 ± 10	425 ± 40	433 ± 30
	10		4		7	8	
					15		
Group 2 (n =24)	432 ± 20	420 ± 30	424 ± 20	428 ± 30	427 ± 20	409 ± 30	442 ± 20
	12		4		18	33**	
					15		
Group 4 (n =24)	416 ± 20	431 ± 30	423 ± 30	424 ± 20	411 ± 20	417 ± 30	443 ± 20
	15		1		6	26*	
					32**		

TABLE XXXIII

Mean MUSCLE N/DNA RATIOS in groups of rats rested for 0, 2 or 4 weeks after an 8-week exercise period, according to the level of exercise, replicate or level of dietary protein.

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Group 0 (n = 24)	35.3 ± 2.7	34.4 ± 2.7	34.4 ± 3.3	35.3 ± 2.0	35.7 ± 1.5	35.1 ± 3.8	33.8 ± 2.4
	0.9		0.9		0.6	1.3	
					1.9		
Group 2 (n = 24)	34.1 ± 2.1	35.3 ± 2.6	34.5 ± 2.1	34.9 ± 2.7	34.4 ± 1.6	36.4 ± 3.0	33.3 ± 1.3
	1.2		0.4		2.0	3.1**	
					1.1		
Group 4 (n = 24)	35.9 ± 1.6	34.8 ± 3.3	34.6 ± 2.8	36.1 ± 2.3	36.2 ± 1.9	36.3 ± 3.0	33.5 ± 1.8
	1.1		1.5		0.1	2.8**	
					2.7**		

TABLE XXXIV

First order interactions observed on muscle nitrogen, DNA and nitrogen/DNA ratios.

	Groups of rats	E x rep	E x D	E x R	rep x D	rep x R	D x R
Muscle N (g.%)	All groups		*		**	*	
	Group 0 (R_0)						
	Group 2 (R_2)						
	Group 4 (R_4)						
Muscle DNA (mg.%)	All groups	*					
	Group 0 (R_0)						
	Group 2 (R_2)		*				
	Group 4 (R_4)	**					
N/DNA ratio	All groups	*					
	Group 0 (R_0)						
	Group 2 (R_2)						
	Group 4 (R_4)	**					

and level of dietary protein and according to the experimental period. One will notice that the level of protein in the diet significantly affected the nitrogen concentration of muscle only in rats of group 0 (R_0), while it affected the percentage of DNA in muscle and the N/DNA ratio only in rats of groups 2 (R_2) and 4 (R_4). Differences in replicate were not the cause of any variation in the DNA concentration nor in the N/DNA ratio during any of the 3 periods, except for N/DNA in group 4 (R_4) in which a significantly higher ratio was found for rats of replicate II. An interaction between exercise and level of protein (table XXXIV) in the diet was observed in the concentration of DNA during period II (group R_2). In the course of the first rest period (period II), the DNA concentration of the muscles of exercised rats was higher than that of cage-idle animals only when the latter consumed maintenance or super-maintenance levels of protein. During period III, a replicate-exercise interaction was noted both in DNA and in the N/DNA ratio. This reflected the fact that exercised animals in replicate I showed a smaller concentration of DNA in muscle and a higher N/DNA ratio than control rats during this period, while the contrary was true for rats included in replicate II.

G - Nitrogenous components of skin

The mean percentage of nitrogen in the skin is given in table XXV, that of DNA and collagen nitrogen, in table XXXV and the mean N/DNA and non-collagen N/DNA ratios, in table XXXVI. Full results on these skin components and

ratios are found in the Appendix (tables 13-A, 14-A, 15-A, 16-A and 17-A). The level of exercise had no effect on any of these variables. If one separates the results according to the experimental period, however, such as in tables XXXVII, XXXVIII, XXXIX, it can be seen that exercise was associated with a significant decrease in the nitrogen content of the skin in group 2 (R_2). Significant exercise-replicate interactions (cf. table XL) occurred in the percentage of DNA as well as in the N/DNA ratio in rats of group 4 (R_4). In replicate I, the skin of exercised animals contained a lower percentage of DNA than that of control animals, but the opposite was seen in rats of replicate II.

Replicate influenced the nitrogen and the DNA concentration of the skin practically to the same extent, so that the N/DNA ratio was similar in both replicates. A significantly higher percentage of collagen nitrogen was found in replicate II but the ratio of non-collagen N/DNA in the two replicates remained within the limits of error variation.

The percentage of protein in the diet was associated with significant differences in the nitrogen and DNA of the skin and in its N/DNA ratio. Animals on diet 2 had less nitrogen in their skin than those on either diet 1 or diet 3. DNA was significantly lower in the skin of rats on diet 3, with the result that the N/DNA was significantly higher in this group of rats compared to those on diets 1 or 2. In group 4 (R_4), i.e. the group of rats that was

last to be sacrificed, a higher concentration of DNA and a lower N/DNA ratio were found compared to either group 0 (R_0) or group 2 (R_2). But the length of the experimental period did not significantly affect the percentage of collagen nitrogen nor the non-collagen N/DNA ratio in the skin.

TABLE XXXV

Mean SKIN DNA (mg./100 grams skin #) and collagen nitrogen (g./100 grams skin #) according to the level of exercise, replicate, level of dietary protein or length of rest period.

	Level of exercise		Replicate		Level of dietary protein			Length of rest period		
	E_1	E_0	I	II	D_1	D_2	D_3	R_0	R_2	R_4
DNA (mg./100 grams dry, fat-free skin) (n=72)	667±80	660±50	641±50	685±60	673±60	681±60	636±50	641±50	650±60	699±60
	7		44		8	45**		9	49**	
					37*			58**		
Collagen N (g./100 grams dry, fat-free skin) (n=72)	10.34±.66	10.61±.69	10.27±.68	10.68±.64	10.48±.77	10.26±.58	10.68±.65	10.48±.67	10.65±.70	10.29±.67
	0.27		0.41*		0.22	0.42		0.17	0.36	
					0.20			0.19		

dry, fat-free skin.

TABLE XXXVI

Mean SKIN NITROGEN/DNA and NON-COLLAGEN NITROGEN/DNA RATIOS, according to the level of exercise, replicate, level of dietary protein or length of rest period.

	Level of exercise		Replicate		Level of dietary protein			Length of rest period		
	E_1	E_0	I	II	D_1	D_2	D_3	R_0	R_2	R_4
N/DNA (n = 72)	24.1±2.8	24.2±1.8	24.5±2.5	24.1±2.2	23.8±1.8	23.3±1.9	25.6±2.7	24.8±2.0	24.9±2.7	22.9±1.7
	0.1		0.4		0.5		2.3**	0.1		2.0**
						1.8**			1.9**	
Non-collagen N/DNA (n = 72)	8.4±1.2	8.1±0.8	8.3±1.2	8.2±1.0	8.1±1.0	8.1±0.7	8.5±1.3	8.3±1.3	8.3±1.1	8.1±0.7
	0.3		0.1		0		0.4	0		0.2
						0.4			0.2	

TABLE XXXVII

Mean SKIN NITROGEN of groups of rats rested for 0, 2 or 4 weeks after an 8-week exercise period, according to the level of exercise, replicateor level of dietary protein.

(grams N/100 grams dry, fat-free skin)

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Group 0 (n = 24)	15.87 ± .41	15.73 ± .38	15.52 ± .26	16.08 ± .29	15.77 ± .44	15.71 ± .42	15.92 ± .34
	0.14		0.56**		0.06	0.21	
					0.15		
Group 2 (n = 24)	15.86 ± .52	16.13 ± .36	15.72 ± .38	16.27 ± .36	16.10 ± .49	15.81 ± .41	16.08 ± .46
	0.27*		0.55**		0.29	0.27	
					0.02		
Group 4 (n = 24)	15.96 ± .55	15.91 ± .60	15.47 ± .37	16.40 ± .22	15.97 ± .49	15.76 ± .70	16.08 ± .50
	0.05		0.93**		0.21	0.32	
					0.11		

TABLE XXXVIII

Mean SKIN DNA of groups of rats rested for 0, 2 or 4 weeks after an 8-week exercise period, according to the level of exercise, replicate or level of dietary protein.

(milligrams DNA/gram, dry, fat-free skin)

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Group 0 (n = 24)	643 ± 50	640 ± 50	629 ± 50	653 ± 40	662 ± 40	651 ± 40	610 ± 40
	3		24		11	41	
					52		
Group 2 (n = 24)	647 ± 80	652 ± 50	633 ± 70	666 ± 50	667 ± 50	674 ± 40	608 ± 80
	5		33		7	66	
					59		
Group 4 (n = 24)	710 ± 80	688 ± 30	661 ± 30	737 ± 70	690 ± 80	718 ± 80	689 ± 30
	22		76**		28	29	
					1		

TABLE XXXIX

Mean SKIN NITROGEN/DNA RATIOS in rats allowed to rest for 0, 2 or 4 weeks after an 8-week exercise period, according to the level of exercise, replicate or level of dietary protein.

	Level of exercise		Replicate		Level of dietary protein		
	E_1	E_0	I	II	D_1	D_2	D_3
Group 0 (n = 24)	24.8 ± 2.0	24.7 ± 2.0	24.9 ± 2.2	24.7 ± 1.8	23.9 ± 1.8	24.2 ± 2.0	26.2 ± 1.5
	0.1		0.2		0.3	2.0	
					2.3		
Group 2 (n = 24)	24.9 ± 3.6	24.8 ± 1.7	25.1 ± 3.4	24.6 ± 2.1	24.2 ± 1.6	23.5 ± 1.5	26.8 ± 3.6
	0.1		0.5		0.7	3.3	
					2.6		
Group 4 (n = 24)	22.7 ± 2.1	23.2 ± 1.2	23.5 ± 1.1	22.4 ± 2.0	23.4 ± 2.0	22.1 ± 1.7	23.4 ± 1.1
	0.5		1.1		1.3	1.3	
					0		

TABLE XL

First order interactions observed on skin nitrogen, DNA, and nitrogen/DNA ratios.

	Groups of rats	E x rep	E x D	E x R	rep x D	rep x R	D x R
Skin N (g.%)	All groups Group 0 (R_0) Group 2 (R_2) Group 4 (R_4)						
Skin DNA (mg.%)	All groups Group 0 (R_0) Group 2 (R_2) Group 4 (R_4)	*					
Skin N/DNA ratio	All groups Group 0 (R_0) Group 2 (R_2) Group 4 (R_4)	*					

VII DISCUSSION

Each set of results presented in section VI will be discussed separately before an attempt is made to integrate these results into a general pattern of immediate and prolonged effects of exercise on blood serum, muscle, and skin of rats fed various levels of protein.

A - Feed intake, body weight, and feed efficiency ratios.

The depressing effect of exercise on appetite and on growth observed in this study suggests that a stressful situation was created when the exercise regimen was first imposed on the rats. According to the standards set by Mayer *et al.* (1954) and by Thomas and Miller (1958), a physical effort of this length (3 hours per day) and intensity (1.6-mile walk at a speed of 47 feet per minute) would not be considered strenuous and should not have depressed the appetite of the animals. Differences in the type of diet, in the room temperature or in the construction of the exercise apparatus could be evoked to explain the discrepancies in the results. One important difference in the experimental conditions between the study reported by Mayer *et al.* (1954) and the present investigation is in the length of the exercise and rest periods within the same day: while in the first case 5 minutes' rest were inserted between each 10 minutes of exercise, a period of rest of this length was allowed only after 25 minutes of walking in the second case. In the present study, the apparent stress was overcome within 4 or 5 weeks at which time the appetite of the animals improved causing a marked increase in weight for the remaining 3 or 4 weeks of the exercise period. The persistance of large food intakes in exercised animals even after cessation of activity is in accordance with a similar observation

by Thomas and Miller (1958). There is a lag in the adjustment of the appetite to the nutritional needs of the animal, no matter whether these needs are suddenly increased or decreased.

Weight gains were closely related to feed intakes in rats receiving the same treatment combination. No improvement in feed or energy utilization could be observed before the end of the 8 weeks of exercise, and the gains of weight observed during this period were entirely due to the increase in feed intake. Increases in weight gains among exercised rats during the two rest periods (periods II and III) correspond to both an increase in feed intake and an improvement in feed efficiency. Exercise appears to exert a delayed sparing action on energy utilization for at least 2 weeks after activity is stopped and perhaps longer, especially if the protein intake is adequate. The loss of weight and decrease in feed efficiency observed in animals on diet 2 during period III (cf. tables XIV and XVI) may have been the consequence of treatment or of some undiagnosed ill-health in some members of this group. The decline in vigor observed in these animals is reminiscent of the condition developed in rats on diet 3 and might have been the result of a slight but chronic protein deficiency.

The higher feed intake of the rats on the 7.5% casein diet (diet 2) may represent an effort of the animals to obtain more energy, because of the lower caloric content of their diet compared to diet 1 (cf. table IX), or to obtain more protein. Since the caloric intake of these rats was higher (cf. table XI) than that of rats fed the 15% casein diet, the second hypothesis is more probable. This is another indication that the level of protein

provided by diet 2 (15 mg. of protein per Calorie) may not have been adequate for rats of this age which, though adults, were still growing slowly. The severe inadequacy of diet 3 with respect to protein made it impossible for the rats consuming this diet to compensate for the lack of protein by an increase in feed intake. Quite the contrary, rats on this diet just barely maintained or gradually lost their appetite.

Weight gains were highest (approximately 14% of initial weight), and energy utilization best, in rats consuming diet 1. Animals on diet 2 gained an average of 11% of their initial weight in the 12-week experimental period, and their feed efficiency was second best. Finally, the use of diet 3 caused a weight loss of about 8% of the initial weight and was associated with the lowest level of energy utilization. These results reinforce the opinion that the 7.5% casein diet was inadequate, at least for optimal growth and feed efficiency in rats of this age and stage of development.

The rats in replicate I ate more food than those in replicate II perhaps because the caloric content of their diets was slightly lower (cf. table IX). This would not explain, however, why their caloric intake was higher than that of replicate II during period II of the experiment. One simple reason for their higher feed intake is their larger size. This could also explain why their efficiency in using calories was lower than that of replicate II during periods II and III: assuming that their energy requirement was higher because of a larger body mass, a smaller proportion of the calories consumed would be used for growth. An average gain in weight of 4.3% throughout the experiment was registered by the

rats in replicate I, and an average gain of 7.0% by animals in replicate II. These results suggest that rats of replicate II might have been younger, physiologically, than rats in replicate I. It may be pertinent to recall that the rats included in replicate II, although of the same strain and age as those in replicate I (cf. section V-B) were obtained from a different source, the first source being unable to supply the animals at the time they were needed.

B - Weights of organs and tissues.

The hypertrophy of the heart observed in the exercised rats was of the order of 5%: this result is in agreement with those reported by Steinhaus (1931b) in dogs, and by Hearn and Wainio (1956) in rats (cf. table VI). But the absolute weight of the heart was less than one-half of that of rats of comparable size used by Hearn and Wainio (1956) (table VII). Without the details of the procedure used by the latter in removing the heart, it is difficult to suggest any explanation for this difference other than the type of exercise, swimming being the exercise to which Hearn and Wainio subjected their rats. The fact that the increase in heart size was not proportional to the increase in body weight is in accordance with the observation of Steinhaus (1931a). But it is not possible from the present data to confirm this author's opinion that the size of a heart that has hypertrophied as a result of exercise regresses when the animal is allowed to rest.

Up to a certain extent the dry, fat-free weight of the liver followed body weight, but there was a tendency for the rats exercised for 8 weeks but not allowed to rest, to have smaller livers in proportion to their body weights compared to their

controls. This is in agreement with the results obtained by Christensen (1963a) in animals exercised for 4 weeks. If one assumes that exercise and rest do not alter the nitrogen concentration of the liver, this result can be interpreted as a decrease in the total amount of nitrogen in the liver in the exercised rats, with a tendency for this depletion to be made up during a subsequent rest period. But no further increase in the size of liver was observed if the rest period was extended from 2 to 4 weeks.

Muscle size was very closely proportional to body size, much more, in fact, than liver size. The only effect of rest on the size of the muscles of rats that had been exercised was to make it possible for them to reach the same weight as muscles of cage-idle animals. No tendency to hypertrophy could be observed in the gastrocnemius muscle neither as an immediate or delayed effect of exercise. According to Leathem (1964) the gastrocnemius is one of the muscles that respond the least quickly to any stimulus. It may be that one only period of exercise of 8 weeks' duration followed by 4 weeks of rest was not long enough for this muscle to show hypertrophy similarly to that observed by Christensen (1963a) in rats subjected to several 4-week periods of exercise and rest. The absolute sizes of the gastrocnemii muscles of both exercised and control animals were very close to those reported by Gollnick and Hearn (1961).

C - Composition of blood serum.

The absence of effects of exercise on serum proteins(cf. table XXVII), either immediately or after rest, comes in contradiction with the

results obtained by Christensen (1963a) who reported a decrease in serum proteins following a 4-week period of exercise, and this whether the rats had or had not been subjected to previous intermittent periods of exercise. The length of the exercise period used in the present study, i.e. 8 weeks instead of 4 weeks, may be responsible, at least to a certain extent, for this difference in results. A longer exercise period may allow sufficient time for the rats to recover from the immediate effects of an increase in activity, and a loss in the albumin fraction may be compensated by an increase in the globulin fraction and/or by a reduction in the catabolism of serum proteins, similarly to what occurs in long-term protein malnutrition in humans (Picou and Waterlow, 1962).

The first hypothesis: an increase in serum globulins, is supported by the observation that the levels of total globulins and of the main component of this fraction, γ globulin, tended to be slightly higher in the exercised rats than in the controls (cf. figure 7), resulting in lower A/G ratios in exercised animals. The finding of a decrease in serum globulins and of an increase in the serum A/G ratio in both exercised and idle animals during period III (cf. table XXVIII) throws some doubt on the validity of relating these changes to exercise. It is possible that serum globulins are influenced by some other factor to which exercised animals are more sensitive. But since even during period III, exercised rats had a higher proportion of globulins in their serum than control rats, exercise is not to be discarded as a possible cause of the above-mentioned differences in the serum protein

fractions.

The effect of the level of dietary protein on serum proteins and on the A/G ratio is similar to that reported in the literature (Lippman, 1948; Weimer et al., 1959a and b; Ambegaonkar and Chandran, 1959; Wannemacher et al., 1963). The increase in the proportion of globulins in serum proteins found in rats fed the low protein diet indicates that the loss of serum proteins that occurred as a consequence of protein deficiency was the result of a loss of albumin, the amounts of globulins and of γ globulin per 100 grams of serum being approximately the same on all levels of proteins (cf. figure 7).

Serum protein values of non-exercised rats (cf. table XXVI) were the same as those reported by Christensen and above those obtained by other workers (cf. table II). The A/G ratio for the same group of animals was also similar to that observed by Christensen (1963a) for idle rats, but higher than that found in normals rats by Weimer et al. 1959a).

D - Composition of muscle.

No significant change in the relative or absolute nitrogen or DNA content of muscle, nor in its N/DNA ratio can be ascribed to exercise (cf. tables XXV and XXX), either as an immediate or late effect. The significant increase in the percentage of nitrogen that occurred after 4 weeks of rest was observed in the idle group as well as in the exercised group (cf. table XXXI), and could therefore not be related to exercise. The small difference (not significant) between the N/DNA ratios of exercised and non-exercised animals during period III (cf. table XXXIII) was caused almost entirely by a higher ratio in exercised animals consuming

diet 3 over idle animals on the same level of protein. This would suggest that exercised animals fed a low-protein diet could maintain their protein reserves better than cage-idle animals but more evidence is needed to support this statement.

This absence of effect of exercise on muscle composition, contrasting with the results obtained by Christensen (1963a) could be related to the following factors:

1) the length of the exercise period;

2) the age of the animals;

3) the type of exercise, i.e. continuous versus discontinuous.

It was suggested with respect to the results obtained on blood serum that an exercise period of 8 weeks may allow adjustments in the metabolic processes, adjustments which do not take place within 4 weeks, and that would cancel short-lasting effects of exercise on tissue composition. The same reasoning may apply to muscle and one may assume that the increase in muscle nitrogen and DNA following 4 weeks of exercise and reported by Christensen (1963a) may be a temporary effect of exercise and may not show after 8 weeks. On the other hand, the only animals in which such an increase was demonstrated by Christensen were those that had been trained prior to the experimental period, i.e. who had been subjected to 3 periods of 4 weeks of exercise, separated by rest periods, before the study began, bringing the total exercise period up to 16 weeks. The difference between the two experimental situations lies either in the length of the exercise period, (16 weeks instead of 8) or in the presence of several rest periods. The present data offer no basis for discarding either one of these hypotheses.

The second factor: age, could also be evoked to explain the difference in results between the two studies. At the time they were sacrificed, the rats used in this experiment were 6 to 7 months old, while those included in Christensen's work were approximately 11 months old. But the fact that it is the older animals, i.e. those observed by Christensen, that exercise affected more markedly, contradicts the general observation that younger individuals are usually more sensitive to external influences than older ones. It is therefore not likely that the age of the experimental animals accounts for the difference in their response to exercise.

Finally, the alternation of exercise and rest periods may induce changes in the mechanism of synthesis and catabolism of protein and of DNA that result in increasingly higher dispositions to hypertrophy and perhaps to hyperplasia in the muscles that participate in the exercise. As early as 1931, Steinhaus had noticed (Steinhaus, 1931b) that long sustained effort would not cause hypertrophy of skeletal muscle, contrarily to short intense exercise periods interrupted with frequent rests. In the present experiment, the effort imposed was moderate, the exercise period, long and was followed by only one continuous period of rest of 4 weeks. Such conditions produced no evident signs of hypertrophy and no increase in the nitrogen or DNA content of muscle. In the light of Christensen's results, one is inclined to think that shorter exercise and rest periods repeated several times would be more successful in stimulating the synthesis of nitrogenous components in muscle.

If one considers the increase in muscle DNA associated with the sub-maintenance level of protein (cf. tables XXX and XXXII) as an indication of a loss of other muscle constituents, then the fall in the N/DNA ratio (cf. tables XXX and XXXIII) observed in the same group of rats may represent a decrease in the labile nitrogen of this tissue. It is to be noted that the muscle N/DNA ratio was significantly lower in rats on diet 3 (low-protein) than in those fed diets 1 or 2 (high- or maintenance- protein) only during the two rest periods. Also of interest is the observation that the muscle N/DNA ratio in the rats given the low-protein diet was higher in the exercised rats than in the cage-idle animals in all three periods of the experiment (cf. Appendix table 12-A). These findings could be related to the opinion (Munro et al., 1953; Yuile et al., 1959b; Allison et al., 1963) that during stress (in this case physical exercise) muscle tissue supplies amino-acids to maintain labile protein reserves. Further data are needed, however, to support the opinion that, if dietary protein is inadequate, exercised animals maintain their protein reserves at a higher level than non-exercised animals.

The finding that the total amount of DNA per muscle fell as the dietary protein was decreased contradicts the observations of Mandel et al.(1949) and of Christensen (1963a) and the opinion that, in general, the DNA content of a tissue is stable after maturity is attained (Allison and Fitzpatrick, 1960). But it is in agreement with the results reported by Mendes and Waterlow (1958) who ascribed this decrease in the quantity of DNA per muscle to a decrease in the number of cells.

The protein contents of the muscle of idle rats is similar to that reported by Gollnick and Hearn (1961) and by Christensen (1963a) in normal or control rats. If the present results are calculated in terms of grams of protein per 100 grams fresh muscle, the mean protein content of the muscle of idle rats on diet 1 and 2 is 21.2, a figure which resembles closely those of 21.4 obtained by Christensen (1963a) and of 21.9, by Gollnick and Hearn (1961). The mean DNA content of the muscle of control rats consuming maintenance or high levels of protein in the present study was 94.9 mg. per 100 grams fresh weight, compared to 123.9 mg. (Christensen, 1963a) and 84.6 mg. (Mendes and Waterlow, 1958). It was therefore within the range of previous observations.

E - Composition of skin.

Neither exercise nor the length of the rest period are believed to have had a direct influence on the composition of the skin in nitrogen, collagen nitrogen, or DNA. The significant difference in the percentage of nitrogen between exercised and idle rats of group 2 (R_2) is (cf. table XXXVII), in fact, the result of a higher value in the idle rats of group 2 (R_2) and not of a decrease in the percentage of nitrogen in the skin of the exercised animals. It is doubtful that this difference has any biological significance.

The marked increase in DNA and corresponding decrease in the N/DNA and, to a lesser extent, in the non-collagen N/DNA ratios associated with the longest rest period (4 weeks) (cf. tables XXXV and XXXVI) could not be prolonged effects of exercise because they were observed in both idle and exercised rats. This increase in the percentage of DNA and consecutive decrease in N/DNA and non-collagen N/DNA ratios were slightly more pronounced in the

exercised than in the cage-idle group (cf. tables XXXVII, XXXVIII and XXXIX) and might reflect a relative decrease in constituents of the skin other than DNA. It is interesting to note that Christensen (1963a) also observed a lower N/DNA in the skin of animals that had been subjected to several intermittent exercise periods but not in those that had been through one 4-week period of exercise only.

If time and, to a certain extent, exercise resulted in a decrease in skin labile nitrogen as reflected on the N/DNA ratio, a deficiency in dietary protein had an opposite effect because of the significantly lower percentage of DNA in the skin in the animals consuming the low-protein diet, compared to those fed maintenance or high levels of protein. A slower rate of synthesis of DNA or the higher proportion of collagen, not affected by diet (cf. table XXXV) could, perhaps account for this finding. If one accepts the first hypothesis, one may also suppose that when the experimental period is prolonged to 12 weeks, the synthesis of both DNA and other nitrogenous components are restrained to the same extent which would explain why the N/DNA ratio of group 4 (R_4) does not show any difference with various levels of dietary protein (cf. table XXXIX).

The lower percentage of nitrogen in the skin of rats consuming diet 2, as compared to those on diet 1, was found in all 3 groups of animals: group 0 (R_0), group 2 (R_2) and group 4 (R_4), although the difference was not significant when these 3 groups were considered separately (cf. table XXXVII). Again, this might be interpreted as an indication that the 7.5% casein diet (diet 2) was not quite adequate to maintain maximal levels of all nitrogenous components in the skin, but not low enough to interfere with the

synthesis of DNA, as would be the case with the 3.75% casein diet.

The skin of idle rats consuming normal or super-maintenance levels of protein contained 85.5% protein on a dry, fat-free basis and 19.6% on a fresh basis. These values are very close to those obtained by Christensen (1963a) for normal rats fed similar diets and slightly higher than those reported by Stanier (1957) for fresh skin, and by Elkinton and Widdowson (1959) for dry, fat-free skin (cf. table IV). The collagen content of the skin of the same rats, in the present study, was 56.4% for the dry, fat-free tissue and 12.9% for the fresh tissue. This is lower than the figure given by Kao and McGavack (1959) for total collagen in the skin of 8-month old female rats, but more than that found for soluble collagen by the same authors. Differences in the preliminary treatment of the tissue prior to the determination of collagen may explain this discrepancy. The DNA content of the skin of the control rats was surprisingly high, compared to values obtained on normal animals by Christensen (1963a) and by Rodesh and Mandel (1958). It was calculated to be 667.3 mg. per 100 grams dry, fat-free skin and 153.0 mg. per 100 grams fresh skin which is about 1.5 times the value obtained by these workers (cf. table IV). Neither the literature reviewed nor the present data offer any basis for relating this difference to the treatments or to the procedure used in this experiment.

F - Integrating discussion.

The 8-week period of exercise imposed on young adult rats in this experiment had immediate effects on the animals' feed intake, growth and heart size, the first two being significantly reduced for the first 4 to 5 weeks of the study, and the latter showing a significant increase. Delayed effects were also observed, mostly on energy utilisation which was sensibly increased in the 4 weeks' rest period that followed the 8-week exercise period. Effects on the nitrogenous composition of blood serum, muscle and skin were, however, slight and in practically all cases, statistically non-significant. A tendency was noted in the exercised animals to have a higher proportion of globulins and of γglobulin in their serum, and in those that were allowed to rest, a lower albumin-globulin ratio. In the skin of the exercised animals, the N/DNA ratios tended to be lower after rest in the exercised rats fed diets 1 or 2 than in the control rats given that same level of protein, and this was the result of larger concentrations of DNA in the skin of the former group of animals, compared to the latter.

These two trends are in line with the results obtained by Christensen (1963a), although the differences observed between exercised and non-exercised rats were not as pronounced in the present study. Contrary to the findings of this worker, no increases in the nitrogen and the DNA content of muscle were observed as a consequence of exercise. Possible reasons for this difference have been stated in the first part of the discussion.

The length of the exercise period and of the rest periods used in this study seem particularly important in the light of the findings on feed intake, body weight and feed efficiency. The marked improvement in feed intake and weight gain noted after 4 to 5 weeks of exercise offers an indication that the 8-week exercise period of this experiment could be divided into 2 phases: first, a 4 to 5 weeks period of adaptation during which the organism would show signs of stress and probably corresponding to an increase in the output of adreno-corticoid hormones, causing an intensification of protein catabolism and of nitrogen excretion. With the end of this adjustment period, nitrogen retention would gradually increase and tissue nitrogen would return to normal levels, the more labile proteins reaching these values more rapidly than the more stable proteins. It is believed that eight weeks were sufficient for most of the labile protein stores of the body to be replenished, as indicated by the similarity of the values obtained after 8 weeks of exercise in exercised and control rats for serum proteins, A/G ration, muscle N/DNA, skin N/DNA and non-collagen N/DNA.

But this length of time (8 weeks) did not allow liver or muscle to grow to a size comparable to that of control animals. The percentage of DNA in muscle was also affected and did not quite reach the same values in exercised rats as in idle rats after this first period of the trial. Rest was the factor that permitted liver and muscle of the exercised group of animals to attain the same weight as the cage-idle group, and rest might have permitted muscle DNA and skin DNA to increase in concentration. As a result of this increase in DNA, the N/DNA ratio in muscle and skin had a tendency to fall, at least during the first 2 weeks of rest, in spite of the stability of the numerator of this ratio. The 4 weeks of rest were,

however, ineffective in producing an increase in either nitrogen or DNA in muscle, over and above the amount found in control animals. In order to observe this phenomenon, an exercise period of more than 8 weeks and/or more than 4 weeks of rest would appear necessary. The fact that energy utilization of exercised rats greatly improved during rest inclines one to think that the presence of several rest periods is probably the determining factor in the development of hypertrophy or hyperplasia in muscle following an increase in physical activity. Some support is given to the hypothesis that one of the delayed effects of exercise on muscle would be to allow rats fed diets inadequate in protein to maintain protein reserves at a higher level than cage-idle animals.

Both the 15% and the 7.5% casein diets appear to have been sufficient for body protein stores to be maintained during the 8 weeks of exercise. However, the 7.5% casein diet gave results that were slightly inferior to those obtained with the 15% casein regime and, with respect to serum proteins and albumin/globulin ratio, the difference between the results obtained on these two diets was significant. For this reason and because higher feed and caloric intakes and lower energy utilization were observed with the 7.5% casein diet, this diet is considered insufficient for maintaining maximal levels of protein in the tissues and for maximal feed efficiency in rats of this age, sex and activity.

One unexpected result associated with diet was the decrease in total DNA per muscle with a decrease in the level of dietary protein. If future experiments on a larger number of animals confirm this finding, one could question the validity of using N/DNA ratio in muscle as a measure of protein reserves in this tissue when

low-protein diets are used.

The replicate effect was important in this experiment although conditions of experiment were similar in each of the two replicates. Results tend to indicate that animals in replicate II were younger, not chronologically, but physiologically, than those used in the first replicate. This belief is supported by the fact that animals in replicate II had lower final weights and smaller livers than those in replicate I, although their weight gains and feed efficiency were higher. The percentage of nitrogen and DNA in the muscles and skin of the former group of animals (replicate II) as well as the collagen content of their skin were higher, and the serum proteins and serum globulins, lower than those of rats included in replicate I. In general, exercise had more pronounced effects on replicate II than on replicate I.

The reaction to the treatments imposed was often different in the various tissues studied: blood serum, muscle and skin. For example, the effect of the dietary protein level on the percentage of nitrogen and of DNA was in opposite directions in muscle and in skin. And labile protein reserves, as represented by the albumin/globulin and N/DNA ratios, increased in blood serum and decreased in the skin during the last rest period (period III). As found by other workers in this field, blood serum appeared more sensitive to treatment than muscle and skin. In both serum and muscle, 4 weeks of rest were sufficient to equalize ratios of labile/stable nitrogen (albumin/globulin in the serum and N/DNA in muscle) in exercised and control animals. In neither of the three tissues: serum, muscle and skin, 8 weeks of exercise and 4 weeks of rest

appreciably altered the percentage of nitrogen.

Significant interactions between treatments were found practically only in connection with replicate, which can be explained by the above discussion on the differences between replicates. Exercise-length of rest period interactions were observed in connection with liver weight, weight gains, weight gain/100 Calories, nitrogen/weight gain and DNA per muscle. As indicated previously, these interactions reflect a delayed effect of exercise on these variables.

VIII SUMMARY

In summary, the following observations were recorded immediately after an 8-week period of physical exercise, or after a 2- or a 4-week rest period following the exercise period, in young adult female rats fed sub-maintenance, maintenance or super-maintenance levels of protein:

A - Effects of exercise

1 - Effects on feed intakes, body weight and feed efficiency ratios.

a - Immediate effects of exercise:

- Exercise was associated with a reduction in the voluntary feed intake continuing for 4 to 5 weeks after the beginning of the trial. This lack of appetite resulted in a retardation of growth.
- After 4 to 5 weeks, both feed intake and body weight gradually increased in the exercised animals, so that the final weight of those that were killed immediately after this 8-week exercise period was practically the same as that of control animals.

b - Delayed effects of exercise:

- During the 4 weeks of rest, and especially during the first 2 weeks, exercised animals significantly increased their feed intake and their efficiency in utilizing food energy.
- As a result, the final weights of the exercised rats that were allowed to rest after the initial 8 weeks of exercise were equal or superior to those of cage-idle animals.

2 - Effects on liver, muscle, and heart weights.

a - Immediate effects of exercise:

- After the initial exercise period of 8 weeks, the weight of the liver and of the gastrocnemius muscle of the exercised animals had not reached that attained by control animals.
- Heart weight was significantly higher in exercised than in control rats.

b - Delayed effects of exercise:

- The liver weight of the exercised rats consuming high- or average-protein diets, and muscle weight of exercised rats consuming the high-protein diet increased through the 4 weeks of rest, to equal or exceed those of the cage-idle animals.
- The muscle weight of exercised rats fed the average-protein diet decreased in the last 2 weeks of rest and was inferior to that of the controls.
- Both liver and muscle weights of exercised animals receiving the low-protein diet gradually decreased during these 4 weeks.
- The hypertrophy of the heart observed in exercised rats was maintained through the 4 weeks of rest.

3 - Effects on blood serum:

- Total serum proteinswere not significantly affected by exercise either immediately or after rest.
- But exercised animals showed a tendency to higher proportions of serum globulins and gamma-globulin during exercise and

rest periods, and to lower albumin/globulin ratio during the 4 weeks of rest.

4 - Effects on muscle:

- No significant change in the nitrogen and in the DNA content of the gastrocnemius muscle, or in muscle nitrogen/DNA ratio could be related to exercise either as an immediate or late effect.
- In the animals consuming high or average levels of protein, labile protein reserves, as measured by nitrogen/DNA ratio tended to drop during the first 2 weeks of rest and to be restored during the last 2 weeks.
- Rats fed the low-protein diet appeared to be able to maintain muscle N/DNA ratio at a higher level than cage-idle animals during the whole 12 weeks of trial.

5 - Effects on skin:

- The nitrogen, the collagen nitrogen, and the DNA composition of the skin were not affected significantly by exercise either immediately or after rest.
- However, when the animals were given high or medium levels of dietary protein, a slightly lower nitrogen/DNA ratio was observed in the exercised group than in the control group after 2 and after 4 weeks of rest. This trend was not apparent in the non-collagen nitrogen/DNA ratio.

B - Effects of diet

- The nitrogenous composition of muscle and of skin were sensibly the same when the diet contained 15% casein and when it contained 7.5% casein.

- But the serum proteins, the albumin/globulin ratio were higher, and the weight gains and the energy utilization were better on the high-protein than on the medium-protein diet, in both exercised and cage-idle animals.
- The adequacy of the 7.5% casein diet for rats of this age, strain, and stage of development is questioned.

IX CONCLUSIONS

The pattern of changes occurring in adult rat tissues as a result of physical exercise appears to depend on a number of factors among which the length of the exercise period and the length and frequency of rest periods play an important role. The hypothesis that protein metabolism is modified by exercise and that this effect lasts after exercise has stopped, is verified in the observations that the feed efficiency ratio was greatly increased and that the albumin/globulin ratio in the serum, the nitrogen/DNA ratio in muscle and in skin tended to fall during the rest period that followed the 8 weeks of exercise imposed to the animals in this study. The levels of these tissue components were reestablished, at least in muscle and in serum, after 4 weeks of rest. This would indicate that the changes observed are reversible.

It is believed that the prolongation of the exercise period to 8 weeks, in this experiment, resulted in obscuring somewhat the picture of the effects of exercise, through allowing the body to make adjustments in its nitrogenous components whenever the protein intake was adequate. Future research on this problem could benefit from dividing the 8 weeks of exercise into 2 periods of 4 weeks, the first being one of adaptation, and the second showing the effects of exercise on trained animals. It would also be of interest to observe the effect of another 4-week period of exercise that would follow a period of rest of the same length.

The full effects of the level of dietary protein on the results could be observed only in the two rest periods included in the experiment. Because the appetite was poor in the exercised rats during most of the 8-week exercise period, this group of animals could not take advantage of any benefit that might have resulted from an increase in their total

daily protein intake. Moreover, the appetite of the rats consuming the low-protein diet was poor during the whole experiment, and these rats, even when exercised, never consumed as much protein as the idle rats fed a moderate level of protein. In these rats, the effects of the protein deficiency predominated and masked the possible influence of exercise on the nitrogenous composition of tissues. It might be sufficient, in future studies of this type, to use only two levels of dietary protein: one which is barely adequate or slightly below requirements, and one which provides an amount of protein which is undoubtedly sufficient to satisfy the needs of the animals.

Serum albumin/globulin ratio and nitrogen/DNA ratio in muscle and skin were found to be useful, but not entirely satisfactory criteria of protein nutrition in this study. The lack of stability of serum globulin and, to a certain extent, of muscle DNA, indicates the need for further research on the validity of using these ratios as measures of labile protein reserves in the body. The difficulty of using nitrogen balance as a method of investigation in cases where the experimental animals are confined for a certain length of time every day to an exercise apparatus, practically eliminates this technique for measuring protein status in studies involving physical exercise. Other possible criteria include the determination of total carcass nitrogen and that of daily urinary creatinine as a measure of muscular mass. A very recent publication by Wannemacher * proposes a new method for the evaluation of protein reserves called **Protein reserve index** (PRI). If this criterion proves to be as reliable as it appears to be from the preliminary report on its use, it will no doubt be most welcomed by investigators in the field of protein nutrition who

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* Fed. Proceedings Abstracts vol. 25, p.605, abstract no. 2344, 1966

have been long waiting for a simple yet valid tool for evaluating the protein status of individuals. This method might have the advantage of giving a more complete picture of the availability of labile nitrogen in the whole body, over methods where it is measured in one or a few tissues only. This feature would be especially valuable in investigations on the question of protein requirements where the question is not so much whether exercise or any other factor lowers the labile protein reserves in one or the other of the body compartments, but whether this factor, through lowering the nitrogen stores, can affect the normal physiological functions of the organism. The final answer to the problem of protein requirement for exercise is therefore linked very closely with those of the role of labile protein reserves in the body, and of the fundamental relationship between the activity of a cell and its requirement for protein.

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Table 1-A

VITAMIN DIET FORTIFICATION MIX

(used at the 1% level)

Vitamin	grams per 100 grams mix
Vitamin A acetate (500,000 I.U./gram)	0.488
Vitamin D_3 (1654 I.U./gram)	17.150
δ-1,$\propto$ tocopherol acetate	2.120
thiamine hydrochloride	0.285
riboflavin	0.285
niacin	1.270
calcium pantothenate	0.855
pyridoxine hydrochloride	0.285
choline chloride	21.270
inositol	1.420
folic acid	0.258
biotin	0.006
menadione	0.634
vitamin B_{12} (0.1% mix)	0.480
alphacel	53.194

Table 2-A

DIETARY PROTEIN REQUIREMENTS OF RATS
assuming a source of dietary protein of high quality (B.V. 100)

(Warner, 1962)

	Maintenance adult requirement	Growth requirement
mg. protein/gross Calorie	10	57-20 (average: 29)
% net protein in the diet (air dry basis)	4	28-10 (average: 12)
grams net protein/day		
- female	0.52	1.2 - 1.9
- male	0.76	1.1 - 2.4

Table 3-A

INITIAL WEIGHTS

(grams per rat)

Level of exercise	Replicate	Group 0				Group 2				Group 4				Row totals
		D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	279	295	282		275	285	308		316	265	313		
		287	302	293		302	286	298		260	293	287		
					1738				1754				1734	5226
	II	264	272	272		261	261	255		250	267	260		
		270	250	265		262	250	247		252	258	260		
					1593				1536				1547	4676
	Sub-total	1100	1119	1112	3331	1100	1082	1108	3290	1078	1083	1120	3281	9902
E_0 (idle)	I	292	286	275		279	283	297		271	277	269		
		276	301	287		285	276	299		306	301	311		
					1717				1719				1735	5171
	II	262	262	256		251	256	260		251	249	245		
		257	259	263		256	251	264		264	259	249		
					1559				1538				1517	4614
	Sub-total	1087	1108	1081	3276	1071	1066	1120	3257	1092	1086	1074	3252	9785
Column totals		2187	2227	2193	6607	2171	2148	2228	6547	2170	2169	2194	6533	19687

Table 4-A

FINAL WEIGHTS

(grams per rat)

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	303	312	247		329	348	265		341	313	281		
		310	305	291		335	301	292		298	350	272		
					1768				1870				1855	5493
	II	285	294	241		327	302	229		304	295	246		
		292	241	249		315	288	227		286	285	237		
					1602				1688				1653	4943
	Sub-total	1190	1152	1028	3370	1306	1239	1013	3558	1229	1243	1036	3508	10436
E_0 (idle)	I	319	279	260		291	284	293		295	310	227		
		322	353	299		344	334	266		334	320	231		
					1832				1812				1717	5361
	II	323	306	240		280	300	243		288	273	228		
		283	293	249		325	292	244		312	282	224		
					1694				1684				1607	4985
	Sub-total	1247	1231	1048	3526	1240	1210	1046	3496	1229	1185	910	3324	10346
Column totals		2437	2383	2076	6896	2596	2449	2059	7054	2458	2428	1946	6832	20782

Table 5-A

HEART WEIGHTS (fresh)

(milligrams per rat)

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	936	809	588		866	864	742		921	834	806		
		838	979	911		816	814	849		853	1014	785		
					5061				4951				5213	15225
	II	959	888	807		944	929	696		892	1000	833		
		822	769	736		917	931	696		826	891	704		
					4981				5113				5146	15240
	Sub-total	3555	3445	3042	10042	3543	3538	2983	10064	3492	3739	3128	10359	30465
E_0 (idle)	I	848	812	700		832	761	807		809	816	653		
		799	847	713		806	806	790		730	882	722		
					4719				4802				4612	14133
	II	815	933	756		832	909	797		868	895	730		
		879	846	807		871	835	707		798	832	686		
					5036				4951				4809	14796
	Sub-total	3341	3438	2976	9755	3341	3311	3101	9753	3205	3425	2791	9421	28929
Column totals		6896	6883	6018	19797	6884	6849	6084	19817	6697	7164	5919	19780	59394

Table 6-A

LIVER WEIGHTS (dry, fat-free)

(milligrams per rat)

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_3	Sub-totals	D_1	D_2	D_3	Sub-totals	D_1	D_2	D_3	Sub-totals	
E_1 (exercised)	I	1895	1692	1347		2617	2036	1800		2846	1878	1984		
		2184	1545	1467		2043	1818	1561		1994	2965	1845		
					10130				11875				13512	35517
	II	1867	1747	1359		2207	2393	1741		2137	2247	1462		
		1625	1543	1374		2337	1783	1627		1761	2106	1264		
					9514				12088				10977	32579
	Sub-total	7571	6527	5546	19644	9204	8030	6729	23963	8738	9196	6555	24489	68096
E_0 (idle)	I	2045	1529	1974		2213	2011	2144		2454	1847	1610		
		2103	2423	2116		2492	2338	1830		2315	2285	1692		
					12190				13028				12203	37421
	II	2062	1935	1505		1800	2013	1626		1859	1739	1414		
		1867	1933	1727		1832	2023	1640		2151	2113	1322		
					11029				10934				10598	32561
	Sub-total	8077	7820	7322	23219	8337	8385	7240	23962	8779	7984	6038	22801	69982
Column totals		15648	14347	12868	42863	17541	16415	13969	47925	17517	17180	12593	47290	138078

Table 7-A

MUSCLE WEIGHTS (dry, fat-free)

(milligrams per rat)

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	722	786	623		871	856	687		892	779	710		
		866	775	721		845	775	856		750	796	641		
					4493				4890				4568	13951
	II	870	910	779		951	812	654		886	837	782		
		845	744	732		808	873	717		948	783	693		
					4880				4815				4929	14624
	Sub-total	3303	3215	2855	9373	3475	3316	2914	9705	3476	3195	2826	9497	28575
E_0 (idle)	I	971	822	727		850	847	711		885	815	646		
		886	796	727		841	840	683		791	786	737		
					4929				4772				4661	14362
	II	947	884	775		855	805	739		917	927	698		
		922	867	770		929	836	770		815	829	664		
					5165				4934				4849	14948
	Sub-total	3726	3369	2999	10094	3475	3328	2903	9706	3408	3357	2745	9510	29310
Column totals		7029	6584	5854	19467	6950	6644	5817	19411	6884	6552	5571	19007	57885

Table 8-A

BLOOD SERUM NITROGEN (1)

(grams %)

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	1.18	1.23	1.03		1.26	1.04	1.01		1.21	1.16	1.01		
		1.16	1.04	1.10		1.34	1.19	1.15		1.16	1.24	0.93		
					6.74				6.99				6.71	20.44
	II	1.11	1.09	0.91		1.07	1.03	0.80		1.16	0.95	0.86		
		1.04	0.99	0.88		1.11	0.96	0.94		1.22	0.88	0.86		
					6.02				5.91				5.93	17.86
	Sub-total	4.49	4.35	3.92	12.76	4.78	4.22	3.90	12.90	4.75	4.23	3.66	12.64	38.30
E_0 (idle)	I	1.19	1.07	1.16		1.30	1.29	0.99		1.07	1.08	1.05		
		1.23	1.16	1.01		1.18	1.23	1.10		1.19	1.16	0.90		
					6.82				7.08				6.45	20.35
	II	1.02	0.99	0.87		1.06	1.03	0.93		1.09	0.89	0.77		
		1.12	0.99	0.85		1.08	0.99	- *		1.08	1.09	0.85		
					5.84				—				5.77	—
	Sub-total	4.56	4.21	3.89	12.66	4.62	4.54	-	—	4.43	4.22	3.57	12.22	—
Column totals		9.05	8.56	7.81	25.42	9.40	8.76	-	—	9.18	8.45	7.23	24.86	—

* missing value.

(1) corrected for NPN

Table 9-A

BLOOD SERUM ALBUMIN / GLOBULIN RATIO

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	0.54	0.48	0.38		0.67	0.62	0.35		0.83	0.69	0.30		
		0.54	0.56	0.41		0.64	0.56	0.53		0.58	0.80	0.44		
					2.91				3.37				3.64	9.92
	II	0.82	0.58	0.48		0.68	0.49	0.38		0.85	0.66	0.52		
		0.81	0.60	0.49		0.65	0.43	0.44		0.72	0.53	0.56		
					3.78				3.07				3.84	10.69
	Sub-total	2.71	2.22	1.76	6.69	2.64	2.10	1.70	6.44	2.98	2.68	1.82	7.48	20.61
E_0 (idle)	I	0.63	0.46	0.60		0.70	0.55	0.42		0.75	0.49	0.47		
		0.63	0.61	0.63		0.57	0.48	0.47		1.01	1.08	0.41		
					3.55				3.19				4.21	10.95
	II	0.52	0.50	0.38		0.77	0.73	0.41		0.85	0.68	0.57		
		0.75	0.58	0.46		0.82	0.58	-*		0.81	0.65	0.50		
					3.20				-				4.06	-
	Sub-total	2.53	2.15	2.07	6.75	2.86	2.34	-	-	3.42	2.90	1.95	8.27	-
Column totals		5.24	4.37	3.83	13.44	5.50	4.44	-	-	6.40	5.58	3.77	15.75	-

* Missing value.

Table 10-A

MUSCLE NITROGEN

(grams %)

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_2	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	14.72	14.45	14.51		14.63	14.48	14.45		14.63	14.35	14.54		
		14.22	14.54	14.63		14.47	14.36	14.79		14.36	14.83	14.63		
					87.07				87.18				87.34	261.59
	II	15.22	14.93	14.87		14.82	15.34	14.92		15.34	15.44	15.47		
		14.92	15.26	14.84		14.06	15.16	14.62		14.82	15.70	14.98		
					90.04				88.92				91.75	270.71
	Sub-total	59.08	59.18	58.85	177.11	57.98	59.34	58.78	176.10	59.15	60.32	59.62	179.09	532.30
E_0 (idle)	I	14.89	14.41	14.11		14.76	14.57	14.80		14.53	14.35	14.52		
		14.62	14.29	14.18		14.70	14.71	14.39		14.64	14.79	14.69		
					86.50				87.93				87.52	261.95
	II	15.28	15.52	14.72		14.84	15.01	15.19		15.12	15.78	15.08		
		15.11	14.94	14.84		14.83	14.87	14.59		15.25	15.23	14.50		
					90.41				89.33				90.96	270.70
	Sub-total	59.90	59.16	57.85	176.91	59.13	59.16	58.97	177.26	59.54	60.15	58.79	178.48	532.65
Column totals		118.98	118.34	116.70	354.02	117.11	118.50	117.75	353.36	118.69	120.47	118.41	357.57	1064.95

Table 11-A

MUSCLE DNA

(milligrams %)

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	430	343	437		447	370	450		420	400	417		
		413	467	427		407	450	430		377	380	407		
					2517				2554				2401	7472
	II	397	430	437		450	460	453		420	440	447		
		417	430	417		427	420	420		400	440	447		
					2528				2630				2594	7752
	Sub-total	1657	1670	1718	5045	1731	1700	1753	5184	1617	1660	1718	4995	15224
E_0 (idle)	I	430	447	460		440	400	420		400	470	470		
		400	453	370		413	417	447		450	417	473		
					2560				2537				2680	7777
	II	413	407	447		400	387	470		390	390	437		
		440	420	470		430	367	447		427	397	447		
					2597				2501				2488	7586
	Sub-total	1683	1727	1747	5157	1683	1571	1784	5038	1667	1674	1827	5168	15363
Column totals		3340	3397	3465	10202	3414	3271	3537	10222	3284	3334	3545	10163	30587

Table 12-A

MUSCLE NITROGEN / DNA RATIO

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	34.2	42.1	33.2		32.7	39.1	32.1		34.8	35.9	34.9		
		34.5	31.1	34.3		35.6	31.9	34.4		38.1	39.0	35.9		
					209.4				205.8				218.6	633.8
	II	38.3	34.7	34.0		33.0	33.4	32.9		36.5	35.1	34.6		
		35.8	35.5	35.6		32.9	36.1	34.8		37.1	35.7	33.5		
					213.9				203.1				212.5	629.5
	Sub-total	142.8	143.4	137.1	423.3	134.2	140.5	134.2	408.9	146.5	145.7	138.9	431.1	1263.3
E_0 (idle)	I	34.6	32.3	30.7		33.5	36.4	35.3		36.3	30.5	30.9		
		36.6	31.1	38.3		35.6	35.3	32.2		32.5	35.5	31.1		
					203.5				208.2				196.8	608.5
	II	37.0	38.1	32.9		37.1	38.8	32.3		38.8	40.5	34.5		
		34.3	35.6	31.6		34.5	40.5	32.6		35.7	38.4	32.4		
					209.6				215.9				220.3	645.8
	Sub-total	142.5	137.1	133.5	413.1	140.7	151.0	132.4	424.1	143.3	144.9	128.9	417.1	1254.3
Column totals		285.3	280.5	270.6	836.4	274.9	291.5	266.6	833.0	289.8	290.6	267.8	848.2	2517.6

Table 13-A

SKIN NITROGEN

(grams %)

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	15.71	15.46	15.90		15.90	14.97	15.27		15.84	15.15	15.82		
		15.12	15.44	15.87		15.73	15.66	15.67		15.39	15.21	15.52		
					93.50				93.20				92.93	279.63
	II	16.58	15.92	16.22		16.38	15.59	16.65		16.14	16.34	16.59		
		15.75	16.07	16.37		16.75	15.79	15.98		16.40	16.45	16.71		
					96.91				97.14				98.63	292.68
	Sub-total	63.16	62.89	64.36	190.41	64.76	62.01	63.57	190.34	63.77	63.15	64.64	191.56	572.31
E_0 (idle)	I	15.73	15.48	15.35		16.09	15.98	16.11		15.55	14.65	15.32		
		15.35	15.14	15.72		15.24	16.08	15.94		15.47	15.65	16.10		
					92.77				95.44				92.74	280.95
	II	16.07	15.74	15.71		16.09	16.23	16.52		16.24	16.08	16.13		
		15.85	16.45	16.23		16.65	16.16	16.46		16.74	16.56	16.47		
					96.05				98.11				98.22	292.38
	Sub-total	63.00	62.81	63.01	188.82	64.07	64.45	65.03	193.55	64.00	62.94	64.02	190.96	573.33
Column totals		126.16	125.70	127.37	379.23	128.83	126.46	128.60	383.89	127.77	126.09	128.66	382.52	1145.64

Table 14-A

SKIN DNA

(milligrams %)

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D1	D2	D3	Sub-total	D1	D2	D3	Sub-total	D1	D2	D3	Sub-total	
E_1 (exercised)	I	623	577	667		667	650	680		683	650	648		
		680	680	563		693	705	460		607	650	647		
					3790				3855				3885	11530
	II	627	690	607		710	737	640		853	850	710		
		713	650	630		623	637	567		697	823	700		
					3917				3914				4633	12464
	Sub-total	2643	2597	2467	7707	2693	2729	2347	7769	2840	2973	2705	8518	23994
E_0 (idle)	I	600	637	607		620	622	562		623	667	700		
		637	713	556		607	665	670		673	700	673		
					3750				3746				4046	11542
	II	703	657	600		670	667	670		710	717	743		
		710	607	647		747	710	617		670	683	683		
					3924				4081				4206	12211
	Sub-total	2650	2614	2410	7674	2644	2664	2519	7827	2676	2767	2809	8252	23753
Column totals		5293	5211	4877	15381	5337	5393	4866	15596	5516	5740	5514	16770	47747

Table 15-A

SKIN NITROGEN / DNA RATIO

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	25.2	26.8	23.8		23.8	23.0	22.5		23.2	23.3	24.4		
		22.3	22.7	28.2		22.7	22.2	34.1		25.4	23.4	24.0		
					149.0				148.3				143.7	441.0
	II	26.4	23.1	26.7		23.1	21.2	26.0		18.9	19.2	23.4		
		22.1	24.7	26.0		26.9	24.8	28.1		23.5	20.0	23.9		
					149.0				150.1				128.9	428.0
	Sub-total	96.0	97.3	104.7	298.0	96.5	91.2	110.7	298.4	91.0	85.9	95.7	272.6	869.0
E_0 (idle)	I	26.2	24.3	25.3		25.9	25.7	28.7		24.9	22.0	21.6		
		24.1	21.2	28.3		25.1	24.2	23.8		23.0	22.4	23.9		
					149.4				153.4				137.8	440.6
	II	22.9	24.0	26.1		24.0	24.3	24.6		22.9	22.4	21.7		
		22.3	27.1	25.1		22.3	22.8	26.7		25.0	24.2	24.1		
					147.5				144.7				140.3	432.5
	Sub-total	95.5	96.6	104.8	296.9	97.3	97.0	103.8	298.1	95.8	91.0	91.3	278.1	873.1
Column totals		191.5	193.9	209.5	594.9	193.8	188.2	214.5	596.5	186.8	176.9	187.0	550.7	1742.1

Table 16-A

SKIN COLLAGEN NITROGEN

(grams %)

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	D_1	D_2	D_3	Sub-total	
E_1 (exercised)	I	10.85	11.27	11.07		9.96	9.75	10.21		10.00	9.41	10.74		
		9.71	10.70	9.51		9.00	9.95	10.31		10.43	9.51	10.31		
					63.11				59.19				60.40	182.70
	II	9.91	9.44	9.76		10.52	10.80	10.75		9.95	10.02	9.55		
		11.07	10.57	10.89		11.71	10.56	11.63		10.84	10.32	11.25		
					61.64				65.96				61.93	189.53
	Sub-total	41.54	41.98	41.23	124.75	41.19	41.06	42.90	125.15	41.22	39.26	41.85	122.33	372.23
E_0 (idle)	I	11.23	9.41	10.61		11.37	10.82	10.68		10.23	9.86	9.17		
		10.82	9.45	11.17		9.49	10.66	11.14		9.64	10.05	11.09		
					62.69				64.16				60.04	186.89
	II	10.81	10.46	11.52		11.98	10.67	10.80		9.78	9.95	10.88		
		9.80	10.88	10.64		11.07	10.52	11.18		11.38	11.23	11.45		
					64.11				66.22				64.67	195.00
	Sub-total	42.66	40.20	43.94	126.80	43.91	42.67	43.80	130.38	41.03	41.09	42.59	124.71	381.89
Column totals		84.20	82.18	85.17	251.55	85.10	83.73	86.70	255.53	82.25	80.35	84.44	247.04	754.12

Table 17-A

SKIN NON-COLLAGEN NITROGEN / DNA RATIO

Level of exercise	Replicate	Group 0 (R_0)				Group 2 (R_2)				Group 4 (R_4)				Row totals
		D1	D2	D3	Sub-total	D1	D2	D3	Sub-total	D1	D2	D3	Sub-total	
E_1 (exercised)	I	7.80	7.27	7.24		8.90	8.03	7.44		8.55	8.84	7.83		
		7.95	6.97	11.31		9.71	8.10	11.65		8.16	8.77	8.06		
					48.54				53.83				50.21	152.58
	II	10.64	9.41	10.64		8.26	6.50	9.24		7.25	7.44	9.92		
		6.55	8.45	8.69		8.08	8.22	7.68		7.99	7.45	7.79		
					54.38				47.98				47.84	150.20
	Sub-total	32.94	32.10	37.88	102.92	34.95	30.85	36.01	101.81	31.95	32.50	33.60	98.05	302.78
E_0 (idle)	I	7.50	9.53	7.81		7.63	8.29	9.65		8.55	7.18	8.66		
		7.11	7.98	8.18		9.47	8.16	7.16		8.66	8.00	7.44		
					48.11				50.36				48.49	146.96
	II	7.49	8.03	6.98		6.13	8.34	8.55		9.10	8.55	7.07		
		8.53	9.17	8.64		7.46	7.94	8.56		8.00	7.82	8.34		
					48.84				46.98				47.88	143.70
	Sub-total	30.63	34.71	31.61	96.95	30.69	32.73	33.92	97.34	34.31	31.55	30.51	96.37	290.66
Column totals		63.57	66.81	69.49	199.87	65.64	63.58	69.93	199.15	66.26	64.05	64.11	194.42	593.44

STATEMENT OF CLAIM OF ORIGINAL WORK

Previous work had shown that prolonged muscular exercise affects the nitrogen status of rats, as indicated by changes in labile nitrogenous components of the body and even in muscle DNA, which is generally considered stable. Moreover, it had been demonstrated that alternative periods of exercise and rest have a more pronounced effect on body nitrogen than a single period of exercise. The hypothesis formulated in the present study is that rest may be the factor that allows some otherwise hidden changes in body nitrogen to become noticeable or to be intensified, and that the level of dietary protein may influence the results. The disclosure of these changes, the length of time necessary for their appearance, and the time required for the same body components to return to pre-exercise levels are the object of the present investigation.

The author claims as an original contribution to knowledge the following observations that form part of the experimental work on which this thesis is based:

1 - observations on the immediate effects of a 56-day continuous period of exercise on the collagen content of skin;

2 - observations on the delayed effects (after 2 or 4 weeks of rest) of a 56-day continuous period of exercise and of the simultaneous effect of exercise and dietary protein level on:

   a) feed efficiency and nitrogen efficiency ratios;

   b) total serum proteins and serum albumin/globulin ratio;

   c) levels of nitrogen and of DNA in skeletal muscle;

   d) levels of nitrogen, collagen nitrogen, and DNA in skin.