of a transpeptidation reaction, leading to the formation of an *N*-substituted trityrosine intermediate, followed by hydrolysis.

4. Peptic digestion of *N*-benzyloxycarbonyl-L-glutamyl-L-tyrosine led to the formation of tyrosine, tyrosyltyrosine and *N*-benzyloxycarbonyl-L-glutamic acid. *N*-Benzyloxycarbonylglutamyltyrosyltyrosine appeared as intermediate. A scheme describing the hydrolysis and transpeptidation reactions taking place in this case was constructed with the help of results obtained with *N*-benzyloxycarbonyl-L-glutamyl-L-tyrosyl-L-tyrosine and *N*-benzyloxycarbonyl-L-glutamyltri-L-tyrosine on digestion with pepsin.

5. Peptic digestion of *N*-benzyloxycarbonyl-L-phenylalanyl-L-tyrosine yielded tyrosine and tyrosyltyrosine, the latter being the result of a transpeptidation reaction.

6. Incubation of benzyloxycarbonyl-L-glutamyl-L-tyrosine amide with pepsin yielded *N*-benzyloxycarbonylglutamic acid and tyrosine amide. No transpeptidation was observed with this substrate.

7. The compounds *N*-benzyloxycarbonyl-L-phenylalanine, *N*-benzyloxycarbonyl-L-glutamic acid, *N*-benzyloxycarbonyl-L-tyrosine and *N*-benzyloxycarbonylglycine were shown to act as acceptors for the terminal tyrosine residue of the substrates, *N*-benzyloxycarbonyl-L-tyrosyl-L-tyrosine, *N*-benzyloxycarbonyl-L-glutamyl-L-tyrosine, acting as donors in the presence of pepsin.

8. Analysis of the pepsin-catalysed transpeptidation reaction showed that it involves the transfer of a tyrosine amino moiety of a donor to an acceptor with a free carboxyl group. It may thus be classified as a transpeptidation of the amino transfer type.

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Biochemical Adaptation as a Response to Exercise

1. EFFECT OF SWIMMING ON THE LEVELS OF LACTIC DEHYDROGENASE, MALIC DEHYDROGENASE AND PHOSPHORYLASE IN MUSCLES OF 8-, 11- AND 15-WEEK-OLD RATS

By M. K. GOULD AND W. A. RAWLINSON

Department of Biochemistry, University of Melbourne, Australia

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Although adaptation to physical training has received extensive study from the aspects of performance and physiological effects (Åstrand, 1956), there is little information on this effect at the cellular level. It is significant that the comprehensive review of enzyme adaptation by Knox, Auerbach & Lin (1956) makes no reference what-

ever to enzymic changes accompanying athletic training.

Most of the published work in this field stems from the Leningrad Physical Culture Research Institute (Yakovlev, 1950; Yampolskaya & Yakovlev, 1951; Yampolskaya, 1952). These workers claim that muscle analyses after training have

shown increases in the concentrations of glycogen, phosphagen, ascorbic acid and glutathione, and increases in the 'phosphorolytic activity' and in the activity of hexokinase, lactic dehydrogenase and succinic dehydrogenase. On the other hand, Hearn & Wainio (1956) found no change in the concentration of succinic dehydrogenase in both heart and skeletal muscle of rats subjected to a more strenuous exercise regimen than that for those of the Russian workers. They concluded that the concentration of this enzyme was normally sufficient to cope with all demands on the animal.

The present paper is the first report of studies being undertaken in this Laboratory to explore further the effects of training. Lactic dehydrogenase, malic dehydrogenase and phosphorylase were chosen as enzymes of importance in the energy-producing system of rat muscle. In addition we have extended the work to include the possible effects of growth and maturity upon the adaptive response.

In their review Knox *et al.* (1956) stress the importance of the reference base when studying adaptation. Considering that a change in activity measured relative to the protein mass constitutes enzymic adaptation, we have selected non-collagen nitrogen as the most satisfactory basis in these initial tests.

EXPERIMENTAL

Materials

Distilled water prepared in all-glass apparatus was used throughout. Reduced coenzyme I (DPNH) was from Nutritional Biochemicals Corp., U.S.A. Pyruvic acid (British Drug Houses Ltd.) was triply distilled under reduced pressure. Oxaloacetic acid was synthesized according to the method of Wohl & Oesterlin (1901) and stored at -15° . Immediately before use a 0.03 M-solution in 0.06 M- NaHCO_3 was prepared. Cysteine hydrochloride and glycogen were from British Drug Houses Ltd., glucose 1-phosphate (dipotassium salt) from L. Light and Co. Ltd. and adenosine 5'-phosphate from Roche Products Ltd. All solutions were stored at -15° .

Methods

Male rats of the Wistar strain were used. One week before experiments began, litter mates were sorted into two groups of approximately equal weights, each group comprising nine animals. Food and water were freely available throughout the experiments. The animals were fed on the normal colony diet, a commercial cube preparation (Barastoc Products, Melbourne, Australia) having the following stated percentage composition: carbohydrate, 50; protein, 24; fat, 7; salts, 1; crude fibre, 4; moisture, 10; and including supplements to provide an adequate vitamin supply.

Three series of experiments were conducted on animals 8, 11 and 15 weeks old. As one animal of the second series died after earmarking, its corresponding control was removed, reducing both groups to eight animals. At their

specified ages animals of the experimental groups commenced a programme of exercise consisting in swimming for 30 min. daily, for six 5-day weeks, in water at $27-30^{\circ}$. Body weights were recorded each week. Twenty-four hours after completing their exercise programme the animals were stunned by a blow on the head and decapitated. Muscle was quickly removed from the biceps region of the hind leg and chilled in ice. A '1:50' homogenate was prepared by slicing about 400 mg. of tissue thinly with a scalpel and blending it in 50 vol. of water in a Potter-Elvehjem apparatus (Potter & Elvehjem, 1936). Care was taken to ensure uniformity of sampling when pipetting such homogenates.

Lactic dehydrogenase assay. The spectrophotometric method of Wroblewski & La Due (1955) was employed, with a Beckman DU spectrophotometer. However, to ensure adequate substrate levels it was necessary to increase the concentration of sodium pyruvate in the cuvette to 3.3 mM. A portion (5.0 ml.) of the 1:50 homogenate was diluted to 250 ml. and 0.1 ml. of this dilute homogenate was incubated at 25° in the cuvette with 0.25 ml. of DPNH solution (1 mg./ml.) and 2.45 ml. of 0.05 M-phosphate buffer, pH 7.2 (28 vol. of 0.05 M- KH_2PO_4 + 72 vol. of 0.05 M- K_2HPO_4). After 20 min. the reaction was started by the addition of 0.2 ml. of 0.05 M-sodium pyruvate, pH 7.2, and the decrease in extinction at 340 m μ followed at 1 min. intervals. One dehydrogenase unit is defined as the amount of enzyme which brings about a decrease in extinction of 0.001/min. under the above conditions; the rate of change in extinction is proportional to the concentration of enzyme for changes in extinction less than 0.04/min.

Malic dehydrogenase assay. A spectrophotometric method adapted from that of Ochoa (1955) was employed. The assay differed from that described above for lactic dehydrogenase only in that 0.2 ml. of 1:2500 homogenate was assayed and 0.25 ml. of 0.03 M-sodium oxaloacetate served as the substrate. The final volume of contents in the cuvette was again 3.0 ml.; the rate of change in extinction is proportional to the concentration of enzyme if the rate does not exceed 0.025/min.

Phosphorylase assay. The method of Illingworth & Cori (1953) was employed with slight modifications as follows: 5.0 ml. of the 1:50 homogenate was mixed with 10.0 ml. of 0.08 M-sodium glycerol β -phosphate buffer, pH 6.8, containing NaF at a concentration of 0.4 M. A portion (2.0 ml.) of this enzyme-buffer mixture was diluted with an equal volume of a freshly neutralized (pH 6.8) solution of 0.06 M-cysteine. A portion (0.5 ml.) of this suspension was incubated at 30° with 0.3 ml. of 3.3% (w/v) glycogen for 10 min., and the reaction started by the addition of 0.2 ml. of solution containing 64 mm-glucose 1-phosphate and 5 mm-adenylic acid (pH 6.8). At 5, 10 and 15 min., samples (0.2 ml.) were taken and assayed for inorganic phosphate by the method of Fiske & Subbarow (1925), in an EEL (Evans Electroelenium Ltd.) photoelectric colorimeter with the red OR1 filter. Activity was expressed in the units of Illingworth & Cori (1953).

Non-collagen nitrogen. Non-collagen nitrogen was separated by the method of Lilienthal, Zierler, Folk, Buka & Riley (1950). The alkali extract was digested according to the method of Chibnall, Rees & Williams (1943) and the ammonia in the digest estimated, with the micro-still described by Archibald (1943). The details were as follows:

5.0 ml. of 1:50 homogenate was mixed with 50 ml. of 0.05 N-NaOH, left overnight at 21° and filtered. A portion (5.0 ml.) of this extract was digested on an electrically-heated rack overnight with 2 ml. of concentrated H₂SO₄ and 0.5 g. of selenium catalyst. The digest was diluted to 50 ml., 5.0 ml. samples were neutralized in the still with 2.5 ml. of 4N-NaOH and, after a 6 min. distillation, the distillate was diluted to 40 ml. To 10 ml. samples, 0.5 ml. of Nessler's solution was added and, after 5 min., the colour read in the EEL photoelectric colorimeter with the blue OB10 filter.

RESULTS

Table 1 lists details of initial weights and weight gains. In all cases the exercised animals gained considerably less weight than did their corresponding controls. This weight-difference appeared as early as 1 week after exercise had commenced and was maintained throughout the experiment in all the age groups.

The results of the enzyme assays are shown in Table 2. If the enzyme activities of the exercised groups are compared with those of the controls, in only two cases—the lactic dehydrogenase levels of the 8-week-old series and the phosphorylase of the 15-week-old series—are statistically significant differences shown. In both these cases the degree of significance ($P < 0.05$) is considered insufficient to constitute a positive effect. Similarly, no differences were found between the corresponding groups of the three series.

DISCUSSION

The results are in agreement with those of Hearn & Wainio (1956), both as regards weight changes and the absence of any change in enzyme concentration. That this absence of change is not merely an indication of an inadequate exercise programme is shown by the weight differences induced (Hearn & Wainio, using identical exercise, also demonstrated increased weights of heart ventricle and adrenal) and the fact that the training programme employed by the Russian workers (Yakovlev, 1950) was less strenuous than that used in this study. The animals in the Russian experiments were exercised for 30 days by swimming for 1 min. on the first day, this increasing by 1 min./day to a total of 30 min. on the final day.

The cause of the disparity between the Russian results, on the one hand, and those of Hearn & Wainio and ourselves, on the other, would appear to be the difference in assay methods employed. The techniques of short-duration, optimum-condition assay described above result in activities which are proportional to the concentration of enzymes studied. The techniques used in the Russian experiments involve long incubations with relatively large samples of tissue brei. It is doubtful whether the activities so obtained were proportional to enzyme concentration, but would depend rather on the presence of endogenous substrates and other

Table 1. *Average initial weights and weight gains of male Wistar rat control groups and groups exercised by swimming 30 min. daily for 6 weeks*

Age (weeks)	Group	No. of animals	Initial wt. (g.) (mean $\pm$ s.e.)	Wt. gain (g./week) (mean $\pm$ s.e.)
8	Exercised	9	193 $\pm$ 16	9.9 $\pm$ 2.7
	Control	9	191 $\pm$ 19	16.3 $\pm$ 3.7
11	Exercised	8	274 $\pm$ 15	8.6 $\pm$ 3.1
	Control	8	272 $\pm$ 20	12.7 $\pm$ 3.6
15	Exercised	9	316 $\pm$ 21	3.7 $\pm$ 1.3
	Control	9	309 $\pm$ 35	8.6 $\pm$ 3.3

Table 2. *Enzyme levels in skeletal muscle of male Wistar rats*

For details and definition of units see Methods.

Age at start of experiment (weeks)	Group	No. of animals	Lactic dehydrogenase (mean $\pm$ s.e.†)	Malic dehydrogenase (mean $\pm$ s.e.†)	Phosphorylase (mean $\pm$ s.e.†)
8	Exercised	9	13.7 $\pm$ 7.0*	6.1 $\pm$ 1.4	128.0 $\pm$ 14.6
	Control	9	10.7 $\pm$ 4.0	5.1 $\pm$ 2.5	123.6 $\pm$ 11.3
11	Exercised	8	12.5 $\pm$ 4.8	4.5 $\pm$ 1.3	123.0 $\pm$ 10.5
	Control	8	12.2 $\pm$ 4.2	5.3 $\pm$ 2.1	125.8 $\pm$ 15.2
15	Exercised	9	13.8 $\pm$ 4.3	4.9 $\pm$ 1.1	132.0 $\pm$ 9.2*
	Control	9	12.1 $\pm$ 4.4	4.8 $\pm$ 1.4	122.1 $\pm$ 9.1

* Exercised *vs.* control: $P < 0.05$.

† Units/mg. of non-collagen nitrogen.

† Units (thousands)/mg. of non-collagen nitrogen.

substances in the tissue samples, which might act as activators or inhibitors. Such activities would reflect more the overall metabolic potential of the disrupted muscle. Here perhaps lie the reasons for the apparent contradiction in results. For while the Russian results indicate that the metabolic potential of muscle has been enhanced by training, thus demonstrating biochemically a phenomenon which has been well defined physiologically, the concentrations of enzymes intimately concerned with the utilization of carbohydrate energy sources remain unaltered. The metabolic activity of the muscle would then depend on the mobilization of the enzyme systems by control mechanisms. Yampolskaya (1952) has shown that the 'phosphorolytic activity' (that is the hexose phosphate produced from glycogen after a 2 hr. incubation at 37°) of muscle taken from trained rats is considerably greater than that from untrained animals. After the rats had swum for 3 min. both values were increased. When the animals were anaesthetized with Amytal, thus depressing the effect of the central nervous system, the resting value for the trained animal fell to a figure only slightly greater than that of the control (which remained unaltered). After the anaesthetized animals had undergone electrically-stimulated exercise, similar in intensity to the 3 min. swim, the values for both trained and untrained rats were only slightly elevated. These results indicate the extent to which control mechanisms may be responsible for the activity increases reported by the Russian workers. Thus the adaptive responses would appear to involve regulatory mechanisms rather than any change in enzyme levels.

SUMMARY

1. Male rats (Wistar strain) 8, 11 and 15 weeks old were exercised by swimming 30 min. daily for six five-day weeks.
2. The exercised animals gained significantly

less weight than their control groups. Muscle assays revealed no differences in the levels of lactic dehydrogenase, malic dehydrogenase and phosphorylase, either between exercised or control groups or between the three different age groups.

3. While other workers have shown that training enhances the metabolic activity of muscle, the constancy of enzyme levels reported here suggests that the adaptive response involves other controlling factors.

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Biochemical Adaptation as a Response to Exercise

2. ADENOSINE TRIPHOSPHATASE AND CREATINE PHOSPHOKINASE ACTIVITY IN MUSCLES OF EXERCISED RATS

By W. A. RAWLINSON AND M. K. GOULD

Department of Biochemistry, University of Melbourne, Australia

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In continuation of our previous work (Gould & Rawlinson, 1959), the effect of training on two of the enzymes concerned with the process of muscular contraction, adenosine triphosphatase and creatine phosphokinase, was studied. As both of these

enzymes require calcium for maximum activity, availability of Ca^{2+} ions might serve as a means of regulating their activity [DuBois & Potter (1943) suggest that this is true for adenosine triphosphatase], and so the activities of these enzymes