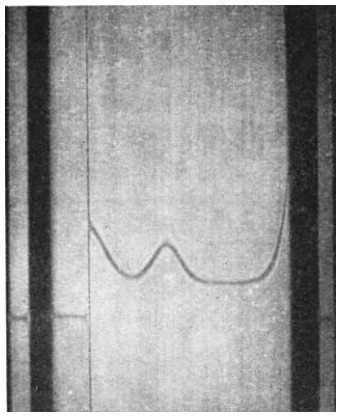

Fig. 1. Ultracentrifuge pattern of *Torulopsis* lipase. Picture taken 120 min after 60,000 r.p.m. was reached. The computed S_{20w} is 3·4 *S*

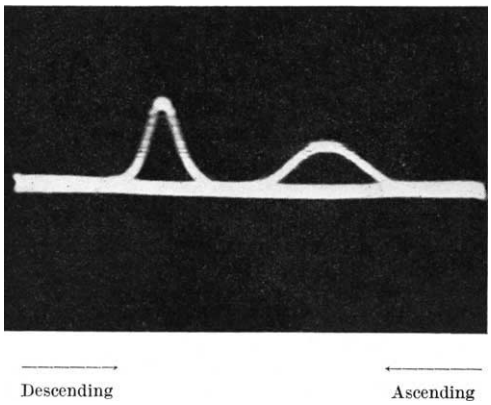

Fig. 2. Electrophoretic pattern of *Torulopsis* lipase at *p*H 6·9 (6 m.amp, 4 h)

broth. The extracellular lipase was also obtained in a highly purified state from the culture broth by the use of ammonium sulphate fractionation, DEAE-cellulose chromatography and 'Sephadex *G*-100' gel filtration. The highly purified enzyme preparation was found to be essentially homogeneous by such criteria as sedimentation in the ultracentrifuge and free electrophoresis.

The culture grown overnight on peptone medium was intensively aerated for 40 h in a soybean medium at 30° C. The lipase activity was assayed at *p*H 6·5 by the method of Yamada *et al.*[11]. The culture supernatant was precipitated at 60 per cent saturated ammonium sulphate, and precipitate was centrifuged at 4,000 r.p.m. for 30 min. The precipitate was then dissolved in 0·01 M phosphate buffer, *p*H 5·9, and dialysed against the same buffer for 2 days at 5° C. The dialysate was absorbed on DEAE-cellulose at *p*H 5·9. After washing with 0·1 M sodium chloride in 0·01 M phosphate buffer, *p*H 5·9, the DEAE-cellulose bed was extracted with 0·2 M sodium chloride in the same buffer. This fraction was dialysed against 0·01 M phosphate buffer, *p*H 5·9, and subjected to chromatography on DEAE-cellulose (linear salt gradient, 0–0·15 M sodium chloride in 0·01 M phosphate buffer, *p*H 5·9, column dimension 2 × 60 cm, flow rate 30 ml./h). Chromatography of the dialysed lipase fraction on DEAE-cellulose was then performed again, under the same conditions. The effluent fraction, partially purified with DEAE-cellulose, was dialysed against 0·01 M ammonium acetate buffer, *p*H 4·9, and lyophilized. Further purification of this partially purified enzyme preparation was attempted using a 'Sephadex *G*-100' column (3 × 70 cm) buffered with 0·01 M ammonium acetate buffer, *p*H 4·9, at a flow rate of 5 ml./h. The yield of the lipase through this purification procedure was more than 30 per cent and the specific activity was about 230 times that of the initial solution.

Analysis of the purified lipase performed on the ultracentrifuge at 60,000 r.p.m. in acetate buffer, *p*H 5·05, at a concentration of 2 per cent showed a single component with an S_{20w} of 3·4 *S* (Fig. 1).

Electrophoretic analysis of the purified lipase showed apparent homogeneity at *p*H 2·0, 3·0, 5·1 and 6·9. Fig. 2 shows the electrophoretic pattern at *p*H 6·9. The isoelectric point has been found to be below *p*H 2·0 in acetate buffer.

These experiments with purified lipase indicate that the enzyme has the following properties and specificity: the *p*H optimum for hydrolysis of some substrates (tributyrin, triolein, olive oil) is 6·5; the lipase is quite stable at *p*H 3–8 at 37° C for 1 h; temperature optimum is 45° C; the lipase is stable at temperatures of up to about 65° C at *p*H 5·0 for 10 min, but rapid inactivation occurs at temperatures above 70° C. The order of hydrolysis rate of some triglycerides by highly purified lipase is as follows: tributyrin > triolein > tristearin > tripalmitin > triacetin, trilaurin. Triacetin and trilaurin were not hydrolysed at all. The lipase activity can be inhibited by such metal ions as Fe^{++}, Fe^{+++}, Cu^{++}, Cd^{++}, Ag^{+} (0·005 M, *p*H 5·0). Meanwhile, the enzyme activity can be inhibited by cyanogen bromide, *N*-bromosuccinimide, iodoacetic acid, bromoacetic acid, diazobenzene sulphonic acid and sodium lauryl sulphate. Photo-oxidation of lipase with ultra-violet light in the presence or absence of a catalyst (such as methylene blue or riboflavin) resulted in the rapid inactivation of the enzyme. It seems that the activity of lipase requires methionine and histidine as essential groups.

A detailed account of the isolation and properties of this unique protein will appear elsewhere.

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PHYSIOLOGY

Muscle Glycogen Synthesis after Exercise: an Enhancing Factor localized to the Muscle Cells in Man

IT is well known that glycogen is utilized during muscular work, but there is very little information available about the resynthesis of glycogen after exhaustive exercise. Goldstein[1] has shown that a humoral factor, which decreases the blood glucose concentration, is released during exercise. Furthermore, it is known that the insulin requirement decreases in diabetic subjects during exercise.

In a recent study of the resynthesis of muscle glycogen after exercise (glycogen values down to zero) we found that a carbohydrate-rich diet increased the muscle-glycogen content far above the basal values (1·0–2·0/100 g fresh muscle). In order to clarify whether a humoral factor similar to the Goldstein factor or a local factor in the muscle cells is in operation, we performed the following experiment.

Two normal subjects (J. B. and E. H.) were placed on either side of a bicycle ergometer. Exercise was performed with one leg while the other leg was in complete rest. Thus J. B. worked with his left leg and E. H. worked simultaneously with his right leg. The load was 1,200 kpm/min. Periods of exercise from 5 to 20 min were interrupted by rest periods of the same duration. The exercise was continued until both subjects were completely exhausted (that is, unable to work continuously for more than 1 min). After this procedure, biopsies of the quadriceps femoris muscles of both legs were performed, and the glycogen content was determined in the specimens[2–4]. During the rest of the day and on the following two days both subjects consumed a diet which consisted almost exclusively of carbohydrates. J. B.'s caloric intake was 2,200 kcal per day (35 kcal/kg body-weight), and E. H.'s was 2,600 kcal per day (33 kcal/kg).

Muscle biopsies on both legs were performed in the morning after 1, 2 and 3 days on a carbohydrate diet, and glycogen was determined in the muscle specimens. A comparison of the exercised leg with the 'resting' leg should disclose any local enhancement of the glycogen resynthesis.

The results are shown in Fig. 1.

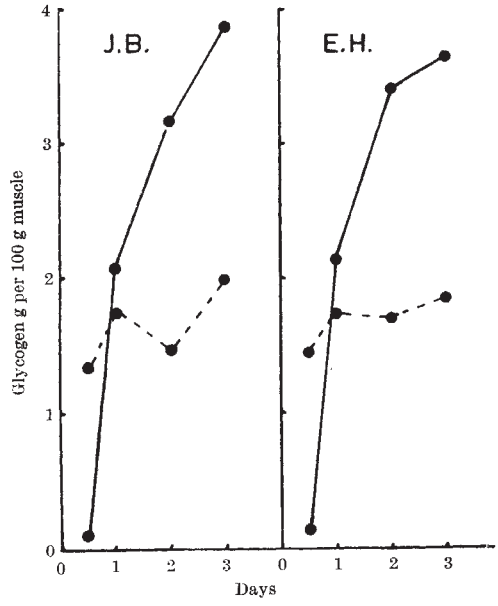

Fig. 1. Glycogen content in exercised (———) and 'resting' (— — — —) leg. Biopsies were performed in both legs of the two subjects at approximately the same time (within 15 min) on each occasion

The muscle glycogen content in the exercised leg was very low immediately after exercise, while the resting leg had a normal glycogen content. The effect of the carbohydrate diet was drastic. After 1 day the glycogen content in the exercised leg had risen from practically zero to higher values than in the resting leg, where only a small increase of the glycogen content was found. The glycogen content of the exercised leg continued to rise dramatically during the following 2 days on the carbohydrate diet, until the values were about twice as high as those in the other leg. The glycogen changes showed the same pattern and were of the same magnitude in both subjects.

From this experiment it can be concluded that exercise with glycogen depletion enhances the resynthesis of glycogen. The factor operates locally in the exercised muscle and the effect persists for at least 3 days.

The nature of the mechanisms involved is unknown. It could be that a stimulation of one or more of the factors directly involved in glycogen synthesis takes place or that an effect is provided on the cell membrane, stimulating glucose uptake. It is possible that some of the beneficial effects of exercise in normal as well as in diabetic subjects are mediated by this factor, thus promoting the storage of carbohydrate as glycogen instead of fat.

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Soluble Saccharin Preference: a Nutritive Basis for Persistence

ANIMALS will drink more of a saccharin solution than water, and will run a maze or bar-press for a saccharin reward. It has been suggested that since saccharin is non-nutritive, such behaviour may be hedonic rather than drive reducing[1]. Sheffield and Roby, and Carper and Polliard maintain that the taste of saccharin is a primary re-inforcer, because hungry animals do not extinguish their response to it as would be expected for an acquired reward related to drive or need reduction[2–4].

Since it is the sodium salt of saccharin ($C_6H_4CONSO_2Na$. 'soluble' saccharin) which is regularly used, the validity of ignoring nutritive effects is questionable. The Na^+ ion is an essential food item, and rats and mice on the usual laboratory chow show a preference for weak sodium chloride solutions to water[5,6]. Thus the persistence in preferring sodium saccharin (Na-saccharin) to water might depend on the re-inforcing value of sodium.

The 'insoluble' saccharin ($C_6H_4CONHSO_2$) is sufficiently soluble to sweeten water to the extent used in motivational studies[7]. It was expected that if the Na^+ ion is indeed a factor in preference, animals should respond differentially to equimolar Na-saccharin and saccharin. Accordingly, eighteen *NIH/N* albino male mice were tested with one or the other of these solutions. Preference was determined by the two-bottle choice technique, the experimental solution paired with water. Consumption was measured to the nearest 0·1 ml. daily. Sides were alternated every other day and stainless steel drinking tubes were alternated daily. In this way the results were controlled for side preference and/or tube preference. Animals had 'Purina' laboratory chow constantly available.

The results are summarized in Figs. 1 and 2. Equimolar concentrations ($8·2 \times 10^{-3}$ M) of saccharin and Na-saccharin are each preferred to water ($P < 0·001$). The Na-saccharin group maintained a high level of preference over a three-week interval (Fig. 1) and maintained a significantly increased ($P < 0·001$) daily fluid intake (Fig. 2). On the other hand, mice on saccharin showed a decreasing preference, and total fluid consumption (which initially rose) was soon equal to the volume taken when water was in both drinkers. Following this period, all the mice received water from both drinkers for three days. Then the total fluid consumed by both groups was the same (Fig. 2).

On the 23rd day concentrations were doubled ($1·6 \times 10^{-2}$ M) for both the saccharin and Na-saccharin animals. The next day both groups showed preference for the