bile salts (pancreatic lipase characteristics) and being inhibited by protamine and pyrophosphate (lipoprotein lipase characteristics). 1M-NaCl either activated, inhibited or had no effect, depending on the conditions of assay. By the use of specific inhibitors for one or other enzyme, the differing optimum pH values for each of the two types of lipolytic activity were demonstrable. From these observations it might be assumed that both preparations consisted of a mixture of two separate enzymes. However, incubation with NaF and protamine in combination did not result, as would be expected in this case, in complete inhibition, but merely in the degree of inhibition equal to that caused by protamine alone. Similarly, in the presence of Ca^{2+} and albumin, where 1M-NaCl gave 40% inhibition (characteristic of lipoprotein lipase) and fluoride 65% inhibition (characteristic of pancreatic lipase), the inhibition in the combined presence of NaF and 1M-NaCl was only 69%. These observations suggest that the two types of lipolytic activity are mediated by a single enzyme.

Further evidence for this was obtained by using Sephadex chromatography. Each pancreatic preparation gave a single chromatographic peak with a molecular weight of 800 000 when run through Sephadex G-200, but after acetone treatment a delipidized product in each case gave a second peak of much smaller molecular weight (15 000–20 000). This molecule also demonstrated the mixed lipolytic activities associated with the parent molecule.

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Fat-Body Lipogenesis during Development of the Adult Male Desert Locust

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Changes in fat-body lipogenesis measured by acetate incorporation into lipid and changes in associated enzymes have been followed during development of the adult male desert locust (*Schistocerca gregaria*). Lipid accumulates in the fat-body during the first 8 days after emergence, the major period of accumulation occurring between the sixth and the eighth day. There is a rapid increase in $[\text{U-}^{14}\text{C}]$ acetate incorporation into lipid between the fifth and seventh day after a period of intensive feeding between emergence and 5 days. Similar results were obtained for $[\text{U-}^{14}\text{C}]$ glucose, $[\text{U-}^{14}\text{C}]$ trehalose and $[1,5\text{-}^{14}\text{C}_2]$ citrate incorporation into lipid. The incorporation of $[1,5\text{-}^{14}\text{C}_2]$ citrate into lipid implies the functioning of the citrate-

cleavage pathway in the conversion of carbohydate into lipid.

'Malic' enzyme, which is considered to be important in the production of NADPH for lipogenesis in mammalian tissues (Kornacker & Ball, 1965), increases in activity between emergence and 4 days, exhibiting a pronounced peak at 4 days. This pattern is similar to those of phosphofructokinase and pyruvate kinase, which both increase in activity after emergence, and phosphofructokinase in particular has a pronounced peak of activity at 4 days. Isocitrate dehydrogenase (NADP-dependent) and glucose 6-phosphate dehydrogenase, which may also be important in the production of NADPH for lipogenesis, increase in activity between emergence and 2 days, the increased activity being maintained until after 8 days. ATP citrate lyase is the key enzyme in the citrate-cleavage pathway. The activity of this enzyme is constant between emergence and 4 days and then increases to give a peak of activity between the sixth and the eighth day. The developmental pattern for this enzyme is very similar to the pattern of acetate incorporation into lipid.

The scheme of Kornacker & Ball (1965) involving coupling of ATP citrate lyase and 'malic' enzyme requires parallel changes in activity of these enzymes during changes in lipogenesis. Since there is no correlation between the changes in activity of ATP citrate lyase and 'malic' enzyme during development it is unlikely that this pathway operates in the locust. 'Malic' enzyme may function to produce malate from pyruvate during increased glycolysis. Intramitochondrial oxaloacetate derived from the malate could be used to condense with acetyl-CoA to provide citrate for lipogenesis. NADPH for lipogenesis may be provided by glucose 6-phosphate dehydrogenase and isocitrate dehydrogenase.

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Rat Liver β -Xylosidase, a Lysosomal Membrane Enzyme

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β -Xylosidase activity, as determined with *o*-nitrophenyl and *p*-nitrophenyl β -D-xylopyranosides, is widely distributed in animal tissues

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(Fisher, Whitehouse & Kent, 1967). In rat liver this enzyme is concentrated in a particulate fraction (A. J. Barrett & J. T. Dingle, cited by Fisher, Higham, Kent & Pritchard, 1966). Treatment of an aqueous suspension of a mitochondrial-lysosomal pellet, prepared by centrifuging at 10 000g for 20 min., freezing and thawing, sonication or Triton X-100 (0.1%, v/v) followed by recentrifugation, resulted in most of the β -xylosidase activity remaining in the supernatant, together with the β -galactosidase activity, assayed with *o*-nitrophenyl β -D-galactopyranoside (Sellinger, Beaufay, Jacques, Doyen & de Duve, 1960). This release of β -xylosidase was prevented when the pellet was treated in citrate-phosphate buffer, pH 5 (McIlvaine, 1921), whereas the release of β -galactosidase was unimpaired. It is suggested that the β -xylosidase is a membrane-bound enzyme that can, under certain conditions, be apparently solubilized.

The β -xylosidase has been partially purified by use of this effect (Higham, Kent & Fisher, 1968). It was obtained as a clear solution with a specific activity about 300 times that of the tissue and was not sedimented by centrifugation at 10⁵g for 60 min.

Some properties of this soluble preparation have been investigated. A single enzyme hydrolyses *o*-nitrophenyl and *p*-nitrophenyl β -xylosides and also *p*-nitrophenyl β -D-glucopyranoside as shown by the competitive inhibitor glucono-1,4-lactone, giving similar K_i values when measured against the three substrates (0.38, 0.25 and 0.25 mM respectively), and by mixed-substrate experiments (Thorn, 1949). This agrees with the suggestion by Beck & Tappel (1968) that the lysosomal membrane β -glucosidase (assayed with *p*-nitrophenyl glucoside) and β -xylosidase are one enzyme. It should be noted that the preparation had only slight β -glucosidase activity towards *o*-nitrophenyl β -glucoside.

The β -xylosidase had no action on synthetic xylose peptides containing the β -O-xylosyl-serine or -threonine linkage. Incubation of the enzyme with β -O-xylosides of serine, threonine, glycyl-serine, seryl-glycine and glycylseryl-glycine at pH 3.5, 5 and 7 at 37° for 24 hr. gave no release of xylose as judged by the methods of Park & Johnson (1949) and Benedict (1931). This is in marked contrast with an activity present in *Helix pomatia* digestive juices, which will hydrolyse these compounds (Fisher & Kent, 1968).

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Acid Phosphatases in Rat Heart

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Since mammalian heart contains few lysosomes (Tappel, Sawant & Shibko, 1963), this tissue would not be expected to contain a large amount of acid phosphatase, a typical lysosomal enzyme (de Duve, 1963). To test this hypothesis, male adult Sprague-Dawley rats were killed and their hearts were chopped with scissors and ground in acid-washed sand with 1 ml. of 0.28 M-sucrose-EDTA, pH 7.2 (Cleland & Slater, 1953). The homogenate was filtered through muslin, 40 vol. (v/w) of sucrose-EDTA was added, and the whole was centrifuged at 2400 rev./min. in an MSE 18 High Speed centrifuge (69181 angle rotor) at 4° for 10 min. The pellet was discarded and the supernatant was centrifuged at 20 000 rev./min. in a Spinco model L preparative ultracentrifuge (N 50 rotor) for 30 min. The pellet was resuspended in sucrose-EDTA and frozen and thawed six times before the enzyme assays were performed. Acid phosphatase activity was measured by incubating samples of the pellet and of the supernatant at 38° for 20 min. in 100 mM-acetate buffer containing 5 mM-disodium *p*-nitrophenyl phosphate (Kilsheimer & Axelrod, 1957) or 10 mM- β -glycerophosphate as substrate. In the latter case, inorganic phosphorus was determined by the method of Chen, Toribara & Warner (1956). Sodium fluoride was used as an inhibitor, and the chlorides of Mg²⁺, Ca²⁺, Na⁺ and K⁺ were used as activators, at concentrations between 1 and 100 mM. Proteins were determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

With *p*-nitrophenyl phosphate as substrate the activity in the supernatant was 2243 nmoles of *p*-nitrophenol liberated/hr./mg. of protein, and was four times that of the pellet. Its pH optimum was between 5.6 and 5.8. The activity was slightly enhanced by 10 mM-Mg²⁺; it was unaffected by K⁺ or Na⁺, slightly inhibited by 10 mM-Ca²⁺ and only