acid and lysine of the walls but little or no diaminopimelic acid. Hydrolysis of the extract gave glucose, identified with Glucostat reagent, and two or three other sugars, which have not yet been identified. No structure was detected after treatment of walls (5mg.) with papain (2mg. in 5ml. of 0·05M-phosphate buffer, pH 7·0) or with trypsin (2mg. in 5ml. of 0·05M-phosphate buffer, pH 7·0).

Walls (100mg.) from the 31hr. culture were suspended in 6M-urea (50ml.) at 20° for 1hr. and then recovered by centrifuging as before. The urea extract was dialysed against distilled water and freeze-dried. Electron microscopy of the residual walls showed that some but not all of the structure had been removed. The extract contained thin layers or strings of structural material (see Plates 1*c* and 1*d*). Prolonged extraction (20° for 30hr.) caused complete disintegration of the walls.

Walls (77mg.) were suspended in water (100ml.) at 100° for 25min. They were then centrifuged and freeze-dried; these 'boiled' walls (71mg.) did not show fine structure. A sample (23·7mg.) was stirred with 6M-urea solution (12ml.) at 20° for 1hr. The remaining walls were centrifuged and washed with water (yield, 12mg.); areas possessing regular structure were observed on some of these walls.

These observations show that, contrary to the earlier suggestion (Baddiley, 1964; cf. Martin, 1966), the structured component of the cell wall is not a typical mucopeptide. The removable material is rich in aspartic acid and lysine and contains little or no muramic acid, glucosamine or diaminopimelic acid. It is not yet known whether the neutral sugars present in extracts are associated with the protein or polypeptide, or whether these are present in a separate polysaccharide component. The disappearance of regular structure on boiling, and its subsequent partial reappearance on brief treatment with urea, suggest that heating may cause reversible changes in the arrangement of the structured component.

Murray & Nermut (personal communication) have recently examined the structured layer in strains of *B. polymyxa* and will describe their results elsewhere.

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Glycerol Kinase Activities in Rat Heart and Adipose Tissue

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Wieland & Suyter (1957) reported that glycerol kinase (ATP–glycerol phosphotransferase, EC 2.7.1.30) was absent from adipose tissue and heart muscle of the rat. These authors assayed glycerol kinase activity by coupling to α-glycerophosphate dehydrogenase and measuring NAD reduction at pH 9·5. During studies on glycerol kinase from rat liver the *in vitro* half-life of this enzyme at 25° was found to be only 6·5min. at pH 9·8, compared with 3·5hr. at pH 7·5. It therefore seemed possible that failure to demonstrate even a low activity of glycerol kinase in adipose tissue and muscle might have been due to rapid inactivation by the high pH of the assay conditions. Recently Newsholme, Robinson & Taylor (1967) described a sensitive radiochemical technique for measuring the activity of glycerol kinase, and this method has now been used to demonstrate the presence of this enzyme in rat adipose tissue and heart.

Rat hearts and epididymal fat pads were cut into small pieces and homogenized in 2vol. of 1% (w/v) KCl and 1mM-EDTA at 2° in a Silverson homogenizer (Silverson Machines Ltd., London, S.E. 1)

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Table 1. *Glycerol kinase activities in rat heart and adipose tissue, and the effects of AMP, ADP, DL-α-glycerophosphate and ATP-regenerating systems*

The glycerol kinase activities in homogenates and supernatants were assayed as described in the text. The glycerol concentration was 0·05 mM for supernatant experiments, 1·0 mM for experiments with adipose-tissue homogenates and 0·05–1·0 mM for heart homogenates; the ATP (or UTP) concentration was 6·0 mM. In Expts. 3, 6 and 7 the enzymes creatine phosphokinase and pyruvate kinase were present at a final concentration of 0·16 mg./ml.; in Expt. 7, 4 mM-K^+ was included in the incubation medium. In Expt. 3, glycerol kinase activities are reported as means ± S.E.M., with the numbers of animals used in parentheses. For supernatants each activity represents the mean of three determinations with a reproducibility of 8%.

	Nucleotide substrate				Glycerol kinase activity	
Expt. no.	ATP	UTP	Additions to incubation medium	Final concn. of addition (mM)	Heart (μmoles/g. fresh wt./hr.)	Adipose tissue
(*a*) Homogenate						
1	+		—	—	0·19	0·12
2		+	—	—	0·28	0·12
3	+		Creatine phosphate (and creatine phosphokinase)	10	0·65 ± 0·05 (4)	0·24 ± 0·03 (3)
					(counts/min. incorporated into product/min./20 μl. of supernatant)	
(*b*) Supernatant						
4	+		—	—	111	103
5		+	—	—	148	94
6	+		Creatine phosphate (and creatine phosphokinase)	10	240	125
7	+		Phosphoenolpyruvate (and pyruvate kinase)	5	228	121
8	+		AMP	0·5	77	81
9	+		AMP	1·0	64	73
10	+		AMP	2·5	42	55
11	+		ADP	1·0	85	69
12	+		ADP	2·5	62	50
13	+		ADP	5·0	51	42
14	+		DL-α-Glycerophosphate	5·0	—	19
15		+	DL-α-Glycerophosphate	5·0	21	—

for heart and Teflon homogenizer for adipose tissue. These homogenates were used without further treatment for assay of glycerol kinase activity (Table 1). Supernatants were prepared from homogenates by centrifugation: adipose-tissue homogenate was first centrifuged at 300*g* for 5 min. at 0° and the top layer of lipid was removed; this supernatant and the heart homogenate were then centrifuged at 27 000*g* for 15 min. at 0°. The supernatants were passed through a Sephadex G-25 column immediately before use; the column was equilibrated with assay buffer (without ATP or glycerol).

Samples (20 μl.) of supernatant or homogenate were incubated with assay buffer (final volume 120 μl.), which contained 6 mM-ATP (or UTP), 4 mM-$MgCl_2$, 1 mM-EDTA, 25 mM-NaF, 20 mM-mercaptoethanol, 100 mM-tris at pH 7·5, and various concentrations of [^{14}C]glycerol (specific activity 8·35 or 23·6 μc/μmole). The assay procedure was identical with that of Newsholme *et al.* (1967), except that the DEAE-cellulose papers were counted in a Beckman liquid-scintillation spectrometer (model 1650). The glycerol kinase initial activities reported were obtained from progress curves, which were linear for 4 min. with heart homogenates and for 30 min. with adipose-tissue homogenates at 37° (in the presence of an ATP-regenerating system); with heart homogenates the activity of glycerol kinase was independent of glycerol concentration between 0·05 and 1·0 mM. However, 1·0 mM-glycerol was required for maximum activity of this enzyme in adipose-tissue homogenates (to avoid excessive dilution by tissue glycerol). The progress curves for both supernatants were linear for 30 min. at 37° and the glycerol concentration could be lowered to 0·05 mM without decreasing the activity. No activity was observed in the absence of ATP, or after boiling the extracts for 2 min. α-[^{14}C]Glycerophosphate, whether assayed radiochemically or enzymically, could be recovered quantitatively after incubation with homogenates or supernatants in the incubation buffer (minus glycerol).

The maximum activities of glycerol kinase detected were 0·65 and 0·24 μmole/g. fresh wt. of tissue/hr. at 37° for heart and adipose tissue

respectively (Table 1). Homogenates were preferred for measurements of maximum activity as there was less chance of losing activity during preparation; but homogenate and supernatant fractions generally gave the same total activity. The maximum activities were obtained in assay systems that possessed creatine phosphokinase or pyruvate kinase ATP-regenerating systems. Although UTP is a less effective substrate than ATP for liver glycerol kinase, the activities of the enzymes from heart and adipose tissue are at least as high with UTP as with ATP (in the absence of an ATP-regenerating system). These two observations could be explained by the inhibition of glycerol kinase by AMP and ADP (Table 1); the presence of adenylate kinase (in the extract) results in the formation of AMP from ATP and ADP involved in the glycerol kinase reaction. The replacement of ATP by UTP, or the presence of an ATP-regenerating system, decreases the rate of formation of AMP and ADP during the incubation.

The inhibitions of glycerol kinases from heart and adipose tissue by AMP, ADP and DL-α-glycerophosphate (Table 1) are similar to those observed for glycerol kinase from rat liver (J. Robinson & E. A. Newsholme, unpublished work). Also, the K_m values for glycerol of the enzymes in supernatant fractions from adipose tissue and heart are similar to that of the liver enzyme, i.e. approx. 10^{-5}M. These observations provide evidence that the enzyme activity that is measured radiochemically is in fact glycerol kinase. Further evidence for this has been obtained by measuring the production of α-glycerophosphate enzymically; the glycerol kinase assay was stopped by addition of $HClO_4$, the solution was neutralized with $KHCO_3$ and the α-glycerophosphate was estimated enzymically (Hohorst, 1963) with a recording fluorimeter (Dalziel, 1962). The radiochemical and the fluorimetric assays of glycerol kinase activities in heart and adipose tissue agreed to within 5%.

The inability of Wieland & Suyter (1957) and of Margolis & Vaughan (1962) to detect glycerol kinase in these tissues may be explained by the low activity of this enzyme. The activities reported in the present paper probably represent the lower limit of enzyme activity detectable with the usual spectrophotometric assay, particularly if no precautions were taken to lower the AMP and ADP concentrations in the extract. Treble & Mayer (1963), who used a radiochemical assay method for glycerol kinase, reported an activity of this enzyme in mouse adipose tissue of a similar order to that now reported for rat.

The presence of glycerol kinase activity in heart and adipose tissue calls into question the accuracy of estimations of rates of lipolysis based on glycerol release. In particular, observed changes in the rate of glycerol release from heart and adipose tissue could be explained by changes in the rate of lipolysis, or glycerol kinase activity, or both. In the present investigation precautions were taken to measure maximum glycerol kinase activities, but, in the absence of detailed knowledge of the properties of the muscle or adipose-tissue enzymes, such maximal activities must remain provisional. Nevertheless, if starvation produced inhibition of glycerol-kinase activity in heart and adipose tissue, this could account (assuming the activities in Table 1) for 81% and 15% of the observed increase in glycerol release by the perfused heart and incubated fat pad respectively (Vaughan, 1962; Garland & Randle, 1964). Thus the increased utilization of endogenous fatty acids by the perfused heart from a starved animal could be explained equally well at the present time by a decrease in the activity of glycerol kinase as by an increase in the activity of a lipase. However, the increased rate of release of fatty acids from adipose tissue from starved animals could not be explained completely by decreasing the activity of glycerol kinase; other factors that produce smaller changes in the rate of glycerol release might be explained by effects on this enzyme.

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