TABLE I

OXIDATIONS IN CELL-FREE YEAST EXTRACTS PREPARED WITH THE MILL

3 ml extract (= *ca.* 50 mg dry wt) in phosphate buffer pH 7. 400 μM substrate. Cofactors: $MnCl_2$: 10 μM; DPN: 160 μg; Cyto. *c*: 200 μg; ATP and A5P: 4 μM each.

Substrate	*Cofactors*	μlO_2 *taken up for the following periods from the commencement of reading:*			
		0–10 min	*30–40 min*	*60–70 min*	*110–120 min*
None	$MnCl_2$, DPN, cyto. *c*, ATP, A5P	43	29	18	15
Ethanol	None	265	103	35	11
Ethanol	$MnCl_2$	294	165	106	72
Ethanol	DPN	292	175	57	19
Ethanol	DPN, cyto. *c*	242	206	80	16
Ethanol	DPN, cyto. *c*, $MnCl_2$	254	206	120	87
Ethanol	ATP, A5P	157	114	64	29
Succinate	None	248	132	66	45

The machine may be made in any desired size and used for preparing fairly large quantities of respiring yeast extracts. Although the dry weights of extracts are not as quantitatively reproducible as with the shaker, there is good agreement between individual runs on different days. The machine should also be useful for disintegrating other micro-organisms.

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Amino acid metabolism in locust tissues

BARRON AND TAHMISIAN[1] reported the presence of glutamate/alanine transaminase in cockroach muscle. We have now shown that transaminations between a large number of α-keto- and α-amino-acids are catalysed by preparations of fat-body, malpighian tubules and gut wall of the Desert Locust, *Schistocerca gregaria* Forsk., and have found a possible link between carbohydrate and nitrogen metabolism in the occurrence of glutamic dehydrogenase activity in fat-body.

The enzyme preparations were obtained by dissecting out the appropriate tissue under ice-cold insect Ringer solution and homogenizing in ice-cold phosphate buffer, pH 7.4, using a Potter-Elvehjem homogenizer. For detection of transamination, the enzyme preparations were incubated at 37° C with the appropriate substrates (initial conc. $M/20$) and at a tissue concentration of *c.* 30 mg wet wt./ml; the reactions were followed qualitatively by paper chromatography.

As in mammalian tissues, the alanine/glutamate and aspartate/glutamate reactions were found to be the ones most rapidly catalysed by fat-body preparations, and both have been shown to reach an equilibrium position starting with either amino-donor and keto-acid. These two transaminases have also been shown in malpighian tubules and mid-gut wall preparations. Glutamic acid was also formed, but more slowly, when fat-body preparations and α-ketoglutarate were incubated with each of the following amino-acids: glycine, valine, leucine, cysteine (anaerobically), methionine, threonine, serine, phenylalanine, tryptophane, histidine, arginine, lysine, and ornithine. No glutamic acid was detected when proline or hydroxyproline were used. A very active arginase was present in the fat-body preparations, so that the apparent arginine/glutamate

transamination may have been due to ornithine formed from the arginine. An approximate quantitative estimate of enzymic activity was obtained by running known amounts of glutamate in parallel on the chromatograms, and it was found that glutamate was formed from α-ketoglutarate and aspartate at a rate of about 200 μM/g wet wt. tissue/hour, from alanine at about half this rate and from the other amino-acids at about one tenth the rate. These rates are of the same order of magnitude as those found by AWAPARA AND SEALE[2] for rat kidney. The pH optimum for the glutamate/alanine reaction was 6.5–8, a value similar to that reported for the same reaction in mammalian liver. Pyridoxal phosphate increased the activity of dialysed homogenates and cell fractions, but not of whole homogenates. A fat-body preparation which had been dialysed to remove traces of glutamate and then fortified with pyridoxal phosphate was shown to contain a leucine/alanine transaminase, an enzyme previously found by ROWSELL[3] in vertebrate tissues. Undialysed homogenates would form aspartate from alanine and oxalacetate, but the ability to do so was lost on dialysis and restored on addition of traces of glutamate, so that the aspartate synthesis in this case presumably resulted from the linking of two glutamate-dependent transaminases. A transamination between glutamine and pyruvate was also demonstrated; the enzyme involved appeared to be analogous to the glutaminase II of ERRERA[4], since no glutaminase I activity could be demonstrated.

HIRD AND ROWSELL[5] reported that whereas the glutamate/aspartate and glutamate/alanine transaminations were catalysed by both mitochondrial and soluble fractions of rat liver, the other transaminases were located only on the mitochondria. We have carried out a cell fractionation of fat-body, using a method essentially that of HOGEBOOM, SCHNEIDER AND PALLADE[6] and found the same distribution of activities. The mitochondrial preparations were seen by phase contrast microscopy to be composed mainly of isolated filamentous bodies 1–2 μ long and 0.5 μ wide. The recovery of activity of the whole homogenate in the cell fractions appeared to be quantitative.

We have shown spectrophotometrically that the oxidation of reduced DPN by cytochrome *c* is catalysed by fat-body preparations, and that addition of glutamate brings about the reduction of cytochrome *c* in the presence of DPN and cyanide. Fat body homogenates (20 mg/ml) were centrifuged for a short time at low speed and the lipid layer discarded. Initial concentrations in the incubation mixture were: 0.1 M K phosphate buffer, pH 7.3; glutamate, $1.5 \cdot 10^{-2} M$; DPN $2 \cdot 10^{-4} M$; KCN, $5 \cdot 10^{-4} M$; nicotinamide, 35 μg/ml; cytochrome *c*, $4 \cdot 10^{-6} M$; semicarbazide, 350 μg/ml; and tissue 1.1 mg/ml. The optical density increment at 550 mμ and about 10° C was 0.015/5 min which corresponds to the reduction of $5 \cdot 10^{-10}$ mols of cytochrome *c*/ml. A recovery of 90% of the glutamate/cytochrome *c* reductase activity of the whole homogenate was obtained in the mitochondrial fraction. The mitochondrial system was shown to be DPN specific and to be completely inhibited by antimycin A (1 μg/ml). AGRELL[7] has reported the occurrence of glutamic dehydrogenase in the abdomen of *Calliphora erythrocephala* Meig. and SACKTOR[8] has demonstrated a DPN cytochrome *c* reductase in flight muscle sarcosomes of *Musca domestica* L. HEARFIELD AND KILBY[9] have evidence for the occurrence of some of the enzymes of the citric acid cycle in locust fat-body tissue, so that the present demonstration of a glutamic dehydrogenase suggests that nitrogen metabolism in the fat-body can be linked with carbohydrate metabolism through α-ketoglutarate as in mammalian tissues. Evidence for the operation of the citric acid cycle in whole insect homogenates has been given by various workers (*e.g.*[1,7]).

The occurrence in the insect fat-body of the enzymes mentioned above (and others such as D- and L-amino-acid oxidases which will be reported upon later) supports the view of WIGGLESWORTH[10] that the fat-body may be important, not only in storage, but also in the intermediary metabolism in the insect.

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