constant at 100%. No changes were observed in the median buoyant densities of either NADH- or NADPH-cytochrome *c* oxidoreductase, but the sedimentable fractions of both were decreased (22% and 16% respectively).

The specific activities of these enzymes also showed interesting changes during encystment. Increases were observed in the activities of *N*-acetyl- β -glucosaminidase, catalase and NADH- and NADPH-cytochrome *c* oxidoreductases, whereas acid phosphatase activity decreased throughout encystment. There were no changes in cytochrome oxidase activity.

These results will be discussed in their relation to the physiological and structural changes that are known to occur in amoebae during cellular differentiation.

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An Assay for Liver Glutamine Aminotransferase

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In glutamine aminotransferase reactions 2-oxoglutarate formed is rapidly hydrolysed to 2-oxoglutarate and ammonia (Meister, 1965), and commonly glutamine aminotransferase has been assayed in crude tissue preparations by measuring the increase in ammonia formation from glutamine after keto acid addition, i.e. correcting for glutaminase activity (e.g. Beaton, 1954).

There are disadvantages to this procedure: difficulties may be encountered in measuring small amounts of ammonia; the increase in ammonia production with keto acid may be only a small proportion of that for glutamine alone; and spurious aminotransferase activity will be recorded if glutaminase is activated by the keto acid (see, e.g., Kvamme, Tveit & Svenneby, 1965).

We have assayed by measuring the accumulation of 2-oxoglutarate. Incubations at 37° under nitrogen included 0.02M-L-glutamine, 0.02M-2-oxoisocaproate (4-methyl-2-oxopentanoate), 0.05M-sodium borate buffer, pH 7.8, and liver homogenate. Heat-inactivated samples were analysed spectrophotometrically for 2-oxoglutarate with glutamate dehydrogenase. With ammonia and glutamate dehydrogenase, 2-oxoisocaproate itself slowly re-oxidizes NADH; nevertheless on assaying 2-oxoglutarate standards in the presence of equal

samples of a heat-inactivated aminotransferase incubation linear calibration curves were obtained with the expected increments, measuring extinction decreases (at 340nm.) for the 5 min. period after the addition of glutamate dehydrogenase.

Liver contains glutaminase, and liberated glutamate could produce 2-oxoglutarate by transamination with keto acids, giving an overestimation of glutamine aminotransferase activity. Borate buffer was employed to minimize glutaminase activity (Roberts, 1960), and 2-oxoisocaproate since it is a poor substrate for liver transamination with glutamate but among the most active with glutamine (Meister & Tice, 1950).

But it was essential to obtain some measure of the glutamate contribution. A glutamine aminotransferase incubation was made with rat liver, and a similar incubation without glutamine but with glutamate injected at a rate simulating glutaminase activity (based on rat liver assays). Rates of 2-oxoglutarate formation were 19 and 1.5 μ moles/hr./g. of liver from glutamine and glutamate respectively. With hamster liver comparable rates were 27.5 and 1.8 μ moles/hr./g.

Negligible 2-oxoglutarate production was observed with glutamine or 2-oxoisocaproate as sole substrate.

Formation of leucine (ninhydrin estimation after separation on paper) and 2-oxoglutarate were equivalent in mouse liver assays; to the same extent ammonia formation (glutamate dehydrogenase estimation) exceeded that from glutamine alone.

Linear progress curves were obtained with incubations up to 90 min., giving rates proportional to liver concentration up to 1.5% (w/v) with mouse, rat or rabbit.

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