Escherichia coli A.T.C.C. 12408 by using a spectrophotometer (air blank). Other experimental conditions were similar to those published by Janda & Work (1971) or to those used by Janda *et al.* (1971).

Determination of the groupings in non-fractionated culture supernatants was accurate and reproducible. The standard deviation was $0.6\mu\text{g}$ of a relevant lipopolysaccharide preparation. This preparation was similar to others (S, 0.2–0.4%; glucose, 8.5–9.6%, w/w). Use of the same standard in a phenol- H_2SO_4 test (Knox *et al.*, 1966) for the determination of lipopolysaccharide allowed the comparison of these procedures.

The results agreed if the samples were taken from later stages of lysine-limited fermentations (within 5% relative), but significant discrepancies (up to 25%) were found in samples from earlier stages. The discrepancies between the methods applied to fractions of the fluid samples and of the preparation were up to 95%. Their trend was continuous and followed an elution pattern (the fractions were obtained by molecular sieving on a Bio-Gel 0.5m column, with water as eluent).

Certain fractions of the preparation caused variations in wavelengths of the shift. These could be decreased by urea treatment of the preparation before it was subjected to sieving (no effect on the total grouping content was observed). The remaining variations resembled spectra previously demonstrated (Janda & Work, 1971) for endotoxins representing various stages in biosynthesis of the lipopolysaccharide of *Salmonella*.

Similar spectral variations were detected in certain fractions of all fluids. The variations appeared to be related to fermentation stages, e.g. a fraction causing a secondary shift at 650nm (like glycolipid from *Salmonella minnesota* mR 595) was relatively increased at the end of a lysine-limited fermentation.

The total final grouping content in fluids from exponentially growing cultures (in non-limited fermentations) was about 2% of the content found in the limited ones. There the groupings were accumulated into fluids by cells in an unbalanced growth. Penicillin G (100mg/l added at the start of a stationary growth phase) prevented a secondary growth (see discussion in Work, 1971), but the accumulation continued and reached normal production.

Solvolysis of the preparation (99% dioxan for 60min at 100°C) resulted in loss of the detectable groupings and its toxicity for mice.

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The Interpretation of Abrupt Transitions in Lineweaver-Burk Plots

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Lineweaver-Burk plots for glutamate dehydrogenase (Dalziel & Engel, 1968) and glucose 6-phosphate dehydrogenase (Muto & Uritani, 1970) show abrupt transitions between two or more linear sections. With decreasing values of $1/s$, at each transition the slope increases and the intercept decreases, both approximately twofold. The enzymes appear to pass to forms with successively higher V_{max} and higher K_m . Dalziel & Engel (1968) explained the data for glutamate dehydrogenase by invoking negatively interacting NAD^+ -binding sites. Schwert (1970) doubted whether such a model would give abrupt transitions between sections differing so little in slope and intercept. This prompted reinvestigation of the conditions governing such transitions.

Engel (1970) suggested that eqn. (1) might yield abrupt transitions:

$$\frac{e}{V_0} = \frac{n_0 + n_1 s + n_2 s^2 \dots n_q s^q}{d_1 s + d_2 s^2 \dots d_q s^q} \quad (1)$$

if the values of s giving $n_2 s^2 = n_0$ and $d_2 s^2 = d_1 s$ approximately coincided, and similarly the values giving $n_3 s^3 = n_1$ and $d_3 s^3 = d_2 s^2$ etc. Successive sections would approximately obey:

$$\frac{e}{V_0} = \frac{n_0 + n_1 s}{d_1 s}, \quad \frac{e}{V_0} = \frac{n_1 + n_2 s}{d_2 s} \quad \text{etc.}$$

This situation, in which each term is significant over some range of s , is designated Case 1.

In Case 2 d_2, d_4, d_6 etc. are chosen to render the corresponding terms always small relative to at least one adjacent term. Linear sections obey:

$$\frac{e}{V_0} = \frac{n_0 + n_1 s}{d_1 s}, \quad \frac{e}{V_0} = \frac{n_2 + n_3 s}{d_3 s} \quad \text{etc.}$$

In Case 3 coefficients are chosen to effectively eliminate $d_2 s^2$ and $d_3 s^3$, $d_5 s^5$ and $d_6 s^6$, $d_8 s^8$ and $d_9 s^9$ etc. and also $n_2 s^2$, $n_5 s^5$, $n_8 s^8$ etc. Linear sections obey:

$$\frac{e}{V_0} = \frac{n_0 + n_1 s}{d_1 s}, \quad \frac{e}{V_0} = \frac{n_3 + n_4 s}{d_4 s} \quad \text{etc.}$$

Three conditions for abrupt transitions may be stated: (i) corresponding switches in dominance of terms within numerator and denominator must

approximately coincide; (ii) successive transitions must not overlap; (iii) a transition must coincide approximately with the intersection of the two theoretical lines.

Exploration with a desk-top computer reveals that Cases 2 and 3 can yield single abrupt transitions. Case 1 cannot.

It has been possible to fit the data for glutamate dehydrogenase equally well with either Case 2 or Case 3 equations. Case 2 represents a simpler model and constitutes the minimal assumption needed to account for the data.

Case 2 for glutamate dehydrogenase requires an equation containing terms in S^7 . Physical interpretation of the mathematical deductions raises interesting questions with regard to the catalytic mechanism and the number of NAD^+ -binding sites.

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The Acylation of Endogenous Lysophospholipids in Isolated Rat Liver Microsomal Fraction: Influence of Glutathione and Ethylenediamine-tetra-acetate on this Process

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Although the acylation of various exogenous monoacylphosphoglycerides by rat particles has been extensively studied, less attention has been paid to the acylation of the endogenous lysophospholipids present in these membranes or generated during the incubation. Because lipids integrated in a membrane might not have the same micellar structure as that of similar substances dispersed in a watery phase and might therefore exhibit different susceptibility to enzymes, it was decided to reinvestigate this acylation process by incubating liver microsomal fraction with trace amounts of ^{14}C -labelled fatty acids and the necessary cofactors without the addition of any acyl acceptor.

The incubation conditions were essentially as described by Possmayer *et al.* (1969) except that the concentration of radioactive fatty acids was $4\mu\text{M}$ and that no glycerol 3-phosphate was added; the medium contained 7mg of protein/ml. Measurement of specific radioactivities at the zero time gave an estimate of the dilution factor, for each radioactive tracer, by the endogenous unlabelled fatty acids. The

dilution factor was 12 for palmitate acid and 4 for linoleate. Hence the amount of fatty acid incorporated into phospholipids was much greater than could be simply derived from the quantity of radioactivity recovered in the latter substances and the amount of radioactive fatty acids added to the medium. Over 95% of the labelled linoleate taken up into phosphatidylcholine was present at the 2-position, indicating the good positional specificity of this microsomal system.

Linoleate was incorporated preferentially into phosphatidylcholine, whereas radioactive palmitate taken up into phospholipids was distributed almost equally between phosphatidylcholine and phosphatidylethanolamine. The fairly high percentage of radioactive palmitate incorporated into the fully saturated phosphatidylcholines correlated with the fact that 20% of this labelled saturated fatty acid introduced into total phosphatidylcholines was recovered at the 2-position. This might perhaps reflect the predominance of the 1-acyl isomer of lysophosphatidylcholine over the other lysophosphoglycerides if one assumes that there was no absolute specificity in the acylation, as was suggested by Van Den Bosch *et al.* (1967).

EDTA and GSH were shown to inhibit the acyl transfer. However, when the concentration of these inhibitors was not greater than 5mM, a selective impairment of the transfer of palmitate into phosphatidylethanolamine was usually observed and this was accompanied by an increased incorporation of fatty acids into triglycerides. Cholesterol esterification was strongly inhibited in all cases. Experimental evidence showed that both inhibitors may act indirectly via the stimulation of a lipase, resulting in a noticeable accumulation of various acyl acceptors, which could then compete with the lysophospholipids for the acyltransferase. The triglyceride breakdown induced by EDTA was moderate but did increase the pool of diacylglycerols; on the other hand, GSH markedly stimulated the hydrolysis of triglycerides, causing some monoacylglycerol to accumulate and leading to a significant enlargement of the free fatty acid pool. However, a direct inhibition of the acyl transfer by higher concentration of inhibitors could not be ruled out. The selective decrease of the palmitate incorporation into phosphatidylethanolamine might indicate that the concentration of the 2-monoacyl isomer of lysophosphatidylethanolamine in the microsomal membranes is much smaller than that of the other lysophospholipids.

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