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A COMPARATIVE STUDY OF THE REVERSIBILITY OF THE REACTION CATALYSED BY BACTERIAL LIPOAMIDE DEHYDROGENASE

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SUMMARY

1. Extracts of *Moraxella lwoffi* and *Escherichia coli* were found to contain high levels of diaphorase activity with NADH or NADPH as electron donor and DCIP or ferricyanide as electron acceptor.

2 With *M lwoffi* extracts lipoamide could replace the artificial acceptors with NADH but not NADPH as electron donor No such activity was found with *E coli* extracts.

3 The reversibility of the reaction catalysed by lipoamide dehydrogenase (NADH. lipoamide oxidoreductase, EC 1 6 4 3) was studied in extracts of 12 different species of bacteria

4 The results suggested that there may be a correlation between the fermentative capacity of the organism and the sensitivity of its lipoamide dehydrogenase to inhibition by NADH

5. NAD-linked lactate dehydrogenase (L-lactate: NAD oxidoreductase, EC 1.1 2.3) was present in high levels in extracts of fermenting organisms but weakly active in or entirely absent from those of non-fermenting organisms.

6. It is suggested that both enzymes may be involved in the metabolic control processes which govern the capacity of an organism to adapt to changes in the oxygen tension of its environment.

INTRODUCTION

The discovery by MASSEY[1] that the classic flavoprotein known as "STRAUB's[2] diaphorase" behaves as a powerful lipoamide dehydrogenase has led to the demonstration that the lipoamide dehydrogenase component of the pig heart α-ketoglutarate dehydrogenase complex and STRAUB's diaphorase are one and the same enzyme[3,4] (NADH lipoamide oxidoreductase, EC 1 6 4 3)

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In a comparative study of bacterial diaphorase enzymes extracts of *Moraxella lwoffi* and *Escherichia coli* were fractionated on Sephadex columns and the fractions tested for their ability to oxidise NADH and NADPH using either potassium ferricyanide or 2,6-dichlorophenolindophenol (DCIP) as electron acceptor (J D MCGARRY, unpublished results) It was felt desirable, therefore, to examine these extracts for their ability to oxidise NAD(P)H when lipoamide acted as electron acceptor

The results of these experiments prompted a more detailed investigation of the lipoamide dehydrogenase in extracts of 12 different species of bacteria. This report describes some of the findings of this work

MATERIALS AND METHODS

Organisms—maintenance and growth

The following organisms were used. *Moraxella lwoffi* (NCIB 8250), *Pseudomonas ovalis* Chester (strain used by KORNBERG, GOTTO AND LUND[5]) *Pseudomonas fragi* (NRRL B-25), *Pseudomonas fluorescens*, PC 4 (Achromobacter species used by DAGLEY, CHAPMAN AND GIBSON[6]), *Escherichia coli* strain W (NCIB 8666), *Escherichia coli* strain 106, *Escherichia aurescens* (NCIB 8714), *Escherichia freundii* (NCIB 3735), *Klebsiella aerogenes* (NCTC 418), *Proteus vulgaris* strain L10, *Proteus mirabilis* (NCIB 5887) and *Proteus morganii* (NCIB 232) *Ps fluorescens*, *E coli* 106 and *P vulgaris* L10 were stock laboratory strains All organisms were maintained on nutrient agar slopes at 4° and grown aerobically at 30° in 500-ml batches of Oxoid Nutrient Broth No. 2 (2 5%). Cells were harvested in the late logarithmic phase of growth and washed with ice-cold 0.05 M potassium phosphate buffer (neutralised to pH 7.0 with NaOH)

Preparation of cell-free extracts

Washed cells were suspended in buffer to a density of approx. 25 mg dry weight per ml. The suspension was passed twice through a pre-cooled Aminco pressure cell which produced a pressure of 20 000 lb/inch^2 The resulting extract was centrifuged at 10 000 × *g* and 2° for 20 min to remove unbroken cells and large particulate debris The supernatant fraction (S_1) was centrifuged at 120 000 × *g* for 1 5 h yielding a clear yellow supernatant fraction (S_2).

Although practically free from particulate NADH oxidase (NADH:O_2 oxidoreductase) S_2 from most organisms contained substantial levels of a soluble NADH oxidase Protein concentration of S_1 and S_2 was determined using the biuret method[7] and was usually in the region of 20 mg/ml.

Enzyme assays

Pyruvateoxidase (pyruvate.O_2 oxidoreductase) was measured at 37° with the YSI Biological Oxygen Monitor (Yellow Springs, Ohio). The reaction volume was 3 ml and contained: 0 05 M potassium phosphate buffer (pH 7 0), 0 5 mM NAD^+, 0 1 mM coenzyme A, 0 01 M sodium pyruvate and 0.2–0 5 ml of S_1 The mixture was allowed to stir for 4 min before the probe was introduced and a trace taken. The results have been corrected for oxygen uptake in the absence of pyruvate

The following enzymes were assayed at 340 mμ in the Unicam SP 800 recording spectrophotometer.

NADH oxidase. The test cell contained in a total volume of 3 ml: 0.05 M po-

tassium phosphate buffer (pH 7 0), 0 2 mM NADH and a suitable volume of S_1 The decrease in absorbance was measured against a reference cell containing all components except NADH

Lactate dehydrogenase (L-lactate NAD oxidoreductase, EC 1 1.1 27) In order to eliminate the effect of NADH oxidase the assay was carried out anaerobically This was achieved by gassing the 2 8 ml of reaction fluid for 2 min with O_2-free nitrogen gas before adding 0 2 ml of suitably diluted extract The cuvette cap was immediately placed in position and the reaction followed by recording the decrease in absorbance. The test cell contained in 3 ml: 0 05 M potassium phosphate buffer (pH 7.0), 0 2 mM NADH, 6 6 mM sodium pyruvate and extract The reference cell lacked NADH. Under these conditions no oxidation of NADH took place in the absence of an added electron acceptor

Lipoamide dehydrogenase In the text the term "lipoamide dehydrogenase" has been used to describe the enzyme which catalyses the following reactions

$$NAD^+ + \text{reduced lipoamide} \underset{\text{Reaction 2}}{\overset{\text{Reaction 1}}{\rightleftharpoons}} NADH + H^+ + \text{lipoamide}$$

$$NAD^+ + \text{reduced lipoic acid} \underset{\text{Reaction 4}}{\overset{\text{Reaction 3}}{\rightleftharpoons}} NADH + H^+ + \text{lipoic acid}$$

Experimental conditions are given in legends to figures

MATERIALS

NAD^+, $NADP^+$, NADPH and coenzyme A were purchased from the Boehringer Corporation (London) Ltd NADH, DL-6,8-thioctic acid, DL-6,8-thioctic acid amide and sodium pyruvate were from Sigma London Chemical Co Thioctic acid and thioctic acid amide were converted to their reduced forms by the methods of GUNSALUS, BARTON AND GRUBER[8] and REED *et al.*[9], respectively Both the oxidised and reduced forms were used as 0 04 M solutions in methanol All other reagents were of the highest purity commercially available

RESULTS

Extracts of *M lwoffi* and *E. coli* 106 exhibited high diaphorase activity with all 4 combinations of electron donors (NADH and NADPH) and electron acceptors (ferricyanide and DCIP) When lipoamide replaced these artificial acceptors it was found that *M lwoffi* S_1 and S_2 catalysed equally rapidly the oxidation of NADH, NADPH was oxidised very slowly by comparison No such activity was observed with *E coli* S_1 or S_2 When *M lwoffi* S_2 was subjected to column chromatography on Sephadex G-150 the lipoamide dehydrogenase was totally recovered in the "void volume" and clearly separated from the bulk of the NADH DCIP oxidoreductase activity which was eluted later as 4 peaks The enzyme was not however entirely separated from the NADH:ferricyanide oxidoreductase, which was eluted as 2 peaks of activity

As a result of intensive studies over the past 10 years, notably by L. J REED's group in Austin, Texas and V. MASSEY in Sheffield, England and Michigan, it has become clear that the oxidation of the α-keto acids, pyruvate and α-ketoglutarate, in

both animal tissues and microorganisms is accomplished by multienzyme complexes, a common component of which is the enzyme lipoamide dehydrogenase It seemed odd therefore that Reaction 2 should be active in extracts of *M. lwoffi* and apparently absent from those of *E coli*, especially when S_1 from both organisms could be shown to catalyse the rapid oxidation of pyruvate and so presumably contained the pyruvate dehydrogenase complex

M lwoffi is known to be strictly aerobic in its metabolism and is unable to ferment carbohydrates[10] On the other hand, *E coli* is facultatively anaerobic and its fermentation capacity has been well documented Other work in this laboratory has revealed several important metabolic differences between these 2 classes of bacteria (unpublished data) and so a number of representatives from each class was chosen at random from laboratory stocks and their extracts (S_1) tested for activity in Reaction 2 At the same time such extracts were tested for their ability to oxidise pyruvate and NADH and for their content of NAD-linked lactate dehydrogenase because of the close association of these activities with pyruvate metabolism In Table I the organisms

TABLE I

COMPARISON OF SOME ENZYME ACTIVITIES IN BACTERIAL EXTRACTS

Specific activity is expressed as μmoles of O_2 consumed per h per mg of protein at 37° for pyruvate oxidase and as μmoles of NADH oxidised per h per mg of protein at 20° for others

	Organism	*Specific activity*			
		Pyruvate oxidase	*Lactate dehydrogenase*	*NADH oxidase*	*Lipoamide dehydrogenase (Reaction 2)*
Group 1 (non-fermenters)	*Moraxella lwoffi*	42	0	25	10
	Pseudomonas fluorescens	24	0 6	7 7	5 2
	Pseudomonas ovalis Chester	50	0 4	7 5	7 7
	PC 4	13	0	7 1	10
Group 2 (fermenters)	*Escherichia coli* 106	101	36	17	0
	Klebsiella aerogenes	95	38	12	0
	Proteus vulgaris	102	41	20	0 4

studied have been divided into 2 groups, 1 and 2, on the basis of their fermentative capacity. *Ps ovalis* Chester and *Ps fluorescens* have been classified as non-fermenters by STANIER[11] and Dr P W. TRUDGILL of this department, respectively. PC 4 has also been placed in this class as a result of exhaustive fermentation tests by Dr. MURIEL RHODES of the Botany Dept , University College of Wales, Aberystwyth In contrast to the members of Group 1 those of Group 2 are well-known fermenting organisms and belong to the class of facultative anaerobes

The results show that S_1 of all organisms oxidised pyruvate; surprisingly the fermenters were more active in this respect All oxidised NADH and again, with the exception of *M lwoffi*, the fermenters were more active A striking difference between the 2 groups was revealed by the distribution of lactate dehydrogenase which was present in high levels in the fermenters but weakly active in or entirely absent from the non-fermenters Equally striking was the finding that extracts of the non-fermenters rapidly catalysed Reaction 2 whereas those of the fermenting organisms appeared to be inactive in this respect It must be emphasised that the values quoted for NADH

oxidase, lactate dehydrogenase and lipoamide dehydrogenase are only estimates calculated from initial reaction rates, as it is unlikely that the enzymes were saturated with respect to NADH at the concentration used (0.2 mM) Thus, they may be taken to represent lower limits of activity

Reversibility of lipoamide dehydrogenase reaction

At this stage of the investigation a survey of the literature revealed that several workers using purified lipoamide dehydrogenase from various sources have encountered difficulty in demonstrating Reaction 2, though in all cases Reaction 1 was easily demonstrable (see DISCUSSION). Therefore, the results shown in Table I suggested that in extracts of fermenting organisms the reaction catalysed by lipoamide dehydrogenase can proceed only in the direction of NADH formation (as is considered to be the case *in vivo*) whereas in extracts of the non-fermenters it is freely reversible Accordingly, a second survey was undertaken, this time with a larger number of organisms, in which the S_2 fraction of each extract was tested for its ability to catalyse Reactions 1, 2, 3 and 4 The organisms studied were those listed in Table I together with *Ps. fragi* (shown to be a non-fermenter by Dr P. W TRUDGILL) and a number of additional facultative anaerobes.

As will be seen, extracts of the fermenting species displayed abnormal reaction kinetics, a finding which made it impossible to express the results in a meaningful, quantitative manner However, it became apparent that certain species yielded extracts with remarkable similarities in their reaction kinetics For these reasons the organisms have been grouped as shown below and, because there was little variation in the reactivity between members of the same group, only the results obtained with one member from each group have been quoted The representatives chosen were *M. lwoffi* (Group 1), *P. vulgaris* (Group 2*a*) and *E. coli* 106 (Group 2*b*).

Group 1 (non-fermenting species)	*Group 2 (fermenting species)*	
	a	*b*
Moraxella lwoffi	*Proteus vulgaris*	*Escherichia coli* 106
Pseudomonas ovalis Chester	*Proteus mirabilis*	*Escherichia coli* W
Pseudomonas fluorescens	*Proteus morganii*	*Escherichia aurescens*
Pseudomonas fragi		*Escherichia freundii*
PC 4		*Klebsiella aerogenes*

Reaction 1

Fig 1 shows that at pH 7 0 Group 1 extracts catalysed Reaction 1 at an initially rapid rate which levelled off at a stage representing approx. 45% reduction of the NAD^+ That a true equilibrium had been attained was indicated by the fact that addition of more NAD^+ or reduced lipoamide at this point resulted in a further increase in absorbance. With Groups 2*a* and 2*b* the initial reaction rate was similar to that given by Group 1 but then, in contrast to Group 1, they both adopted a very much slower and prolonged rate. Over the time period used in these assays the rate of NAD^+ reduction in the absence of added reduced lipoamide was negligible

At pH 7 8 reaction rates were faster and the final concentration of NADH

produced was higher than at pH 7 0. This was not surprising since inspection of the equation for Reaction 1 shows that one of the products is a proton and therefore as the pH of the reaction fluid is increased the equilibrium position will lie further in the direction of NADH formation The observations with Group 2 extracts outlined above agree, in general, with those made by HAGER[12], GUNSALUS[13] and KOIKE, SHAH AND REED[14] using the purified enzyme from *E coli*.

Reaction 2

Fig 2 shows that Group 1 extracts catalysed Reaction 2 at a rapid rate to a position representing approx 90% oxidation of NADH In sharp contrast to these, Group 2*b* extracts showed no reaction The behaviour of Group 2*a* extracts appeared

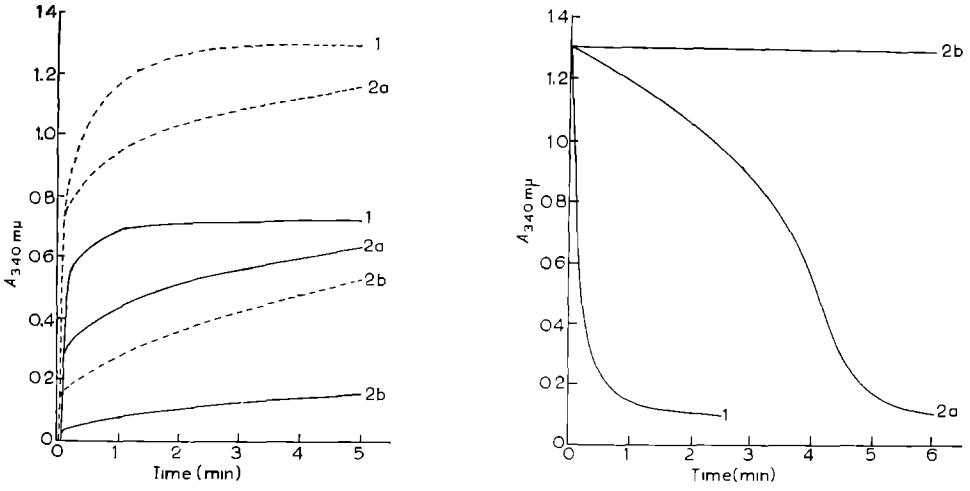

Fig. 1 Reaction 1 Reactions carried out anaerobically The test cell contained in a total vol of 3 ml 0 05 M potassium phosphate buffer, 0 26 mM NAD^+, 1 33 mM reduced DL-lipoamide and 0 2 ml of extract (S_2 fraction, approx 4 mg protein) The reference cell lacked NAD^+ pH of reaction fluid was 7 0 (———) and 7 8 (------) (1), (2*a*) and (2*b*) refer to grouping of extracts (see text)

Fig 2 Reaction 2 Reactions carried out anaerobically The test cell contained in a total vol of 3 ml 0 05 M potassium phosphate buffer (pH 7 0), 0 2 mM NADH, 1 33 mM DL-lipoamide and 0 2 ml of extract (S_2 fraction, approx 4 mg protein) The reference cell lacked NADH (1), (2*a*) and (2*b*) refer to grouping of extracts (see text)

to fall in between that of the other 2 groups, in that the rate of the reaction increased with time, as shown by the sigmoid nature of the recording trace By using different initial concentrations of NADH it could be shown that this lag in the reaction was not a function of time but a result of the inhibitory action of NADH These results are shown in Fig. 3 which also illustrates the effect of increasing the pH of the reaction mixture to 7 8 When the initial concentration of NADH was raised from $2 \cdot 10^{-4}$ M (as in Fig 2) to $2\,6 \cdot 10^{-4}$ M the initial rate of reaction at pH 7 0 was slower and the time taken to reach maximum velocity was longer. On the other hand, when the initial concentration of NADH was decreased to $7\,2 \cdot 10^{-5}$ M the reaction proceeded without lag at an extremely rapid rate This inhibition of the enzyme by higher concentrations

of NADH could be largely relieved by increasing the pH of the reaction fluid to 7 8. KOIKE, SHAH AND REED[14] found a similar effect of pH when studying Reaction 2 with the purified *E coli* enzyme

The inhibitory effect of NADH was most pronounced in Group 2*b* extracts, there being 100% inhibition at a concentration of $2 \cdot 10^{-4}$ M In fact, it was necessary to lower the NADH concentration to the region of $3 \cdot 10^{-5}$ M before a reaction could be observed By carrying out the reaction aerobically using a concentration of NADH which was initially inhibitory it was possible to demonstrate the onset and gradual

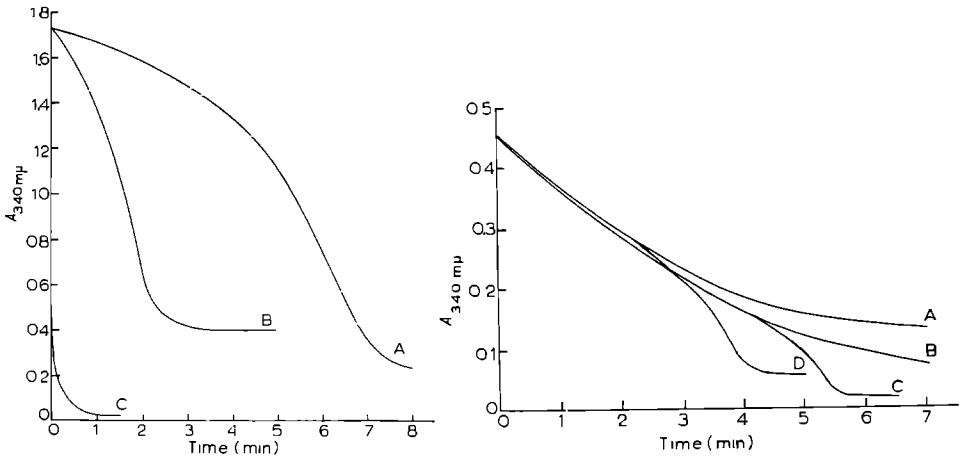

Fig 3. Effect of NADH concentration and pH on Reaction 2 of Group 2*a* extracts Reactions carried out anaerobically $2 \cdot 6 \cdot 10^{-4}$ M NADH and pH 7 0 (curve A), 2 6 10^{-4} M NADH and pH 7 8 (curve B), $7 \cdot 2 \cdot 10^{-5}$ M NADH and pH 7 0 (curve C) Other conditions as in Fig 2

Fig 4 Effect of NADH concentration and pH on Reaction 2 of Group 2*b* extracts Reactions carried out aerobically Initial concentration of NADH was $7 \cdot 2 \cdot 10^{-5}$ M. pH of reaction fluid was 7 0 (curve C) and 7 8 (curve D) Other conditions as in Fig 2 Curves A and B show removal of NADH at pH 7 8 and 7 0, respectively, in the absence of lipoamide

acceleration of Reaction 2 as the NADH was continuously removed through the action of NADH oxidase (Fig. 4) Here again the reaction was favoured by a higher pH.

Reactions 3 and 4

When oxidised or reduced lipoic acid replaced oxidised or reduced lipoamide, respectively, in the assay mixtures it was found that all extracts catalysed Reaction 3 at a slower rate than Reaction 1, though Group 2 extracts were invariably more active in this respect As expected, the effects of changing the pH of the reaction mixtures from 7 0 to 7 8 mirrored those found with Reaction 1 Reaction 4 was catalysed very slowly by Group 1 and not at all by Group 2 extracts even at NADH concentrations in the region of 10^{-6} M

Effect of NAD$^+$ and EDTA on Reaction 2

It has been shown by MASSEY and coworkers (see ref. 15) that the pig heart enzyme has a requirement for NAD$^+$ in Reaction 2. MISAKA, KAWAHARA AND NAKA-

NISHI[16] suggested that upon preincubation with NADH the yeast enzyme is reduced and subsequently inactivated by trace metals contaminating the buffer solution This inactivation could be prevented by chelating agents such as EDTA

Therefore, using extracts of *E coli* and *E. freundii*, Reaction 2 was carried out in the presence of 0.13 mM NAD^+ and 2 mM EDTA Under normal assay conditions no relief of the NADH inhibition could be observed. Unfortunately, higher concentrations of NAD^+ were not tested, had this been done it is possible that the NADH inhibition would have been relieved (see ref 17)

DISCUSSION

As mentioned earlier, investigations have been in progress in this laboratory into certain metabolic differences between the strictly aerobic and facultatively anaerobic bacteria or, more accurately, between the fermenting and non-fermenting species of bacteria An indication that one such difference might be in their relative content of NAD-linked lactate dehydrogenase comes from the results shown in Table I Although there may be numerous reasons why one organism can effect the fermentation of a sugar such as glucose and another not, a major difference between the two types is that the former is equipped to use pyruvate (or a product of the anaerobic metabolism of pyruvate) as electron acceptor for the regeneration of NAD^+ from NADH produced in the Embden–Meyerhoff glycolytic sequence, the latter type of organism is unable to do this A common end product of microbial fermentation is lactic acid which arises from the reaction between NADH and pyruvic acid catalysed by lactate dehydrogenase It might be expected then that the fermenting organism would be more heavily endowed with this enzyme than the non-fermenter Although only a small number of representatives from each class was tested the results obtained would lend support to this view

The reverse relationship held with regard to the ability of extracts to oxidise NADH using lipoamide as electron acceptor (Reaction 2) Since it is generally accepted that *in vivo* this reaction proceeds in the direction of NADH formation (*i e* Reaction 1) these findings suggested that another difference between the two types of bacteria may lie in their ability to catalyse this reaction in the opposite direction Several reports have appeared in recent years describing the inhibitory effect of NADH on lipoamide dehydrogenase The very elegant and intensive studies by MASSEY and coworkers (for review see ref. 15) revealed that the presence of NAD^+ in the reaction mixture is a prerequisite for the pig heart enzyme to show activity in Reaction 2 It appears that the function of NAD^+ is to prevent the NADH from converting the enzyme into its fully reduced form in which state it is catalytically inactive Similar observations were made by MISAKA, KAWAHARA AND NAKANISHI[16] and by WREN AND MASSEY[18] using the purified enzyme from baker's yeast KOIKE, SHAH AND REED[14] produced evidence for the inhibition of the *E. coli* enzyme by NADH and although under certain circumstances they succeeded in demonstrating Reaction 2 they failed to show Reaction 4. NOTANI AND GUNSALUS[19] had previously shown that the *E. coli* enzyme would not catalyse Reaction 4 if the NADH concentration exceeded $6 \cdot 10^{-6}$ M

A more detailed study of Reactions 1–4 with a larger number of organisms yielded results similar in several aspects to those of other workers referred to above For example, extracts of the fermenting organisms catalysed Reaction 1 at an initially

rapid rate which shifted to a much slower and prolonged rate. Moreover, increasing the amount of extract by 4-fold caused little difference in the shape of the reaction trace. These findings suggested that when NADH reached a critical concentration, inhibition of the enzyme took place This concentration was found to be higher for the Proteus species than for the others and in all cases was higher at pH 7.8 than at pH 7 0 (Fig 1) In contrast to these, extracts of non-fermenting organisms catalysed the reaction rapidly to a point of true equilibrium which, as expected, allowed a higher concentration of NADH to be formed at pH 7 8 than at pH 7 0 The differential inhibitory effect of NADH on the bacterial enzyme was demonstrated more clearly in studies of Reaction 2 (Fig 2). In this case NADH at a concentration of $2 \cdot 10^{-4}$ M had no effect on the enzyme from Group 1 organisms, partially inhibited that from Group *2a* and completely inhibited that from Group *2b* Higher concentrations of NADH were tested only with Group *2a* extracts in which case the inhibition was even more severe. When the concentration of NADH was decreased either by putting less in the cuvette (Fig 3) or through the action of NADH oxidase (Fig. 4) the inhibition was relieved. As might have been predicted from the kinetics of Reaction 1, the inhibitory concentration of NADH was found to be much lower with Group *2b* than with Group *2a* extracts The effect on Reaction 2 of increasing the pH from 7 0 to 7.8 was tested on only one member of Group 1, *viz. M lwoffi*, very little difference in reaction rate was observed From thermodynamic considerations this reaction would be favoured by a lower pH and yet the opposite effect was found with Group 2 extracts (*cf* KOIKE, SHAH AND REED[14]) This strange effect remains unexplained

Oxidised and reduced lipoic acid proved to be poor replacements for their amide derivatives when used with Group 1 extracts, but here again there was no evidence for an inhibitory effect of NADH On the other hand, Group 2 extracts, though they were less active in Reaction 3 than in Reaction 1, showed no activity in Reaction 4 even at NADH concentrations in the region of 10^{-6} M However, such low concentrations represented an absorbance in the region of 0 006 at 340 mμ and without more careful work using scale expansion equipment it cannot be said with certainty whether a slow reaction was occurring.

In his review of the properties of lipoyl dehydrogenase enzymes MASSEY[15] suggested that the enzyme from all sources is probably inhibited by excess NADH, the enzymes differing in the degree of inhibition obtained The findings presented here would strongly support this view. Relatively little work has been done on the nature of the inhibition of the bacterial enzyme, nor has it been the author's intention to attempt an explanation of this curious phenomenon Rather, it is considered that, unless a most extraordinary coincidence has occurred with regard to the bacterial species chosen for study and the observed pattern of enzyme activity, the results of the present investigation would signify a correlation between the fermentative capacity of an organism and the sensitivity of its lipoamide dehydrogenase to high concentrations of NADH Indeed, the notion of such a relationship might be strengthened when considered in the light of the report by HANSEN AND HENNING[20] on the regulation of pyruvate dehydrogenase activity in *E coli* by NADH These workers calculated the NADH concentration in aerobically and anaerobically-grown *E coli* to be 0 06 and 0.13 μmole per g wet cell paste, respectively It is noteworthy that in the present investigation the concentration of NADH found to inhibit the lipoamide dehydrogenase from *E. coli* was of the same order of magnitude

Obviously there must exist in the microbe world some metabolic controls other than those governing the formation of cytochromes and respiratory enzymes which determine whether an organism will be able to adapt to changes in the oxygen tension of its environment. As indicated earlier, one such controlling factor may be the organism's content of lactate dehydrogenase Although it can be dangerous to extrapolate from conditions prevailing in experiments with cell-free extracts to those in the intact cell, it is tempting to speculate that the reaction catalysed by lipoamide dehydrogenase constitutes another locus of control

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REFERENCES

1 V MASSEY, *Biochim Biophys Acta*, 30 (1958) 205
2 F B STRAUB, *Biochem J*, 33 (1939) 787
3 V MASSEY, *Biochim Biophys Acta*, 37 (1960) 314
4 R L SEARLS AND D R SANADI, *J. Biol Chem*, 236 (1961) 580
5 H L KORNBERG, A M GOTTO AND P LUND, *Nature*, 182 (1958) 1430
6 S DAGLEY, P J CHAPMAN AND D T GIBSON, *Biochem J*, 97 (1965) 643
7 A G GORNALL, C J BARDAWILL AND M M DAVID, *J Biol Chem*, 177 (1949) 751
8 I C GUNSALUS, L S BARTON AND W GRUBER, *J Am Chem Soc*, 78 (1956) 1763
9 L. J. REED, M KOIKE, M E LEVITCH AND F R LEACH, *J Biol Chem*, 232 (1958) 143
10 C A FEWSON, *J Gen Microbiol*, 46 (1967) 255
11 R Y STANIER, *J Gen Microbiol*, 43 (1966) 159
12 L P HAGER, Ph D Thesis, University of Urbana, Illinois, 1953
13 I C GUNSALUS, in W D MCELROY AND B GLASS, *The Mechanism of Enzyme Action*, Johns Hopkins, Baltimore, 1954, p 545
14 M KOIKE, P C SHAH AND L J REED, *J Biol Chem*, 235 (1960) 1939
15 V. MASSEY, in P D BOYER, H LARDY AND K MYRBACK, *The Enzymes*, Vol 7, Academic Press, New York, 2nd ed, 1963, p 275
16 E MISAKA, Y KAWAHARA AND K NAKANISHI, *J Biochem.*, 58 (1965) 436
17 C H WILLIAMS, JR, *J Biol Chem*, 240 (1965) 4793
18 A WREN AND V MASSEY, *Biochim Biophys Acta*, 122 (1966) 436
19 G W NOTANI AND I C GUNSALUS, *Federation Proc*, 18 (1959) 295
20 R G HANSEN AND U HENNING, *Biochim Biophys Acta*, 122 (1966) 355