the respiratory control ratio has been shown to be affected in rat liver mitochondria[12]. However, it has been recently reported that polyamines inhibited both NADPH-dependant and ascorbate-dependant lipid peroxidation in rat liver microsomes, possibly through binding to phospholipids[13]. The discrepancy with the present observations may be due to the different reaction conditions. The nonenzymic ascorbate-induced lipid peroxidation is widely distributed in the various subcellular fractions of the rat brain, whereas the enzymic NADPH linked peroxidation in brain microsomes is capable of forming only small amounts of lipid peroxides[3]. Also, in our studies peroxidation was initiated by the addition of ascorbic acid alone and nonspecific peroxidation, if any, was eliminated by the use of BHT. In addition, the phospholipid content of rat brain mitochondria is higher (530 nmoles/mg protein) than the reported values of 322 nmoles/mg protein for rat liver microsomes[14]. Moreover, MDA production during the peroxidation of membranes varies among the different types of tissues, chiefly due to the different amounts of PUFA present in the different tissues. In fact, Iwata and coworkers[15] have recently shown stimulation of thiamine diphosphatase activity in rat brain microsomes by ascorbic acid induced peroxidation in membrane lipids. Thus, further studies appear to be warranted to explain the present findings.

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2 E.D. Wills, Biochem. J. *113*, 315 (1969).
3 S. Bishayee and A.S. Balasubramanian, J. Neurochem. *18*, 909 (1971).
4 G.L. Plea and H. Witschi, A. Rev. Pharmac. Tox. *16*, 125 (1976).
5 E.D. Wills, Biochem. J. *123*, 983 (1971).
6 C.W. Tabor and H. Tabor, A. Rev. Biochem. *45*, 285 (1976).
7 R.O. Sinnhuber, T.C. Yu and T.C. Yu, Food Res. *23*, 626 (1958).
8 G.L. Miller, Analyt. Chem. *31*, 964 (1959).
9 B.P.F. Adlard, Biochem. Soc. Trans. *2*, 281 (1974).
10 G. Haase and W.L. Dunkley, J. Lipid Res. *10*, 561 (1969).
11 B. Tadolini, Biochem. biophys. Res. Commun. *92*, 598 (1980).
12 R.J. Chafee, R. M. Arina and R.H. Rochelle, Biochem. biophys. Res. Commun. *86*, 293 (1979).
13 M. Kitada, K. Igarshi, S. Hirose and H. Kitagawa, Biochem. biophys. Res. Commun. *87*, 388 (1979).
14 A. Colbeau, J. Nachbaur and P.M. Vignais, Biochim. biophys. Acta *249*, 462 (1971).
15 H. Iwata, T. Matsuda, S. Maeda and A. Baba, Biochim. biophys. Acta *583*, 159 (1979).

On the production of 12α-hydroxysteroid dehydrogenase from *Clostridium group P*, strain C48-50 ATCC 29733

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Summary. The production of 12α-hydroxysteroid dehydrogenase of *Clostridium group P* strain C48-50 was optimized when the organism was grown in the presence of 2% fructose and 0.1% dithiothreitol. It appears that an initial redox potential of less than −160 mV (achieved by autoclaving in the presence of dithiothreitol, dithioerythritol or cysteine) is important in the production of this enzyme.

Nicotinamide adenine denucleotide phosphate dependent (12α-HSDH) active against bile acids can be demonstrated in mixed fecal cultures[1], in *Clostridium leptum*[2] and in a number of non-fermentative clostridia[3]. Two of the above organisms, a *Clostridium leptum*[2] and a *Clostridium group P* organism[3,4] have been isolated, which contain 12α-HSDH in the total absence of 3α- or 7α-HSDH. Cell-free preparations of the latter clostridium have been used in the quantification of 12α-OH groups in bile[5] and fecal[6] extracts.

Recent failures to obtain cell-free preparations of high specific activity as reported earlier[4] have prompted us to investigate the effect of various reducing agents on the production of 12α-HSDH from *Clostridium group P*. In this communication, we report the effect of various reducing agents in the medium on the growth of *Clostridium group P*, the initial redox potential (Eh) value and the yield of 12α-HSDH.

Materials and methods. Clostridium group P strain C48-50 was grown for 96 h at 37 °C in 10 ml volumes of brain heart infusion (BHI) broth in 15 ml culture tubes as described earlier (except with reducing agent included). Glucose or fructose were added to the medium to give a final concentration of 2.0%; and a reducing agent was added to give a final concentration of 0.1% before autoclaving the medium for 20 min. Final Eh values of the medium (of duplicate tubes) were measured using an Orion platinum redox electrode (mold 96-78) attached to an Orion pH/Eh meter. A reference standard of 0.5% cysteine giving an Eh value of +25 mV was employed[7].

Cell-free peparation of 12α-HSDH were prepared as before[4] and 12α-HSDH was assayed as before[4] except

Table 1. Effect of reducing agents and sugars on the growth of *Clostridium group P* strain C48-50, production of 12α-HSDH and Eh value of the medium

Addition to medium	Absorbance of culture at 660 nm*	Units of 12α-HSDH per 10 ml culture	Initial Eh value of medium (mV)
1 No additions	0.20	0.6	−100
2 TG alone	0.22	0.6	−140
3 DTT alone	0.28	0.8	−250
4 Fructose alone	1.3	1.0	−100
5 TG + fructose	1.4	10.5	−145
6 TG + glucose	1.4	8.5	−147
7 Cyst + fructose	1.3	14.5	−165
8 Cyst + glucose	1.3	11.1	−170
9 DTE + fructose	1.8	12.5	−240
10 DTE + glucose	1.8	7.0	−240
11 DTT + fructose	1.9	15.0	−255
12 DTT + glucose	1.8	7.0	−255
13 'Aged' TG + fructose	1.4	1.0	−135
14 'Aged' TG + glucose	1.4	0.81	−137

* Growth measured after 96 h.

10^{-3}M deoxycholic acid was used as substrate. Viable counts of cultures were performed by serial dilution of cultures using human blood agar plates.

Results and discussion. Results are summarized in tables 1 and 2. The optimal condition for growth of the organism and production of 12α-HSDH thus far tested appear to occur in the presence of 2% fructose and 0.1% dithiothreitol (DTT) while drastic losses in enzyme activity are evident if either of these components are deleted. The sugar appears to be taking part in a neutral fermentation not yet characterized[4] while the addition of reducing agent assures a low initial Eh value. The deletion of reducing agent or inclusion of aged thioglycollate (TG) (stock powder kept on the shelf for approximately 5 years) allows the organisms to grow equally well but drastically reduces the yield of 12α-HSDH. It appears that one can predict the success in producing enzyme simply by the appearance, odor and behaviour of the cells at stationary phase and appearance of the cell-free preparation (table 2). Circumstantial evidence suggests that the initial Eh value is important in the production of the enzyme and other features of the culture but less critical in determining the cell number (table 2). Other factors such as amounts of oxidized TG in the 'aged' TG may also be of some importance in enzyme production.

A similar Eh-microbial enzyme effect has been observed by Bokkenheuser et al.[8] who showed in *Eubacterium lentum* that 3α-HSDH was present in low amounts but 21-dehydroxylase (active against corticosteroids) was very active at an initial Eh value of -280 mV. In contrast, at an initial Eh value of about -150 mV, the 21-dehydroxylase was depressed but the 3α-HSDH enhanced. The organisms grew well under both conditions.

The author recommends the use of 2% fructose and 0.1% DTT in BHI broth and then freshly autoclaving the resulting broth for the routine production of 12α-HSDH from *Clostridium group P* strain C48-50.

Table 2. Culture properties of *Clostridium group P*, strain C48-50 and 12α-HSDH levels under 2 growth conditions

Number	Cells grown with fructose and DTE in BHI broth	Cells grown with fructose and 'aged' TG in BHI broth
1	Cells suspended throughout the culture	Cells precipitated on the bottom of culture
2	Meaty-fruity odor of culture	Unpleasant 'rotting' odor of culture
3	Centrifuged pellet white	Centrifuged pellet pinkish
4	Cell-free preparation clear-yellow	Cell-free preparation slightly pinkish
5	Viable count $= 5 \cdot 10^{10}$ organisms/10 ml culture	Viable count $= 6 \cdot 10^{10}$ organisms/10 ml culture
6	HSDH level 12-16 units/10 ml culture	HSDH level < 1.0 unit/10 ml culture

1 I.A. Macdonald, G.R. Webb and D.E. Mahony, Am. J. clin. Nutr. *31*, S233 (1978).
2 J.N. Harris and P.B. Hylemon, Biochim. biophys. Acta *528*, 148 (1978).
3 D.E. Mahony, C.E. Meier, I.A. Macdonald and L.V. Holdeman, Appl. envir. Microbiol. *34*, 419 (1977).
4 I.A. Macdonald, J.F. Jellett and D.E. Mahony, J. Lipid Res. *20*, 234 (1979).
5 I.A. Macdonald, C.N. Williams and B.C. Musial, J. Lipid Res. *21*, 281 (1980).
6 M.J. Crowell and I.A. Macdonald, Clin. Chem. *26*, 1298 (1980).
7 L.DS. Smith, in: The Pathogenic Anaerobic Bacteria, p.10. Ed. A. Balows. C.C. Thomas Publishers, Springfield, Ill. 1975.
8 V.D. Bokkenheuser, J. Winter, S.M. Finegold, V.L. Sutter, A.E. Richie, W.E.C. Moore and L.V. Holdeman, Appl. envir. Microbiol. *37*, 1001 (1979).

Deoxyribonuclease immobilized on the erythrocytes

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Summary. Bovine pancreatic deoxyribonuclease (DNase I) was immobilized on human erythrocytes with several procedures. DNase immobilized on the erythrocytes by chromic chloride showed DNase activity in vitro. Other binding procedures inhibit the immobilized DNase activity.

In patients with systemic lupus erythematodes (SLE), especially in those with anti-DNA antibodies, a fall in complement levels is often associated with the development of nephritis. In addition, some patients with serum antibodies to DNA develop fever and nephritis as the antibody disappears and is replaced by free DNA, a sequence which presumably represents immune complex formation and deposition followed by antigen (DNA) excess. Zöllner reported that sera from SLE patients with high anti-DNA antibody contents had a low DNase activity and sera with low or even absent anti-DNA antibody contents had high DNase activity[1]. These results indicate the usefulness of DNase administration in vivo to digest DNA in the sera of the patients with SLE. For clinical use in future, we immobilized pancreatic DNase (DNase I) on human erythrocytes by several procedures and studied the enzyme activity of the immobilized DNase.

Materials and methods. Freshly obtained human blood was washed 4 times with 0.9% NaCl to remove plasma and buffy coat.

a) Chromic chloride method. DNase immobilized on erythrocytes by the chromic chloride method of Jandle[2]. 0.5 ml of a 50% suspension of erythrocytes, 1 ml of a 2.5 µM solution of $CrCl_3$ and 2.5 ml of a DNase (4000 units/ml, Sigma Chemical Co.) in 0.9% NaCl were mixed and allowed to stand at 20 °C for 1 h. The erythrocytes were subsequently washed 6 times with 0.9% NaCl and finally suspended in phosphate buffered saline pH 7.4(PBS).

b) DIDS method. 3 ml of DNase (4000 units/ml) plus 0.5 ml of a 50% erythrocytes in PBS were incubated for 10 min at 4 °C with 0.5 mg of 4,4'-diisothiocyano-2,2'-stilbene disulfonate (DIDS) in the dark[3]. The cells were washed 5 times and suspended in PBS.