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.

c) EDCI method. 3 ml of DNase (4000 units/ml) plus 0.1 ml of a 50% erythrocytes in PBS were incubated for 1 h at room temperature with 100 mg of 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDCI)[4]. The cells were washed 5 times and suspended in PBS.

Control studies were performed with same procedure without immobilizing agents. 1 ml of DNA (200 mg Na-DNA/dl containing 0.05 M $MgSO_4$ in PBS) plus 1 ml of PBS were incubated with 1 ml of the erythrocyte suspension (Ht 50%) at 37 °C for 1 h and centrifuged. 1 ml of 3M trichloroacetic acid was added to the supernatants. The mixtures were left in an ice water bath for 15 min then filtered through Whatman No.42 paper. Aliquots of the filtrate were analyzed by the diphenylamine procedure.

Results and discussion. DNase immobilized on the erythrocytes by chromic chloride showed DNase activity in vitro, but other immobilizing procedures inhibited the DNase activity. About 10% of added DNase activity was immobilized on the erythrocytes. No significant hemolysis was observed and DNase activity did not decrease after several incubations (table).

DNase activities of erythrocyte immobilized DNase

	Units/0.1 ml of erythrocytes
Control	90
Immobilized DNase	
$CrCl_3$ method, 1st incubation	430
2nd incubation	450
3rd incubation	400
DIDS method	110
EDCI method	80
Free DNase (4000 units/ml)	390 (0.1 ml of DNase solution)

Considerable evidence exists to suggest that complexes of DNA and anti-DNA antibodies play a role in the pathogenesis of nephritis in SLE. A significant correlation exists between the occurrence of high titers of DNA-anti-DNA antibody complex and the severity of clinical disease and immunoglobulin deposit in the kidney. Some sera from SLE patients with nephritis, when treated with DNase, show an increase in anti-DNA antibody levels. This fact suggests that DNA has been bound in vivo to the antibody as an immune complex.

Pancreatic DNase degrades DNA mainly to a mixture of oligonucleotides, and in vivo administration of DNase to the patients with SLE may be useful to digest not only free DNA but also antibody-bound DNA. Digestion of free DNA may prevent the anti-DNA antibody formation and digestion of antibody bound DNA may decrease the immune complex.

For clinical use in future, human pancreatic deoxyribonuclease must be purified to prevent immunological reactions.

1 E.J. Zöllner, B. Heicke and R.K. Zahn, Z. klin. Chem. Biochem. *12*, 459 (1974).
2 J.H. Jandle and R.L. Simmons, Br. J. Haemat. *3*, 19 (1957).
3 T. Kitao and K. Hattori, Thromb. Haemost. *43*, 70 (1980).
4 H.M. Johnson, J. Brenner and H.E. Hall, J. Immun. *97*, 791 (1966).

Tissue survey of mammalian modulator-dependent protein kinases

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Summary. The levels of modulator-dependent protein kinases and protamine-dependent protein kinase(s) in various tissues of adult mice were compared. Cerebellum contained the highest levels of both modulator-dependent protein kinases and protamine-dependent protein kinase(s), whereas skeletal muscle contained no detectable enzymes. The lung and the ileum were also rich in modulator-dependent protein kinases, while other tissues were poor sources of these enzymes.

At least 2 protein kinases[2-4] from mammalian sources have been shown to be stimulated in vitro by either crude protein kinase modulator (PKM)[5-8] or by partially purified stimulatory modulator[7,8]. While investigating these 2 modulator-dependent protein kinases, other investigators have reported protamine- or histone-stimulable protein kinase(s)[9], which will be called protamine-dependent protein kinase(s). In this paper, we have addressed the question of whether modulator-dependent protein kinases and protamine-dependent protein kinase(s) are the same enzymes by comparing the levels of modulator-dependent protein kinases and protamine-dependent protein kinase(s) in various tissues of adult mice.

Materials and method. [γ-^{32}P] ATP was purchased from New England Nuclear; protamine chloride (grade V) was obtained from Sigma. Adult male ICR mice (each weighing 25.0±2.9 g) were used exclusively. Crude protein kinase modulator was prepared from liver extracts of ICR mice by boiling and trichloroacetic acid-precipitation[5-8]. Crude protein kinase preparations were as follows. Appropriate amount of fresh tissues from ICR mice were homogenized in 3 volumes of ice-cold 50 mM potassium phosphate buffer, ph 7.0, using a glass-teflon homogenizer. The homogenate was centrifuged for 15 min at 30,000×g. The supernatant fluid (crude extract) was filtered through 2 layers of glass wool to remove fat, and then appropriately diluted with potassium phosphate (50 mM, pH 7.0) before use as an enzyme source. The standard assay system[10] for protein kinase activity was modified over the past studies in a final volume of 0.2 ml to contain phosphate buffer, pH 7.0, 10 µmoles; varied amount of endogenous substrate protein; $MgCl_2$, 2 µmoles; [γ-^{32}P] ATP, 1 nmole containing about 1.2×10^6 c.p.m. with or without stimulator, protein kinase modulator (50 µg) or protamine (4 µg); appropriate amount of crude protein kinase preparations. The reaction was carried out for 10 min at 30 °C. 1 µU of the enzyme was defined as that amount of enzyme that transferred 1 pmole of ^{32}P from [γ-^{32}P] ATP per min to recovered substrate protein under the assay conditions.

Results. The levels of modulator-dependent protein kinases and protamine-dependent protein kinase(s) were examined in 8 different tissues (table). The cerebellum contained by far the highest modulator-dependent protein kinases and protamine-dependent protein kinase(s) activites. In con-