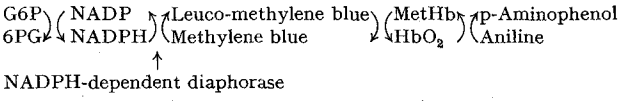
known to be NADPH generating systems in red cells[7,8].

Methods. ACD blood was washed 3 times with isotonic saline after removal of serum and buffy coats. The red cells thus obtained were suspended in a solution containing 110 mM NaCl, 10 mM glucose, 30 mM sodium phosphate (pH 7.0), 5 mM KCl, 1 mM $MgCl_2$ and 10 mM aniline hydrochloride (Hematocrit values are 35%), and incubated at pH 7.0, 37 °C for 4 h with or without 10 μM methylene blue. The samples were taken out for analyses of p-aminophenol and deproteinized with 4.6% trichloroacetic acid. The determination of p-aminophenol was followed in accordance with the method of Mieyal and Blumer[9].

Results and discussion. The figure shows the aniline hydroxylation by human red cells for 4 h. The production of p-aminophenol was linearily increased with time in the absence and presence of methylene blue. Especially, in the presence of catalytic amounts of methylene blue (10 μM), the aniline hydroxylation by red cells was much accelerated. From this figure, the rate of aniline hydroxylation in red cells suspension was calculated to be 0.9 μM/h in the absence of methylene blue. In the presence of methylene blue, the aniline hydroxylating activity was accelerated as much as 33 times and the rate of aniline hydroxylation was calculated to be 30 μM/h. This value is comparable to the rat liver microsomal aniline hydroxylating activity[3,10], though a direct comparison of the activity is difficult.

The stimulation of methemoglobin reduction by NADPH-dependent diaphorase has been shown in the presence of methylene blue[11]. This phenomenon is considered to be due to the continuous supply of NADPH by methylene blue through activation of the pentose phosphate shunt of red cells. Taking account of this fact and the reports mentioned above[2,3], the following scheme, as one possibility, may be proposed for the aniline hydroxylation by red cells.



However, another possibility cannot be eliminated that aniline hydroxylation may be coupled with other hemoproteins such as P-450, though it has not been detected in red cells yet.

The plausible explanation of the acceleration of aniline hydroxylation by methylene blue is probably due to the stimulated generation of NADPH coupled with the well-known activation of the pentose phosphate shunt by this dye. Thus present results suggest that the electron transport systems from NADPH to aniline are operating in red cells, though the detailed information for the intermediate step is obscure in our experiment.

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Changes in phospholipid composition of *Nocardia polychromogenes* during temperature adaptation

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Summary. The effect of growth temperature on the phospholipid composition of Nocardia polychromogenes has been examined. When the growth temperature was decreased from 37 °C to 27 °C, there was a large decrease in phosphatidyl ethanolamine with an increase in cardiolipin and phosphoinositides. These changes are discussed in context with the control of membrane fluidity.

There is now substantial evidence that phospholipids play a major role in the structure and functions of biological membranes[2,3]. The importance of the apolar portions of membrane lipids has recently been emphasized by investigations with Mycoplasma laidlawii[4] and with mutants of E. coli[5] which cannot synthesize unsaturated fatty acids. In contrast, little information is available regarding the importance of the polar moieties of phospholipids to the fluid properties of the cell membrane. We have now studied the effect of growth temperature on the phospholipid composition of Nocardia polychromogenes. This organism contains cardiolipin, phosphatidyl ethanolamine and phosphoinositides as its major phospholipids[6]. This report pertains to the changes in the distribution of phospholipids in Nocardia polychromogenes grown at 37 °C and 27 °C.

Materials and methods. Nocardia polychromogenes were grown in a medium containing glucose, beef extract and peptone[7]. Cells initially grown at 37 °C were used as inocula for 27 °C cultures. Cells were grown for 8 days. Extraction and purification of lipids were as described previously[6,7]. The separation, isolation, characterization and quantitation of phospholipids were as detailed in previous publications[8,9].

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Results and discussion. The effect of growth temperature on the phospholipid composition is given in the table. Compared with cells grown at 37 °C, significant differences were noted in the proportion of the different classes of phosphatides at 27 °C. It is known that changes occur in the fatty acid composition of complex lipids in microorganisms, plants and animals in response to growth temperature[10,11]. As a general rule, the composition of fatty acids is modified in the direction of lower average melting points as the growth temperature is lowered. We have now observed significant changes in the phospholipid compositions of cells grown at 27 °C as compared to cells grown at 37 °C. Similar to our observations, a decrease in phosphatidyl ethanolamine and an increase in cardiolipin has been observed in Vibrio cholerae with decreasing growth temperatures[12]. Ethanolamine phosphoglycerides have also been shown to decrease in Tetrahymena pyriformis with a drop in temperature[13]. In Clostridium butyricum, changes were seen in the phospholipid composition when cells were grown either at lower temperature[14] or in the presence of exogenous unsaturated fatty acids[15]. When the growth temperature was decreased from 37 °C to 25 °C, the proportion of glycerol phosphoglycerides increased at the expense of ethanolamine and N-methylethanolamine phosphoglycerides[14]. When the medium was supplemented with oleate at 37 °C, there was a decrease in the content of ethanolamine plus N-methylethanolamine plasmalogens with a corresponding increase in the glycerol acetals of these plasmalogens[15]. It was suggested that such changes may be involved in the maintenance of membrane fluidity. It is also known from model membrane studies that changes in the phospholipid head groups can have an effect on fluidity[16,17]. It therefore seems possible that the differences in phospholipid distribution found in Nocardia polychromogenes grown at high and low temperatures are a feature of adaptive processes to regulate membrane fluidity.

Composition of phosphatides of Nocardia polychromogenes grown at 37 °C and 27 °C

Phospholipid classes	Percent of total phospholipid (mean ± SD) 37 °C	27 °C
Inositol containing phospholipids	33.1(±2.1)	45.0(±3.3)
Phosphatidyl ethanolamine	25.9(±1.4)	12.8(±0.7)
Cardiolipin	32.7(±1.8)	38.7(±2.3)
Unknown phospholipids	8.4(±0.7)	3.3(±0.8)

At 37 °C and 27 °C, 4 different batches were analyzed. The differences observed are statistically significant.

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A study of broad bean α-amylase isoenzymes

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Summary. 2 α-amylase isoenzymes were isolated from cotyledons of germinated broad bean seeds. The examination of the 2 isoenzymes isolated by column chromatography on DEAE-cellulose and sephadex showed that these isoenzymes have the same behaviour towards soluble starch, amylose, amylopectin, heavymetal ions, and high temperature, and differ only in their mol.wt and their electrophoretic mobility.

The α-amylase (α-1,4 glucan 4-glucanohydrolase, E.C. 3.2.1.1.) is one of the enzymes involved in the starch hydrolysis of the living tissues, splitting the 1,4-bonds of the starch. For complete hydrolysis of the starch, the presence of R-enzyme (splitting the 1,6-bonds of the starch) is required in addition to-α-amylase. β-Amylase (E.C. 3.2.1.2.) splitting also the 1,4 bonds of the starch occur in the plants and sometimes in the same tissues where the α-amylase occurs. The physiological role of these 2 amylases (α and β) has not yet been fully understood. In addition the amylases occur in isoenzymes both in plants[1–7] and animals[8–11]. In the cotyledons of broad bean, the α-amylase occurs in 2 isoenzymes. In order to contribute to the further understanding of the significance of these isoenzymes, the 2 isoenzymes from the broad bean were isolated and examined separately.

Materials and methods. Seeds of broad beans (Vicia faba var. aquadulce) were grown and the cotyledons from 10-days-old plants were used as the source of the isoenzymes. For the enzyme assay, 1 ml of soluble starch solution (300 mg/100 ml acetate buffer 0.1 M, pH 6.0 + 10 mM $CaCl_2$) was incubated at 30 °C with 1 ml of the properly diluted enzymic solution. After 1–5 min, 1 ml of the iodine reagent[12] and 5 ml water were added and the A570 μm was read and transformed to mg of starch by proper standard curve. The hydrolyzed starch (mg) per min under the above conditions defines one enzymic unit. The specific activity is defined as the units/mg of protein measured by the method of Lowry et al.[13].

Electrophoresis. Polyacrylamide disc gel electrophoresis was used. For the preparation of gel, the following reagents were used: polyacrylamide (6.4% w/v) in a buffer consisting of Tris (6 g) and glycine (28.8 g)/l pH 8.6 and containing soluble starch (0.25% w/v). NNN'N'-tetramethylethylene diamine (0.15% v/v) and ammonium persulfate (0.05% w/v). After running the gels vertically at 4–5 mA/gel, they were incubated for 15 min at 30 °C in 0.1 M acetate buffer pH 5.6 containing 10 mM $CaCl_2$.