fective agent of this type. While 5'-deoxyriboflavine[15] and riboflavine-5'-sulfate[16] have marginal anticoccidial activity, in general, with the exception of the benzyl compounds, variations from the ribityl group appear unprofitable. Modest activity against E. tenella is observed in tests of N-methyl-N-2-propynylbenzylamine, or pargyline (**13**), a compound which functions by k_{cat} inhibition of flavine-mediated monoamine oxidase[17].

While 8-amino- and 8-dimethylamino-8-norriboflavines (**7**, **8**) were known previously[18,19], the latter as the antibiotic roseoflavine, the 8-methylamino analog (**6**) had not been described when we completed its synthesis, but the compound was reported subsequently as a photolysis product of roseoflavine[20]. Of the 3 amines, **6** is the best coccidiostat, since it is better tolerated than the equipotent 8-amino compound (**7**). Compound **6** [m.p. 307–310 °C; UV_{max} (pH 7) 487 nm (ε 40,500), 306 (8900), 254 (49,900)] and **8** [m.p. 273–277 °C; UV_{max} (pH 7) 506 nm (ε 31,800), 314 (7600), 258 (39,000)][21] were obtained by amination (150fold excess of amine, DMF, 100 °C, 1 h) of 8-chloro-8-norriboflavine[22].

9-Azariboflavine (**9**) [m.p. 255–258 °C; UV_{max} (pH 7) 436 nm (ε 16,700), 312 (4900), 265 (32,500)] was prepared by reacting 2-chloro-5,6-dimethyl-3-nitropyridine[23] with D-ribitylamine, according to the procedure of Israel[24], to give 5,6-dimethyl-3-nitro-2-D-ribitylaminopyridine, m.p. 140–142.5 °C, followed by hydrogenation (Pt, MeOH) of the nitro group and condensation (C_5H_5N, 55 °C, 2 h) of the diamine with 5,5-dichlorobarbituric acid[25].

The 10-(3-chlorobenzyl) analog (**12**) [m.p. 302–305 °C; UV_{max} (MeOH) 440 nm (ε 9800), 347 (8000), 265 (28,200)] was prepared from 4,5-dinitro-o-xylene, 3-chlorobenzylamine and alloxan by a standard three-step procedure[26,27].

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Aniline hydroxylation in the human red cells

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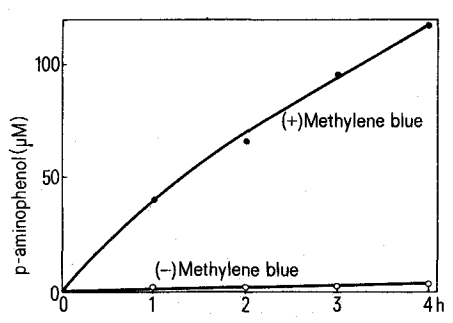
Summary. The aniline hydroxylation in human red cells was studied and the hydroxylating activity was much accelerated in the presence of methylene blue.

The hydroxylation of such drugs as aniline has been shown to be catalyzed by microsomal cytochrome P-450 systems in a variety of mammalian tissues since the discovery by Estabrook et al.[1]. However, the contribution of the red cells to this drug hydroxylation has been considered to be negligible because of the deficit of microsome.

Recently Juchau and Symms showed the aniline hydroxylating activity of human hemoglobin[2]. Furthermore Mieyal et al. indicated that hemoglobin can be substituted for P-450 in aniline hydroxylation by microsomal monooxygenase systems[3].



Aniline hydroxylation by red cells in the absence and presence of methylene blue.

On the other hand, human red cells seem to have an intracellular circumstance similar to microsome. The NADH- and NADPH-dependent diaphorases and cytochrome b_5, which are comparable to those of microsome with regard to function and structure, have been recognized in human red cells by many authors[4–6], though it is unclear whether these proteins are derived from the microsome in the stages of the erythroblasts. These results suggest the possibility that red cells are capable of hydroxylating drugs such as aniline.

In spite of these possibilities, the detailed study for aniline hydroxylation by intact red cells has not been reported. This paper deals with the aniline hydroxylation in human red cells and the effect of methylene blue as an activator for the pentose phosphate shunt, which is

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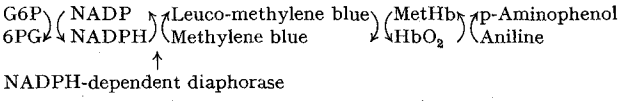
known to be NADPH generating systems in red cells[7,8].

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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