the reduction of enzyme-bound flavin by NADPH, as suggested by the decrease in absorbancy at 455 mμ on addition of NADPH. Under anaerobic conditions, a NQ, also bound by the enzyme, will accept electrons from the reduced flavin to form NQH· and then NQH_2. If it is assumed that the NQH· is very labile to oxygen and rapidly reoxidizable to NQ by oxygen, probably because of its special linkage to the enzyme protein, it will be understandable that NQ mediates, in the presence of oxygen, the aerobic oxidation of NADPH without intermediate formation of NQH_2. The semiquinone form of *p*-benzoquinone may not be oxidizable by oxygen for reasons still to be investigated. The participation of a radical, monodehydroascorbate, has also been suggested in the ascorbate-stimulated oxidation of NADH by adrenal microsomes[13].

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The oxidation–reduction potential of cytochrome b_5 in soluble and particulate form with reference to the role of lipid

The oxidation–reduction potential is one of the important properties of respiratory enzymes. It generally determines the position of the enzymes in respiratory chains and contributes to understanding their function in the living cells. Concerning the potential of cytochrome b_5, a microsomal hemoprotein, several conflicting values have been reported. The potential determined first by YOSHIKAWA[1,2] with dog- and rabbit-liver preparations was shown to be −0.13 V. Later, STRITTMATTER AND BALL[3] obtained the same value with a rat-liver particulate suspension. The cytochrome from rabbit-liver microsomes was solubilized and purified by VELICK AND STRITTMATTER[4] who estimated its oxidation–reduction potential to be +0.02 V. In the Symposium on Hematin Enzymes held at Canberra[5], the cause of the discrepancy was subjected to debate, but no clear answer was obtained. The object of the present study is to clarify this question.

Cytochrome b_5 was purified from beef liver essentially in the same way as described by STRITTMATTER[6]. The oxidation–reduction potentials were estimated by the dye method with nile blue, methyl capri blue and methylene blue, the E_0' values of which at pH 7.0 are −0.13 V, −0.06 V and +0.01 V, respectively. The measurement was carried out at pH 7.0 and at 23°. With clear solutions, the cytochrome and dye were placed in a Thumberg-type cuvette filled with nitrogen gas and stoppered with rubber through which dithionite solution was introduced from a syringe. The per cent reductions of the pigment and dye were estimated spectrophotometrically, and the potential of cytochrome b_5 for half-reduction, E_0', was calculated. With turbid solutions, the mixture with dye was placed in a test tube and covered with

TABLE I

OXIDATION-REDUCTION POTENTIALS OF CYTOCHROME b_5 AT DIFFERENT STAGES OF PURIFICATION PROCEDURE

Stage	E_0' (V)
Microsomes	−0.14
Lipase treatment	−0.01
Acid precipitation	+0.02
DEAE-cellulose chromatography	+0.02

liquid paraffin. Dithionite solution was added drop by drop from a syringe, until the absorption band of reduced cytochrome b_5 appeared. At this point, the per cent reduction of the dye was estimated by comparing the color intensity with a series of standard dye solutions, while the reduction grade of the cytochrome was observed by the use of Zeiss hand spectroscope[1,2]. These two methods gave practically identical results.

In Table I are given the oxidation–reduction potentials of cytochrome b_5 at different stages of purification. The pigment in microsomes showed a low potential, −0.14 V, as reported by previous investigators. When the microsome preparation was treated with lipase, the potential shifted to a higher level, +0.01 V. As seen in Table II, treatment of microsomal preparation with other agents, such as cholate or peptidases, brought about no appreciable change in the E_0'.

TABLE II

EFFECT OF TREATMENT OF MICROSOMAL PREPARATION WITH VARIOUS AGENTS UPON THE POTENTIAL OF CYTOCHROME b_5

Agent	E_0' (V)
Pancreatic lipase (purified according to Willstätter), pH 8.1, 37°, 3 h	+0.01
Sodium cholate (1%), pH 7.5, 0°, 30 min	−0.13
Trypsin (Mochida Pharmaceutical Co.), pH 8.6, 23°, 24 h	−0.13
Bacterial proteinase (Nagase Ind. Co.), pH 7.5, 23°, 24 h	−0.14

In the next series of experiments, the influence of the addition of various substances to purified cytochrome b_5 was examined. The results are given in Table III. Among the substances so far tested, only lipid which was extracted from beef-liver microsomes with chloroform–methanol (1 : 1) lowered the potential to nearly the same level as that of particulate-bound microsomal pigment.

These findings suggest that the much lower potential of cytochrome b_5 in particulate form may be attributed to some sort of combination of the hemoprotein with lipid in microsome (presumably phospholipid). The exact nature of the combination is not clearly understood, but the crude lipase contained in the enzyme

TABLE III

EFFECT OF ADDITION OF VARIOUS SUBSTANCES ON THE POTENTIAL OF CYTOCHROME b_5 IN PURIFIED FORM

Substance added	*The amount of added substance (mg/mg cytochrome b_5)*	E_0' (V)
None		+0.02
Microsomal lipid	14	−0.12
Bovine serum albumin	150	+0.02
Protamine	180	+0.01
Sodium cholate	63	+0.01
Cellulose powder	40	+0.01
Cellite	16	+0.02
Alumina C_γ	11	+0.01

preparation from hog pancreas splits the lipid component of the particulate cytochrome and solubilizes it. The participation of mitochondrial lipid in electron-transfer mechanism has been discussed recently[7]. Our experimental results might suggest an important role of lipid in microsomal function.

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