only when the acceptors were glucose-1-^{14}C in the form of the free hexose or as a monosaccharide moiety of an oligosaccharide. With lactose-*U*-^{14}C, the donor molecule, galactose, is labelled resulting in a marked increase in oligosaccharide radio-activity (Fig. 2).

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HÆMATOLOGY

Methæmoglobin Reduction through Cytochrome B_5

HUENNEKENS *et al.*[1] purified an erythrocyte fraction containing hæm which was able to catalyse the reduction of methæmoglobin by reduced triphosphopyridine nucleotide in the presence of methylene blue. A single enzyme was believed to be responsible for the electron transport from reduced triphosphopyridine nucleotide to the dye, and from the dye through hæm to methæmoglobin. Although reduced diphosphopyridine nucleotide-cytochrome *c* reductase from pig heart and reduced triphosphopyridine nucleotide-cytochrome *c* reductase from yeast do not reduce methæmoglobin, it was shown by Cromier and Rostorfer[2] that bacterial extracts with diaphorase activity, as well as milk xanthine oxidase, do reduce methæmoglobin in the presence of methylene blue, suggesting that diaphorases could also act similarly to methæmoglobin.

One of us previously isolated from pig liver[3] (H. R. Mahler, and I. Raw, unpublished work) also a cytochrome, identical to cytochrome b_5. Rapid purification of the cytochrome was achieved by treating the ammonium sulphate fraction, obtained in a manner previously reported[3], with 'DEAE'-cellulose at *p*H 6 in 0·001 *M* potassium phosphate buffer, to adsorb most of the cytochrome, leaving behind the hæmoglobin. The cytochrome was then eluted with *M*/4 potassium phosphate buffer, *p*H 7 and further purification was obtained by chromatography in hydroxyapatite[3], and irradiation at 0° C., for 3 hr. with short-wave ultraviolet light ('Mineralight' model *V*–41) to destroy residual reductase.

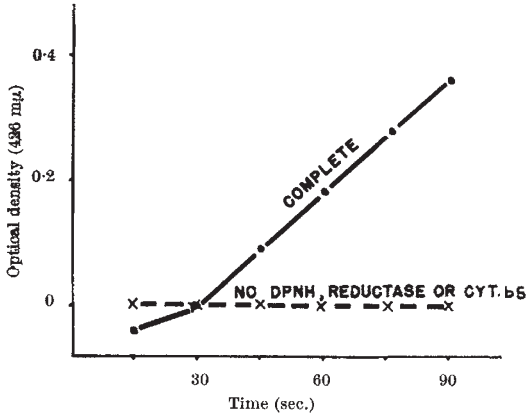

Fig. 1. Reduction of methæmoglobin by diphosphopyridine nucleotide. The system contained in 2·3 ml. $1·4 \times 10^{-4}$ moles of methæmoglobin (prepared or ferricyanide reduction of crystaline horse hæmoglobin) 0·5 γ of diphosphopyridine nucleotide-cytochrome b_5 reductase $SA = 60$, $1·4 \times 10^{-6}$ moles cytochrome b_5 (or 10^4 moles methylene blue) and 2×10^{-3} moles 426, and $\Delta\varepsilon$ assumed to be 57×10^6 (ref. 5). Methæmoglobin was added at 0 time after pre-incubation of the other components for 2 min., at 25° C.

A specimen of reduced diphosphopyridine nucleotide-cytochrome b_5 reductase from pig liver was used and prepared as previously described[4]. This enzyme did not reduce methæmoglobin directly.

Fig. 1 shows that methæmoglobin is reduced by reduced diphosphopyridine nucleotide in the presence of the reductase and cytochrome b_5. As expected, methylene blue cannot be substituted for the cytochrome. The rate of reaction is, within experimental error, equal to the rate of cytochrome b_5 reduction, and linear after the first 30 sec.

Present results agree with Huennekens *et al.*[1] that methæmoglobin can be reduced by a reduced hæmoprotein. It is interesting to note that the best preparation of erythrocyte methæmoglobin reductase reduced about 0·03 μmole/min./mgm. of protein, whereas a rate of 200 μmole/min./mgm. of protein is obtained with purified reduced diphosphopyridine nucleotide cytochrome b_5 reductase from pig liver. This would suggest that the erythrocyte methæmoglobin reductase may be a similar multi-enzymatic system, but lacking one fully active component.

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Banding of Hæm to Protein in Hæmoglobin and Myoglobin

RECENTLY it was postulated that in hæmoglobin and myoglobin the hæm iron is bound to the apoprotein other than through histidine imidazole groups[1]. This conclusion, which does not appear to be warranted by evidence at present available, is based on the assumption that the *p*H at which half the hæm is bound to the protein moiety can be identified with the pK_L of the binding group. There is no theoretical reason why this should be so.

Assuming the binding between the hæm and the protein to involve co-ordination between the iron atom and a ligand group on the protein, then *p*H (50 per cent) is related to pK_L, to the stability constant (K_s) of the complex and to the total ligand ($[L]_T$) and metal ($[M]_T$) concentrations, in the following manner :

$$p\text{H (50 per cent)} = pK_L + pK_s - \log_{10}(L_T - \tfrac{1}{2} M_T - {}^1/_{Ks})$$