

## New Pigments Derived from Vitamin B<sub>12</sub> by the Use of a Gram Negative Organism

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We wish to report the bacterial conversion of cyanocobalamin into several different coloured substances by a gram negative rod isolated from soil. The gram negative rod has been classified as *Aerobacter aerogenes*.

The microorganism was grown in a synthetic fluid medium containing 0.8 % glucose, salts and 0.4 mg cyanocobalamin per ml. After incubation at 37°C for 12 days the culture was centrifuged and the supernatant extracted with phenol. The phenol layer was separated by centrifugation, washed with water to remove salts and diluted with ether. Unchanged vitamin B<sub>12</sub> and the pigments were then re-extracted into water.

By electrophoresis in N acetic acid the extracted material separated into two coloured substances with mobilities different from that of unchanged cyanocobalamin. They both carried a positive charge and exhibited a mobility of 0.75 (I) and 0.25 (II), respectively, relative to aquo-cobalamin.

Substance (I) had a brownish-yellow colour, while substance (II) was yellow. The yield of these two substances was very low and only substance (I) has been further investigated. It was purified by descending chromatography in the usual *sec.* butanol water-1 % acetic acid-0.01 % KCN solvent. In this solvent it had an  $R_F$  of 0.35 whereas the  $R_F$  of cyanocobalamin was 0.25.

When dissolved in distilled water it exhibited the following absorption maxima, 262, 342 and 474  $m\mu$ , and when examined in 0.1 M KCN the brownish-yellow substance turned pink with a concomitant shift in the maxima to 280, 344 and 494–502  $m\mu$ . This shift in colour and maxima, as with cyanocobalamin, was shown to be reversible. In water it showed the absorption ratios  $O.D._{342}/O.D._{474} = 1.0$  and  $O.D._{342}/O.D._{262} = 0.4$ , and in 0.1 M KCN  $O.D._{344}/O.D._{494} = 1.0$  and  $O.D._{344}/O.D._{280} = 0.6$ . The absorption spectrum resembles that of cyanocobalamin, the main difference being the lack of the large maximum in the region 300–400  $m\mu$ .

The presence of Co as indicated by the absorption spectrum in KCN was verified by the use of <sup>58</sup>Co labeled cyanocobalamin.

Substance (I) was purified twice by electrophoresis in N acetic acid and twice by paper chromatography in the previously mentioned solvent and showed then small but definite activity in the *Lactobacillus leichmannii* and *Euglena gracilis* test. Since the presence of contaminating vitamin B<sub>12</sub> cannot be completely excluded, the microbiological activity of the substance must be interpreted with some caution.

No conversion was found with *E. coli* or several gram positive bacteria tested (*B. subtilis*, *B. cereus*, *Str. faecalis* and *Staph. aureus*).

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## A Cytochemical Study of Enzymes in the Alveolar Phagocytes

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Alveolar phagocytes of several vertebrates have been studied using histochemical techniques. These cells have been shown to exhibit a strong activity of acid phosphatase, non-specific esterase, cathepsin and leucine aminopeptidase. They were also found to contain a high activity of DPNH diaphorase as well as succinic dehydrogenase. Furthermore, the enzymatic activity of eight out of the nine histochemically demonstrable pyridine nucleotide-linked dehydrogenases was found to be intense,  $\beta$ -hydroxybutyrate dehydrogenase activity, however, being absent in the lungs of all the species studied. This obvious blockage of fatty acid breakdown in alveolar phagocytes may be correlated with some pathological changes in the lungs.

The DPNH diaphorase activity was also demonstrated in the fetal mouse lungs. Here, the staining reaction was, however, localized in the endodermal cells only; thus, towards the end of the foetal life, the forming alveoli were found to be filled with cells of moderate enzyme activity, whereas the mesodermal connective tissue cells exhibited no enzyme activity at all. This finding strongly suggests that the alveolar cells are derived from the alveolar epithelium, *i.e.* of endodermal origin.