A COBALAMIN GLUTATHIONE COMPLEX

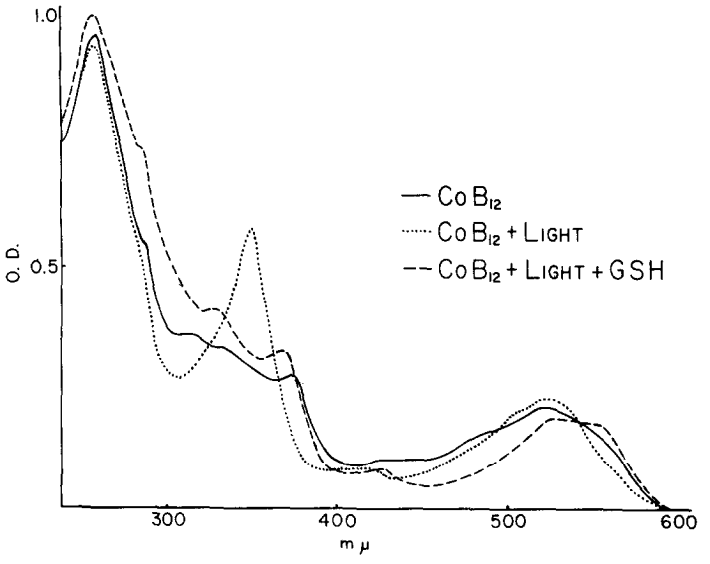
J. W. Dubnoff
Laboratory of Neurological Research
Loma Linda University
Los Angeles County Hospital
Los Angeles, California 90033

Received June 15, 1964

In a study of naturally occurring forms of vitamin B_{12} a light sensitive B_{12} derivative was isolated which proved to be identical with the dimethylbenzimidazole coenzyme (DMBC) isolated by Barker and co-workers (1960). Glutathione (GSH) added during the early stages of purification appeared to prevent the change in spectrum induced by light. However, experiments with spectrally pure B_{12} coenzyme revealed significant differences in the two spectra and the product no longer reacted to light. Aquocobalamin (B_{12b}) also reacted with glutathione to give the same spectrum above 350 mμ. This reaction was unique to glutathione. Homocysteine, cysteine and mercaptoethanol converted aquocobalamin to B_{12r} (Diehl and Murie, 1952).

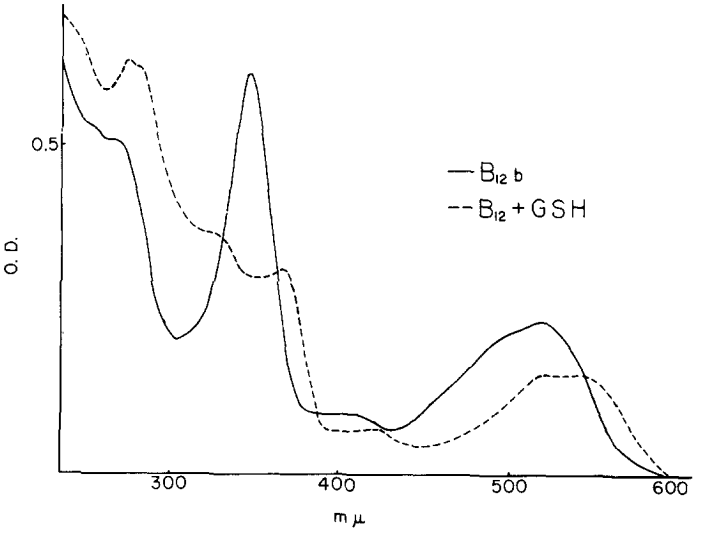
All experiments described in this paper were carried out under yellow sodium light. The B_{12} solutions were buffered with 0.1 M sodium phosphate, pH 7.0. The concentration of glutathione was 10^{-3} M. The DMBC coenzyme was isolated from Propionibacterium freudenreichii kindly supplied by Robert Fisher of the Bioferm Corp. B_{12b} was prepared by aerating an acid solution of cyanocobalamin under illumination.

Fig. 1. shows the spectrum of DMBC coenzyme before and after a 10 minute exposure to a daylight Multiray desk lamp at a distance of 10 cm. Addition of glutathione reduced the peak at 351 mμ, characteristic of aquocobalamin within 5 minutes and a peak at 370 mμ appeared.



If glutathione was added before light exposure, the 375 mμ peak of the DMBC coenzyme shifted to 370 mμ without the formation of the intermediate 351 mμ peak. The product was put on a Dowex 50X-2 column and eluted with 0.05 M sodium acetate buffer at pH 5.0. This compound was inactive in the diglycoll dehydrase system of Abeles (1961) and was insensitive to light. The 260 mμ peak remained, indicating theat the adenine moiety had not split off in the presence of glutathione.

The reaction of glutathione with aquacobalamin is shown in Fig. 2.; it resembles that with the light exposed DMBC coenzyme. The 290 mμ

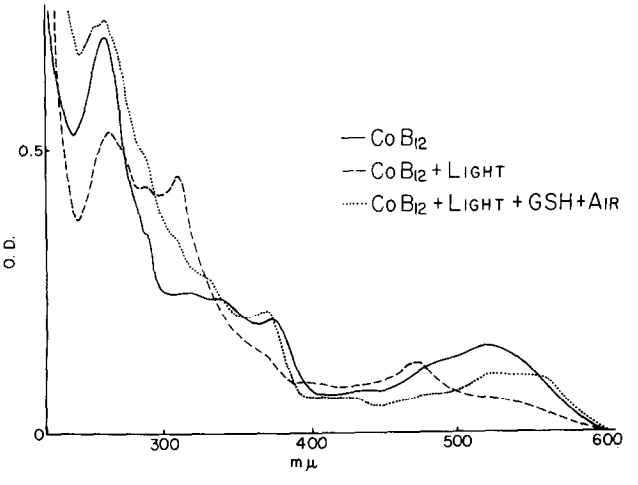


peak was sharper suggesting a loosening of the dimethylbenzymidazole bond to cobalt.

If equimolar adenosine was added to aquocobalamin and glutathione the spectrum was in all respects similar to that shown in Fig. 1. However, adenosine could be separated from the new compound on Sephadex 50. The isolated compounds formed from B_{12b} or the DMBC coenzyme were quite stable to air even though one would expect some catalysis of glutathione oxidation under these circumstances (Dubnoff and Phillips, 1959; Peel, 1963).

Several preparations of DMBC gave a 355 mμ peak on exposure to light. These reacted only slowly with glutathione. Likewise, samples of B_{12b} with a 355 mμ peak did not react. After standing 24 hours in solution the peak shifted to 351 mμ and reacted rapidly with glutathione. This shift in absorption maximum of B_{12b} has been reported by George, et al. (1960).

The addition of glutathione to the DMBC coenzyme, either before or after exposure to light under helium did not prevent the production of B_{12r}, as described by Barker and Brady (1961) and by Powelkiewicz, et al. (1960), (Fig. 3.). After admission of air the spectrum changed very slowly to that of the glutathione complex, compared with the fast reaction with glutathione shown in Fig. 1. There was no evidence for



the intermediate formation of B_{12b}.

If p-mercuribenzoate was added in excess of the glutathione, the spectrum immediately reverted to that of aquocobalamin. This occurred even under helium. The isolated aquocobalamin glutathione product reacted with one equivalent of p-mercuribenzoate, evidence that a definite compound had formed without the loss of the SH group of glutathione.

The superficial resemblance of the DMBC spectrum to that of B_{12r} has been suggested as evidence that the cobalt is in a reduced state in this compound. The data presented here show that the spectrum of the glutathione cobalamin complex closely resembles that of DMBC. These spectral changes brought about by addition or removal of glutathione do not appear to involve oxidation or reduction.

This research was supported by a research grant from the U. S. Public Health Service (AM 06716).

Since this paper was completed a report on a glutathione cobalamin complex by F. Wagner and K. Bernhauer appeared in the Annals of the New York Academy of Sciences, 112, 580 (1964).

References

Abeles, R. H. and Lee, H. A., Jr., J. Biol. Chem., 236, 2347 (1961).

Barker, H. A., in Second European Symposium on Vitamin B_{12} and the Intrinsic Factor. H. C. Heinrich, Ed., Enke Verlag, Stuttgart, Germany.

Barker, H. A., Smyth, R. D., Weissbach, H., Toohey, J. I., Ladd, J. N., and Volcani, B. E., J. Biol. Chem., 235, 480 (1960).

Brady, R. O. and Barker, H. A., Biochem. Biophys. Res. Commun. 4, 373 (1961).

Cooley, G., Ellis, B., Petrow, V., Beaven, G. H., Holliday, E. R., and Johnson, E. A., J. Pharm. and Pharmacol. 3, 271 (1951).

Diehl, H., and Murie, R., Iowa State Coll. J. Sci., 26, 555 (1952).

Dubnoff, J. W. and Phillips, L., Fed. Proc. 18, 219 (1959).

George, P., Irvine, D. H. and Glouser, S. C., Ann. N. Y. Acad. Sci., 88, 393 (1960).

Pawelkiewicz, J., Bartosinski, B. and Walerych, W., Bull. Acad. Polonaise Sciences, CI. II, 8, 123 (1960).

Pawelkiewicz, J., Bartosinski, B. and Walerych, W., Bull. Acad. Polonaise Sciences, CI. II, 8, 433 (1960).

Peel, J. L., Biochem. J., 88, 296 (1963).