

Table 1. TOTAL IODINE CONTENTS OF THE EYE TISSUES FROM FIVE NORMAL SUBJECTS ($\mu\text{g}/100 \text{ G}$)

Tissues	Mean	Fresh tissue $\pm S.D.$	Range	Mean	Dry tissue $\pm S.D.$	Range
Optic nerve	0.84	0.48	6.2-7.5	26.98	0.85	26.5-27.4
Lens	11.02	0.62	10.5-12.0	43.80	0.24	43.0-44.2
Uvea	18.92	1.38	17.3-20.5	104.64	0.82	103.8-105.8
Cornea	9.90	0.75	9.0-10.8	51.82	0.28	51.5-52.2
Sclera	2.70	0.47	2.0-3.2	8.96	0.26	8.6-9.2
Vitreous humour	5.44	0.31	5.1-5.9	387.92	2.48	385.5-391.5
Conjunctiva	19.04	1.63	17.2-20.8	74.08	0.69	73.0-74.8
Orbicular muscle	23.70	0.98	22.5-25.0	94.56	0.62	94.0-95.6
Orbital fat	26.24	1.56	24.3-28.0	82.96	0.93	82.0-84.2
Aqueous humour	3.42	0.32	3.0-3.8			

that iodine which is not bound to protein in the blood serum. The orbital fat contains an appreciable amount of iodine. Azevedo and De Jorge¹ had found high contents of mineral elements, especially sodium, phosphorus and copper, in the orbital fat, and these findings may suggest some physiological function of this fat in the mineral exchange of the eye.

F. B. DE JORGE
N. KARA JOSÉ

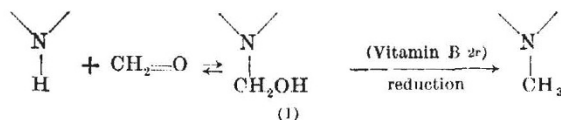
Department of Medicine and
Department of Ophthalmology,
University of São Paulo,
Brazil.

¹ Azevedo, M. L., and De Jorge, F. B., *Ophthalmologica*, **149**, 43 (1965).

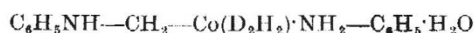
² Bird, R., and Jackson, D. J., *Clin. Chem.*, **8**, 389 (1962).

Synthesis of Methyl Groups catalysed by Vitamin B₁₂ *in vitro*

THE use of Raney metal catalysts for the reductive methylation of amines with formaldehyde and hydrogen is well known¹. We wish to report that this reaction is also catalysed by vitamin B₁₂ *in vitro*, and thus is potentially of biochemical importance. For example, a 1:1 mixture of aniline and formaldehyde in methanol and water absorbs hydrogen at room temperature and 1 atm. of pressure if a solution of vitamin B₁₂ is added. *N*-methyl-aniline is formed in significant yield (1).

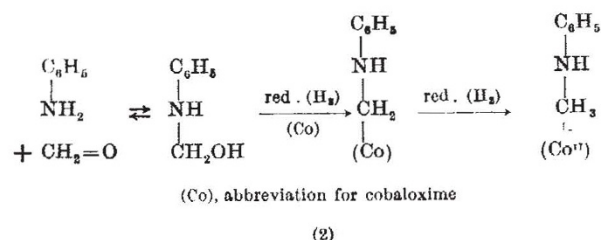


Bis-dimethylglyoximate cobalt (II) complexes such as $\text{Co}(\text{D}_2\text{H}_2) \cdot 2\text{H}_2\text{O}$ ('Diaquocobaloxime (II)')² (D = dianion of dimethylglyoxime) which may be regarded as simple models of vitamin B₁₂ were similarly found to be catalytically active. Aniline could also be substituted by *N*-methylaniline which was converted to *N,N*-dimethylaniline under analogous conditions. In order to isolate or detect intermediates of this reaction, the reductive alkylation of aniline was also carried out using stoichiometric amounts of the reactants. When 0.4 moles of aniline and 0.25 moles of formaldehyde (in 40 per cent aqueous solution) were added to a suspension of 0.20 moles of $\text{Co}(\text{D}_2\text{H}_2) \cdot 2\text{H}_2\text{O}$ in 1 l. of methanol under 1 atm. of hydrogen, 0.1 mole of hydrogen was rapidly absorbed on stirring and the cobaloxime dissolved completely. On addition of 1 l. of water a crystalline complex precipitated in a yield of 94 per cent, which was dissolved in methanol and reprecipitated by adding water. After drying at 0.1 mm of mercury 25° C it analysed for $\text{C}_{21}\text{H}_{29}\text{N}_5\text{O}_4\text{Co} \cdot \text{H}_2\text{O}$, corresponding to



The complex decomposed in mineral acid to Co^{++} , dimethylglyoxime, aniline, formaldehyde and traces of *N*-methylaniline. On heating to 100° C in pyridine solution methylaniline and aniline were formed. With

mercaptans such as CH_3SH , methylaniline was formed in an amount near to the expected stoichiometric quantity. This suggested the presence of a Co—C bond, since substituted alkylcobaloximes (and -cobalamins) are usually cleaved under reducing conditions³. The complex cannot be isolated if the cobaloxime is employed in catalytic amounts, because the reductive cleavage of the Co—C bond readily occurs with molecular hydrogen in the presence of cobaloxime (II) as the electron transfer catalyst; it thus may be regarded as a reactive intermediate in the catalytic reductive methylation (2):



Under essentially identical conditions methylthioethers were obtained from thiols, formaldehyde, and vitamin B₁₂, or the cobaloximes as the catalysts of the reduction. It is still not known whether these reactions also occur *in vivo*. A vitamin B₁₂ requirement for the reductase converting 5,10-methylene-tetrahydrofolate into 5-methyl-tetrahydrofolate has not yet been established. It is conceivable, however, that vitamin B₁₂ may promote the *de novo* synthesis of 5-methyl-tetrahydrofolate in connexion with the enzymatic methionine formation. Even if the actual reductase should prove B₁₂ independent, the cobinamide containing enzyme could serve as an auxiliary system.

G. N. SCHRAUZER*
R. J. WINDGASSEN

Shell Development Company,
Emeryville,
California.

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* Present address: Department of Chemistry, University of California, San Diego, Revelle College, La Jolla, California 92038.

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² Schrauzer, G. N., and Windgassen, R. J., *Chem. Ber.*, **99**, 602 (1966).

³ Schrauzer, G. N., and Windgassen, R. J., *J. Amer. Chem. Soc.*, **88**, 3738 (1966).

Pitfalls in Electron Spin Resonance Spectrometry in Biological Tissues

WE have been following with interest the papers concerning electron spin resonance (ESR) signals in biological materials published by various workers in the USSR, the United Kingdom, and the United States¹⁻⁶. Since the published results in selected normal and tumorous tissues conflict in reporting the presence or absence of an ESR signal at about 2.004 g, we investigated several of the variables in the detection of the signal and its amplitude. We feel it is important for workers in the biological and pathological field to follow standard procedures and to be