

## THE INABILITY OF THIOLS TO REDUCE COBALAMINS IN THE ABSENCE OF A METAL ION \*

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It has been reported that cysteine, thioglycolic acid and other thiols are able to reduce cobalamins [1-3]. Since reduced cobalamins are reoxidized by molecular oxygen, cobalamins are able therefore to catalyze the oxidation of thiols in the presence of air [4]. We have found, however, that the reduction of hydroxocobalamin ( $B_{12a}$ ) by thiols is indirect and is mediated by a metal ion present as an impurity in the  $B_{12a}$  sample.

Reduction of  $B_{12a}$  has been followed by recording the spectral curve in the range 270-400 m $\mu$  by a Beckman DK2A spectrophotometer. Quartz cuvettes converted in Thunberg tubes, provided with 2 side arms, were used. In the central cavity was placed 0.09  $\mu$ moles of  $B_{12a}$  (Pierrel, Milano) dissolved in 2 ml  $H_2O$  and 0.5 ml 0.02 M acetate buffer pH 4.2. In one side arm was placed 0.45  $\mu$ moles cysteamine hydrochloride dissolved in 0.3 ml  $H_2O$ . In the other side arm was placed 0.2 ml of 0.5 M glycine-NaOH buffer pH 8.9. The tube was evacuated with a water pump and refilled with argon three times.

Fig. 1 shows the spectral curve of  $B_{12a}$  at pH 4.2 (aquocobalamin  $\lambda$  max 350 m $\mu$ ). When cysteamine is added at pH 4.2 the curve  $B_{12a}$  changes to that reported for the complex of  $B_{12a}$  with thiols [3,4]. Under the present conditions the complex is formed slowly and reaches its maximum in 20 minutes. No reduction of  $B_{12a}$  is observed at pH 4.2. When the pH of the solution containing the complex has risen from 4.2 to 8.9 by the addition of glycine-NaOH buffer,  $B_{12a}$  is reduced as seen by the slow change of the spectrum to that of  $B_{12r}$  [5]. The reduction is almost complete in 60 minutes. If an experiment similar to that described

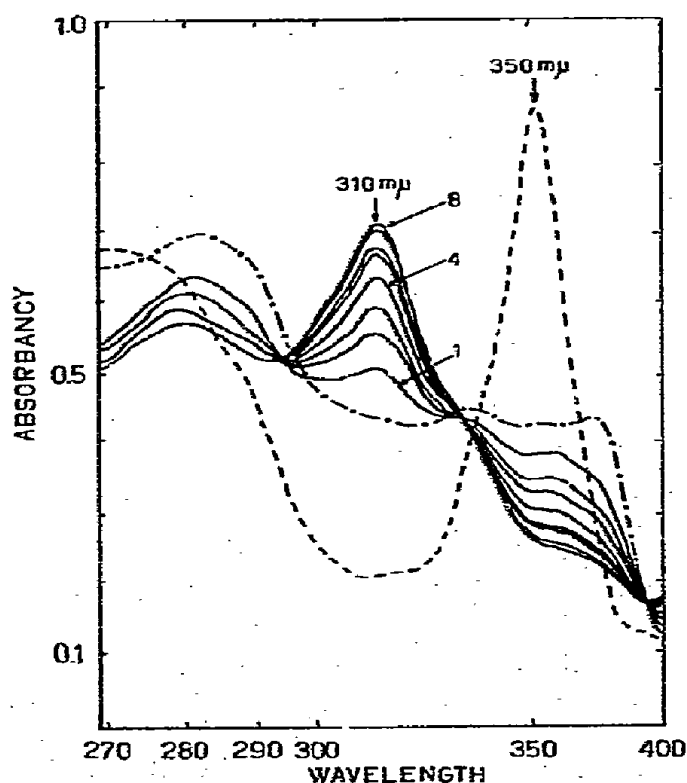


Fig. 1. —  $B_{12a}$  in  $H_2O$ ; - - - - - cysteamine- $B_{12a}$  complex, 20 min after the addition of cysteamine at pH 4.2; —  $B_{12r}$  formation after increasing the pH solution to 8.9; 1 = after 2 min; 2 = after 4 min; 3 = after 6 min; 4 = after 10 min; 5 = after 20 min; 6 = after 30 min; 7 = after 45 min; 8 = after 60 min.

above is carried out in the presence of  $2.5 \times 10^{-3}$  M EDTA, the complex thiol- $B_{12a}$  at pH 4.2 is again produced, but the addition of glycine-NaOH buffer does not cause the reduction to  $B_{12r}$ . Taking this as a pre-

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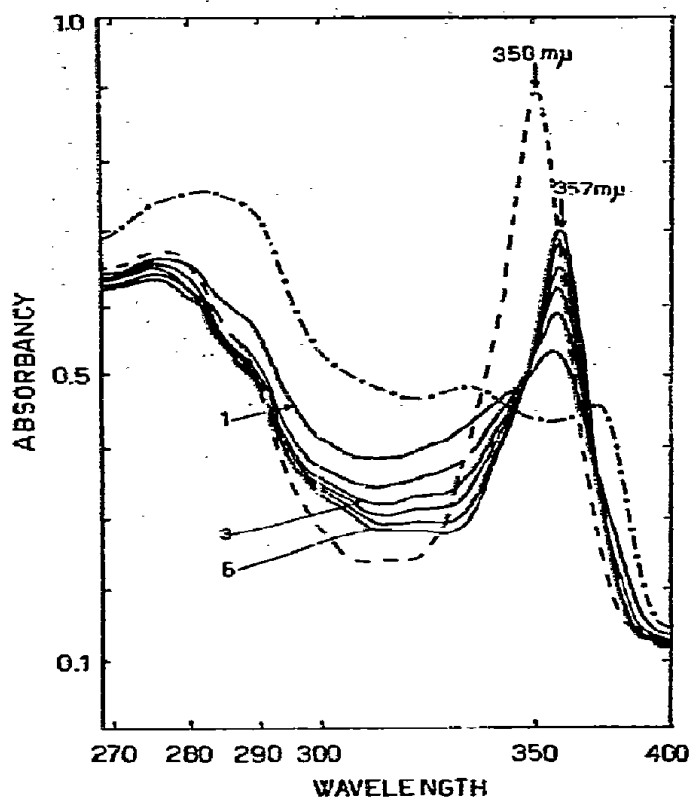


Fig. 2. ———  $B_{12a}$  after passage through a chelating resin, dissolved in  $H_2O$ . - - - - - cysteamine- $B_{12a}$  (passed through a chelating resin) complex, 20 min after the addition of cysteamine. ——— splitting of the cysteamine- $B_{12a}$  complex, after rising the pH to 8.9; 1 = after 2 min; 2 = after 4 min; 3 = after 10 min; 4 = after 20 min; 5 = after 30 min; 6 = after 60 min.

sumptive evidence for the necessity of a metal ion impurity as an intermediate for the reduction of  $B_{12a}$ , we have eliminated eventual metals by passage of the  $B_{12a}$  solution through a chelating resin (Dowex A1 in the  $Na^+$  form). Using the solution of  $B_{12a}$  passed through the chelating resin, the complex thiol- $B_{12a}$  at pH 4.2 is again produced upon the addition of cysteamine (fig. 2); however, when the pH of the solution is raised to 8.9 no reduction occurs. In the place of the curve of  $B_{12r}$ , the curve is changed slowly to that of  $B_{12a}$  at pH above 7. This observation indicates that the alkalization in the absence of a metal ion produces the cleavage of the thiol- $B_{12a}$  complex in the place of reducing  $B_{12a}$  to  $B_{12r}$ . Identical results have been obtained when  $B_{12a}$  free from contaminating metals was prepared using paper electrophoresis in 0.5 M acetic acid. In order to establish whether the suppression of

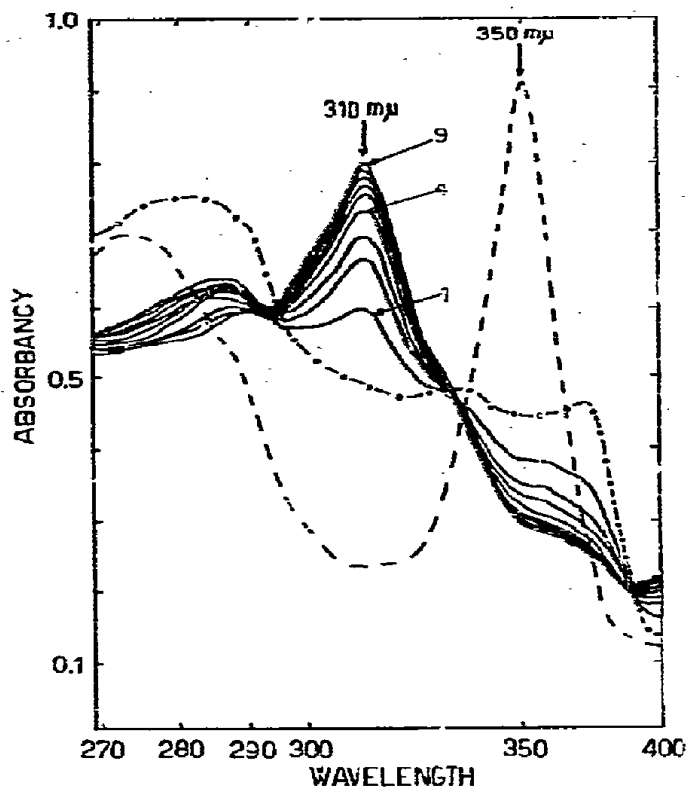


Fig. 3. - - - - -  $B_{12a}$  after passage through a chelating resin, dissolved in  $H_2O$ . - - - - - cysteamine  $B_{12a}$  (passed through a chelating resin) complex 20 min after the addition of the cysteamine. ———  $B_{12r}$  formation, after addition of pH 8.9 buffer in the presence of  $Cu^{++} 3 \times 10^{-7} M$ . 1 = after 2 min; 2 = after 4 min; 3 = after 6 min; 4 = after 8 min; 5 = after 10 min; 6 = after 20 min; 7 = after 30 min; 8 = after 45 min; 9 = after 60 min.

the reduction of  $B_{12a}$  by cysteamine has to be related with the removal of a metal ion, the experiment reported in fig. 2 has been repeated in the presence of  $Cu^{++}$  ions. As seen in fig. 3, the addition of a trace of  $Cu^{++}$  ions reintroduces the reduction of  $B_{12a}$  by cysteamine.  $Fe^{+++}$ ,  $Co^{++}$ ,  $Ni^{++}$  and  $Zn^{++}$  have been assayed in the place of  $Cu^{++}$ , without success. The necessity for a metal ion has been observed also when cysteine or mercaptoethanol were used in the place of cysteamine.

In the light of the results reported above it appears that the reduction of  $B_{12a}$  by thiols is indirect. The presence of a metal ion is necessary and a possible explanation is that the thiol reduces the metal which in its turn reduces the cobalamin. That this is a reasonable explanation has been checked by establishing

that, under the conditions of the present experiments,  $\text{Cu}^+$  in the form of  $\text{CuCl}$  dissolved in 0.25 M  $\text{KCl}$  actually reduces  $\text{B}_{12a}$  to  $\text{B}_{12r}$  in the absence of a thiol at a very fast rate.

Although the addition of a trace of  $\text{Cu}^{++}$  ions may replace the metal removed by the passage of the  $\text{B}_{12a}$  solution through the chelating resin, we do not so far have any evidence to establish which metal is actually present as an impurity in  $\text{B}_{12a}$  catalysing the reduction of the cobalamin by thiols.

#### References

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