Aqueous humour vitamin B₁₂ and intramuscular cobalamins

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Previous work on the vitamin B₁₂ content of the aqueous humour (Phillips, Ainley, van Peborgh, Watson-Williams, and Bottomley, 1968) suggests that the levels found in a series of mainly elderly patients (mean: 29.9 pg./ml.) are of the same order as those found in cerebrospinal fluid (mean: 21 pg./ml.) (Kidd, Gould, and Thomas, 1963).

As an extension of this study, the response of aqueous vitamin B₁₂ levels to injected cyanocobalamin and hydroxocobalamin was examined.

Methods

The patients were admitted for extraction of senile cataracts, and were therefore mostly over 60 years of age. Between 1 and 3 hours before operation ten patients were given 1 mg. cyanocobalamin (Cytamen, Glaxo) and ten patients were given 1 mg. hydroxocobalamin (Neocytamen, Glaxo) by intramuscular injection. In a second group of patients the same doses were given 15 to 20 hours before operation, in twenty cases cyanocobalamin and in seventeen hydroxocobalamin. At operation aqueous humour for analysis was removed by a dry-sterilized syringe and cannula, with care to minimize contamination by blood; just before or just after operation venepuncture was performed for estimation of serum B₁₂.

Total vitamin B₁₂ in serum and aqueous humour was estimated by biological assay with *Euglena gracilis*, and the values from patients given cyanocobalamin were compared with those from patients given hydroxocobalamin.

Results (Table and Figs 1 and 2)

A canonical analysis was done on the data and a discriminant function was constructed, based on the vitamin B₁₂ concentration in serum and in aqueous. The difference between

Table *Mean Vitamin B₁₂ in aqueous humour and serum*

<i>Interval after injection (hrs)</i>	<i>Form of Vitamin B₁₂ given</i>	<i>Number of Patients</i>	<i>Mean aqueous total Vitamin B₁₂ (pg./ml.)</i>	<i>Mean serum total Vitamin B₁₂ (pg./ml.)</i>
1-3	Cyanocobalamin	10	560.9	27,915
	Hydroxocobalamin	10	184.6	37,493
15-20	Cyanocobalamin	20	270.8	3,132
	Hydroxocobalamin	17	176.8	18,904

the groups given cyanocobalamin and hydroxocobalamin was tested by an "approximate test" of Bartlett (1938). For details of the principles underlying this method and the statistical techniques used, see Seal (1964).

FIG. 1 Distribution of cyanocobalamin and hydroxocobalamin between serum and aqueous humour 1 to 3 hours after injection

FIG. 2 Distribution of cyanocobalamin and hydroxocobalamin between serum and aqueous humour 15 to 20 hours after injection

The two groups were significantly different at a high level. For those given vitamin B₁₂ 1 to 3 hours before operation, the significance level was 0.1 per cent. For those given vitamin B₁₂ 15 to 20 hours before operation the significance level was 0.05 per cent. The difference in the significance levels is likely to be due mainly to the fact that there were more individuals in the second group.

Discussion

The results suggest that cyanocobalamin enters the aqueous humour from the blood much more readily than does hydroxocobalamin. Both in the group treated 1 to 3 hours before operation and that treated 15 to 20 hours before, the mean aqueous vitamin B₁₂ level was higher after cyanocobalamin than after hydroxocobalamin, although the serum level was higher after hydroxocobalamin than after cyanocobalamin. However, at 1 to 3 hours, the difference between the two groups in serum vitamin B₁₂ was less marked than at 15 to 20 hours, probably because injection of cyanocobalamin produces almost as high a rise in serum vitamin B₁₂ initially as does hydroxocobalamin; but in the former case the rise is more transient so that the serum level has fallen considerably by 15 to 20 hours. This observation on serum levels has been made by several other workers (Herbert, Zalusky, and Skeggs, 1963; Hertz, Østergaard Kristensen, and Hoff-Jørgensen, 1964), who found a greater excretion of vitamin B₁₂ in the urine in the few days following treatment with cyanocobalamin than with hydroxocobalamin. It may be attributed to the greater binding of hydroxocobalamin to plasma proteins, which would tend to impede both its entry into the eye and its passage through the glomerular membrane.

The values obtained probably underestimate the true difference in penetration of the two forms of vitamin B₁₂ into the aqueous, since removal of aqueous by paracentesis would tend to produce transudation of protein-rich fluid from the capillaries of the iris and ciliary body ("plasmoid aqueous"). Because of the greater protein-binding power of hydroxocobalamin (Hertz and others, 1964) the overestimate for aqueous vitamin B₁₂ might be greater after injection of hydroxocobalamin than after cyanocobalamin.

The levels of vitamin B₁₂ in the aqueous humour may not be a measure of the levels in the vitreous or in the posterior chamber if diffusion occurs from the relatively permeable iris vessels; however, because the aqueous levels are similar to those of the cerebrospinal fluid (Phillips and others, 1968; Kidd and others, 1963) it seems likely that vitamin B₁₂ accompanies secreted aqueous.

The greater ease with which cyanocobalamin enters the aqueous is consistent with the view (Mackenzie and Phillips, 1968), loosely called the "Trojan horse" hypothesis, that a toxin might enter the eye in the aqueous, diffuse posteriorly through the vitreous, and affect the retina from its inner surface. The existence of a flow of organic anions pumped from the vitreous through the retina (Cunha-Vaz and Maurice, 1967) may increase the risk. It is known that the reversible reaction:

can take place *in vitro*. In tobacco amblyopia, conversion of serum hydroxocobalamin to cyanocobalamin by the cyanide in tobacco smoke (if such a reaction occurs *in vivo*) might enable the cyanide radicle to enter the eye in larger amounts as cyanocobalamin, diffuse through the vitreous, and, especially at the macula, be decomposed by light to yield hydroxocobalamin and free cyanide, the latter affecting the central retina directly. Of course, photolysis could release cyanide no matter what route cyanocobalamin had taken to reach the retinal receptors or nerve fibres.

Rather against that hypothesis is the observation that injections of cyanocobalamin produce a little improvement in tobacco amblyopia (Chisholm, Bronte-Stewart, and Foulds, 1967), although much less and more slowly than hydroxocobalamin; however, some hydroxocobalamin is usually present in solutions of cyanocobalamin, especially if exposed to light, and the response of serum levels and presumably tissue levels to injection of the two forms is different. It should also be noted that we have not been able to distinguish between cyanocobalamin and hydroxocobalamin in aqueous and serum, and our assumption that these are the specific forms of vitamin B₁₂ responsible for the raised total vitamin B₁₂ levels in aqueous and serum after intramuscular injection of the two compounds may not be justified; further work on this aspect is in progress.

Summary

Intramuscular injection of 1 mg. cyanocobalamin produced a higher level of total aqueous vitamin B₁₂ as measured by biological assay with *Euglena gracilis* than 1 mg. hydroxocobalamin, in patients having cataract extractions 1 to 3 hours after injections (ten patients in each group) and 15 to 20 hours afterwards (twenty and seventeen patients respectively), in spite of a higher serum level after hydroxocobalamin. In tobacco amblyopia (as in any toxic amblyopia), aqueous humour could form a route of entry to the eye for cyanide (or other toxin) which could then pass towards the retina *via* the vitreous. Cyanocobalamin

is a cyanide-carrier which this work has shown to gain relatively easy entry to the eye, where it may be broken down (*e.g.* by light) to produce free cyanide ("Trojan horse" hypothesis).

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