IN VITRO STUDY OF THE VITAMIN B₁₂-BINDING CAPACITY IN EYE TISSUES

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The vitamin B₁₂-binding capacity in bovine, pig and rat eye tissue extracts have been investigated *in vitro*. The B₁₂-binding capacity in decreasing order was: cornea, retina, optic nerve and lens in bovine eye tissue extracts; cornea, retina, optic nerve and lens in pig eyes; and cornea, optic nerve and lens in rat. There are differences in B₁₂-binding capacity in individual eye tissue extracts. However, no interspecies differences in the B₁₂-binding capacity were found among bovine, pig, and rat eye tissue extracts, especially in the cornea, lens, retina and optic nerve *in vitro*.

The effects of vitamin B₁₂ (B₁₂) on eye tissue have been investigated in many ways from the biochemical and clinical aspects; however, much less information on its affinity to eye tissue is available.

The bulk of B₁₂ in serum or tissue is normally found in a bound form with a protein carrier (1). The carrier protein is normally in an unsaturated state as it is able to bind additional B₁₂ when added *in vivo* or *in vitro* (2-5).

In the present study, the authors have attempted to investigate B₁₂-binding capacity in bovine, pig, and rat eye tissue extracts *in vitro* using a ⁵⁷Co-cyanocobalamin.

MATERIALS AND METHODS

Bovine and pig eyes obtained from a local abattoir were kept at 4°C and used within 6 hr after sacrifice. Wistar strain rats weighing about 200 g were used. Eye tissue extracts were prepared according to OKUDA's report concerning the determination of B₁₂-binding capacity in the rat stomach (6). The cornea, lens, conjunctiva, iris, vitreous humour, chorioidea, sclera, aqueous humour, retina and optic nerve of the bovine eye, along with the cornea, lens, retina and optic nerve of pig eyes and the cornea, lens and optic nerve of rat eyes were used in this experiment. The lens, iris, chorioidea, vitreous humour and retina were homo-

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genized with an aliquot of physiological saline solution at 4°C using an Elvehjem homogenizer or Dounce microhomogenizer. Hard tissues such as the cornea, conjunctiva, sclera and optic nerve were minced with a scissors and homogenized in a Waring Blendor with an aliquot of physiological saline solution at 4°C. The homogenate was centrifuged at 3,000 rpm for 20 min. The pH of the opalescent supernatant fluid was found to be 6–7. Each supernatant was measured for its N content, and determination of B₁₂-binding capacity was carried out in the following ways.

Supernatant was mixed with an equal volume of radiocyanocobalamin (⁵⁷Co-CN) solution (50 mμg/ml) with a specific activity of 1.1 μCi/μg, purchased from the Radio Chemical Centre, England. The mixtures as well as the controls containing ⁵⁷Co-CN were incubated at 37°C for 60 min and thereafter dialyzed in Visking Cellophane tubes against running tap water at 4°C for 48 hr. The contents of the tube were then transferred quantitatively into a plastic container and its radioactivity was measured with a Well-type scintillation counter (Aloka, TDC-6, PS-9 Tokyo, Japan).

B₁₂-binding capacity of the eye tissue extracts was calculated in terms of undialyzable radioactivity, and nitrogen concentrations of the eye tissues were determined according to LOWRY *et al.* (7).

Eye tissue's B₁₂-binding: N ratio was represented by $1/X \cdot 10^{-3}$

$$X = \frac{\text{Nitrogen content}}{\text{Bound } ^{57}\text{Co-cyanocobalamin}}$$

RESULTS AND DISCUSSION

In the present study, we found that B₁₂ binds with the bovine, pig, and rat eye tissue extracts, and that there are differences in the binding capacity of B₁₂ in each tissue extract of the eye (Table 1).

Concerning the B₁₂-binding capacity of bovine eye tissue extracts, the cornea was the largest, followed by the retina, optic nerve, lens, conjunctiva, iris, chorio-idea, vitreous humour, sclera and finally, the aqueous humour was the smallest.

The binding capacity in the pig eye extracts in decreasing order was cornea, retina, optic nerve and lens. It was also cornea, optic nerve and lens in the rat eye tissue extracts.

It is very interesting that the ranking of the binding capacity of B₁₂ in the bovine, pig, and rat eye tissue extracts was quite similar; that is, cornea, retina, and lens or optic nerve.

The fact that B₁₂ binds, to a considerable degree, with the cornea, retina, lens and optic nerve extract *in vitro* suggests that these values of binding capacity could also be applicable to the affinity to B₁₂, which was administered either orally or locally for therapeutic purpose.

It is well known that the optic nerve is occasionally involved in pernicious

Table 1. Vitamin B₁₂-binding capacity in the bovine, pig and rat eye tissue extracts.

	B ₁₂ -binding capacity (mμg/ml)	N (mg/ml)	Binding N-ratio (×10 ⁻³)
Bovine			
Cornea	3.393±0.926 ^a	0.19±0.05	1/0.056±0.013
Retina	3.922±0.938	0.40±0.17	1/0.102±0.009
Optic nerve	2.177±0.836	0.27±0.09	1/0.124±0.018
Lens	1.757±0.647	0.26±0.02	1/0.148±0.038
Conjunctiva	0.255±0.001	0.04±0.01	1/0.157±0.023
Iris	0.777±0.188	0.15±0.06	1/0.193±0.026
Chorioidea	1.524±0.195	0.32±0.02	1/0.210±0.036
Vitreous humour	0.894±0.065	0.22±0.06	1/0.246±0.045
Sclera	0.545±0.177	0.18±0.02	1/0.330±0.076
Aqueous humour	7.856±1.631	4.25±0.81	1/0.541±0.009
Pig			
Cornea	2.955±0.193	0.26±0.05	1/0.088±0.005
Retina	1.239±0.378	0.14±0.10	1/0.113±0.026
Optic nerve	1.688±0.836	0.27±0.18	1/0.160±0.048
Lens	1.106±0.381	0.24±0.07	1/0.217±0.015
Rat			
Cornea	0.690±0.093	0.02±0.01	1/0.029±0.008
Optic nerve	0.584±0.115	0.07±0.03	1/0.120±0.016
Lens	3.526±0.256	1.10±0.32	1/0.312±0.053

^a Average of three animals with standard deviation.

anemia (8). HEATON *et al.* (9) reported that low serum B₁₂ levels were found in heavy smoker's amblyopia. PHILLIPS *et al.* (10, 11) reported that abnormally low values of B₁₂ in the aqueous humour and serum could be found in patients suffering from tobacco amblyopia and that they improved following treatment with B₁₂. OTAGURO (12) showed that B₁₂ acts effectively on the oxygen consumption due to anaerobic glycolysis in the optic nerve. MIYAURA (13) described that B₁₂ encourages the regeneration of rhodopsin in the retina. These observations indicate that there are close relationships between retinal or optic nervous function and B₁₂.

In spite of the fact that the effects of B₁₂ on the retina or optic nerve have been reported experimentally or clinically, its effect on the cornea or lens is still obscure. The most outstanding findings in this *in vitro* experiment was that the binding capacity of B₁₂ in the cornea and lens extract was larger than those in other eye tissue extracts with the exception of the retina and optic nerve.

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