Change in A^3 Hæmoglobin due to β-Chain

SEVERAL authors[1-3] have shown that the minor anodic fraction of hæmoglobin obtained by electrophoresis at *p*H 8·6 is part of the hæmoglobin modified during the ageing of the red cells. Proof is submitted here that the modification of the electrical charge of hæmoglobin A^3 (Hb^{A_3}) is due only to the modification of the β-chain. This is proved by comparison of the electrophoretic migration of the hybrids obtained between canine hæmoglobin Hb^{CAN} and Hb^A and between Hb^{CAN} and Hb^{A_3}.

Hybridization between Hb^A and Hb^{CAN} has already been described[4]. If it is carried out with equimolar hæmoglobins[5] the results are as follows:

25 per cent of slow hybrid $\alpha^A \beta^{CAN}$
25 per cent of fast hybrid $\beta^A \alpha^{CAN}$

and reconstitution of 25 per cent of human hæmoglobin $\alpha^A \beta^A$ and 25 per cent of canine hæmoglobin $\alpha^{CAN} \beta^{CAN}$.

In our work Hb^{A_3} was obtained by chromatography on 'Amberlite'[6] with No. 5 developer, followed by electrophoresis on starch blocks[7] to eliminate non-hæm proteins.

Hybridization was performed in equimolar ratio as described previously[5].

Examination of the hybrids was accomplished by starch-gel electrophoresis at *p*H 8·6 in borate buffer[8].

In these conditions (Fig. 1), Hb^{A_3} gave two hybrids with Hb^{CAN}: a slow hybrid $\alpha^A \beta^{CAN}$ reacts in the same way as the $\alpha^A \beta^{CAN}$ hybrid. On the contrary a fast hybrid $\beta^{A_3} \alpha^{CAN}$ is faster than $\beta^A \alpha^{CAN}$.

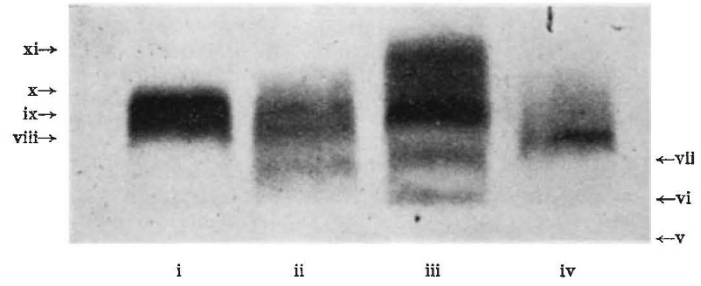

Fig. 1. Hybridization of Hb^{A_3} with Hb^{CAN}. Analysis in starch-gel borate/borate system *p*H 8·7. i, Mixture of Hb^A and Hb^{CAN} marker; ii, hybridization $Hb^A \times Hb^{CAN}$; iii, hybridization $Hb^{A_3} \times Hb^{CAN}$; iv, mixture of Hb^{A_3} and Hb^{CAN} marker; v, commencing line; vi, non-hæm protein; vii, hybrid $\alpha^A \beta^{CAN}$; viii, Hb^{CAN}; ix, Hb^A; x, hybrid $\beta^A \alpha^{CAN}$; xi, hybrid $\beta^{A_3} \alpha^{CAN}$

It seems that the modification of Hb^{A_3} is essentially localized on the β-chain.

Moreover, it has been observed[9] that incubation of Hb^A with glutathione modifies its electrophoretic mobility with augmentation of the ratio of Hb^A.

We have effected hybridization between Hb^{CAN} and Hb^{A_3} artificially induced by glutathione. Results were identical to those already mentioned.

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PATHOLOGY

Optic Neuritis and Encephalomyelitis in Monkey produced with Human Optic Nerve

OPTIC neuritis is an integral part of the encephalomyelitis which rarely follows anti-rabies vaccination with brain preparations[1,6]. This condition in man is an accidental allergic encephalomyelitis and appears to be identical to the experimental allergic encephalomyelitis (EAE) produced in animals by injection of brain. Optic neuritis also occurs in other forms of encephalomyelitis and in multiple sclerosis[2]. The lesions in these demyelinating diseases may be due to an immunological process[3]. If so, an encephalitogenic antigen common to the developmentally related optic nerve, brain and spinal cord may account for the simultaneous appearance of the lesions in these structures. Suggestive evidence of such an antigen has been provided by the demonstration of optic nerve lesions in animals in which EAE was produced by injection of brain[3].

In the investigation reported here we attempted to determine whether the adult human optic nerve contains an antigen common to the brain, cerebellum and spinal cord. Since the brain antigen which causes experimental allergic encephalomyelitis is tissue and not species specific[4], we felt justified in testing in monkeys the encephalitogenic property of human optic nerve.

Optic nerves were obtained from refrigerated cadavers of patients who had no infectious or neurological illness and had been dead not longer than 24 h. Pieces about 1 cm long of the retrobulbar portion of the optic nerves were used. The tissue was ground in distilled water containing 0·25 per cent phenol to make a 33 per cent suspension. Equal amounts of this material and Freund's adjuvant were emulsified with a syringe. The adjuvant was a mixture of equal parts of heavy mineral oil and aquaphor and contained 15 mg heat-killed mycobacteria (*BCG*) per ml. Although the adjuvant is not essential for the production of EAE, it enhances the incidence and severity of the illness[5]. Previous experience has shown that the injection of adjuvant alone or in combination with non-neural tissue does not cause neurological lesions[4,7].

Each of five adult squirrel monkeys received five 0·1-c.c. intracutaneous injections into the abdominal