HAEMATOLOGY

Basic Protein of Normal Human Plasma

BASIC low molecular weight proteins of normal human blood, designated as B_1 and B_2, were recently discovered by Takahashi and Schmid[1]. Acidic and neutral low molecular weight serum proteins and peptides, however, have been known for many years[2–4]. In the present report the purification and partial characterization of one of the basic proteins, B_2, are described.

Fraction VI of pooled normal human plasma was chromatographed on a DEAE-cellulose column at *p*H 5·5 and Γ/2 0·005 (ref. 5). The resulting effluent was passed subsequently through a CM-cellulose column at the same *p*H and ionic strength to adsorb and concentrate the proteins from this solution. Fractional elution of the second column gave the $3S$ γ_1 (ref. 5) and $2S$ γ_2 (ref. 6) globulins and the mentioned basic components. The basic compounds were further purified on a DEAE-'Sephadex *A*-50' column at *p*H 8·4 and Γ/2 0·005. Final purification was achieved by chromatography on a hydroxylapatite column at *p*H 6·8. The resulting B_2-preparation revealed a single component on ultracentrifugation and starch gel electrophoresis at *p*H 8·6 (Fig. 1).

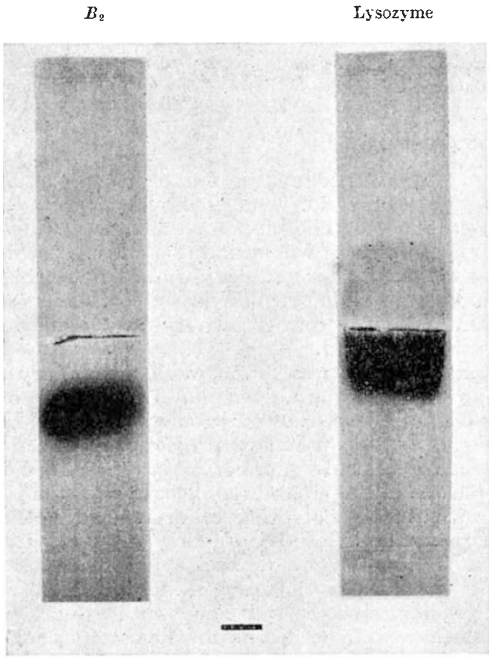

Fig. 1. Starch gel electrophoresis of B_2 in *p*H 8·6, Γ/2 0·01 borate buffer. Amido black was used as stain and lysozyme as reference. The protein moved toward the cathode (—).

The basic protein, B_2, was characterized in terms of some of its major physical chemical and chemical properties. Its molecular weight determined by the Yphantis procedure[7] was found to be approximately 9,000. On ultracentrifugation B_2 sedimented at the rate of 1·3*S*. The isoelectric point of this protein was estimated to between *p*H 10·0 and 10·5. Ultra-violet absorption (Fig. 2) between 250 and 290 mμ revealed a maximum that indicated a very low content of tyrosine and/or tryptophan. Chemical analysis of B_2 showed that its polypeptide moiety accounted for the total weight. Independent measurements confirmed the lack of neutral hexoses, hexosamines, and sialic acid.

We thank Dr. J. McComb, Mr. L. Larsen and their co-workers of the Division of Biologic Laboratories at Massachusetts Department of Health. This investigation

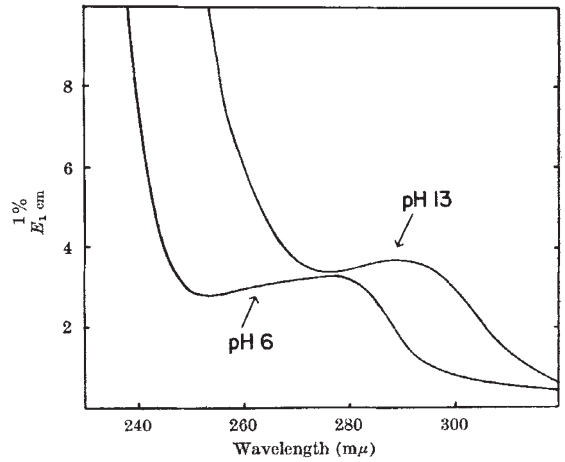

Fig. 2. Absorption curves of B_2 measured at *p*H 6 and 13, respectively.

was supported by grants from the National Institute of General Medical Sciences, U.S. Public Health Service.

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Decrease in the Concentration of Haemoglobin A_2 during Erythroleukaemia

DURING the past decade there have been several descriptions of abnormal haemoglobin in leukaemia[1–4]. The earliest reports emphasized the increase of foetal haemoglobin content in some cases of leukaemia in children and adults[1–3]. The occurrence of haemoglobin H in cases of erythroleukaemia and atypical chronic myeloid leukaemia was also reported[4]. In these cases no alteration in the level of the normal minor haemoglobin fraction, haemoglobin A_2, was noted. We have investigated an erythroleukaemic patient with a very low level of haemoglobin A_2 which developed in the course of leukaemia.

A 63 year old man, first seen in 1963, had clinical and haematological findings of subacute erythroleukaemia with signs of a haemolytic component (reticulocytosis, hyperbilirubinaemia, increased osmotic fragility before and after incubation at 37° C for 24 h). Coombs test and tests for complete antibodies were negative. Haemoglobin analysis with starch gel electrophoresis with borate buffer, *p*H 8·6, and alkali denaturation[1,5] gave normal results, namely foetal haemoglobin less than 1 per cent and no alteration in the content of the normal minor haemoglobin component, haemoglobin A_2 (3·5 per cent). The patient was successfully treated with corticosteroids, and clinical manifestations and haematological findings improved moderately. Nearly 1 year later, a second analysis of haemoglobin was carried out. Repeated determinations of haemoglobin A_2 with different methods such as starch gel electrophoresis[5], starch block electrophoresis[6] and DEAE-cellulose chromatography[7] showed a very low content, namely between 0·29 per cent and 0·5 per cent (Fig. 1). This finding was confirmed by Dr. C. Pik of the Department of Pediatrics, State University of Groningen, and also at the Central Laboratorium of the