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Haemoglobin Stanleyville II (α78 Asparagine → Lysine)

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[WITH SPECIAL PLATE FACING PAGE 95]

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Summary: A fourth observation of haemoglobin Stanleyville II has been made. The affected person was a 17-year-old female from the north-eastern area of the Congo Republic, which is entirely inhabited by Nilotes. The amino-acid substitution was found to be a replacement of the asparagine residue by one of lysine in the 78th position of the 141 of the α-chain.

Introduction

Haemoglobin Stanleyville II was first described in two families from the area where the north-east of the Congo Republic borders on the Sudan (Dherte, Vandepitte, Ager, and Lehmann, 1959). One family belonged to the Budu tribe, which was considered Bantu but which is surrounded by Nilotic tribes; the other family came from the region of the river Uele, where the population is of mixed Bantu–Nilotic stock. The mother of the propositus, from whom the haemoglobin had been inherited, was of mixed Greek–African origin. On electrophoresis at alkaline *p*H on paper and on starch Hb Stanleyville II has the mobility of Haemoglobin S or D; on electrophoresis at *p*H 6 it also resembles Hb D Punjab by not separating from Haemoglobin A. On column chromatography on amberlite resin at *p*H 6 Hb Stanleyville moves, however, behind Hb S and D.

A third family with this haemoglobin was described by Hall-Craggs, Marsden, Raper, Lehmann, and Beale (1964). It was of Nilotic origin and belonged to the Ulur tribe, which lives in the Western Nile region of Uganda. Little material was available, and the condition of the specimens was not very good. However, it was possible to demonstrate that Stanleyville II was an α-chain mutant, and the family study showed independent segregation of Hbs A, S, and Stanleyville II. It was suggested that the mutation was in the region of residues 93–139 of the α-chain. It has now been possible to place the amino-acid substitution within the tryptic peptide comprising residues 62–90 of the α-chain.

Case History

The subject of the present report was a 17-year-old nun, born in Dakwa, which is situated about 400 km. north-north-east of Kisangani (formerly Stanleyville). There are two tribes in this region, the Azande and the Amadi; they are both Nilotes and not Bantu. While in Belgium as a temporary refugee from the rebellion in her part of the Congo Republic she was admitted to hospital for treatment of schistosomiasis, and the abnormal haemoglobin was discovered in the course of her routine examination. A family study was not possible.

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Examination of the Haemoglobin

As in the previous subjects with Hbs A and Stanleyville II there was no anaemia which could be linked with this haemoglobin variant. The variant was discovered and studied originally by starch-block electrophoresis, a discontinuous buffer system (Van Ros, 1966) being used. Otherwise the techniques were the same as those in the two previous investigations (Dherte *et al.*, 1959; Hall-Craggs *et al.*, 1964). The methods used for the isolation of the haemoglobin and the identification of the amino-acid substitution have been summarized recently, and the necessary references have been given by Sick, Beale, Irvine, Lehmann, Goodall, and MacDougall (1967) and Beale (1967).

It was possible to demonstrate that the haemoglobin moved like Hbs S and D on paper and starch-gel electrophoresis at *p*H 8.9 and 8.6 respectively, but that on chromatography on Amberlite resin IRC 50 at *p*H 6 it moved more slowly. It did not separate from A on agar-gel electrophoresis at *p*H 6. It was shown to be an α-chain variant because on starch-gel electrophoresis a second Hb A_2 was noted to be moving behind the normal Hb A_2 as would be expected when the variant haemoglobin differs from Hb A ($\alpha_2^A \beta_2^A$) in its α-chains (Special Plate, Fig. 1). The variant α-chains would give rise both to a variant Hb A ($\alpha_2^X \beta_2^A$) and to a variant Hb A_2 ($\alpha_2^X \delta_2$).

TABLE I.—*Amino-acid Composition (Molar Ratios) of Two New Peptides from Hb Stanleyville II*

	Hb Stanleyville II		Hb A	
	αTpIX-a	αTpIX-b	α62–78	α79–90
Asp	3·9	0·9	5	1
Thr	1·1	0·1	1	0
Ser	0·1	2·2	0	2
Pro	0·9	0	1	0
Ala	3·8	2·9	4	3
Val	3·2	0	3	0
Met	0·8	0	1	0
Leu	1·1	3·2	1	3
Lys	1·0	1·0	0	1
His	0·9	1·9	1	2
Total residues	17	12	17	12

TABLE II.—*Sequence of αTpIX of Hb A and the Suggested Sequence in Hb Stanleyville II*

No. of Residues in α-chain:	62	63	64	68	72	74	75	76	77	78	79	85	87	88	89	90
Hb A TpIX ..	Val	Ala	Asp	Asn	His	Asp	Asp	Met	Pro	Asn	Ala	Asp	His	Ala	His	Lys
Hb Stanleyville:																
TpIX-a ..	Val	Ala	Asp	Asn	His	Asp	Asp	Met	Pro	Lys						
TpIX-b ..											Ala	Asp	His	Ala	His	Lys

The amino-acid composition of α^{A}TpIX is 4 Asp, 2 Asn, 1 Thr, 2 Ser, 1 Pro. 7 Ala, 3 Val, 1 Met, 4 Leu, 1 Lys, 3 His.

The fact that the haemoglobin was an α-chain variant was confirmed also by hybridization with canine haemoglobin when an aberrant $\alpha_2^{human}\beta_2^{canine}$ was observed.

Identification of the Amino-acid Substitution

When the fingerprint (peptide chromatogram) of the purified variant was prepared it was found that the tryptic peptide No. 9 of the α-chain (α^{A}TpIX) was missing (Special Plate, Fig. 2). This peptide comprises residues 62–90 of the 141 residues of the α-chain, of which residue 78 is an asparagine (Hill and Konigsberg, 1962). There is usually present another peptide, α^{A}TpVIII–IX, in which a lysine residue (α^{A}TpVIII) is attached to αTpIX at its N-terminal; this peptide was also absent. Two new discrete peptides were noticed instead (Table I). One was in the acid region just above α^{A}TpIV and stained positive for histidine and methionine, the other showing a more positive charge than α^{A}TpIX stained positive for histidine but not for methionine. Amino-acid analysis showed that this latter peptide had the same amino-acid composition as the last 12 residues of α^{A}TpIX, while the other new peptide had the same amino-acid composition as the first 17 residues of α^{A}TpIX except that there was one aspartic residue less and a lysine residue was found instead (Table II). As this was a tryptic peptide the lysine residue must be expected to be in position 78, where an asparagine is present in the α-chain of Hb A.

The aspartic acid residues in the control peptide from Hb A include those formed during acid hydrolysis of the peptide from asparagine residues α68 and 78. There was also a peptide present in the neutral region which was adjacent to βTpI (residues β1–8) but distinguishable from it by staining positively for methionine. It is assumed that this peptide represented α^{A}TpVIII plus residues 62–78 of the α-chain of Stanleyville II—that is, α Stanleyville TpVIII-a. From these results it can be inferred that a replacement has occurred at position α78 of an asparagine residue by one of lysine, and that the formula of Hb Stanleyville II can be written $\alpha_2^{\text{Asparagine}\rightarrow\text{Lysine}}\beta_2^{A}$.

The replacement occurs in a non-helical region of the α-chain between the E and F helices (EF7), and is situated at the surface of the molecule, where it is not in contact with the haem or with any of the other haemoglobin polypeptide chains (Perutz, 1965). No pathological consequences of this mutation would therefore be expected, and indeed none have been observed.

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Comparison of Piperazine and Tetramisole in Treatment of Ascariasis

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Summary: Tetramisole, a new synthetic anthelmintic, was compared with piperazine in the treatment of ascariasis in African children. Of 100 children receiving tetramisole 94 were cured, and of 100 given piperazine 85 were cured (0.05>P>0.02). Tetramisole was free of side-effects.

Introduction

Tetramisole is a new synthetic anthelmintic developed by Belgian workers. Most of its properties have been summarized recently (Thienpont *et al.*, 1966). Chemically it is the white, crystalline, stable, water-soluble hydrochloride of 2,3,5,6-tetrahydro-6-phenyl-imidazo(2,1-*b*)thiazole with the structure shown opposite.

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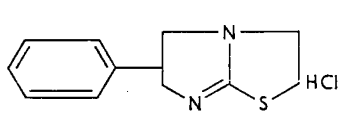
The compound is active in a number of mammalian and avian species against a variety of gastrointestinal and pulmonary nematodes such as *Ascaridia, Capillaria, Heterakis, Chabertia, Bunostomum, Dictyocaulus,* and *Trichostrongylus.* In vitro



low concentrations of tetramisole exert a rapid paralysing action on nematodes which persists for many hours. The drug is inactive against cestodes, trematodes, fungi, or bacteria. It is also devoid of antihistaminic, anticholinergic, adrenolytic, or any other classical pharmacological properties. Acute and chronic toxicity studies in animals, including tests in pregnancy, have revealed a high degree of safety. The LD_{50} in rats is 130 mg./kg. subcutaneously and 480 mg./kg. orally.

M. N. MARSH *ET AL.*: STUDIES OF SMALL-INTESTINAL MUCOSA WITH THE SCANNING ELECTRON MICROSCOPE

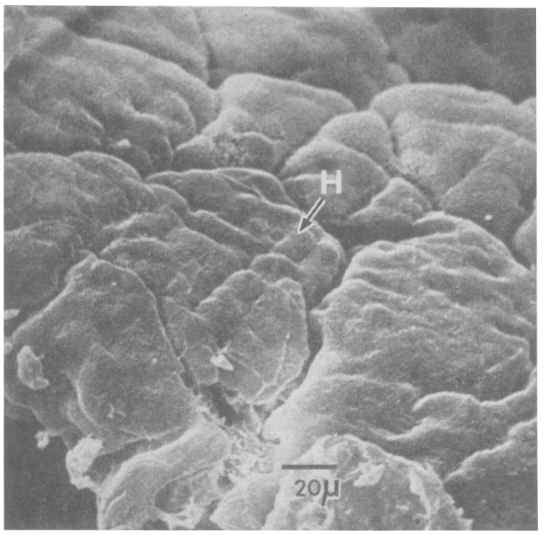


FIG. 5

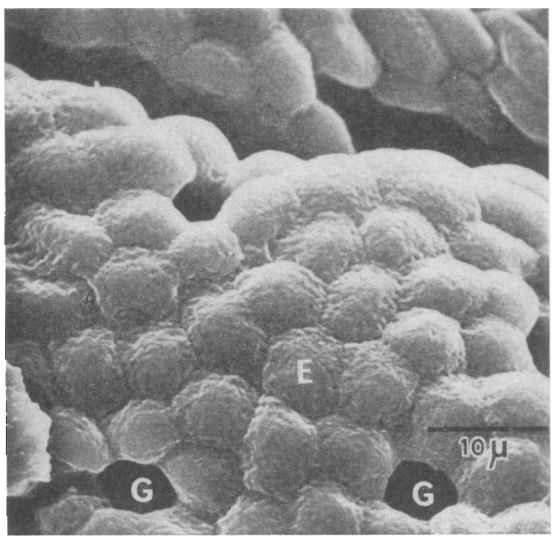


FIG. 6

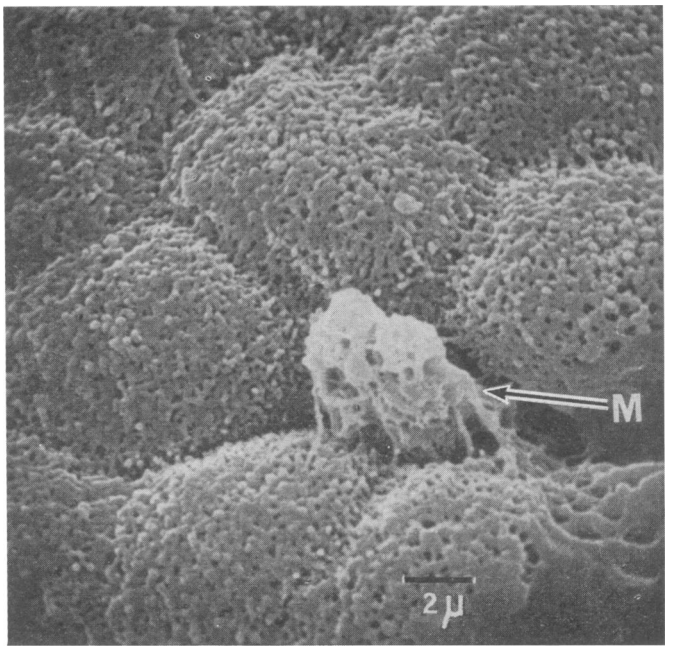


FIG. 7

FIG. 5.—Intestine in coeliac disease. This micrograph illustrates the cracked surface of an apparently villus-free zone. Hemispherical projections are also evident (H).

FIG. 6.—Top surface of a stunted villus in coeliac disease. The surface is apparently covered by spherical epithelial cells (E). Goblet cell orifices (G).

FIG. 7.—Similar area to Fig. 6 but illustrating epithelial cells covered by swollen microvilli. A mucous plug (M) is evident at a goblet cell orifice.

G. VAN ROS *ET AL.*: HAEMOGLOBIN STANLEYVILLE II

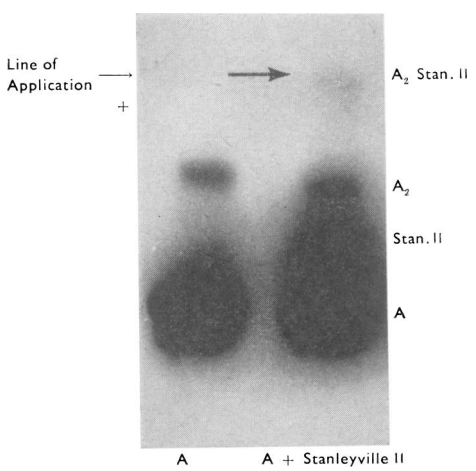


FIG. 1.—Demonstration of two Hb A_2 fractions in the specimen containing Hb A and Hb Stanleyville II (for details see text). On starch-gel electrophoresis at *p*H 8.6 Hb A and Hb A plus Hb Stanleyville are compared. Only one Hb A_2 is seen in the control. When Hb Stanleyville II is also present its variant α-chains give rise to a second Hb A_2. The gel had to be overloaded to visualize the two Hb A_2 fractions, and therefore separation between Hb A and Hb Stanleyville II is poor.

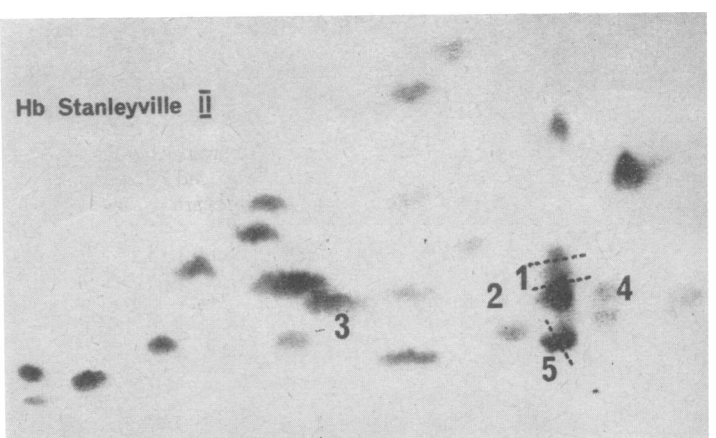


FIG. 2.—The "fingerprint" of Hb Stanleyville II. 1, The area where αTpIX (residues 62–90) of Hb A is absent. 2, The area where αTpVIII-IX (residues 61–90) of Hb A is absent. 3, αTpIX-b of Hb Stanleyville II (residues 79–90). 4, αTpIX-a of Hb Stanleyville II (residues 62–78). 5, A methionine staining peptide which is presumed to be αTpVIII-IX-a of Hb Stanleyville II (residues 61–78).