The unstable haemoglobins and haemolysis

Name Robin Carrell
Haematology Department,
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The unstable haemoglobins and haemolysis

1966-2016

: and the lessons they have taught us

Robin Carrell Dept of Haematology

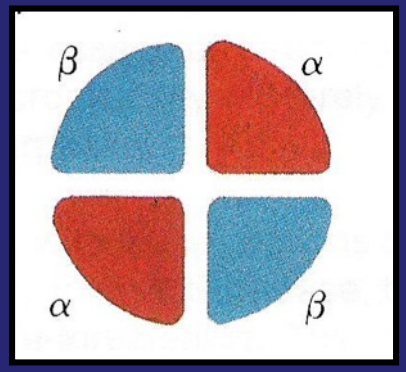
Cambridge Institute for Medical Research University of Cambridge

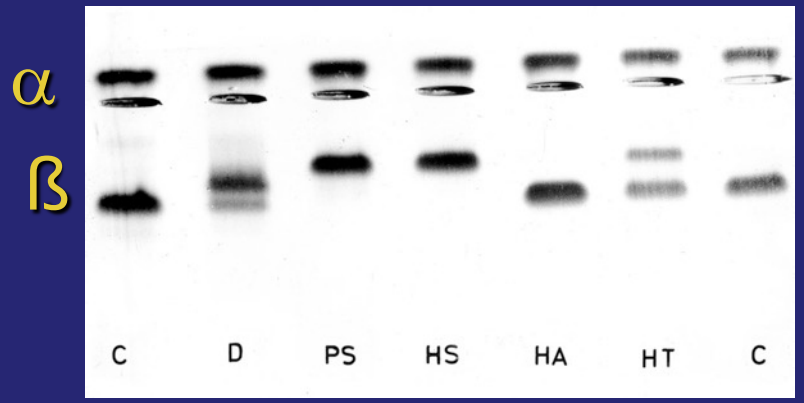
Biochemistry Department, Cambridge 1965

HbS identified by Pauling and Itano in 1949, molecular abnormality defined by Ingram in 1958 .

MRC Abnormal Hb Unit, Cambridge 1965

Lehmann and Ingram further identified the molecular changes in other abnormal haemoglobins, detected by their change in electrophoretic mobility.





The amino acid sequence of the globins could be split into defined tryptic peptides.

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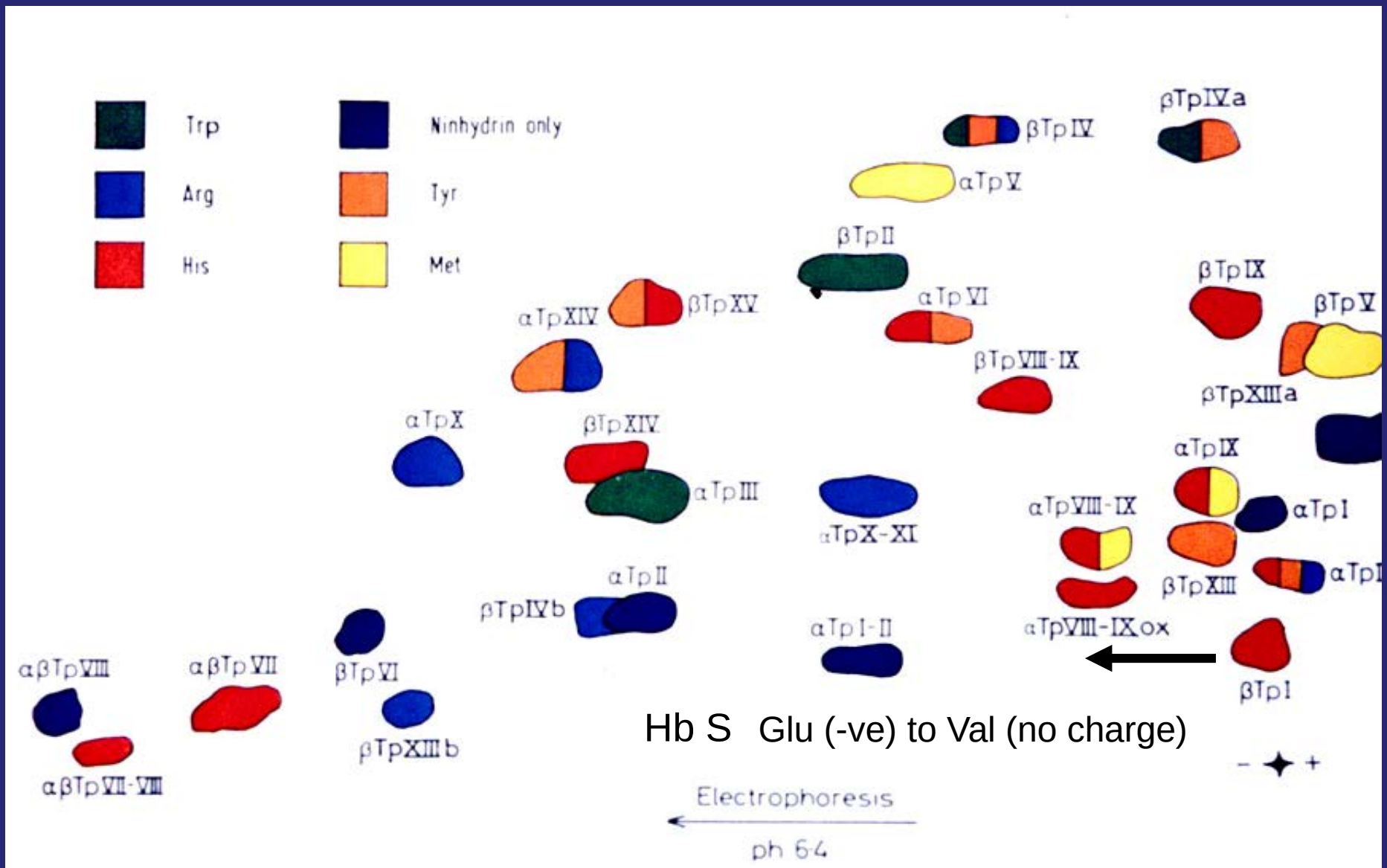
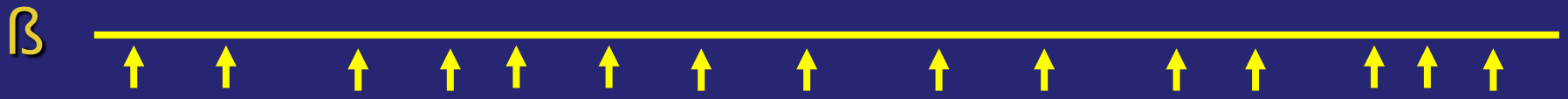
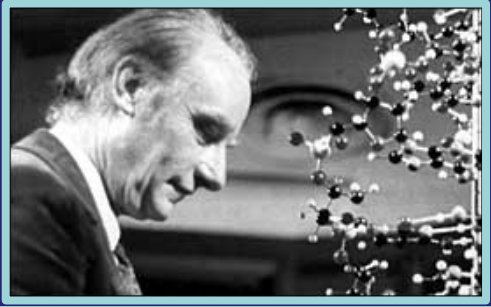


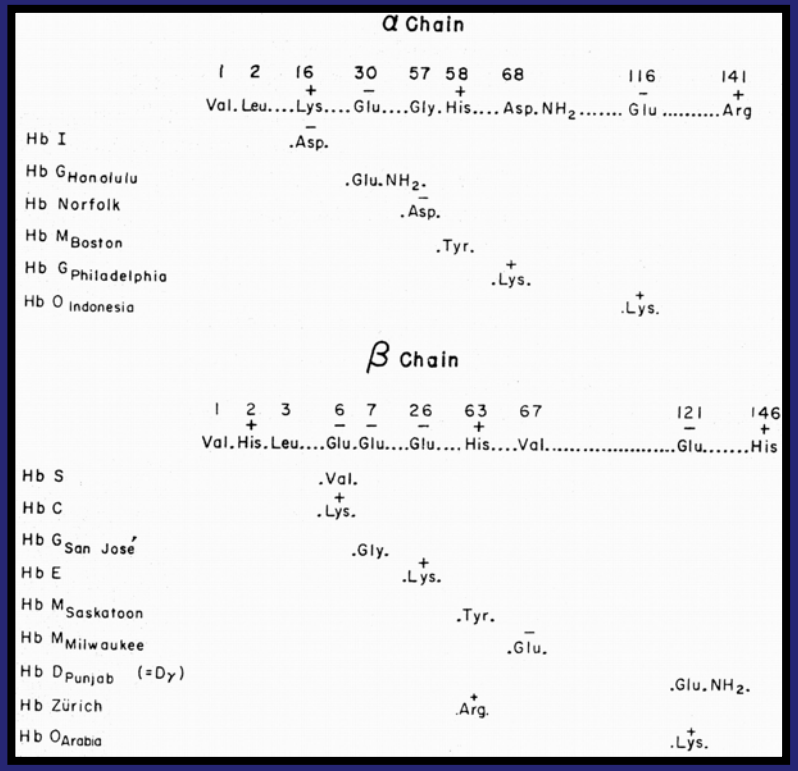
The amino acid sequence of the globins could be split into defined tryptic peptides.



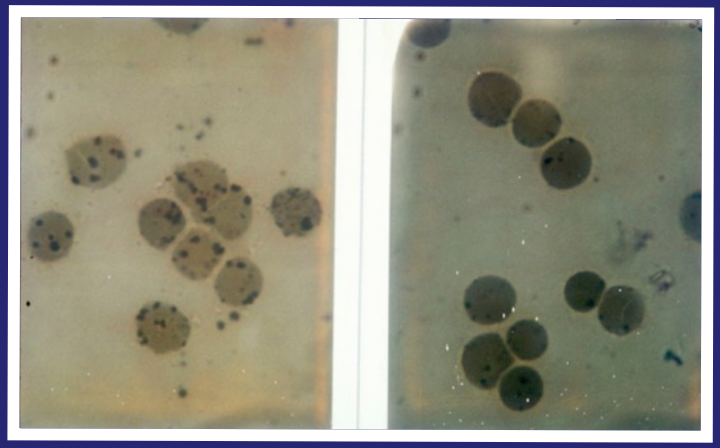
The findings of these abnormal haemoglobins was of intense interest at the time, in human genetics & in establishing the new field of molecular anthropology.

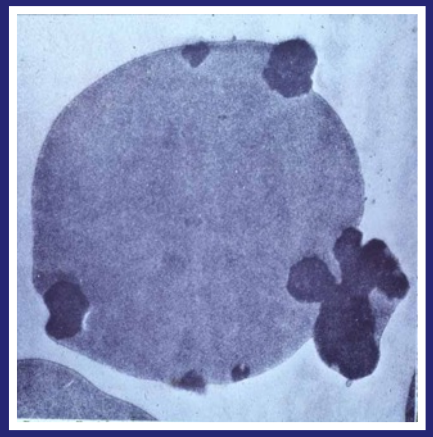


Exciting days! Francis Crick – and the universality of the genetic code



But my interest was in the molecular mechanisms of disease.



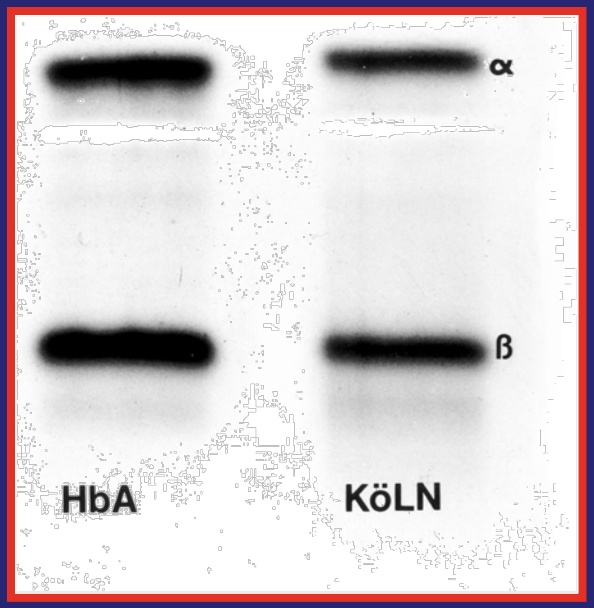


The unsolved cases of the Heinz-body haemolytic anaemias

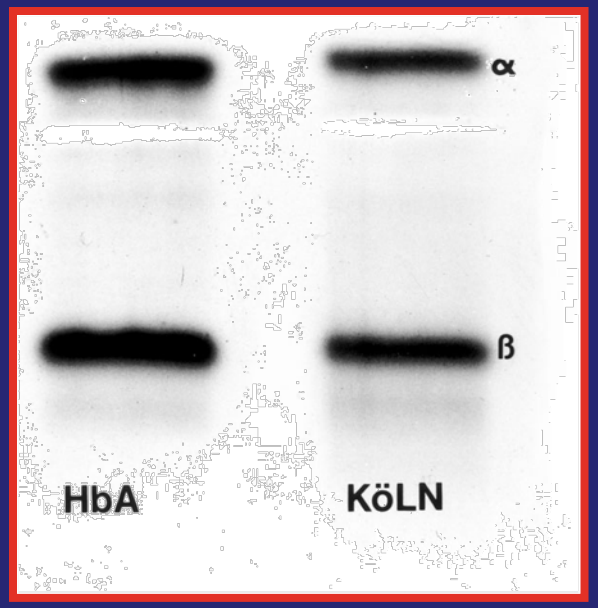
Episodic, sometimes fatal, haemolytic crises, characteristically commencing near age 1 yr in association with incidental fevers.

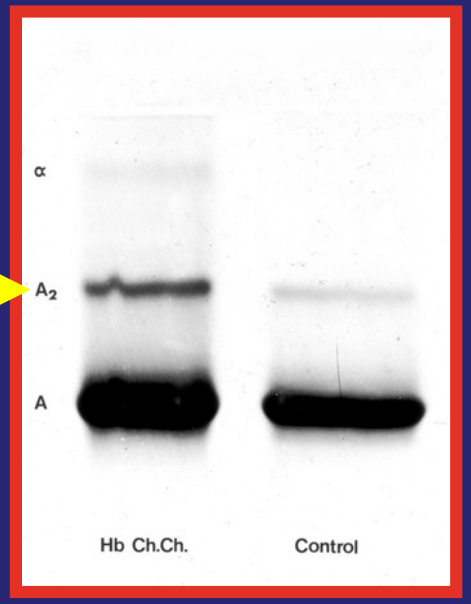
Uncommon but not rare: initial presenting family from Köln.
Other index families: Hammersmith, Glasgow, Sydney, Genova,

Inclusion (Heinz) bodies suggested the presence of an abnormal Hb but numerous investigations had failed to identify an abnormality



Moreover, definitive testing of the index Köln Hb showed no inherent change in electrophoretic mobility. Was an abnormal Hb really present?





there were tantalising clues

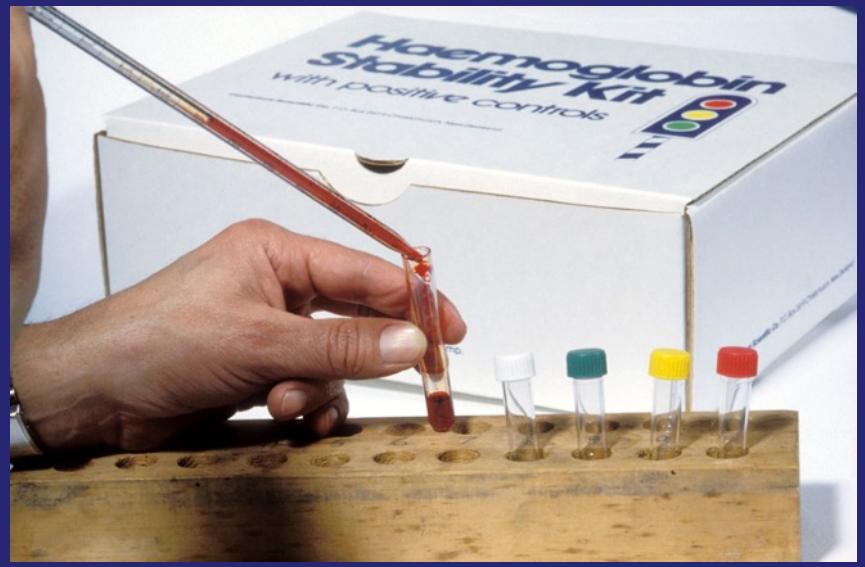
The unstable ß-globin precipitates as inclusion ('HbH') bodies, with a trace excess of α chains and raised HbA_2 - in effect a ß-thal intermedia.

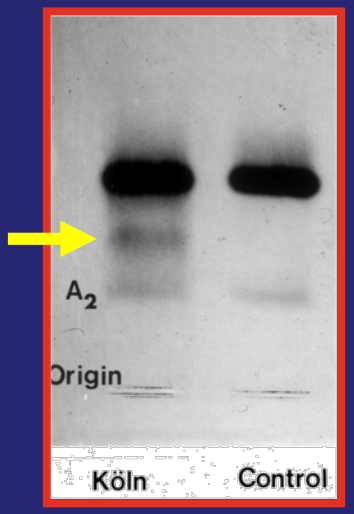
the critical consistent finding was the demonstrable presence of an unstable Hb fraction in all affected family members.

- Heat stability test. Dilute haemolysate incubated for 1 and 2 hours at 50º C *(Grimes Meisler and Dacie).*

The critical clue however was the demonstration of a consistently present unstable Hb component

- Heat stability test. Dilute haemolysate incubated for 1 and 2 hours at 50º C *(Grimes Meisler and Dacie).*
- Isopropanol test. Similar but incubate at 37ºC in 17% isopropanol.





<u>Breakthrough step:</u> to analyse not the trace electrophoretic band but instead the flocculent precipitate.

Tryptic peptide map of the flocculent precipitate

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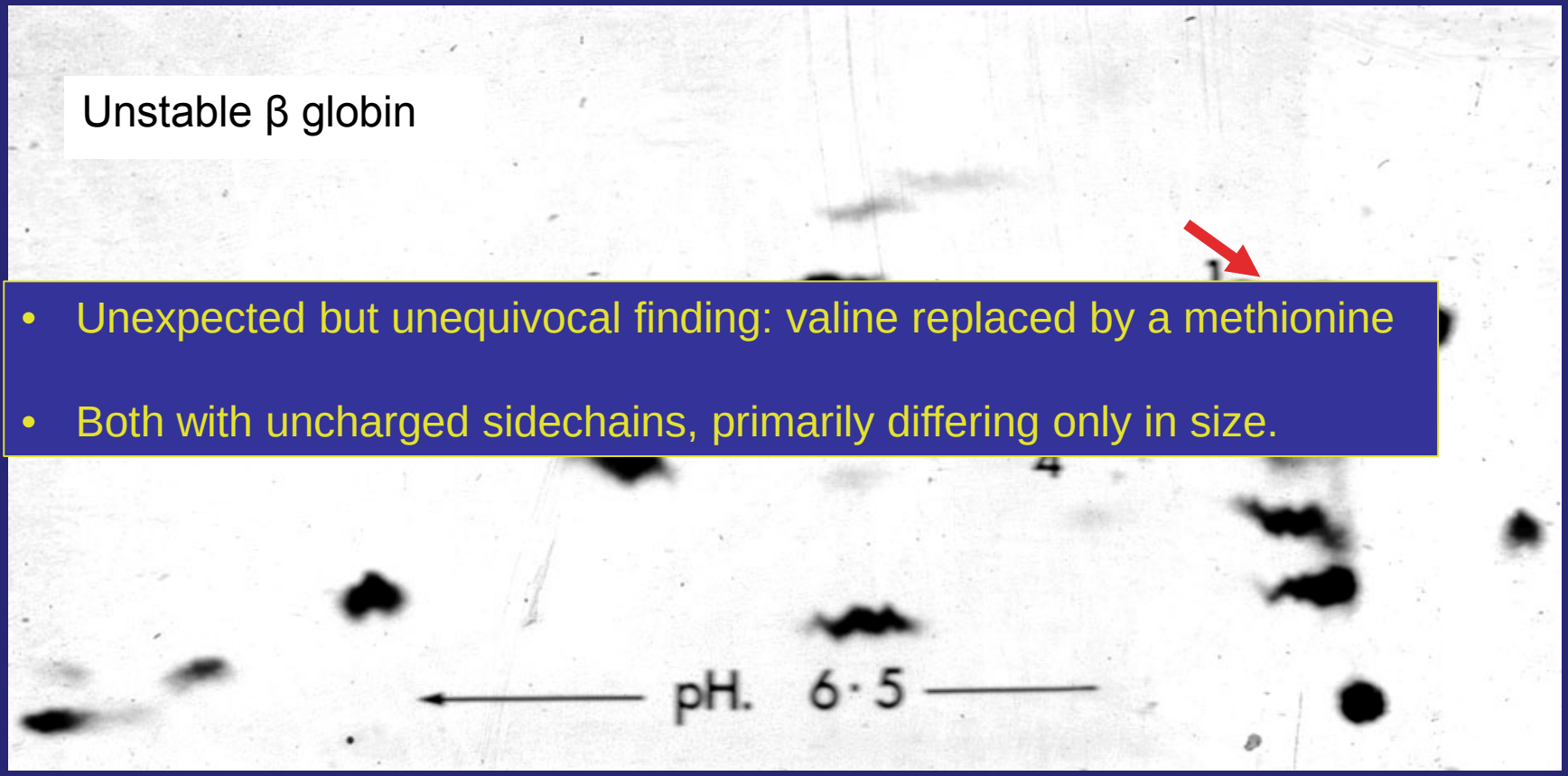


- Unexpected but unequivocal finding: valine replaced by a methionine
- Both with uncharged sidechains, primarily differing only in size.

None of the peptides had shifted electrophoretically…..***but***

Nature May 28 1966

(*Reprinted from Nature, Vol. 210, No. 5039, pp. 915–916, May 28, 1966*)

HAEMOGLOBIN KÖLN (β–98 VALINE → METHIONINE): AN UNSTABLE PROTEIN CAUSING INCLUSION-BODY ANAEMIA

By R. W. CARRELL* and DR. H. LEHMANN

Medical Research Council Abnormal Haemoglobin Research Unit, Department of Biochemistry, Cambridge

AND

DR. H. E. HUTCHISON

University Department of Haematology, Western Infirmary, Glasgow

IN 1964 an unstable human haemoglobin was described

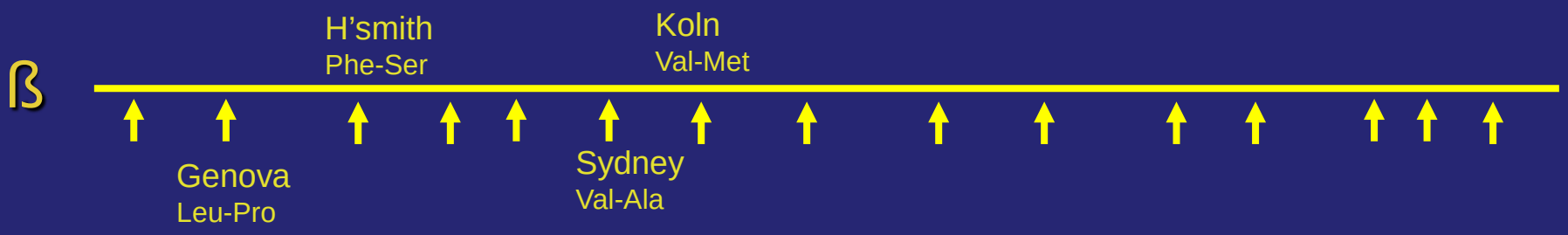
- Unexpected but unequivocal finding: valine replaced by a methionine
- Both with uncharged sidechains, primarily differing only in size.

(haemoglobin A) (Fig. 1). At about this time, a haemoglobin from a German family was investigated which had

The finding was met with scepticism – how could a replacement of an uncharged aminoacid side-chain cause such profound consequences?

behaviour, products of hybridization with canine haemoglobin, and the results of fingerprinting of the soluble peptides after tryptic hydrolysis. The fingerprints were indistinguishable from those of haemoglobin A, and it was concluded that haemoglobin Köln was an abnormal variant of haemoglobin A and not haemoglobin A_2. Because on electrophoresis at alkaline *p*H haemoglobin Köln moved more slowly than haemoglobin A, it was assumed that the part of the molecule carrying the additional positive charge did not appear in the fingerprint of the soluble tryptic peptides, but formed part of the insoluble 'core'—that portion of the haemoglobin molecule which does not go into solution on tryptic

* Supported by a grant from the Wellcome Trust and on study leave from the Pathology Department, Christchurch Hospital, New Zealand.



Confirmation soon came from the solving of other unstable Hbs

But what remained unsolved was how such small changes in a single globin aminoacid, in heterozygotes, could result in a life-threatening haemolytic anaemia?

People remained unconvinced

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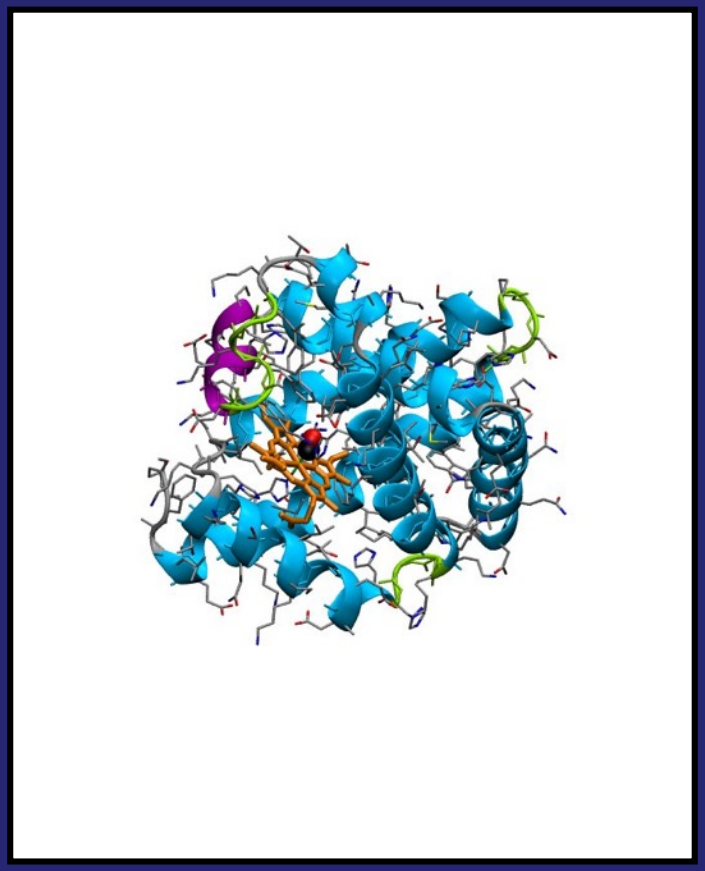
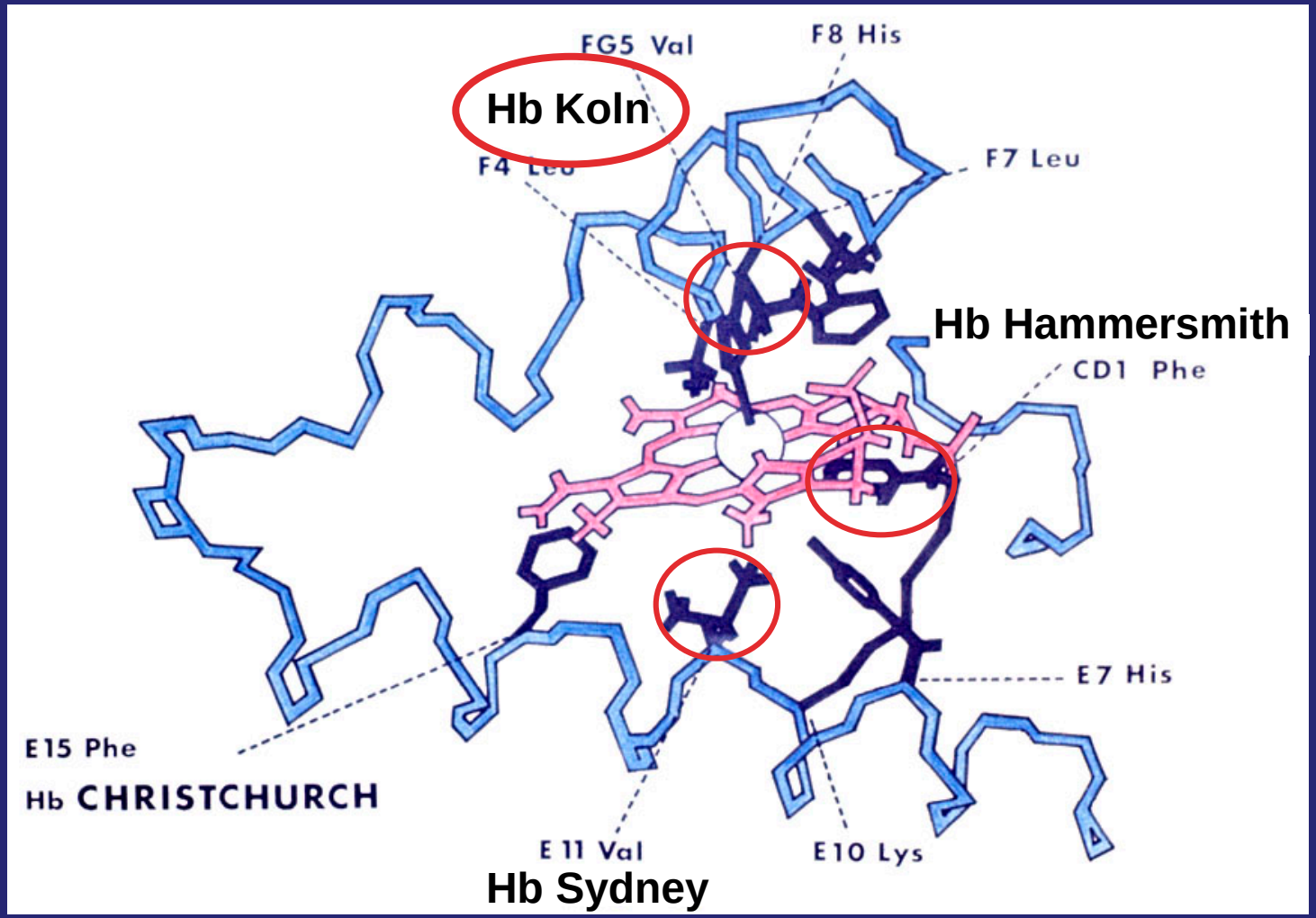
H'smith
Phe-Ser

Koln
Val-Met

Genova
Leu-Pro

Sydney
Val-Ala



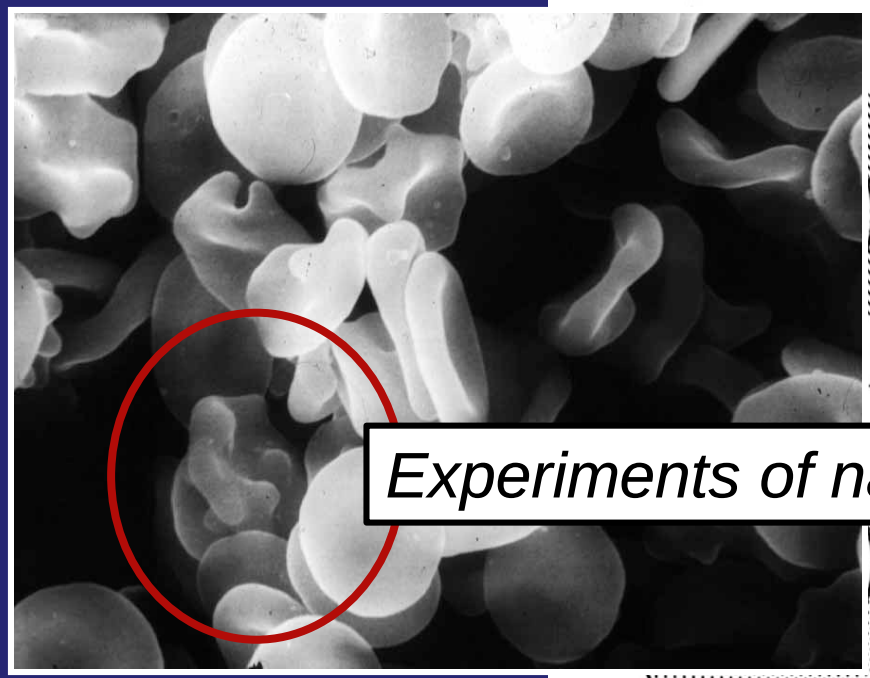


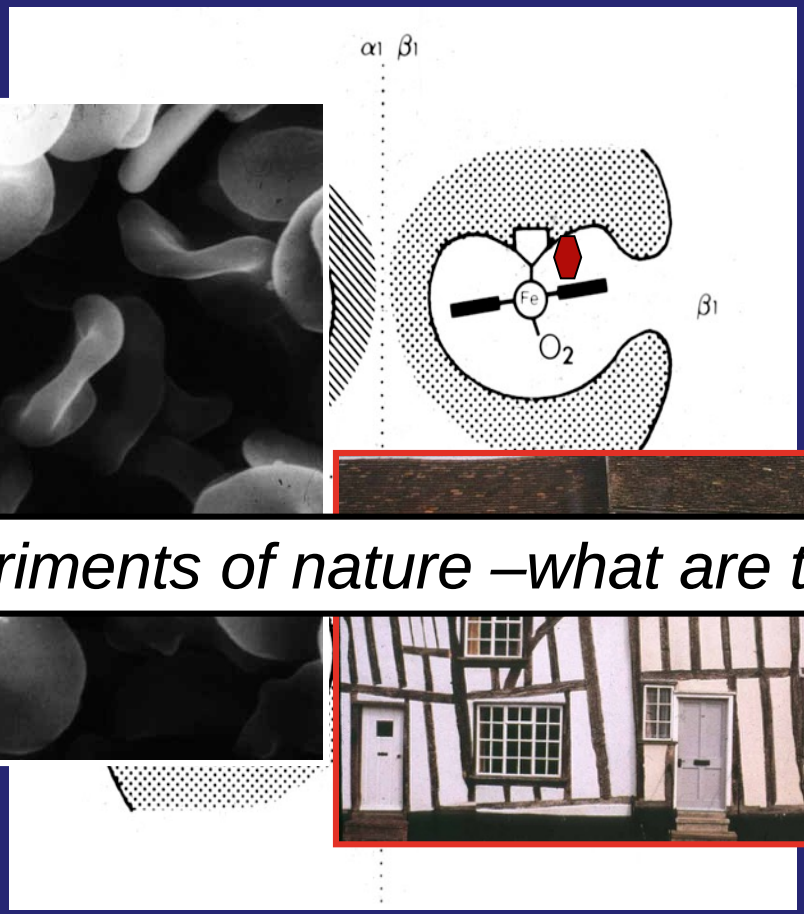
A general pattern of mutations in the Heinz body anaemias: affecting amino acids binding to the haem group and hence the stability of the ß-globin.

Why were the consequences so severe?

Gain of function abnormality

Haemolysis explicable by the pitting of inclusions in the splenic sinusoids





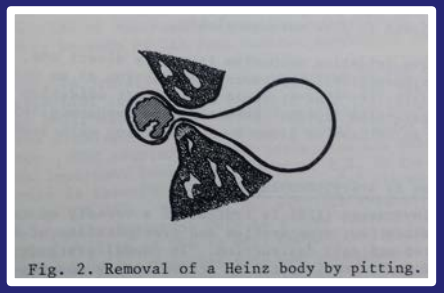
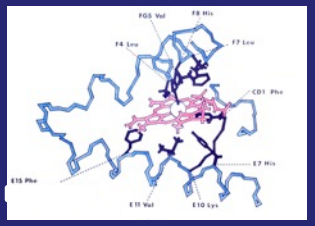
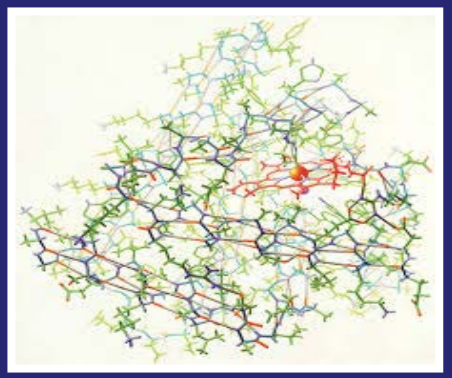


Fig. 2. Removal of a Heinz body by pitting.

Experiments of nature –what are they telling us?

- *In protein and medical science:*

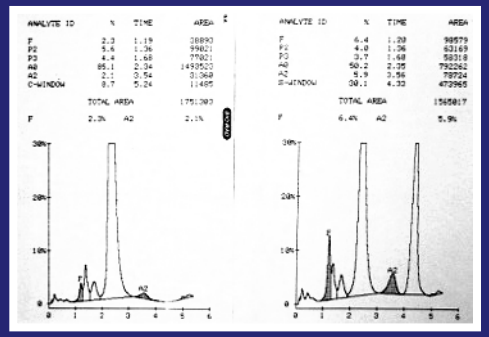




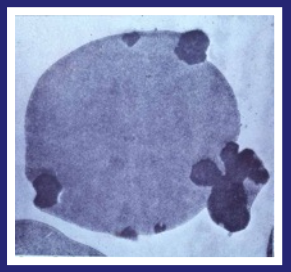
- new understandings of the forces that maintain protein stability

- how the structure of one member protein family is relevant to all others

- the technologies for the stabilisation of Hb, now critical for the standardisation of analysis of Hb fractions, notably HbA1c and HbA_2



Inclusion-(Heinz)-Body Haemolytic Anaemias.



as a clinical syndrome:

- Arise from mutations affecting the haem binding pocket and globin stability.
- Primarily affect ß-globin and hence infant presentation is after 8 months age.
- Gain of function: instability in a single globin gives genetic dominance.
- Onset: inclusion formation triggered by the incidental fevers of infancy.
- haemolysis explicable by the pitting of inclusions in the splenic sinusoids

But this was not all – something else was happening!

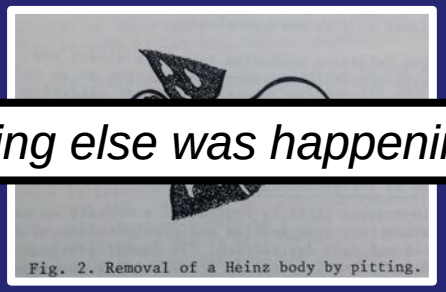
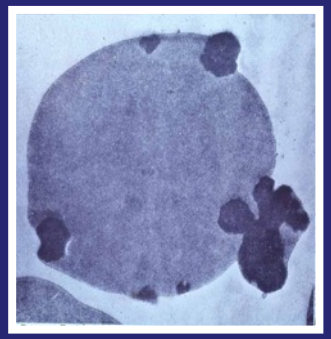
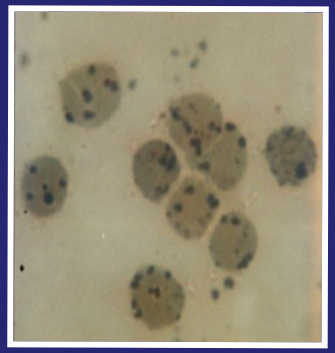


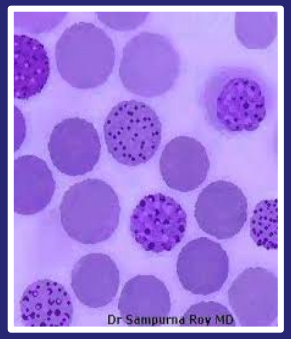
Fig. 2. Removal of a Heinz body by pitting.

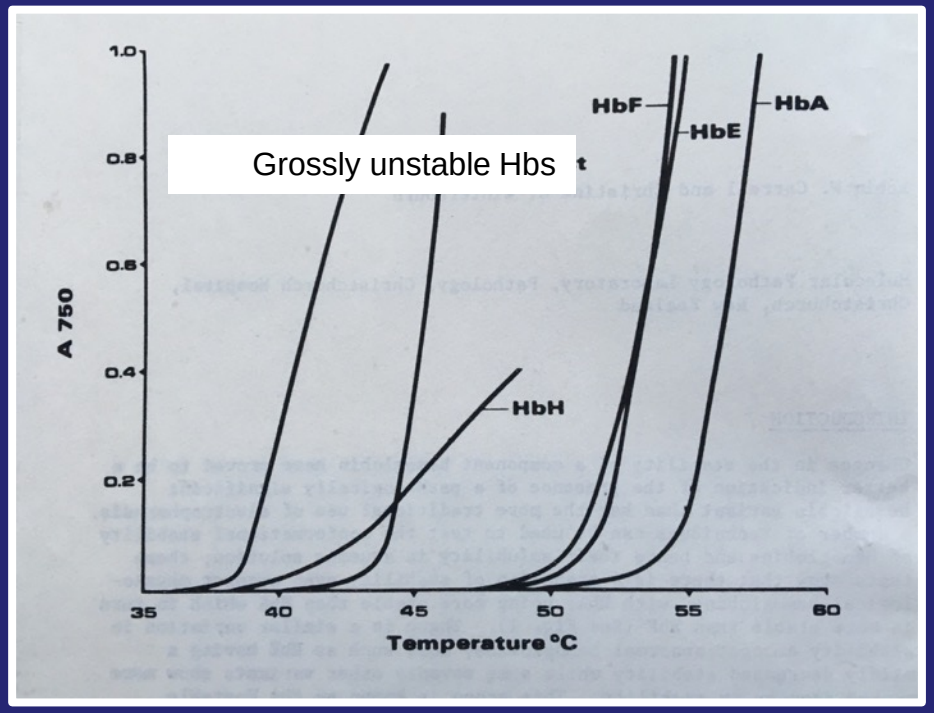
- A clue came from other ‘antimalarial’ (inclusion-body) polymorphisms. HbE, the thalassaemias, G6PD defic, malarial drug activity:

  - *the oxidative haemolytic anaemias*.



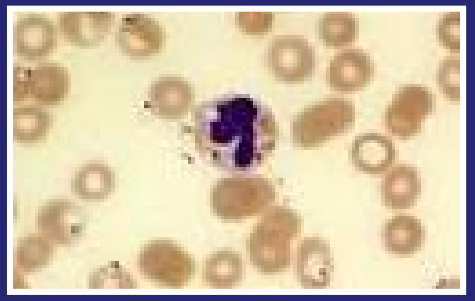


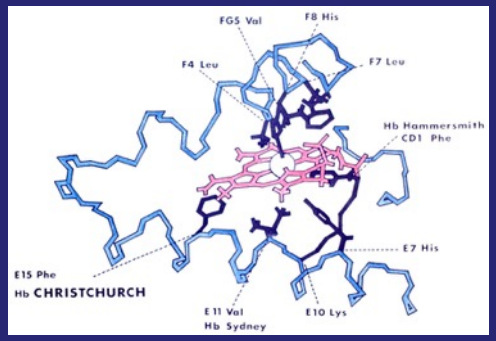


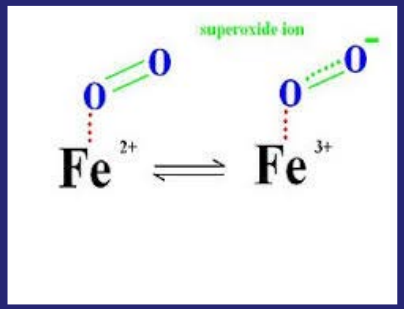


- The shared feature of the antimalarial polymorphisms is an increased free radical production or exposure within the RBC

  Markedly so with the conformational deformation of the haem pocket in the unstable Hbs, allowing the release of activated oxygen radicals.







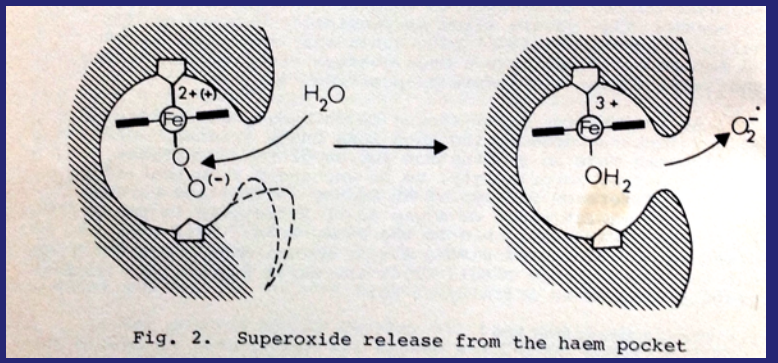
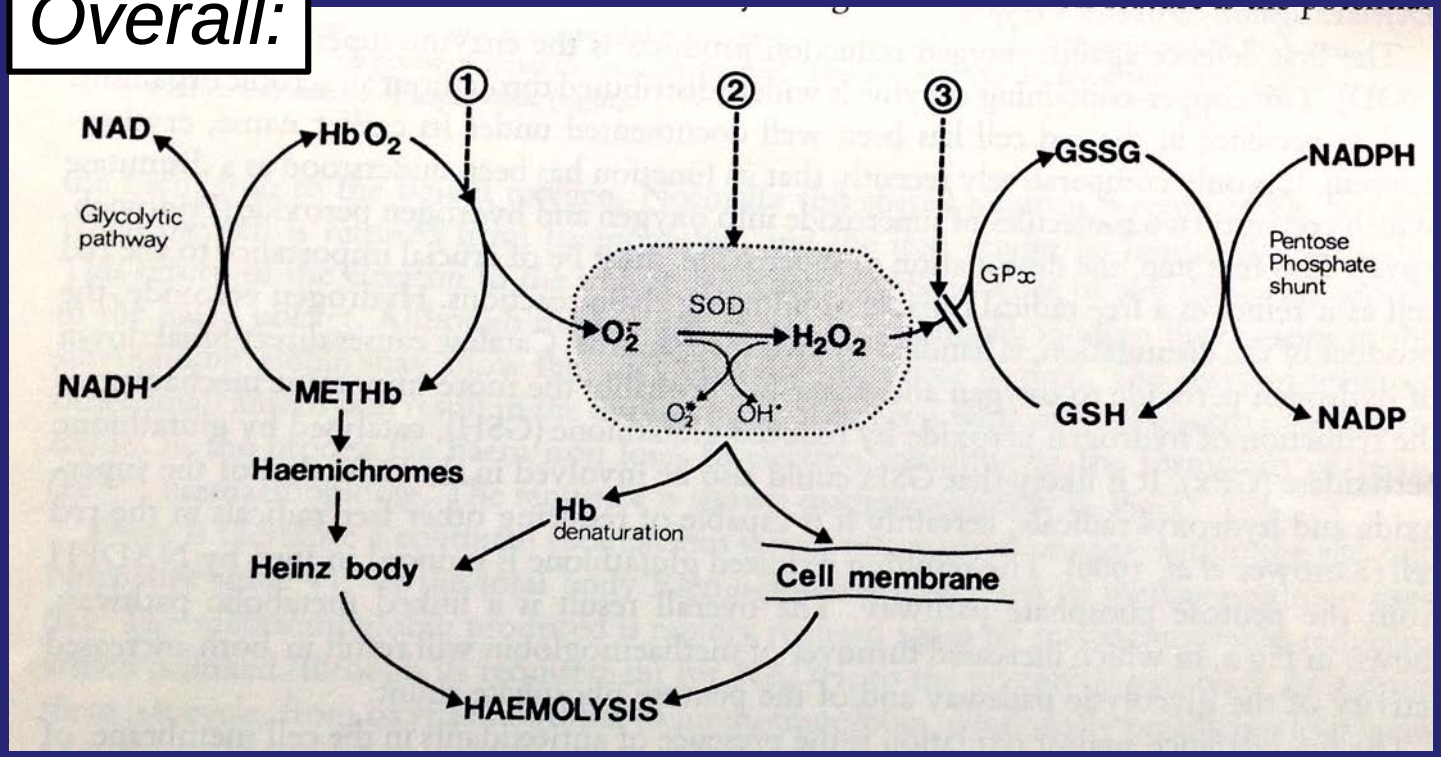
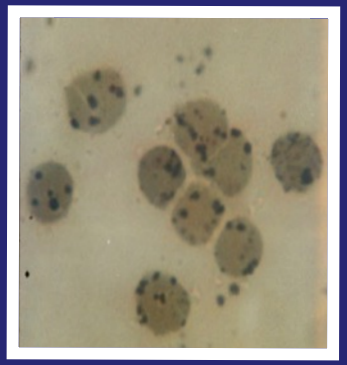


Fig. 2. Superoxide release from the haem pocket

- Opening whole field of intracellular free radical and activated oxygen release.

Overall:





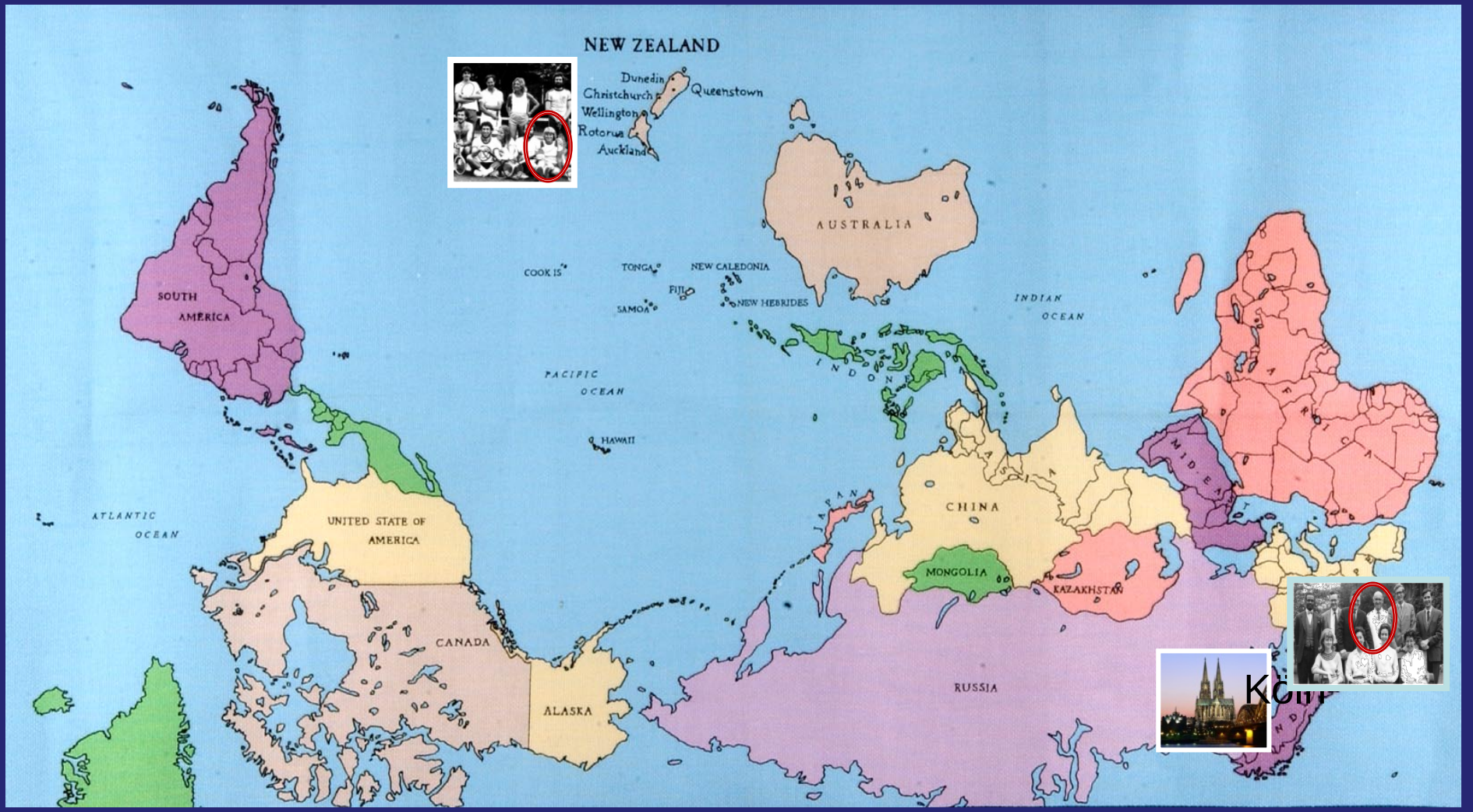
to give haemichrome formation and membrane distortion.

An on-going story that commenced and continues far away:

RBC free radicals and activated oxygen in health & disease

(Christine Winterbourn, et al, ChCh NZ)

With acknowledgements 1966-2016



Hermann Lehmann FRS 1910 – 1985

Founder of the haemoglobinopathies