Considerable differences in susceptibility towards denaturation were observed. Haemoglobin C (β6 Glu → Lys) and haemoglobin S (β6 Glu → Val) show sigmoid 'melting' curves with the point of inflexion at about 40°C. Haemoglobin A shows a point of inflexion some 5°C higher, and haemoglobin A_2 ($\alpha_2\delta_2$) seems to be the most stable of the haemoglobins investigated, 'melting' at about 55°C. Addition of haemoglobin A_2 to haemoglobin C and haemoglobin S (4–10% of the total) caused a large increase in the apparent 'melting' temperature. The effective amount of the haemoglobin A_2, assuming that the stability of the hybrid $\alpha_2\beta\delta$ is the same, cannot account for the full change observed in the denaturation profile.

Fluorescence measurements at the fixed temperature gave complementary results, with haemoglobin A_2 less than 5% denatured in the time in which the fluorescence change for haemoglobins C and S is complete, and haemoglobin A about 50% denatured. The rates of change of fluorescence for haemoglobin C and haemoglobin S are identical. The plot of fractional change of fluorescence with time gives sigmoid curves, which suggests that, unlike myoglobin, denaturation is not first-order (Tanford, 1968; Acampora & Hermans, 1967). At these concentrations the haemoglobins are predominantly in the dimeric form (Guidotti, 1967). All the haemoglobins studied had the same α-chain, hence the denaturation of the α-chain is not the rate-determining step, and if there is no co-operativity the order of susceptibility to denaturation must be $\alpha > \beta^C = \beta^S > \beta^A > \delta$. The denaturation of either chain, unless quenching by the adjacent chain masks completely, should result in some change in the fluorescence, which is not observed in the results for haemoglobin A_2. It is therefore likely that some co-operative effect is being observed.

These experiments suggest that haemoglobin A_2 may be involved in the stabilization of other haemoglobin components, and it is notable that the relative amount of this haemoglobin is increased in sickle-cell-trait carriers (Wrightstone, Huisman & Van Der Sar, 1968).

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The Effect of Organic Phosphates on the Reactions of Haemoglobin and Oxyhaemoglobin (Carboxyhaemoglobin) with Carbon Dioxide

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Many haemoglobins react with CO_2 to form carbamates. The combination at physiological pH values occurs, at least for horse haemoglobin, and presumably also for human and bovine haemoglobin (the other species investigated) with the terminal amino groups of the α and β chains. The reaction is greater with haemoglobin than with oxyhaemoglobin (or carboxyhaemoglobin), i.e. is oxygen-linked. Organic phosphates, e.g. 2,3-diphosphoglycerate and ATP, are also differentially bound (Benesch & Benesch, 1969) and interfere with the oxygen-linked carbamate reactions at physiological pH values (Bauer, 1969), probably by combining at or near the terminal amino groups of the β chains. To test this view we have determined F, the extra carbamate per haem molecule, at various pH values but constant CO_2 partial pressure and temperature (37°C), from the difference in total CO_2 between haemoglobin and oxyhaemoglobin (or carboxyhaemoglobin) measured by a new micro-equilibration technique, for: (1) haemolysed blood; (2) organic-phosphate-free human and bovine haemoglobins; (3) as (2) but with various added amounts of diphosphoglycerate or ATP. The total CO_2 content of the liganded (carboxy) haemoglobin is the same for (1), (2) and (3) at any given pH between 7.0 and 7.6. F for (1) and (3) is half its value for (2) with human haemoglobin. With bovine haemoglobin, F for (1) is the same as for (2). These results confirm that (*a*) organic phosphates under physiological conditions interfere with combination of CO_2 with human haemoglobin, in agreement with Bauer's (1969) views; (*b*) diphosphoglycerate and ATP combine at or near two out of four of the terminal amino groups of the human haemoglobin molecule; (*c*) diphosphoglycerate and ATP (up to the molar ratio, so far investigated, of 2 mol of P_i/haemoglobin tetramer) do not affect the O_2-linked reactions of the other two terminal amino groups; (*d*) there is no apparent reaction between diphosphoglycerate or ATP with oxyhaemoglobin or carboxyhaemoglobin at or near any of the four terminal amino groups. The above values of F agree generally with the results obtained by other methods, i.e. direct chemical determination (Ferguson, 1936) and indirect calculation from the

specific effect of CO_2 on the oxyhaemoglobin equilibrium curve (Rossi-Bernardi & Roughton, 1970).

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Two New Pathological Haemoglobins: Olmsted β141 (H19) Leu → Arg and Malmö β97 (FG4) His → Gln

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We have examined two abnormal human haemoglobins that are representative of two groups of pathological haemoglobins (Perutz & Lehmann, 1968). One of these comprises unstable haemoglobins that are associated with inclusion-body anaemia (Lehmann & Carrell, 1969). These haemoglobins have mutations that result in the haem being less strongly bound and they are more susceptible to oxidation than normal. Many of the unstable haemoglobins have mutations in the lining of the haem 'pocket' (Perutz *et al.* 1968*a*; Perutz, Muirhead, Cox & Goaman, 1968*b*) involving the substitution of a non-polar residue by another of different size or by a polar residue. We have examined haemoglobin Olmsted described by Fairbanks, Opfell & Burgert (1969), which appears to have a Leu → Arg mutation at position 141 (H19) of the β-chain. This is in a position homologous with that of the α-chain-unstable haemoglobin Bibba α136 (H19) Leu → Pro (Kleihauer *et al.* 1968).

Another group of pathological haemoglobins associated with erythraemia (polycythaemia) is characterized by an increased oxygen affinity and decreased haem–haem interaction. Here the mutations are found mainly in the regions of the $\alpha_1\beta_2$ contacts of the tetramer. We have identified a new haemoglobin of this type, Malmö β97 (FG4) His → Gln, discovered in a study of the inheritance of polycythaemia in four generations of a Swedish family.

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Haemoglobin Synthesis in Thalassaemia

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The thalassaemias are a series of genetically determined disorders of haemoglobin synthesis, which all result from a defective rate of production of either the α- or β-peptide chains of haemoglobin. Haemoglobin synthesis *in vitro* has been studied in reticulocytes obtained from thalassaemic patients and compared with that in non-thalassaemic reticulocytes. The parameters measured include the overall rate of α- and β-chain production, the magnitude and properties of any excess of globin chains that are produced, the rate of globin chain assembly and, more recently, the rate of chain initiation.

The results of these investigations indicate that in each form of thalassaemia there is either a partial or total decrease in the rate of synthesis of one of the globin chains. No fragments of chains have been isolated from the cells of these patients. The assembly time of the globin chains has been measured and appears to be normal despite the marked decrease in the rate of chain synthesis. An attempt has been made to examine the distribution of nascent α- and β-chains on polyribosomes of different sizes with the object of studying the process of initiation in thalassaemic cells. Preliminary studies indicate that α- and β-chains from non-thalassaemic individuals are synthesized on different-sized polyribosomes.

The results obtained to date are compatible with a decreased rate of mRNA production of a defect in chain initiation as the underlying genetic defect in some forms of thalassaemia.