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A Possible New Sclerotization Agent in the Cockroach Cuticle

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A variety of sclerotization agents exist in insects (Brunet, 1967). Among these are *N*-acetyldopamine (Karlson & Sekeris, 1962) which has been shown to play an important role in the 'tanning' of the cockroach cuticle (Mills, Lake & Alworth, 1967). Further investigation has shown that [*carbinol*-¹⁴C]-noradrenaline [2-amino-1-(3,4-dihydroxyphenyl)-ethanol] is also incorporated into the cuticle of the American cockroach, *Periplaneta americana* L.

By using conditions both *in vivo* and *in vitro*, it has been found that a number of acidic, basic and neutral compounds arise from noradrenaline. These substances have been separated by high-voltage electrophoresis and paper chromatography. Co-chromatography and comparison with known metabolites has led to their identification, which suggests *N*-acetylation may be an important step.

I propose that β -hydroxylation of dopamine followed by *N*-acetylation may be involved in the synthesis of a previously unreported sclerotizing substance.

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Synthesis of Unstable Haemoglobins

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In subjects heterozygous for Hb-A* and a β -chain-variant unstable haemoglobin, the proportion of the unstable haemoglobin usually comprises 25–35% of the total haemoglobin. In three such cases the synthesis rates of the unstable β -chain and the β^A -chain have been shown to be equal (White & Dacie, 1970; Steadman, Yates & Huehns, 1970). The observation that the unstable haemoglobin

* Abbreviation: Hb, haemoglobin.

comprises less than 50% of the total haemoglobin in these cases suggests that a proportion of this haemoglobin is being removed as insoluble inclusion (Heinz bodies).

There are four unstable haemoglobins, however, present in smaller amounts, 15% or less of the total haemoglobin: Hb-Borås, F4 Leu $\rightarrow$ Arg (Hollender, Lorkin, Lehmann & Svensson, 1969); Hb-Santa Ana, F4 Leu $\rightarrow$ Pro (Opfell, Lorkin & Lehmann, 1968); Hb-Sabine, F7 Leu $\rightarrow$ Pro (Schneider, Veda, Alperin, Brimhall & Jones, 1969); Hb-Köln, FG5 Val $\rightarrow$ Met (Carrell, Lehmann & Hutchinson, 1966). In two patients heterozygous for Hb-Köln the relative rates of synthesis of the $\beta^{Köln}$ -chain and β^A -chain have been measured by incubation of their reticulocytes with [³H]leucine and measurement of the total incorporation of [³H]leucine into the two chains. The ratio of β^A - to $\beta^{Köln}$ -chain synthesized was found to be approx. 4:1 (White & Brain, 1970). The low proportion of Hb-Köln found in heterozygotes is therefore due to defective synthesis of the $\beta^{Köln}$ -chain as well as to its denaturation. This explanation may also be valid for the other three haemoglobins.

In all of these four haemoglobins the region of the amino acid substitutions are close to each other and near the haem-like histidine residue, F8. It is postulated that if an amino acid substitution in this region of the β -chain disrupts the normal structure then it may impede assembly of that chain.

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Thermal Denaturation of Human Haemoglobins

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The thermal denaturation of dilute solutions of various human haemoglobins has been studied by following fluorescence changes at 45°C, and from 'melting' curves by using the percentage change in optical rotation at 233nm with temperature.

Considerable differences in susceptibility towards denaturation were observed. Haemoglobin C ($\beta 6 \text{ Glu} \rightarrow \text{Lys}$) and haemoglobin S ($\beta 6 \text{ Glu} \rightarrow \text{Val}$) 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 A₂ ($\alpha_2\delta_2$) seems to be the most stable of the haemoglobins investigated, 'melting' at about 55°C . Addition of haemoglobin A₂ 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 A₂, 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 A₂ 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 A₂. It is therefore likely that some co-operative effect is being observed.

These experiments suggest that haemoglobin A₂ 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