The eluted proteins combined with but did not precipitate ^{125}I -labelled metmyoglobin. Examination of the eluted antibodies by isoelectric focusing (pH 3–10) in 5% (w/v) polyacrylamide gel (Awdeh, Williamson & Askonas, 1968) revealed a small number of bands; antibodies isolated from different antisera gave similar patterns. By contrast, antibodies to whole myoglobin obtained from the metmyoglobin–Sephadex immunosorbent by elution with *m*-propionic acid gave a very much more complex pattern. The results will be discussed in relation to the structure of the C-terminal antigenic determinant of myoglobin.

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Methine-Bridge Specificity of the Coupled Oxidation of Myoglobin and Haemoglobin with Ascorbate

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Ascorbate-coupled oxidation of haem as the pyridine haemochrome occurs randomly at all four methine bridges, ultimately yielding four isomeric biliverdins (Rüdiger, 1968; Ó Carra & Colleran, 1969a), but similar oxidation of haem in myoglobin is specific for the α -methine bridge (Ó Carra & Colleran, 1969a,b).

Applying previously described analytical techniques (Ó Carra & Colleran, 1969a, 1970; Rüdiger, 1968) we find that when the protohaem moiety of myoglobin is replaced by mesohaem or deuthaem (Smith & Gibson, 1959) the specificity of the α -methine-directed oxidation is largely retained, confirming that the directional effect is attributable to the influence of the protein environment around the haem group.

When coupled oxidation of pyridine haemochrome was carried out in hydrophobic solvents

(e.g. benzene–butan-2-one, 1:1, v/v) rather than in the usual aqueous mixtures, the initial step, hydroxylation of a methine bridge, was considerably accelerated but still random. Such an accelerating effect might account for the specificity for the α -methine bridge in myoglobin since the environment around this bridge seems to be particularly hydrophobic.

Haemoglobin differs in a noteworthy way from myoglobin in directing oxidation partly to the α -methine bridge and partly to the β -bridge (Ó Carra & Colleran, 1969a). The proportion of β -methine oxidation varies with species and is particularly high (about 40%) for human haemoglobin.

Incubation of human haemoglobin with *p*-hydroxymercuribenzoate, which causes its dissociation into monomeric subunits (Bucci & Fronticelli, 1965), did not eliminate the β -methine oxidation. The individual α and β subunits (separated as described by Geraci, Parkhurst & Gibson, 1969) both retained the dual specificity, although the proportion of β -methine oxidation was decreased in both, and was particularly low in the isolated β subunit. The difference in specificity between myoglobin and haemoglobin is not therefore primarily attributable to interchain interactions. Hydrophobic environments might be invoked again, however, as a possible explanation, since the environment around the β -methine bridge in both the α and β subunits appears, in the molecular models of haemoglobin (cf. Perutz, 1969), to be more hydrophobic than in myoglobin, though not as hydrophobic as the environment of the α -methine bridge.

Diphosphoglycerate, which allosterically changes the oxygen-binding characteristics of haemoglobin (cf. Benesch & Benesch, 1969) did not change the specificity of the coupled oxidation significantly, but complexing with haptoglobin (Connell & Smithies, 1959) caused a considerable decrease in the proportion of β -methine oxidation, a fact with possible implications in studies of haemoglobin catabolism (cf. Ó Carra & Colleran, 1969a).

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Observations on Haemoglobin P (Congo Type)

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Haemoglobin P was first reported from Galveston (Schneider & Haggard, 1957) and then from the Congo (Dherte, Lehmann & Vandepitte, 1959). The Galveston type has been identified as β^{117} His $\rightarrow$ Arg (Schneider, Alperin, Brimhall & Jones, 1969). The Congo type was found in several individuals together with haemoglobins A and S (Dherte *et al.* 1959; Lambotte-Legrand, Ager & Lehmann, 1960), and therefore thought to be an α -chain mutant. However, on hybridization it behaves like a β -chain mutant (Gammack, Huehns, Lehmann & Shooter, 1961) and this was also seen on electrophoresis of the separated chains (Chernoff & Pettit, 1964).

Since β^A and β^S are alleles it is impossible for one individual to have haemoglobins A, S and another β -chain variant. The possibility suggests itself that haemoglobin P is a fusion product of an unequal cross-over between the δ - and β -chain genes, producing a chromosome carrying the genes for the δ -chain, a $\beta\delta$ fusion product and normal β -chain. With the $\beta\delta$ product, haemoglobin Lepore, there are no genes for the δ - and β -chains on the same chromosome.

A cross-over between β^{23} and 115 would give a $\beta\delta$ product resembling on electrophoresis haemoglobin P-Galveston (β^{117} His $\rightarrow$ Arg) because the difference in charge between β and $\beta\delta$ would be that between β^{116} His and δ^{116} Arg.

Enough haemoglobin P-Congo was available for peptide chromatography but not for determining amino acid sequences. The tryptic peptides of the aminoethylated globin were 'fingerprinted' in the usual way (Sick *et al.* 1967). Peptide β III (β^{18-30}) was present, but no peptide was found where δ III would be expected; on the other hand the three peptides, δ XIIa, b₁ and b₂, expected from the aminoethylated δ -chain were present with expected staining reactions; BXIIb was absent.

A $\beta\delta$ fusion product, haemoglobin Miyada, has been described by Yanase *et al.* (1968). It resembles the β -chain at least as far as β^{17} , but it contains peptide δ III and is the δ chain from then on.

As haemoglobin P-Congo occurs with a normal A₂ content and can be present with haemoglobins A and S, the order of genes from the N-terminus is $\delta\beta$ rather than $\beta\delta$, otherwise haemoglobin Lepore and not the $\beta\delta$ product would be inherited on a chromosome that also carries δ - and β -chain genes.

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Ethanolamine Plasmalogen Metabolism in Myelinating Rat Brain

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Although brain plasmalogens undoubtedly have a structural function, in very young animals these substances have been shown to be undergoing a rapid catabolism (Debusch, 1964, 1966). Further, although the rate of formation of ethanolamine plasmalogens in myelinating rat brain is relatively greater than at any other time, Ansell (1968) has reported that brain plasmalogenase, which liberates aldehydes from ethanolamine plasmalogens, increases rapidly at this time.

To obtain labelled ethanolamine plasmalogen and glycerophosphatide (EGP) for these studies, [9,10-³H]palmitic acid (1 mCi/animal) was injected intracerebrally into 13-day-old rats and the [³H]EGP was isolated after 2 days incubation. The [³H]EGP was emulsified in Tween 20 and injected intracerebrally into 13-day-old rats (Bickerstaffe & Mead, 1967). After a 2 h incubation, brain lipids were fractionated and the distribution of radioactivity in phospholipids and sphingolipids was determined. Comparative studies were also made with plasmalogen-free [³H]EGP and with [¹⁴C]-palmitic acid.

These studies indicate that the alkenyl groups of ethanolamine plasmalogens are better precursors of the acyl groups of sphingomyelin and cerebroside