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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

than either the acyl groups of phosphatidylethanolamine or free palmitic acid. Sphingosine is not significantly labelled by either EGP-alkenyl or -acyl groups. The relatively poor labelling of lecithin (phosphatidylcholine) in the [^3H]EGP incubations indicates that *N*-methylation of EGP does not occur in myelinating brain. The labelling of sphingolipid acyl groups by EGP-alkenyl groups suggests that the aldehydes derived from EGP become involved in fatty acid chain-elongation processes.

The results below are in accord with this suggestion. (i) Brain EGP-alkenyl groups (almost exclusively $\text{C}_{16:0}$, $\text{C}_{18:0}$ and $\text{C}_{18:1}$) are turning over more rapidly in myelinating rat brain than EGP-acyl groups of corresponding chain length (Debuch, 1964). (ii) Brain contains a mitochondrial aldehyde dehydrogenase of very broad specificity. The K_m for palmitaldehyde is $1.3 \times 10^{-6} \text{ M}$ (Erwin & Deitrich, 1966). (iii) Brain plasmalogenase is associated with the mitochondria, the site for aldehyde dehydrogenase activity and fatty acid chain-elongation processes. Further, these are specific for the C_{16} and C_{18} acids (Aeberhard & Menkes, 1968). (iv) Brain cerebroside acyl groups are formed by chain-elongation processes involving palmitic acid derived from two pools, namely *de novo* from acetate and from 'complex lipids' (Kishimoto & Radin, 1966).

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Separation at Neutral pH of a ^{32}P -Labelled Membrane Protein Associated with the Sodium-plus-Magnesium Ion-Activated Adenosine Triphosphatase from Ox Brain

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The Na^+ -dependent acyl-bound phosphate formed during the hydrolysis of ATP by cerebral microsomes has only been clearly demonstrated after acid-denaturation of the labelled membrane proteins. At neutral pH the nature of the bond has

not been described, leaving open the possibility of an acid-catalysed migration of phosphoryl groups during denaturation at low pH. Attempts to characterize the phosphorylated membrane-component, now widely considered to be an intermediate in the $\text{Na}^+ + \text{K}^+ + \text{Mg}^{2+}$ -activated adenosine triphosphatase (Kanazawa, Saito & Tonomura, 1967; Fahn, Koval & Albers, 1968; Schoner, Beusch & Kramer, 1968), at physiological pH values have been hampered by the lability of the bond.

Sodium dodecyl sulphate was used by Nagano *et al.* (1967) to stabilize and solubilize membrane ^{32}P -labelled protein with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ as precursor. Using 0.1% sodium dodecyl sulphate to terminate the reaction we have confirmed that a Na^+ -dependent increment of 50 pmol of bound P/mg of protein can be isolated from ox brain microsomes, labelled as described by Rodnight, Hems & Lavin, (1966), after separation on Sephadex G-25 at pH 7. Precipitation of labelled protein with acetone and treatment with 0.8M-hydroxylamine at 0°C for 10 min at pH 7 resulted in rapid hydrolysis of the label. Hydrolysis rates over the range pH 2-9 gave a profile characteristic of an acyl phosphate.

A fivefold purification of acyl-labelled protein was achieved with a column of Sepharose 6B; by markers the molecular weight of the component at this stage was estimated at 570 000. After concentration of the labelled protein by rapid ultrafiltration further fractionation was attempted by electrophoresis on polyacrylamide gel (10%, w/v) containing 0.1% sodium dodecyl sulphate, 5M-urea and 0.01% mercaptoethanol at pH 8.7. Twelve protein bands were observed and despite the increased lability of the acyl bond at this pH enough hydroxylamine-sensitive label was present after electrophoresis (40 min) to demonstrate its presence in a single band. From mobilities of markers (Shapiro, Vinuela & Maizel, 1967) the molecular weight of the labelled band was estimated as 102 000.

Incubation of microsomes with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ also results in a Mg^{2+} -dependent transfer of phosphate to protein serine residues (Rodnight & Lavin, 1966). Membrane proteins labelled for 2 min with 1mM- MgATP were solubilized with sodium dodecyl sulphate and fractionated on Sepharose 6B. Three peaks of phosphorylserine labelling were obtained; incorporation of label into the first two peaks was stimulated by $100 \mu\text{M}$ -3':5'-cyclic-AMP (Weller & Rodnight, 1969). The second peak was eluted in the same position as the acyl-labelled protein. On polyacrylamide gels the phosphorylserine labelling was confined to two major protein bands of lower molecular weight than the acyl-labelled band.

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