131 Val-Thr-Asn-Pro-Glu-Arg-Phe-Glu-Pro-Glu-
 141 Leu-Asn-Glu-Val-Asn-Pro-Gly-Glu-Thr-Gln-
 151 Asp-Thr-Ser-Thr-Ala-Arg-Ala-Leu-Val-Thr-
 161 Ser-Leu-Arg-Ala-Phe-Ala-Leu-Glu-Asp-Lys-
 171 Leu-Pro-Ser-Glu-Lys-Arg-Glu-Leu-Leu-Ile-
 181 Asp-Trp-Met-Lys-Arg-Asn-Thr-Thr-Gly-Asp-
 191 Ala-Leu-Ile-Arg-Ala-Gly-Val-Pro-Asp-Gly-
 201 Trp-Glu-Val-Ala-Asp-Lys-Thr-Gly-Ala-Ala-
 211 ----Ser-Tyr-Gly-Thr-Arg-Asn-Asp-Ile-Ala-
 221 Ile-Ile-Trp-Pro-----Pro-Lys-Gly-Asp-Pro-
 231 Val-Val-Leu-Ala-Val-Leu-Ser-Ser-Arg-Asp-
 241 Lys-Lys-Asp-Ala-Lys-Tyr-Asp-Asp-Lys-Leu-
 251 Ile-Ala-Glu-Ala-Thr-Lys-Val-Val-Met-Lys-
 261 Ala-Leu-Asn-Met-Asn-Gly-Lys.

Direct evidence linking residues 54-55, 220-221 and 233-234 is lacking, but the homology makes alternative structures very unlikely. If deletions at 211 and 225 are allowed, 110 residues (43%) are identical in matching positions.

Tetranitromethane is a reagent specific for tyrosine (Sokolovsky, Riordan & Vallee, 1966). After 1 hr. at pH 8.5 with 1 mole of tetranitromethane/mole penicillinase, activity fell 40% and nitrotyrosine was found only at residue 77.

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Breakdown of Pyridine Haemichrome by Liver Extracts

By EMER COLLERAN and P. Ó CARRA. (*Department of Biochemistry, University College, Galway, Irish Republic*)

Pyridine haemichrome is converted by liver extracts into a green product that is readily converted into biliverdin. Nakajima, Takemura, Nakajima & Yamaoka (1963) and Nakajima & Gray (1967) report that this reaction is enzymic, whereas Murphy, Ó hEocha & Ó Carra (1967) and Levin (1967) consider that it is mostly, if not entirely, non-enzymic and possibly similar to the coupled oxidation of pyridine haemichrome with ascorbate.

Re-examining the general extraction and assay procedures of the above authors, we find that the pyridine haemichrome-cleaving activity of dilute liver extracts is labile to heating at 100° for 15 min., giving a semblance of enzymic activity that may partly account for the claims of Nakajima *et al.* (1963). However, the activity survives shorter periods of heating, and dilute solutions of ascorbate

(e.g. 0.03%) display an almost identical lability. Both systems display greater heat-stability at higher concentrations and an identical non-linearity in the relationship between concentration and pyridine haemichrome-cleaving activity. Careful analysis of the complex results gave no indication of an enzymic component in the pyridine haemichrome-cleaving activity of extracts from ox, guinea-pig or rat liver or from guinea-pig or rat kidney.

Whereas coupled oxidation of pyridine haemichrome with ascorbate yields an almost random mixture of the four biliverdin isomers (Rüdiger, 1968), Nakajima & Gray (1967) report that liver extracts specifically cleave pyridine haemichrome to the IX α -isomer of biliverdin. This is also the biliverdin isomer specifically produced *in vivo*. However, using the chromatographic technique described in the accompanying communication (Ó Carra & Colleran, 1969), we can find no difference between the isomeric composition of the products formed with liver extracts or with ascorbate. Both produce mixtures of all four isomers. This result is supported by the results obtained by W. Rüdiger (personal communication) by using a dichromate micro-degradation technique (Rüdiger, 1968).

It seems unlikely therefore that pyridine haemichrome breakdown is a valid model for haem breakdown *in vivo*.

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Coupled Oxidation of Myoglobin with Ascorbate as a Model of Haem Breakdown *in vivo*

By P. Ó CARRA and EMER COLLERAN. (*Department of Biochemistry, University College, Galway, Irish Republic*)

When haemoproteins are incubated with ascorbate at physiological pH and temperature the haem groups undergo coupled oxidation and are released as biliverdin on acidification (cf. Kench & Gardikas, 1952). We have reinvestigated these systems, since the much-studied breakdown of pyridine haemichrome now appears to be an inadequate model for the mechanism of haem breakdown *in vivo* (see