(2) Such extension, if azimuthally independent, would require linear extensions of 34 and 26% to produce the locally maximal values of b of 1.8 and 1.6 respectively[4].

The amount of extension accommodated by listric faults is debatable. Although Montadert *et al.*[5] suggest that listric-faulted blocks on the north Biscay margin, displaying tilts of 20° to 30°, allow some 15% extension, the same seismic reflection data have been interpreted by X. Le Pichon and J.-C. Sibuet (personal communication) to allow extension of 200% to 300%. Both Montadert *et al.* and Le Pichon and Sibuet cite extension as being the primary cause of crustal attenuation in this area, with post-rift subsidence the result of isostatic adjustment to cooling of the lithosphere.

We agree that magmatism during the Jurassic and early Cretaceous was trivial, justifying our assumption that crustal material was conserved. However, the apparent predating by basaltic effusions of the onset of extension by ~10 Myr seems more to support the placing of the stretching event in the Lower Jurassic than to refute the model.

The noted existence of a Permo–Triassic event in the North Sea may require an explanation of subsidence in terms of the superposition of two main stretching episodes. In this case, we would agree that the North Sea crustal thickness may already have been attenuated by the start of the Jurassic phase of activity, in which event, less Jurassic extension of even a one-dimensional type would be required to produce the seismic cross-section.

However, the good agreement between the seismic and gravity estimates of the depth to the Moho suggests that there is indeed crustal attenuation beneath the North Sea Basin, and 4 km of actual post mid-Cretaceous sedimentation is compelling evidence for something other than simple loading of an elastic lithosphere, which predicts a thickened crust[6]. A loading hypothesis would require the existence of a wide, 1,200-m deep depression at the time of the Aptian–Albian unconformity over a somehow previously thinned crust.

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Counting integral numbers of amino acid residues

THE method of "counting integral numbers of amino acid residues per polypeptide chain" described by Creighton[1] is not new either in concept or in execution. The idea of using varying ratios of iodoacetamide and iodoacetate to alkylate proteins and then to determine the number of alkylated cysteine residues by gel electrophoresis was first introduced by Feinstein[2] in 1966. In that original paper starch gel electrophoresis was used. Subsequently, Feinstein and Stott[3] used isoelectric focusing to resolve the differently charged proteins. In both of those papers the 'charge-shift' technique was used to determine the number of free thiol groups on partially reduced proteins.

More recently Singer and I[4] have used 'charge-shift' analysis of fully reduced protein to determine the cysteine content. We have therefore used the method in exactly the same manner as that described by Creighton. Extension of the charge-shift method to other amino acid residues is an obvious possibility. However, the data presented by Creighton relate only to the determination of cysteine residues.

I am most surprised that the previous publications on the charge-shift method should have escaped the attention of both Creighton and the *Nature* referees.

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CREIGHTON REPLIES—The papers cited by Williamson[1–3] describing the 'charge-shift' method of determining cysteine residues, which has become the same in principle as the method I used[4], were unfortunately unknown to me, the referee of my paper, all the colleagues with whom I discussed this matter, and the authors of all review articles and monographs on the subject of amino acid analysis that I could find. A thorough search through the index of *Amino Acid, Peptide and Protein Abstracts* for papers on amino acid analysis also failed to uncover these papers. This ignorance of the development of Feinstein's original work[2] can be attributed to its publication only as a small part of papers dealing with the biosynthesis of immunoglobulins published in an immunological journal[2,3], with no mention of the method in either the titles or abstracts. This is an instructive example of how care must be taken if the attention of the appropriate audience is to be drawn to published work.

Having now studied these papers in detail, I note that the validity of the charge-shift method was not established at that stage, either by confirming the results with an independent, established method or by demonstrating that it gave the correct results with a well characterized protein. It was also used inappropriately with a protein that was electrophoretically heterogeneous so that the results were ambiguous[3]. Demonstrating the validity of this method is especially important because it depends critically on the completeness and the specificity of the reaction of cysteine thiol groups with iodoacetate and iodoacetamide. I demonstrated this by obtaining single electrophoretic bands of unfolded proteins treated with the limiting cases of just iodoacetamide and just iodoacetate[4]. There was then no uncertainty about where to start and where to stop counting the bands generated by reaction with mixtures of the two reagents. The number of bands generated gave the correct number of cysteine residues for six well characterized proteins. When this procedure is used with a new protein, a well characterized protein should always be included as a control, to ensure the specificity and completeness of the reactions.

It is clear that this general method of determining integral numbers of specific amino acid residues is simple, easy, and can give unambiguous results when properly applied. The general approach should be useful for some amino acid residues other than cysteine; the observations of Anderson and Hickman[5] using carbamylation and of M. Hollecker in this laboratory using succinic anhydride[6] demonstrate that it may readily be extended to lysine residues.

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