PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

# Concluding Remarks

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# Concluding remarks

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This is the second time that I have been asked to make the concluding remarks at a Royal Society Discussion Meeting on enzyme mechanisms. The first occasion was in 1966, shortly after D. C. Phillips and his colleagues had solved the structure of the first enzyme, lysozyme, in the Davy Faraday Laboratory at the Royal Institution. It was also the first time that anyone had been able to interpret the catalytic mechanism of an enzyme in stereochemical terms. It seemed rash for me to generalize from the structure of one enzyme, but certain features stood out (Perutz 1967):

> 'We may now ask ourselves why chemical reactions, which normally require powerful organic solvents or strong acids and bases, can be made to proceed in aqueous solution near neutral pH in the presence of enzyme catalysts. Organic solvents have the advantage over water of providing a medium of low dielectric constant, in which strong electrical interactions between the reactants can take place. The non-polar interior of enzymes provide the living cell with the equivalent of the organic solvents used by the chemists. The substrate may be drawn into a medium of low dielectric constant in which strong electrical interactions between it and specific polar groups of the enzyme can occur.'

The work of A. R. Fersht and his colleagues reported at this Discussion has made it possible to quantify these interactions and to show that a single hydrogen bond between the substrate and a charged amino-acid side chain of the enzyme may accelerate the reaction by a factor of the order of $10^5$.

I ended my remarks in 1966 with the words:

> 'Once we understand the stereochemical basis of enzymic catalysis it may become possible to design and synthesize enzymes for specific catalytic functions, for both biological and industrial purposes. I look forward to a future Royal Society Discussion on that subject.'

While I am delighted to have been able to take part in the discussion of which I dreamed 19 years ago, I have no reason to feel content at my foresight. In 1965 I published a paper together with Kendrew & Watson on the relation between secondary structure and amino-acid sequences of haemoglobins, which I hoped to be the first step towards the prediction of structure from sequence (Perutz *et al.* 1965). It would never have occurred to me that this problem would prove so refractive as to be still unsolved 20 years later. On the other hand, if anyone had suggested to me that it would become possible within that period to produce β-globin in *E. coli* and to perform directed mutagenesis on the globin gene, I would have dismissed this as science fiction.

Because we all obtain our support from public funds, we must ask ourselves not only how much the new techniques will contribute to our understanding of enzymatic catalysis but of what practical value they will be to medicine and industry. M. Neuberger's contribution has

given us a glimpse of the tremendous scope for genetically engineered monoclonal antibodies in clinical medicine, and J. A. Wells has shown how an industrially important enzyme can be stabilized by a single genetically engineered amino-acid substitution, with only minor impairment of its activity.

Medically, perhaps the greatest promise of protein engineering lies in the manufacture of pharmacologically active human proteins. Being human, they can exert their effects without eliciting adverse immunological reactions. M. Courtney has told us how he engineered suitable inhibitors of elastase and thrombin by directed mutagenesis of the $\alpha_1$-antitrypsin gene (Courtney 1985; Carrell & Travis 1985). There have been reports of preliminary clinical trials showing that a human protein, tissue plasminogen activator (produced in *E. coli*) dispersed blood clots and restored circulation in coronary thrombosis when injected five hours after the first onset of pain (T.I.M.I. Study Group 1985; European Cooperative Study Group for Recombinant Tissue-type Plasminogen Activator 1985). Another human protein, tumour necrosis factor, has been found to cause necrosis of mammary carcinoma in mice and to kill or arrest the growth of cell lines derived from several kinds of human tumours in tissue culture (Pennica *et al.* 1984). First reports of clinical trials of interferon in the treatment of cancer have been disappointing, but it has recently been useful in the treatment of hairy cell leukaemia (Quesada *et al.* 1984). The production of such proteins in *E. coli* and their perfection by genetic engineering is likely to become an important field of research.

B. S. Hartley has explained that enzymes have a restricted market and that investment of large capital sums in their production may be uneconomic, but this may be true for any catalyst. For example, the economic value of the catalyst in the Haber synthesis of ammonia should not be measured by the tonnes of catalyst sold, but by the tonnes of fertilizers synthesized. Similarly, the economic value of genetically engineered enzymes must be estimated by the value of their reaction products. E. Katchalski-Katzir recently reviewed the enormous scope of enzymes immobilized on columns for the production of a wide range of chemicals and drew my attention to a recent paper showing how the key stages in the synthesis of a new antibiotic can be performed cheaply and effectively by immobilized enzymes (Wolfe *et al.* 1984). Enzymes are also used on a large scale in clinical biochemistry, as in the routine tests for the serum levels of a variety of metabolites. For example, the serum level of creatinin as an indicator of renal function is measured with the aid of four enzymes: creatininase, creatinase, sarcosin oxidase, and peroxidase. The glucose level is measured with the help of glucose oxidase and peroxidase. The cholesterol level is measured with the help of cholesterol esterase, cholesterol oxidase and peroxidase. Note that the last enzyme in each of the chains is peroxidase, which produces a colour reaction by the coupling of two components of a dye. I am told that there is scope for improvement of this enzyme by increasing its specificity for the dyes or perhaps the specificity of the dye for the enzyme.

These few examples illustrate the great scope for enzyme engineering. I hope that it will not take another 19 years before the time is ripe for a Royal Society Discussion on the application of enzyme engineering to medicine, agriculture and industry.





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