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Closing Remarks

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Closing remarks

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Only a bold man would dare attempt a synthesis of the wide-ranging discussions of the last two days, let alone distil from them any general principles of biological recognition. The only sentiment which could command general assent is one of gratitude to the Society, the organizers and the contributors for putting before us such a fascinating collection of papers and letting us sharpen our wits on so much new knowledge. The following remarks will therefore be nothing more than personal footnotes; if they omit reference to much of the work which has been presented, and only mention a few of the issues that have been raised, that is a matter of sheer necessity, not of personal prejudice.

In our first session we began by reviewing the physical chemistry of molecular interactions in terms of quantum mechanics and statistical mechanics. Professor Buckingham warned us against the temptation of regarding molecular association energies as made up of additive contributions from pairs of atoms or ions, and Professor Symons stressed the highly individual structure-forming habits of water, which make it so difficult to interpret its solvation properties in terms of simplified models which treat water as a uniform dielectric, or as a mixture of monomers and high polymers. Professor Truter then introduced us to the structures of the complexes formed by ferrichrome A, nonactin and other biological agents with metal ions of various kinds. She drew attention to the large conformational changes which often accompany complex formation, and suggested that some at least of these agents owe their specificity to their high flexibility. Presumably one should interpret this generalization in terms of an ability of the complexing agent to meet very precisely the stereochemical needs of one particular ion, coupled with an inability to meet the precise needs of other ions without a certain amount of mechanical strain. To take a single example, it is not immediately obvious how the needs of Tl^+ differ from those of the alkali metal ions; but that is because we tend to overlook the effect of ionic polarization on the stability of the solvated ion. The Tl^+ ion, because of its outer electron pair, has a low-lying dipole transition ($6s \rightarrow 6p$) which confers on it a high electric polarizability.† As a result, a set of negative ligands on one side induce a negative charge on the other side, and this will hinder the approach of further ligands on that side – as suggested by the curious stereochemistry of some of Professor Truter's Tl^+ complexes.

The idiosyncrasies of small molecules in determining their 'recognizability' were also discussed in the following session. At the molecular level, one of the most discriminating of all biological systems is the immune system of the vertebrates, the study of which has made enormous strides during the last ten years. We now know that γ globulin is not a single protein capable, like the human hand, of picking up a wide range of differently shaped objects, but a

† Whereas the ionic radii of Tl^+ and K^+ are roughly equal, namely 144 pm (1.44 Å) and 130 pm (1.30 Å) respectively (Pauling 1940), the polarizability of Tl^+ in sodium D light is almost 4 times that of K^+, the respective values being 0.0052 nm^3 (5.2 $Å^3$) and 0.00133 nm^3 (1.33 $Å^3$) (Tessman, Kahn & Shockley 1953). I am indebted to Professor Buckingham for this comment.

whole family of proteins of similar but not quite identical structure. Professor Poljak, in expounding present structural knowledge about the immunoglobulins, drew attention to the fact that in the folded protein the regions of variability, though quite widely separated on the polypeptide chains (of which there are two pairs) are close together in space, at an exposed end of the molecule. By ringing the changes on the amino acid residues in this region, one obtains antibodies against a wide variety of different antigens, so that there is no particular problem in understanding the variety of immune responses which an animal can make, given that it is equipped with the requisite variety of lymphocyte clones, each with its own specific antibody. Considerable interest attaches, however, to the mechanism which produces and sustains the variety in the antibody population; the puzzle is accentuated by the fact that animals can produce antibodies against synthetic antigens which do not occur in nature. On this particular problem members of the conference were notably silent, possibly because the 'instructive' theory of antibody production runs into head-on collision with the 'central dogma' of molecular biology, and the 'somatic mutation' theory fails dismally on quantitative grounds, unless special replicative mechanisms are postulated. It must be admitted, then, that in spite of our new structural knowledge, the molecular biology of the vertebrate immune system is still far from clear.

After Professor Poljak had unveiled the tertiary structure of the immunoglobulins, Dr Dwek explained how electron and nuclear spin resonance can be used for estimating the size of the active site in solution, and Dr Richards convinced many of us that quantum-mechanical calculations can help to indicate what conformational conditions must be satisfied by a small flexible molecule for it to be recognized by an enzyme. Later, Professor Dunitz, in a more empirical vein, brought forward crystallographic evidence that organic molecules can actually be 'frozen' in configurations which have in the past been assumed to occur in the course of chemical reactions. But the most complete mechanistic analysis reported in the second session was that of the catalytic action of trypsin. The reaction mechanism proposed by Dr Blow and Mr Smith on the basis of their crystallographic studies shows very convincingly how a combination of mechanical strain, bond polarization and charge relay effects can suffice to promote such hydrolytic reactions with the smoothness and elegance of a synthesis planned by Sir Robert Robinson.

It was at this point in the meeting that the present author began seriously to doubt whether the customary distinction between 'energetic' and 'entropic' effects of catalysts upon reaction rates might not be a positive hindrance to understanding enzymic catalysis. Ever since the concept of the transition state was introduced into reaction kinetics by Eyring and others it had been argued that the effect of a catalyst on a reaction was to lower the free energy of the transition state by either lowering its energy, or raising its entropy, or both. It was not difficult to understand how the energy might be lowered, by mechanical interaction between the enzyme and the transition state; but how could the entropy be significantly increased? A possible answer was that the required negative entropy was stored initially in some ordered water structure intimately associated with the enzyme, and that when this was required for stabilizing the transition state, the water would 'melt', and thereby increase the entropy of the system as a whole. Such ideas were briefly aired in the course of the meeting, but obviously failed to carry general conviction.

Dr Sutton had pointed out, in the course of the afternoon, that one could not hope to explain the activity of a catalyst by assuming that it merely arranged the *reactants* in a favourable

configuration; if this had any effect at all, it would only be to deplete the population of the transition state. But if the catalyst opened a new *pathway*, previously inaccessible, then the reaction might indeed be greatly accelerated, through the appearance of an entirely new transition state. This observation seems highly relevant to the findings of Dr Blow and Mr Smith. The mechanism which they put forward postulates, in effect, a transition state the like of which would be most unlikely ever to occur in a disordered assemblage of the relevant amino acid residues. In this sense, perhaps, it might be formally correct to assert that the catalysed and uncatalysed reactions have very different entropies of activation; but such a remark would disguise the essence of the matter, namely that the tertiary structure of the trypsin molecule is particularly well adapted to the function which it has evolved to perform – that it is an astonishingly 'well designed' chemical machine.

The second day of the conference was more highly differentiated than the first. Professor Eigen, in his opening remarks, pointed out that biological discrimination can be bought relatively cheaply in the currency of energy; an energy difference of only 6 kJ/mol (1.4 kcal/mol) corresponds to a factor of 10 in relative stability. But to make a number of successive discriminations one needs to spend the energy in smaller amounts, and natural selection has solved this problem by inventing large molecules with many degrees of freedom, each with a relatively small characteristic energy. Highly specific reactions do not necessarily involve substantial conformation changes; antibody–hapten reactions, for example, are often so fast that their rates must be limited by mutual diffusion. Other reactions can, however, gain specificity at the expense of speed – the reaction of K^+ with valinomycin being an example – and this reminds one of the pay-off between speed and accuracy associated with a noisy communication channel. It was, in retrospect, curious that in the course of two days no one referred explicitly to the mathematical theory of communication, although biological recognition is plainly an exercise in the correct identification of messages. Perhaps this was because, in biology, information theory suffers from the same limitations as thermodynamics; no one doubts that it applies, but the difficulty is in applying it!

In the following papers we were introduced to a number of systems which have been subjected to very thorough empirical study. Dr Dalziel's paper on alcohol dehydrogenases showed that the kinetics of enzyme reactions involving the participation of a coenzyme can supply useful information about the interaction between coenzyme and substrate on the enzyme surface. Dr Perham discussed the biological advantages which could be gained from the spatial organization of proteins into complex multimeric structures, and hinted, in his 'hot potato' hypothesis, at the kind of protection that this could afford to unstable intermediates. Dr Sobell's paper brought us up to date on the stereochemistry of dinucleotide complexes. Here, it seemed, was an area of study which would soon throw light not only on the effects of drugs on the conformation of nucleic acids, but also on the molecular mechanisms of information transfer from DNA to the smaller molecules which have to read its sequence. And here again one was impressed by the individuality of the molecular structures which are formed by intercalation, and correspondingly daunted by the prospect of predicting such structures from any general principles, however well founded.

The last four papers raised problems of rather different kinds, scarcely statable in purely molecular terms. Dr Bishop's studies of nucleic acid hybridization showed that we can now begin to identify a given base sequence, and even measure its abundance, in very complex DNA

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preparations such as the genomes of vertebrates; here biological recognition has proved its value as a tool, as well as a phenomenon to be investigated in its own right – rather as happened in the field of immunology. Dr Radda's paper on membranes showed how one can now 'probe' membranes to various depths with carefully designed molecules detectable by their fluorescence or n.m.r. spectra. Moving further afield, Dr Crumpton suggested, on the basis of his studies of the effect of PHA on lymphocytes, that the crucial event triggered by the antigen–antibody interaction is a substantial increase in the permeability of the cell membrane to calcium ions, mediated by protein molecules which extend right through the cell membrane. Finally, with the aid of a time-lapse film which no one who saw it is likely to forget, Dr Gerisch brought into focus the detection problems which must be solved by the cells of the slime mould if they are to fulfil their destiny and aggregate into a multi-cellular organism. Quite apart from the molecular mechanism of cyclic-AMP receptor system, there are two problems: how does the cell measure the gradient across its body; and how is synchronization achieved between the pulses of cyclic-AMP emission from different cells? But it would be superfluous to recapitulate Dr Gerisch's views.

All in all, those who were not already of that opinion must have been convinced by what they heard at the meeting that biological recognition is not merely a physiochemical phenomenon but an inescapably biological one. Quantum mechanics, statistical thermodynamics and chemical kinetics will take us just so far and no further in satisfying our curiosity about the means employed by organisms for discerning what is of interest to them. It would be surprising if this were not so, because evolution is a history of invention. To paraphrase a remark made by Dr Crick: if a cell finds that a certain job has to be done, it invents some gimmick for doing it; so if we want to understand how the job is done, we had better discover the gimmick. General principles will only serve to exclude totally outrageous hypotheses.

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