long exercised protein chemists. Spectroscopic and chemical methods have been devised, and one of the most successful has recently sprung from the discovery that the C-2 ring proteins of histidine side chains can be resolved in proton magnetic resonance spectra, so that in favourable cases the tritration curves of the individual histidine of a protein can be determined.

An altogether different and potentially very useful approach is given a preliminary airing by Matsuo *et al.* (*J. Biochem., Tokyo*, **72**, 1057 ; 1972), who report as follows: the isotope exchange rate of the C-2 imidazole ring proton can be observed by NMR, and follows the *p*K curve of the ring protonation. Thus a plot of the deuteration rate constant against the *p*H gives the imidazole titration curve. Now at acid *p*H, the rate is evidently infinitesimally slow ; thus the exchange rate against tritium can equally well be followed by quenching the reaction at various times with acid, and then determining the degree of titration by scintillation counting. The *p*K of histidine in a tripeptide and of the single histidine residue in lyozyme were determined in this way. After acidifying, the lysozyme solution is passed through a column, and this is followed by acid hydrolysis paper electrophoresis of the product and scintillation counting of the basic amino-acid fraction. Evidently the imidazole side chain is the only one in the protein that retains its tritium through the hydrolysis. The *p*K of his-15 turns out to be *p*H 5.2, whereas in the denatured state it is found to be 7.0. The possibilities will no doubt not be lost on protein chemists, for if this method can be made to work equally well in other situations, and combined with enzymatic hydrolysis and peptide separation, all manner of interesting cases, involving histidines in active sites and the like, could be explored.

The steric accessibility of side chains in proteins is commonly judged by their ability to react with specific reagents. Perham (*Biochem. J.*, **131**, 119 ; 1973) advocates the use of the chromophoric imidoester, methyl picolinimidate, for the determination of available amino-groups in proteins. In TMV protein, the two lysines (53 and 68) react, whereas in the intact virus lys-53 becomes unavailable. In mutants with extra lysines the same situation recurs: a lysine at position 140 remains reactive in the intact virus. In a set of two mutants a lysine at position 9 reacts, whereas one at 33 is reactive in the monomeric protein, but is evidentally engulfed in the structure of the whole virus. There is independent evidence from the position of a heavy-atom label in crystals that position 140 is indeed on the outside of the particle. Position 9 is also expected to occur on the surface, for the first nine residues make up a highly polar segment. At residue 10 hydrophobic character sets in, and the side chains presumably submerge in the structure. In the one mutant it therefore seems as though two out of four lysines, in the intact virus at least, are sequestered. From an energetic viewpoint it should be advantageous if they were to emerge in ion-pair interaction with carboxylate groups. Until comparisons with known X-ray-based structures can be made, the structural implications of anomalous reactivity of side chains must, of course, be approached with caution, as Perham is at pains to stress.

At the conformational level, a new method, minted and burnished in Anfinsen's laboratory, is now on display (Sachs *et al.*, *Proc. US Nat. Acad. Sci.*, **69**, 3790 ; 1972). This depends on the preparation of two kinds of antibodies to a given determinant, or group of determinants, on a protein. One results from immunization with peptide fragments, the other with the whole protein, a specific population of antibodies being

Reiteration of Duck Haemoglobin Genes

In the hands of biochemists reverse transcriptase purified from RNA tumour virus particles has become an invaluable tool for the synthesis of single-stranded DNA complementary to cellular messenger RNAs. The DNA made by reverse transcriptase can be labelled to high specific activities, and for this reason it can be used as a probe for messenger RNAs and their DNA templates in hybridization experiments designed, for example, to measure the expression of a particular gene or the number of copies of that gene per genome. Using this approach Bishop and Rosbash, as they report in *Nature New Biology* next Wednesday (February 14), estimate that in duck cell there may be only two or three copies of haemoglobin genes.

Bishop and Rosbash isolated 10S RNA containing globin messenger from immature duck red blood cells and in the presence of oligo (dT), which acts as a primer for reverse transcriptase, used this RNA as a template for the synthesis of short strands of complementary DNA (cDNA) with a molecular weight of about $1–2\times10^5$. They then used this cDNA to isolate a cDNA-duck cell DNA duplex. Measurements of the rate of reannealing of cDNA-duck DNA and of duck DNA in the presence and absence of cDNA were then made. From the results of these C_0t analyses, and making allowances for the various problems in interpreting such data, Bishop and Rosbash estimate that the reiteration frequencies of duck haemoglobin genes may be as low as two to three.

This estimate is in close agreement with that reported by Bishop *et al.* last year from data obtained from RNA-DNA hybridization experiments ; they concluded that the reiteration frequency of haemoglobin genes in ducks was probably less than five. Such a low degree of reiteration is, of course, in marked contrast to estimates that in sea urchins there may be about 1,200 copies of histone genes.

α Globin mRNA is Lacking in α Thalassaemia

In next Wednesday's *Nature New Biology* (February 14), Grossbard, Terada, Dow and Bank demonstrate that the decreased production of structurally normal α globin chains, typical of α thalassaemia syndromes, can be localized to the level of production of α chain messenger RNA. These workers have investigated the synthesis of α and β globin chains in reticulocytes from non-thalassaemic blood and those from the blood of a patient with haemoglobin H (HbH) disease. The red cells in HbH disease contain a tetramer of four β chains as a result of the relative deficiency of α chain synthesis.

Intact cells from HbH and non-thalassaemic patients were incubated with radioactive leucine, the cells were lysed, globin was prepared and the α and β chains were separated on carboxy-methyl-cellulose (CMC). The ratio of α/β chain synthesis in the intact cells of the patient with HbH disease was 0.55, and the α/β ratio in non-thalassaemic cells was 1.0. Next, mRNA was isolated from HbH and non-thalassaemic blood, and was assayed in a cell-free system from Krebs ascites tumour cells. The products of the cell-free systems were analysed by CMC chromatography and this yielded α/β ratios of 0.79–1.0 for non-thalassaemic cells and 0.13 and 0.17 for HbH cells. The low α/β ratios in HbH blood were not dependent on the concentration of mRNA used.

Thus the decreased production of α globin chains in α thalassaemia is reflected in the chain mRNA associated with polyribosomes. The mRNA in thalassaemic cells, therefore, is either decreased in amount or is abnormal. A similar situation of decreased globin mRNA activity has been reported previously for β thalassaemia.