in its ability to excise thymine dimers. But it does have an abnormally active exonuclease activity which extensively degrades the DNA of mutant cells after ultraviolet irradiation. If this is a result of the mutation to DNA polymerase, it may be that the mutant enzyme is no longer able to synthesize DNA *in vitro*, but retains an ability to degrade DNA *in vivo*, although with altered characteristics.

Trgovčević and Kucan suggest (page 752) that DNA polymerase may be concerned with the degradation of DNA *in vivo*. Irradiation with X-rays or gamma-rays introduces internucleotide chain breaks into the DNA of *E. coli*, and after this the bacterial DNA is degraded quite considerably. They find that the products of degradation induced by gamma-rays are such as to result from an enzyme action which is exercised in *E. coli* only by DNA polymerase. This invites another experiment with the DNA polymerase mutant; it would be interesting to see whether the characteristics of this degradation are affected by the mutation. It seems that definition of which of its many postulated roles DNA polymerase actually fulfils *in vivo* must await a more complete investigation of the precise nature of the polymerase mutation.

ENZYMES

Counting Sites

from our Molecular Biology Correspondent

ENZYMES of which the molecular weights and number of subunits have been in dispute are legion. Particular frustration and bad temper seem to result when the number of subunits determined by molecular weight measurements disagrees with the apparent number of active sites. An interesting case has now been resolved by Mann *et al.* (*Biochemistry*, **9**, 1348; 1970). This is human transferrin, an iron-binding protein present in serum. It is accepted that the molecule has two iron-binding sites with identical characteristics. Reported molecular weights vary between 68,000 and 93,000, and there has also been a choice between the views that the protein consists of a single chain, and that it can be caused to dissociate into two subunits.

The new results give a molecular weight by sedimentation equilibrium of 77,000, which agrees with two out of the range of earlier measurements. The fully denatured, reduced protein examined in guanidine hydrochloride solution has a similar molecular weight, and two other methods involving complete denaturation and dissociation of subunits likewise lead to a value in the 70–80,000 range. It thus appears that transferrin represents an unusual case of a single polypeptide chain possessing two identical functional sites. In terms of precedent this is so unexpected that one is immediately forced to consider whether the protein may nevertheless in reality not consist of two subunits, which are covalently fused. Mann *et al.* prepared tryptic fingerprints, and counted the number of peptides. Subjective as this procedure is apt to be, it still seems that there are altogether too few fragments for the number of trypsin-labile bonds (one for each lysine and arginine) expected on the basis of amino-acid composition. Thus some fifty-four spots can be discerned, whereas seventy-six are expected, and

specific staining for tyrosine, tryptophan and histidine indicated that peptides containing these residues were also present in only half to two-thirds of the expected number. The evidence is circumstantial, for with such a large number of fragments there can be no assurance that all are resolved, but the results are at least consistent with the conjecture hazarded by Mann *et al.* that transferrin may indeed consist of two identical polypeptide segments that have in some way grown together. It may be recalled that covalent association between two subunits of β -galactosidase in wild-type *E. coli* was postulated recently by Goldberg (*J. Mol. Biol.*, **46**, 441; 1969), the covalent link between them being reportedly absent in a mutant.

A protein long the centre of bitter wrangles is myosin. It is now established that it consists of two principal chains and some additional small associated chains. If any lingering doubts remained about the presence of two active sites in myosin, Murphy and Morales (*Biochemistry*, **9**, 1528; 1970) have dispelled them still further: using an ATP derivative with a built-in chromophore that absorbs in the near-ultraviolet, they measure the number of binding sites from the magnitude of the transient absorbance change that accompanies the introduction of the substrate into the protein solution, and find a value of close to 2.

But the greater interest in this work is that the new chromophore contains a thiol group, which, when the pH is raised above 8, will react with a cysteine side chain at or near the active site. The enzymatic activity is then inhibited, and is regenerated only when the disulphide bond which has been formed is broken by addition of excess of an added thiol. Two moles of the chromophore are then again released per mole of myosin. When the light chains of the labelled myosin are stripped off, the label comes with them. This is an agreeable result, for it strongly supports earlier suggestions that a low molecular weight species on the myosin carries or at least closely associates with the ATPase sites.

NUCLEIC ACIDS

Searching for Messengers

from our Cell Biology Correspondent

IDENTIFYING messenger RNAs or their precursors in mammalian cell nuclei is proving as hard as picking out a handful of needles from a haystack. The chief problem is that mammalian nuclei contain a heterogeneous collection of high molecular weight RNAs which have some of the characters of messengers. Apart from their large size and heterogeneity these RNAs undergo rapid turnover in the nucleus; apparently the molecules never migrate to the cytoplasm and never participate in protein synthesis. As a result, in pulse labelling experiments the heterogeneous nuclear RNAs form an effective screen obscuring the detection of the messengers.

A few weeks ago (*Nature*, **226**, 215; 1970) I mentioned the experiments of Darnell and his collaborators which are at least compatible with the idea that in HeLa cells a small fraction of the total heterogeneous nuclear RNAs are precursors of cytoplasmic mRNAs which escape destruction in the nucleus and undergo maturation by cleavage before migrating to the cytoplasm. This hypothesis, attractive if only because it suggests