PROTEIN SYNTHESIS

Doing without Cells

from our Cell Biology Correspondent

THE development of cell free systems derived from eukaryotic cells which support *in vitro* efficient translation of added messenger RNAs is currently a major occupation of many cell biologists. There are, of course, numerous interesting questions which no doubt will be swiftly answered once such systems become available. And to judge from the contents list of the latest issue of the *Proceedings of the National Academy of Sciences*, which is always a reliable barometer of the fashionable areas of research, appreciable progress is being made towards the goal of a mammalian cell free system which is as efficient as that derived from *Escherichia coli*.

Metafora, Terada, Dow, Marks and Bank (*Proc. US Nat. Acad. Sci.*, **69**, 1299; 1972), for example, report that material washed from rabbit reticulocyte ribosomes by 0.5 M KCl, when added to a cell free system derived from Krebs tumour cells, enhances by a factor of some five to ten-fold the efficiency of translation of globin mRNAs in 10S RNA preparations obtained from rabbit, mouse or human reticulocytes. When supplemented by these reticulocyte ribosome washings the Krebs cell free system will incorporate 80 pmol of leucine into globin for every 1 pmol of 10S reticulocyte RNA added. These results mean that this cell free system provides an assay for globin mRNAs which is not only almost as efficient as the frog oocyte system pioneered by Marbaix, Gurdon and their colleagues but also is a good deal more familiar to most biochemists.

The stimulation of translation by the KCl washings of reticulocyte ribosomes is dependent on the addition to the system of mammalian tRNAs and these cannot be replaced by tRNAs from *E. coli*. Presumably, therefore, during the preparation of the Krebs cell system endogenous tRNAs are destroyed, and it seems most likely that the washings from the reticulocyte ribosomes include initiation and/or elongation factors which in the unsupplemented system may be at limiting concentrations. These factors from reticulocytes are not, however, specific for the translation of globin mRNAs, for they also enhance the translation of endogenous messengers and, for example, added encephalomyocarditis virus RNA.

There seems little doubt that in eukaryotic cells, as in bacteria, translation depends on factors which catalyse chain initiation and elongation, and Moon, Redfield and Weissbach (*ibid.*, 1249) describe experiments involving the molecular sieve chromatography of

elongation factors (EF1_A and EF1_B) obtained from calf brain. Factor EF1_A, a multimeric molecule with a molecular weight in excess of 150,000, apparently interacts with GTP to form an EF1_A-GTP complex which in turn will form a ternary complex with amino-acyl-tRNA (Phe-tRNA). During the formation of this ternary complex the elongation factor probably dissociates to yield the lower molecular weight (60,000–80,000) species EF1_B.

Weissbach and his colleagues speculate that as the tRNA binds to the ribosome the aminoacyl-tRNA-EF1_B-GTP complex dissociates and the GTP is hydrolysed to GDP. The liberated EF1_B-GDP, by reacting with GTP, might then be reconverted to EF1_A-GTP. If this scheme proves to be correct—at present it is in many respects speculative—one can conclude that eukaryotic factor EF1_A is functionally equivalent to the two bacterial elongation factors, Tu and Ts.

Levin, Kyner and Acs (*ibid.*, 1234) meanwhile have been investigating the initiation of translation of another set of mRNAs translated in eukaryotic cells—the messengers transcribed off reovirus double stranded genomic RNA. As Levin *et al.* report reovirus mRNAs of all three size classes, which can be made *in vitro* by the endogenous transcriptase present in reovirus cores, form initiation complexes with rat liver 40S and 60S ribosomes and rat liver (35S) Met-tRNA_i after mild treatment with formaldehyde which presumably partially denatures the reovirus RNAs to expose the ribosome binding sites. Furthermore, when puromycin is added, a peptide bond is formed which links methionine to puromycin.

By contrast, rat liver (35S) Met-tRNA_m does not form an initiation complex with reovirus mRNA and rat liver ribosomes until supernatant factors are added, and even in the presence of these factors the formation of the complex can be blocked with fusidic acid at concentrations which do not inhibit formation of initiation complex with (35S) Met-tRNA_i. These data strongly indicate that reovirus mRNAs have AUG initiation codons which are specifically translated by Met-tRNA_i and apparently Levin and his colleagues have used their system to prepare reovirus polypeptides with labelled 5' amino termini.

SOIL SCIENCE

Artificial Conditioners

from a Correspondent

THE first international meeting to be devoted to current work on soil conditioners was held at the University of Ghent (Belgium) from April 17–21. The meeting was the result of renewed interest on soil conditioning, particularly in those parts of the world where the traditional methods of aggregating and stabilizing soils naturally — for example by the use of grass-clover leys — cannot easily be applied.

The first synthetic chemical conditioner, krilium, was introduced in 1952, and some of the early work associated with this substance was reviewed by Professor W. C. Moldenhauer (Iowa State University). Unfortunately the soil conditions necessary for effective action of conditioners were not properly understood in the early days. From time to time excellent results, in

Exercise in Quantum Biochemistry

IN spite of the enormous number of published calculations—usually of a rather simple kind—of charge distributions and other parameters in physiologically active molecules, it is still far from clear that the results lead to realistic explanations of their behaviour. New and Richards, in next Wednesday's *Nature New Biology* (June 14), have attempted a critical test of the ability of molecular orbital theory to represent a purely electrostatic interaction.

New and Richards looked for a system in which the energy of a molecular interaction can be graded through a series of compounds, and in which all energetic contributions other than the coulombic can be disregarded. Such a system is the set of quaternized trimethylammonium compounds, which interact as haptens with antibodies, and were studied in 1946 by Pauling and his

associates. It is assumed that the quaternary ammonium salt interacts electrostatically with a negative centre in the antibody. The interaction energy should then be linearly related to the magnitude of the charge density on the nitrogen, if the entropy of association is the same for all members of the series.

Using the extended Hückel molecular orbital method, New and Richards find that the charge density on the nitrogen varies from a value of about 0.13 when the quaternizing group is *p*-aminobenzyl to about 0.17 when it is α -naphthyl. The calculated results for a series of eight such haptens show a reasonably good correlation with the measured free energies of association. New and Richards regard this as a favourable indication of the usefulness of molecular orbital calculations in accounting for the activities of simple compounds.