and thus should make experiments more reproducible. Although the microbiological state of diets has received some attention in recent years, little has been done about the equally important matter of their formulation.

Protein Turnover

from a Correspondent in Microbiology

THE turnover of endogenous protein is known to be of great importance in the differentiation of micro-organisms. For one thing, it can provide raw material for the synthesis of enzymes and other proteins that will allow growth to continue in a changed environment. Then, by generating amino-acids in non-growing cells, it may provide the energy necessary for cell maintenance. The analyses which have been made of protein turnover in micro-organisms suggest that the rate is low in growing cells and high in those which are not growing. Although there may be an inactive proteolytic system in growing cells, it only becomes activated under certain conditions such as starvation.

Recent results obtained by N. S. Willetts (*Biochem. J.*, **103**, 453 and 462; 1967) have added significantly to understanding of the mechanism of protein turnover. The rates of protein degradation in a strain of *Escherichia coli* were determined by following the release of ^{14}C-valine from prelabelled cells. In deprivation of phosphate, ammonium ion, glucose or leucine, protein degradation proceeded at a common rate of 5 per cent per hour, suggesting that there is a single mechanism induced by any starvation state. The inhibition of protein breakdown by agents such as p-mercuribenzoate supports the hypothesis that breakdown is a consequence of direct enzymatic hydrolysis. Starvation of Mg^{2+} ions, however, produced a lower rate of degradation, 2·9 per cent per hour. Evidence is presented which implicates the Mg^{2+} ion as an essential co-factor for the proteolytic system rather than a prerequisite for rapid amino-acid equilibration. On the nature of the latent state of the proteolytic system in growing cells, Willetts argues that ribosomal peptidases have too restricted a specificity to produce extensive proteolysis and his data give no support for the possibility that NH_4^+ ions, amino-acids or proteins may be inhibitors of the process, as is frequently proposed. Instead, he offers some evidence that the inhibition might be due to an unstable species of RNA.

These investigations also confirm Mandelstam's postulate that individual proteins can vary in their rates of turnover. Willetts has found that proteins synthesized during the regrowth of bacteria long starved of NH_4^+ ions and leucine are very susceptible to degradation on the return of starvation conditions but not during further growth. Furthermore, a protein such as β-galactosidase may differ in its susceptibility to degradation when starved of different nutrients. This phenomenon still awaits an adequate explanation. Finally, some very interesting data are presented on the rates of protein turnover and growth. Experiments in a bactogen continuous culture apparatus have shown that the net rate of protein breakdown is the same (0·6 per cent an hour) during *balanced* growth at any growth rate but is rapid (about 4 per cent an hour) during the transition period following a decrease of growth rate. It is proposed that the rapid rate of degradation at the beginning may be explicable in terms of a small, specific protein fraction with a short half-life. The elucidation of the mechanism that regulates such a preferential turnover of protein during growth offers intriguing possibilities for future experimentation, and will be vital to a fuller understanding of processes of differentiation in living cells.

Cocoonase

from a Correspondent in Cell Biology

COCOONASE is a remarkable proteolytic enzyme produced by silk moths of the genus *Antheraea* for the sole purpose of digesting the protein (sericin) matrix of the cocoon and allowing the emergence of the adult moth at ecdysis. Discovered in 1964 by Kafatos and Williams at Harvard, some of the properties of cocoonase have now been described by Kafatos and collaborators in *J. Biol. Chem.*, **242**, 1477 and 1488; 1967.

Cocoonase is synthesized and accumulated, probably as a proenzyme, in the epidermal cells of the maxillary galeae, vestiges of the functionless mouthparts of the non-feeding adult moth. About two days before ecdysis, highly concentrated droplets of the proenzyme at pH 6·8–7 are extruded on to the surface of the galeae where the proenzyme is converted into active cocoonase and dries down to form a semi-crystalline deposit. Before ecdysis, the enzyme redissolves in a secretion of the labial glands, effectively a buffer solution containing 0·15 M $KHCO_3$ at about pH 8·3. As a result, Kafatos and his colleagues are able to obtain cocoonase simply by dissecting away the cocoon and picking the crystals off the exposed galeae. The natural occurrence of an enzyme in such a pure form is, of course, without precedent.

The unfractionated semi-crystalline cocoonase contains little salt, no carbohydrate, and no free amino-acids. It appears to be homogeneous, but CM cellulose chromatography shows that it contains 80 per cent of active enzyme and 20 per cent of inactive material, probably a mixture of inactive enzyme and a peptide liberated when the proenzyme is activated.

Cocoonase resembles α-chymotrypsin and trypsin in many ways. It has a sedimentation constant of 2·75S, and an estimated molecular weight of 25,000; the corresponding values for α-chymotrypsin and trypsin are 2·56S and 2·5S and 24,000 and 23,000. All three enzymes have maximal activity in the range pH 7–9. All of them are basic proteins. Cocoonase is, however, much the less stable in non-physiological conditions, including low pH, high temperature and in the presence of urea. This may be the result of a comparative deficiency of cross links in the cocoonase molecule, which has only one cystine disulphide bond compared with six in α-chymotrypsin and five in trypsin. Apart from this, the amino-acid composition of cocoonase is very similar to that of trypsin. On the other hand, cocoonase is more stable to auto-digestion at its pH optimum—no doubt an adaptive feature of cocoonase which is subject to prolonged exposure at about pH 8 during ecdysis.

Cocoonase has the characteristic specificity of trypsin, and shows no chymotryptic activity. Heavy metal ions and sulphydryl groups have little inhibitory effect, and experiments with specific organic inhibitors suggest the presence of serine and possibly histidine in the