PROTEINS

Kinds of Kinase

from our Molecular Biology Correspondent

POSTSYNTHETIC modification of proteins is a widespread phenomenon, the best documented form of which is phosphorylation. This in turn has been defined in the most comprehensive detail in relation to its role in certain metabolic pathways, especially glycolysis. For other systems information is more fragmentary, though a little fact has often been made to go a long way by the strenuous exercise of imagination. In sorting out the role of cyclic AMP in regulating glycogen phosphorylation Krebs and his associates have written a new chapter of biochemistry, and this is now being extended to embrace lipolysis. Corbin *et al.* (*J. Biol. Chem.*, **247**, 3736 ; 1972) have isolated the cyclic AMP-dependent kinase from adipose tissue. Its resemblance to the skeletal muscle enzyme is very close. In particular, addition of cyclic AMP brings about dissociation of the protein into its regulatory and catalytic subunits, according to an equilibrium in which the nucleotide binds competitively to the former. The dissociated catalytic subunit then operates as a cyclic AMP-independent kinase, until inhibited by the addition of either its own or another (for example skeletal muscle) regulatory subunit. In terms of enzymatic properties and the stimulation of lipolysis the adipose and muscle kinases are essentially identical, and rat liver kinases of again very similar properties have also been described (Kumon *et al.*, *ibid.*, 3726).

The phosphorylation of all manner of proteins by kinases from different sources has been reported. Eukaryotic ribosomal proteins, for example, are phosphorylated by endogenous kinases. Traugh and Traut (*Biochemistry*, **11**, 2503 ; 1972) find that skeletal muscle kinase is capable of catalysing the phosphorylation of some, though by no means all, of the proteins of *E. coli* ribosomes. The origin of the specificity is obscure: four of the 30S subunit proteins and seven or more of the 50S react in varying degrees, and it is the same proteins that accept a label from isotopic ATP whether they are in the ribosome or in the free state. One protein is most prominently labelled in the large subunit. These findings may or may not have functional significance, for although a cyclic AMP-dependent kinase exists in *E. coli*, it has not been shown that it attacks ribosomes. In eukaryotic ribosomes, the issue seems to be clearer, and it has also been reported that a kinase actually resides in the ribosome itself. Jergil (*Eur. J. Biochem.*, **28**, 546 ; 1972) has been exercised to show that this is a ribosomal protein, in the sense at least that it is not easily dissociated by low salt washing. Working with ribosomes from trout testes, he finds that the kinase is dissociated only at high salt concentration (0.6 M KCl), and that in its characteristics on an ion-exchange column it is distinct from the protamine kinase, which is present in high concentration in these cells. The ribosomal kinase will incorporate phosphoryl groups at serine residues of several ribosomal proteins and of protamines, as well as various histone fractions. There is a mild activation effect by cyclic AMP, of such a low order (10 to 25%), however, that Jergil suggests it might arise from a cyclic AMP-controlled kinase present only as a contaminant. What the substrate might be for which the new kinase lies in wait on the ribosome is an open question.

The modification *in vivo* of histones by phosphorylation and by other means has been recognized for some time, and attempts have been made to relate these effects to the interaction of the histones with the DNA, and thus claim them as transcriptional control mechanisms. Such notions have little basis in experiment, but it seems to be established that substitution of histone side chains is associated with particular stages of the cell cycle. In their most recent report, Louie and Dixon (*Proc. US Nat. Acad. Sci.*, **69**, 1975 ; 1972), working also on trout testes, describe the appearance of a family of ten electrophoretic zones in the arginine-rich histone f2a1 (or histone IV). Nine of these are shown by the incorporation of label to contain modified residues. Both phosphorylation of serines and acetylation of lysines proceed progressively, reach a maximum and then decline. In particular the tritium label initially appears predominantly in the diacetylated species, then in the tri- and tetra-acetylated components, and is only later transferred to the mono- and unacetylated fractions. Louie and Dixon favour the idea that the acetylation and phosphorylation serve to weaken the binding to the DNA, so that the histone is able to search for its correct position, apparently in a kind of annealing process. The deacetylation then liberates new cationic centres, which increase the strength of the electrostatic interaction, and lock the molecule in place. There follow some hypotheses about the specificity of function of different parts of the histone, together with the suggestion that acetylation may drastically change the α-helix-forming propensity of the highly charged N-terminal part of the chain, which one may take or leave according to disposition. Candido and Dixon (*J. Biol. Chem.*, **247**, 3868 ; 1972) have also found that acetylation in trout testes during spermatogenesis is in fact much more extensive than, for instance, in the relatively dormant tissue, calf thymus. In three histone fractions there is acetylation at one or two sites (apart from f2a1, which reacts at four sites). In f2a2 the lysine primarily substituted has been identified, and occurs in a region closely homologous in sequence to a segment of f2a1.

The acetyl and phosphoryl groups of histone f3 of calf thymus have been studied by Marzluff and McCarty (*Biochemistry*, **11**, 2672 ; 1972), who have prepared three cyanogen bromide fragments, taken advantage of the presence of only two methionine residues, favourably spaced in the chain. The largest of those fragments has ninety residues, and contains all the modified side chains, comprising methyllysine, acetyllysine and phosphoserine. Lysine and serine residues in the other fragments are not modified. The authors further show (*ibid.*, 2677) that the modification is incomplete, two lysines being partially acetylated, one partially methylated and two serines partially phosphorylated. This clearly gives a considerable number of permutations for an extensive microheterogeneity.

(In my account of the thermolysin structure (July 14), specificity was wrongly defined. The enzyme hydrolyses the substrate on the imino side of hydrophobic residues.)

REINDEER

Successful AID

ON May 9 this year two reindeer calves were born to two cows which had been artificially inseminated 126 days previously. This is probably the first recorded case of successful artificial insemination of these animals, and the Reindeer Council of the United Kingdom says that mothers and calves are doing well.

The artificial insemination of two cows is the first step in a campaign to propagate reindeer herds in other unlikely parts of the world—as well as to introduce some fresh stock into the herd which has lived in the Cairngorms for the past ten years, without running into difficulties with Britain's quarantine regulations which tend to be fatal to reindeer. The Cairngorm herd has flourished and has even been profitable; by mid-1970 more than eighty reindeer had been bred in Scotland.

There is a tentative plan afoot to introduce reindeer into the Falkland Islands. The chief economic virtue of the animals seems to be that they turn lichen—an otherwise unused natural resource—into meat and leather, without damaging other parts of the natural environment such as trees. But flying reindeer to all the corners of the Earth