labelled with iodo[2-¹⁴C]acetate and digested with trypsin (Thomson, Eveleigh & Miles, 1964). The resulting peptides were purified by ion-exchange and gel-filtration chromatography and by high-voltage electrophoresis. Chymotryptic digests of the major radioactive tryptic peptides were similarly treated. The amino acid sequences of peptides were determined by the 'dansyl'-Edman method (Gray, 1967).

From the sequences obtained, together with amino acid analysis data, it was deduced that the amino acid sequence around the reactive thiol groups of bovine brain creatine phosphotransferase is:

Asx-Tyr-Glx-Phe-Met(Glx,Asx,His,Leu,Gly,Tyr)Ile-Leu-Thr-CMC-Pro-Ser-Asx-Leu-Gly-Thr-Gly-Leu-Arg

where CMC represents *S*-carboxymethylcysteine.

The sequence from isoleucine to arginine is identical with that in the enzymes from rabbit and bovine muscle (Thomson *et al.* 1964, 1968) except that isoleucine substitutes for valine. Arginine phosphotransferase from lobster has a very similar sequence (Thoai, 1968). Further, the sequence from phenylalanine to arginine (except for isoleucine) is likely to be identical with the corresponding sequence in muscle creatine phosphotransferases. The yields of labelled peptides together with kinetic evidence suggests that the chemical environments of the two essential thiol groups of the brain enzyme are identical.

It is confirmed that, despite differences in primary structure in other parts of the molecule, the sequence around the reactive thiol groups of bovine brain creatine phosphotransferase is virtually identical with that of muscle enzyme. This part of the sequence of guanidino phosphotransferases has been substantially conserved throughout their evolution. These enzymes should thus prove invaluable in unravelling evolutionary pathways.

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Study by Mass Spectrometry of Amino Acid Sequences in Peptides containing Histidine

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The choice of a method for the preparation of peptide derivatives suitable for mass spectrometry depends to a certain extent on the composition of the peptides. It is now common practice, besides blocking of the functional groups, to permethylate the peptide bonds. The permethylation of peptides containing histidine, however, gives rise to some problems.

Our procedure is the following. We prepare a protected peptide by dissolution of 0.01 mmol of a peptide in 2 ml of water and subsequently add 0.01 mmol of diethyl pyrocarbonate/acylatable group. The pH is kept constant at 8.0 with a pH-stat for 30 min. After acidification with 1 M-HCl the ethoxycarbonylated component is extracted with ethyl acetate (Kamerling, Heerma & Vliegenthart, 1968). Besides the amino group, the phenolic hydroxyl group of tyrosine, the thiol group of cysteine and the *N*-imidazole group of histidine are also ethoxycarbonylated. The peptide derivative is subsequently permethylated with methyl iodide and methylsulphonyl carbanion as base in dimethyl sulphoxide (Vilkas & Lederer, 1968). The application of this method to histidine-containing peptides leads to the formation of a quaternary base, which remains in the water phase during the extraction with chloroform. We were able to circumvent this problem by cleavage of the imidazole ring of histidine with an excess of diethyl pyrocarbonate. After the reaction of the peptide with the initial amount of diethyl pyrocarbonate an excess of 4 mol of reagent/mol of histidine residue is added in four 1 mol portions at 30 min intervals at constant pH 8.0. This operation splits off the C-2 atom of the imidazole ring as formate, and converts the remaining part of the ring into the 1,2-bis(ethoxycarbonylamino)ethylene group. After acidification the reaction product can be isolated by extraction with ethyl acetate and subsequently be permethylated. The product is now soluble in chloroform and suitable for mass spectrometry.

Histidine itself can also be cleaved by exhaustive treatment with diethyl pyrocarbonate at pH 8.0, yielding 2,4,5-tris(ethoxycarbonylamino)pent-4-enoic acid. This product contains a small percentage of bis(ethoxycarbonyl)histidine. The cleavage reaction is comparable with cleavage of the imidazole ring in histidine methyl ester with benzoyl chloride under Schotten-Baumann conditions (Ashley & Harington, 1930). After permethylation of 2,4,5-tris(ethoxycarbonylamino)pent-4-enoic acid, the mass spectrum showed the presence of exclusively

methyl 2,4,5-tris-(*N*-ethoxycarbonyl-*N*-methyl-amino)pent-4-enoate. We applied the exhaustive treatment with diethyl pyrocarbonate followed by permethylation with success to the following peptides containing histidine: Ala-His; β -Ala-His; His-His; Glu-His-Phe; His-Phe-Arg-Trp-Gly. Ala-His and β -Ala-His could easily be distinguished from each other. Before the reaction of the pentapeptide with diethyl pyrocarbonate it was treated with hydrazine (Shemyakin *et al.* 1967) to convert arginine into ornithine.

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The Effect of Trypsin or Ethylenediaminetetraacetate on the Surface of Cells in Tissue Culture

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Carbohydrate components have frequently been implicated in the properties of the cell surface (see, e.g., Burger, 1969; Hakamori, Teather & Andrews, 1968). One approach has been to study cell-surface materials released when trypsin or EDTA is used to harvest monolayers of cells grown on glass in tissue culture (Kraemer, 1967; Beierle, 1968). We have shown that a permanent line of baby-hamster kidney cells (BHK 21-C13) incorporated radioactivity from [^{14}C]glucosamine over a period of 48 h (5.5-fold increase in cell number and 90% confluent) exclusively into bound hexosamines and sialic acids of the ethanol-precipitable muco-substances. Of the total radioactive muco-substances 20% was in the supernatant after centrifugation of these radioactive cells harvested from glass with 0.1% Difco trypsin (Ca^{2+} and Mg^{2+} present). Similarly 0.5mM-EDTA released 13% of the total radioactive muco-substances.

In determining whether such trypsin and EDTA fractions come from the cell surface it is important to assess increased cell permeability and rupture when harvesting from glass. A sensitive method for doing this is by measuring the [^3H]RNA and [^{14}C]DNA precipitated by trichloroacetic acid from cells grown for 48 h in a medium dual-labelled with [^3H]uridine and [^{14}C]thymidine. Such cells harvested with

0.5mM-EDTA or with tris-buffered saline without Ca^{2+} or Mg^{2+} , lost 7.6 and 13.1% of their cellular RNA respectively, but only 0.7 and 0.3% of their cellular DNA. Compatible with this loss of cellular RNA being due to increased cell permeability, 60% and 76% of the cells were permeable to Trypan Blue (Paul, 1965). Further incubations of the cells in EDTA extracted 63% of the cellular RNA but only 9.2% of the cellular DNA. In contrast cells harvested from glass by trypsin lost 10.4% cellular RNA and 11.4% cellular DNA, consistent with lysis of about 11% of the cells. Less than 1% of the unlysed cells were permeable to Trypan Blue.

These experiments show that the cell-surface components released through trypsin or EDTA harvesting of cells from glass may be heavily contaminated with intracellular material. The importance of Ca^{2+} in membrane permeability has been demonstrated by Lowenstein (1966).

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Effects of Metabolic Inhibitors on Glucagon Release from Isolated Guinea-Pig Islets of Langerhans

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The availability of methods for the isolation of islets of Langerhans from mammalian species in large numbers (Lacy & Kostianovsky, 1967; Howell & Taylor, 1966) has made studies of the metabolism of their constituent cell types possible. The regulation of insulin secretion and of concentrations of some intermediates within the β -cell have already been investigated (Matschinsky & Ellerman, 1968; Montague & Taylor, 1968). In the present studies aspects of the regulation of glucagon secretion from the α -cells of guinea-pig islets were explored by examining the effects of a range of metabolic inhibitors on rates of glucagon release from isolated islets.

Islets of Langerhans were isolated by collagenase digestion of guinea-pig pancreas and groups of ten islets were then incubated for 30 min in a bicarbonate-buffered medium containing glucose (5.5mM) to-