methyl 2,4,5-tris-(*N*-ethoxycarbonyl-*N*-methylamino)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 Ethylenediaminetetra-acetate 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 [1-^{14}C]glucosamine over a period of 48h (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 [H^3]RNA and [^{14}C]DNA precipitated by trichloroacetic acid from cells grown for 48h in a medium dual-labelled with [5-^{3}H]uridine and [2-^{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 30min in a bicarbonate-buffered medium containing glucose (5.5mM) to-