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

By A. ALLEN and CHRISTINE SNOW. (*Department of Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, U.K.*)

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

By J. C. EDWARDS, S. L. HOWELL and K. W. TAYLOR. (*Department of Biochemistry, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, U.K.*)

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-

gether with the various test substances. The glucagon content of the incubation media was then determined by radioimmunoassay. The release of glucagon from the islets in these conditions has been shown to respond to glucose and to amino acids in a similar manner to glucagon release from isolated rat islets and from perfused dog pancreas (Edwards, Howell & Taylor, 1969). Glucagon secretion from guinea-pig islets *in vitro* was found to be inhibited in the presence of low concentrations of fatty acids and of β -hydroxybutyrate. A very small inhibition was also found with high concentrations of glucose (Edwards *et al.* 1969).

More recently the effects of metabolic inhibitors have been studied. Cyanide (1mM), 2,4-dinitrophenol (0.25mM) and iodoacetate (0.4mM) all stimulated the release of glucagon from guinea-pig islets incubated in the presence of glucose alone. Inhibition of release by fatty acids was abolished by 2,4-dinitrophenol but not by iodoacetate.

Release of glucagon therefore is stimulated by a number of cell poisons and agents that disrupt the supply of intracellular energy. It is suggested that, in contradistinction to the release of insulin from the β -cell, expenditure of energy is necessary to retain glucagon within the α -cell.

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Glucose and Glycogen Metabolism in Hypertrophied Denervated Rat Hemidiaphragm

By L. V. TURNER and K. L. MANCHESTER. (*Department of Biochemistry, University College London, Gower Street, London W.C.1, U.K.*)

Section of the phrenic nerve to one half of the rat diaphragm initiates a pronounced, although transient, hypertrophy of the muscle on that side. Feng & Lu (1965), from histological studies, found that the hypertrophy was solely a function of the 'red' fibres of the tissue, the 'white' fibres apparently only being capable of atrophy. Since red fibres and white fibres differ characteristically in their capacity to phosphorylate glucose and to participate in glycogen metabolism (Bär & Blanchaer, 1965), the activities of enzymes associated with these processes were investigated as a function of time after denervation to see if they reflect changing proportions of red cells and white cells in the fibre population.

Expressed per unit weight of tissue, the extractable activity of hexokinase remained largely unchanged for up to 15 days after denervation. During the hypertrophy phase, however, the tissue mass increases by some 30–40%, so that it appears that net synthesis of hexokinase is taking place. The concentration of glycogen in the tissue taken from fed rats was found to fall markedly as a result of denervation: after 3 days the value was 50% of that in the control tissues. Since Buse & Buse (1959) have established that glucose uptake is unaltered during denervation hypertrophy, it seems that glucose entering the cell tends to be metabolized rather than be stored as glycogen. Glycogen phosphorylase activity decreases significantly in the hypertrophied tissue; this decrease is reflected in the higher glycogen contents present in denervated muscle taken from rats starved for 16h before assay. Similarly activities of phosphoglucoisomerase and phosphoglucomutase were both found to have decreased significantly in concentration as soon as 3 days after denervation. These changes in enzyme activities, and especially in the decrease of the phosphorylase/hexokinase activity ratio, are considered to be indicative of a decreasing proportion of glycogenolytic, i.e. white-fibre, metabolism in the hypertrophying tissue.

Activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are found to increase immediately after phrenicectomy, reaching a maximum after 3 days and thereafter declining; this behaviour is compatible with the observed increase in nucleic acid content of the denervated muscle (Manchester & Harris, 1968), and is suggestive of a reorientation of metabolism to direct glucose 6-phosphate towards pentose synthesis.

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Glycerol Metabolism in Perfused Rat Liver

By J. COCHRANE and J. H. OTTAWAY. (*Department of Biochemistry, University Medical School, Teviot Place, Edinburgh EH8 9AG, U.K.*)

Livers from starved female albino rats weighing about 200g were perfused by the Miller technique with haemoglobin-free Ringer solution. The preparations were stable for at least 2h. It was found that glycerol was metabolized at a constant rate by each liver, but that the rates varied from liver to liver for reasons as yet unknown. To achieve a steady perfusate concentration glycerol was added to the medium at the beginning of the perfusion, and from its rate of disappearance a rate of constant infusion of 33% glycerol was calculated that would