APPENDIX

Radioautographic Localization of Labelled Proteins After Incubation of Guinea-Pig Islets of Langerhans with [³H]Tryptophan

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An analysis was made by electron-microscopic radioautography of the distribution of silver grains over storage granules of A₂ and B cells after incubation of isolated guinea-pig islets of Langerhans for 17h in the presence of [³H]tryptophan. A significant concentration of labelled proteins was present in the A₂ cell, but not in the B-cell granules.

As part of a study of glucagon biosynthesis in guinea-pig islets of Langerhans we have investigated the labelling of islet proteins after incubation of the isolated islets *in vitro* for 17h in the presence of [³H]tryptophan (Hellerström *et al.*, 1972, 1974). We report here the intracellular distribution of these labelled proteins after an incubation of this duration as determined by electron-microscopic radioautographs, and in particular the distribution of radioactivity within the storage granules of the A₂ and B cells.

Methods

Isolated guinea-pig islets of Langerhans were incubated in a bicarbonate buffer-saline medium (Gey & Gey, 1936) supplemented by the addition of 3mg of glucose/ml and essential amino acids as described by Eagle (1959) and L-[³H]tryptophan (20 μ Ci/ml), by a procedure described in detail elsewhere (Hellerström *et al.*, 1974). After incubation for 17h at 37°C the medium was removed and the islets were rinsed extensively with incubation medium containing excess of unlabelled L-tryptophan. The islets were then fixed in 3% (w/v) glutaraldehyde (Polysciences Inc., Warrington, Pa., U.S.A.) in 0.1 M-sodium phosphate buffer, post-fixed in osmium tetroxide (Johnson Matthey Ltd., London E.C.7, U.K.) in the same buffer, dehydrated and embedded according to a standard procedure (Lockwood, 1964).

Ultrathin sections were prepared for radioautography by procedures described in detail elsewhere (Howell *et al.*, 1969; Howell & Whitfield, 1973); Ilford L4 emulsion and an exposure period of 4 weeks were used. After development in Kodak D 19 developer and fixation in 20% (w/v) sodium thiosulphate the radioautograms were examined in an AEI EM 6 B electron microscope.

Silver-grain distribution was assessed from prints of areas of tissue selected at random. The relative

volume of the storage granules was assessed with the aid of a grid intersection method (Williams, 1969; Elias *et al.*, 1971) on similar random prints. The observed radioautographic grain distribution was compared with a random distribution by χ^2 analysis.

Results and Discussion

A prolonged period of incubation in the presence of labelled amino acid would be expected to result in labelling of a wide variety of endogenous islet proteins as well as exportable proteins with a more rapid rate of turnover. It was therefore not surprising to find label distributed over a wide variety of cellular structures (Plate 1). It was of particular interest for this study to determine whether there was any significant association of labelled tryptophan-containing proteins with the storage granules of either A₂ or B cells. For this purpose the percentage distribution of 800–1100 grains over A₂ and B granules was assessed from the radioautographs, and was compared with the fractional area of the A₂ and B cell occupied by the storage granules; if the distribution of labelled protein was random, then the percentage of grains present over the granules should not differ significantly. It is clear from Table 1 that the percentage of the total number of grains present that was associated with A₂ cell granules was significantly higher than would have been expected for a random distribution. By contrast the proportion of the grains associated with B-cell granules was significantly less than would be expected from a random distribution (Table 1), suggesting that tryptophan-containing proteins are not an important component of the B granule in the guinea pig.

In view of the association of a tryptophan-containing protein of mol.wt. 9000 with a granule-rich fraction of guinea-pig islets of Langerhans, together with evidence of glucagon-like immunoreactivity of the protein, it seems reasonable to conclude that the tryptophan-containing protein located within the

EXPLANATION OF PLATE I

Electron-microscopic radioautographs of A₂ and B cells of a guinea-pig islet of Langerhans that was incubated for 17 h in the presence of [³H]tryptophan under the conditions described in the text

In the A₂ cell [Plate 1(a) right and Plate 1(b) centre] grains are apparently concentrated over the storage granules, whereas in the B cell [Plate 1(a) left] the concentration of silver grains present is lower and they are not concentrated over the granules. Quantitative analysis of micrographs of this type is presented in Table 1.

Table 1. *Percentage distribution of radioautographic grains over secretion granules in the A₂ and B cells of guinea-pig islets incubated with [³H]tryptophan*

The expected frequency denotes the association between silver grains and storage granules if the distribution of labelled protein were random. Results are means $\pm$ S.E.M., for the numbers of prints analysed given in parentheses. A statistical test was done with the aid of χ^2 analysis.

	Association of silver grains with storage granules in	
	A ₂ cells	B cells
Expected frequency	24.1 $\pm$ 3.0	8.9 $\pm$ 1.7
Observed frequency	38.2 $\pm$ 4	5.6 $\pm$ 1.4
	(11)	(7)
	P < 0.001	P < 0.01

A₂-cell granule may in fact represent the 9000-mol.wt. protein whose characteristics have been described in the accompanying paper (Hellerström *et al.*, 1974).

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