Biochemical Pharmacology, Vol. 21, pp. 695–706. Pergamon Press, 1972. Printed in Great Britain

TRANSPORT AND STORAGE OF 5-HYDROXYTRYPTAMINE IN PANCREATIC β-CELLS

BO HELLMAN, ÅKE LERNMARK, JANOVE SEHLIN and INGE-BERT TÄLJEDAL

Department of Histology, University of Umeå, Umeå, Sweden

(*Received* 17 *June* 1971; *accepted* 20 *September* 1971)

Abstract—To elucidate the role of biogenic amines in insulin secretion, pancreatic islets rich in β-cells were microdissected from obese-hyperglycemic mice and were incubated with ^{14}C-labelled 5-hydroxytryptamine (5-HT). The saturability of uptake and the fact that 5-HT was accumulated to high levels indicated that the β-cells possess a transport system with great capacity for this amine. The initial uptake was not sensitive to glucose or diazoxide.

Efflux of radioactivity from islets preloaded with ^{14}C-labelled 5-HT exhibited complex kinetics suggesting incorporation of the amine into some less mobile compartment of the β-cells. This compartment may be the insulin-containing secretory granules, since homogenization and centrifugation of preloaded islets revealed closely parallel sedimentation profiles for insulin and ^{14}C. The apparent co-sedimentation of insulin and 5-HT probably reflects the ultrastructural organization of the β-cells, as insignificant radioactivities were recovered in the sediments after adding ^{14}C-labelled 5-HT to homogenized islets. Furthermore, gel filtration of insulin on 5-HT-equilibrated Sephadex G-50 did not indicate any great affinity of insulin for the amine.

Neither glucose nor glibenclamide could be shown to mobilize granule-bound 5-HT from intact β-cells. In these experiments insulin release was slow despite a glucose concentration as high as 20 mM. It seems possible that co-storage of insulin and 5-HT reduces the ability of β-granules to undergo normal emiocytosis.

FLUORESCENCE microscopy and autoradiography have established the existence of tryptaminergic mechanisms in the pancreatic islets.[1–7] The distribution of fluorescence in fine cytoplasmic granules suggested that 5-hydroxytryptamine (5-HT) might be stored in the insulin-containing secretory granules of the β-cells.[5] This idea was supported by the electron microscopic demonstration of an intense reaction in the β-granules after treating the islets with glutaraldehyde and potassium dichromate.[6] Since, in addition, 5-HT has been found to modify secretion from the β-cells,[8–11] it seems reasonable to suspect that tryptaminergic mechanisms may play a role in the physiological regulation of insulin release. In the present study we have investigated the transport and storage of ^{14}C-labelled 5-HT in microdissected islets of obese-hyperglycemic mice paying particular attention to the possibility that 5-HT co-exists with insulin in the β-granules.

MATERIALS AND METHODS

Chemicals. Non-radioactive 5-HT creatinine sulphate was obtained from Sigma Chemical Company, St. Louis, Mo., U.S.A., and 5-HT-3′-[^{14}C]creatinine sulphate was from the Radiochemical Centre, Amersham, England. Glibenclamide (*N*-{4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulphonyl}-*N*′-cyclohexylurea) was a gift

from Farbwerke Hoechst A.G., Frankfurt/M., Germany. Diazoxide (7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide) was donated by Schering Corp., Bloomfield, N.J., U.S.A., and reserpine (Serpasil®; 3,4,5-trimethoxybenzoyl methyl reserpate) by Ciba, Basel, Switzerland. Pargylin (*N*-methyl-*N*-2-propynylbenzylamine) was a gift from Abbot Laboratories, Chicago, Ill., U.S.A. Insulin antibodies and [^{125}I]labelled insulin were purchased from the Radiochemical Centre, Amersham, England. Crystalline mouse insulin was prepared by Novo Research Institute, Copenhagen, Denmark. Human serum albumin was from AB Kabi, Stockholm, Sweden, and Sephadex G-50 from Pharmacia Fine Chemicals, Uppsala, Sweden.

Animals and microdissection of islets. Adult mice were obtained from a local stock carrying the gene *ob*. Unless otherwise stated, homozygous obese-hyperglycemic animals were used. Twenty-four hr before killing, seven mice of normal phenotype were intraperitoneally injected with reserpine (5 mg/kg body wt.), and seven controls received a corresponding volume of aqueous vehicle containing 20·6 mg/ml phenylcarbinol. All animals were killed after starving overnight. The pancreas was then quickly excised and immersed in chilled (2°) Krebs–Ringer bicarbonate medium[12] supplemented with 0·3–0·5% human serum albumin and equilibrated with O_2 and CO_2 (95 : 5). In each experiment about 25 fresh pancreatic islets were microdissected free-hand as described by Hellerström.[13]

Incubation, freeze-drying and weighing of islets. Studies of 5-HT uptake were started by pre-incubating islets at 37° for 30–45 min in a non-radioactive medium of the same basic composition as that used during microdissection. Additives to this medium are described in Figures and Tables. After preincubation, batches of 3 islets were transferred to vials containing 200 μl of basal medium supplemented with ^{14}C-labelled 5-HT and other additives as required. If not otherwise stated, the concentration of 5-HT was 5·0 mM (2·0 mc/m-mole). After incubation at 37°, the islets were washed for 4 × 1 min in non-radioactive and ice-cold Krebs–Ringer bicarbonate medium supplemented with 0·3% human serum albumin. Previous studies[14] have revealed that this washing procedure removes about 80 per cent of the extracellular and contaminating label without greatly affecting that in the intracellular space. Table 1 shows the effect of washing on the radioactivity of islets preloaded with 5-HT. The amount of label washed off was independent of the islet content of 5-HT and agrees with that previously noted for islets equilibrated with radioactive sucrose.[14] Control experiments were also performed to examine the extent to which islet radioactivity represented labelled 5-HT. Dansylation and two-dimensional thin-layer chromatography[15] revealed that 5-HT accounted for more than 95 per cent of the islet radioactivity during incubations for up to 2 hr. After washing, the islets were freeze-dried overnight (−40°, 0·001 mm Hg), weighed on a quartz-fibre balance and dissolved by incubation for 45 min in 100 μl Hyamine at room temperature.

In efflux studies, islets were first loaded by incubation for 45 min at 37° in basal medium supplemented with 5·0 mM ^{14}C-labelled 5-HT (2·0 mc/m-mole). After subsequent incubation at 37° in non-radioactive media containing additives as required, the islets were freeze-dried, weighed, and dissolved in Hyamine.

Subcellular distribution of 5-HT. To study the subcellular localization of amine, about 25 islets were incubated for 45 min with 5 mM ^{14}C-labelled 5-HT (2·0 mc/m-mole) and were subsequently homogenized in 250 μl of 0·32 M sucrose adjusted to pH 6 by equilibration with ambient air. Samples were taken for determination of

radioactivity, protein and insulin. Of the remaining homogenate, 200 μl was diluted 1 : 1 with 0·32 M sucrose (pH 6), and differential centrifugation was performed in a Sorvall refrigerated centrifuge at 400 *g* and 5000 *g* for 10 min, and in a Spinco L50 ultracentrifuge at 28,000 *g* for 10 min and at 110,000 *g* for 30 min. The given gravitational forces refer to the middle of small polyethylene microtubes (height 45 mm), which had a capacity of 400 μl and which were placed in perspex inserts shaped to fit the ordinary rotors HB-4 and SW 39. Each fraction was analyzed for radioactivity, protein and insulin.

TABLE 1. EFFECT OF WASHING ON THE ISLET CONTENT OF 5-HT

Time for preloading (min)	Islet content of 5-HT (m-moles/kg dry islet)		
	Unwashed islets	Washed islets	Unwashed minus washed
15	56·7 ± 3·0	39·8 ± 0·8	16·9 ± 3·2
60	117·8 ± 14·6	102·0 ± 12·3	15·8 ± 6·9

After preloading with ^{14}C-labelled 5-HT for 15 min or 60 min, islets were washed for 4 × 1 min in non-radioactive and ice-cold Krebs–Ringer medium. The radioactivities remaining after washing were translated to the equivalent amounts of 5-HT and are given as mean values ± S.E.M. for six experiments.

Gel filtration. The affinity of insulin for 5-HT was investigated by gel filtration at 4°.[16] A column (diameter 0·9 cm, height 60 cm) was packed with Sephadex G-50 and equilibrated with 0·01 M veronal buffer (pH 7·4) supplemented with 5 mg/ml human serum albumin and 0·01 mM ^{14}C-labelled 5-HT giving a radioactivity of 90,000 counts/min/ml. Crystalline ox insulin (400 μg) was applied to the column and was eluted with the radioactive buffer at a rate of 50 μl/min. Fractions of 1 ml were collected for analysis of their radioactivity and insulin content.

Counting of radioactivity. Counting of radioactivity was carried out in a liquid scintillation spectrometer, each vial containing 10 ml scintillation liquid (5 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-2-[5-phenyloxazolyl]benzene in 1 l. of toluene). After correction for blanks and background, the observed counts/min values were translated into m-moles of 5-HT by comparison with external standards counted in parallel with the Hyamine-dissolved islets or subcellular fractions. These standards consisted of 5 μl incubation medium dissolved in 100 μl of Hyamine.

Insulin and protein assays. Insulin was radio-immunologically assayed,[17] using crystalline mouse insulin as reference. Protein was determined according to Lowry *et al.*,[18] using human serum albumin as reference.

Statistical evaluation of results. In experiments designed to test the effects of different substances on 5-HT uptake or efflux, islets from a single animal were simultaneously incubated in test and control media. The statistical probability that any effect of a test substance was due to chance was estimated from the differences between paired observations in a series of identical but separate experiments.

RESULTS

Concentration dependence of 5-HT uptake. As shown in Fig. 1, uptake of 5-HT was clearly saturable. From a double-reciprocal plot of these data the apparent K_m was estimated as about 10 mM and V_{max} as about 1 mole/kg dry islet per hr.

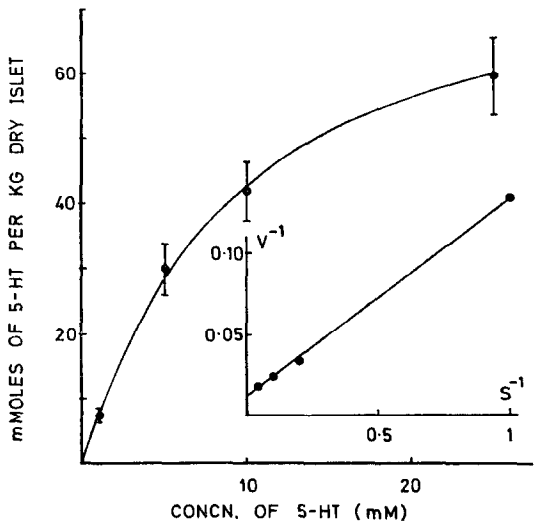

FIG. 1. Concentration dependence of 5-HT uptake by pancreatic β-cells. After preincubation for 30 min with 3 mM glucose, islets were incubated for 5 min with 1–25 mM ^{14}C-labelled 5-HT (0·4–10·0 mc/m-mole) in glucose-free media. The main diagram is an explicit plot of the amounts of 5-HT taken up during these 5 min (mean values ± S.E.M. for four experiments), while the inset is a double-reciprocal representation of the same data.

Time course of 5-HT uptake and efflux. Islets incubated with 5 mM ^{14}C-labelled 5-HT exhibited an accumulation of radioactivity that continued for more than 2 hr (Fig. 2). The presence of the monoamine oxidase inhibitor Pargylin in the medium considerably enhanced this accumulation. After 10 min of incubation in Pargylin-free medium, the radioactivity of washed islets was equivalent to about 20 m-moles of 5-HT/kg dry islet, whereas the corresponding value after incubation with 1 mM Pargylin was 60 m-moles/kg dry islet. Since this effect of Pargylin suggests a slower exit of 5-HT than of 5-HT metabolites, a study was also carried out on the efflux of radioactivity from islets that had been preloaded with ^{14}C-labelled 5-HT. It is evident from Fig. 3 that there was rapid efflux of label from the preloaded β-cells, which shows that the data of Fig. 2 represents net uptake of 5-HT rather than 5-HT entry. It is also apparent from Fig. 3 that the exit of radioactivity did not follow any simple kinetics. The efflux curve appeared to approach an asymptote well above the abscissa, suggesting that part of the 5-HT taken up was incorporated into some less mobile compartment of the β-cells.

The above effect of Pargylin on 5-HT net uptake is consistent with the results of five experiments showing that Pargylin inhibited the efflux of label from the islets preloaded by incubation with 5 mM ^{14}C-labelled 5-HT for 45 min. After subsequent incubation in amine-free medium for 60 min, the radioactivity of control islets was equivalent to 20·9 ± 5·2 m-moles of 5-HT/kg dry islet (mean value ± S.E.M.). When

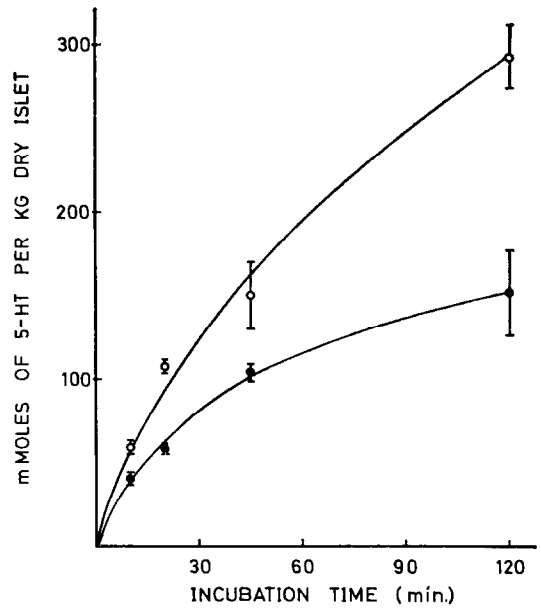

FIG. 2. Uptake of 5-HT with time. After preincubation as in Fig. 1, islets were incubated with 5 mM ^{14}C-labelled 5-HT in glucose-free media containing (○) or lacking (●) 1 mM Pargylin. Mean values ± S.E.M. for three to five experiments.

1 mM Pargylin was present in the final incubation medium, the islets retained a radioactivity corresponding to 35·0 ± 7·7 m-moles of 5-HT/kg dry islet (mean value ± S.E.M.). From the difference between the two groups, 14·2 ± 4·1 m-moles/kg dry islet, it can be estimated that the inhibitory action of Pargylin was significant at the 5 per cent level.

Subcellular distribution of 5-HT and insulin. After incubation for 45 min with 5 mM ^{14}C-labelled 5-HT, islets were homogenized and subjected to differential centrifugation. As much as 40–50 per cent of the total radioactivity in the homogenate was found to sediment with the different particulate fractions. Among these, the 28,000 *g* fraction exhibited the greatest amount of label and the highest specific activity as expressed per unit protein (Fig. 4, Table 2). This fraction was also characterized by the greatest content of insulin and by the highest insulin/protein ratio. With the exception that little insulin was recovered in the postmicrosomal supernatant, the sedimentation profiles for 5-HT and insulin were strikingly similar, suggesting an intimate association between the amine and the protein hormone. The question arose as to whether this association might result from homogenization rather than being a true reflection of β-cell organization. A control experiment was therefore performed in which the ^{14}C-labelled 5-HT (1·46 nmoles; 56 mc/m-mole) was mixed with the homogenate (200 μl) after the disintegration of the islets. Upon subsequent fractionation as much as 85·9 per cent of the radioactivity was recovered in the supernatant. The percentile distribution of label to the 400 *g*, 5000 *g*, 28,000 *g* and 110,000 *g* fractions was only 1·6, 2·1, 2·6 and 1·6. The latter values are explained by the water content of the particulate fractions. A series of similar experiments with ^{3}H-labelled

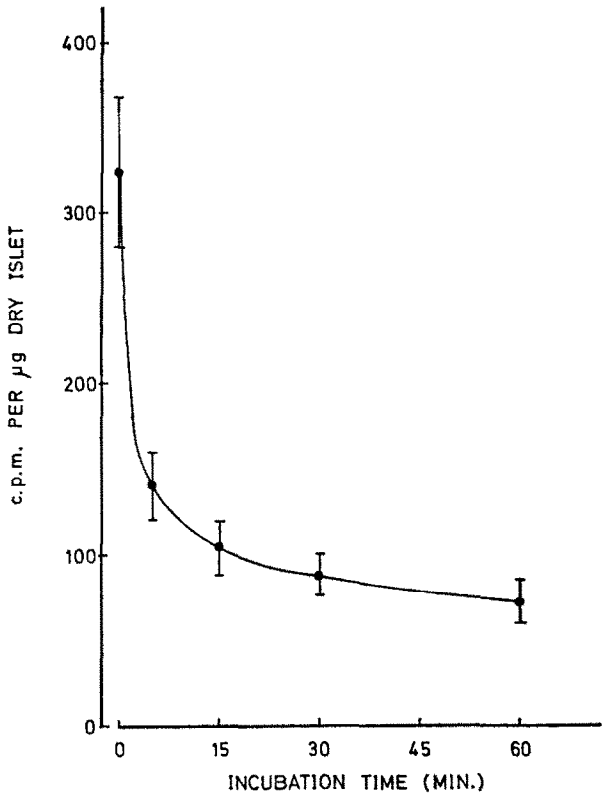

FIG. 3. Efflux of label from islets preloaded with 5-HT. After incubation for 60 min with 5 mM ^{14}C-labelled 5-HT, islets were incubated for different periods of time in amine-free medium. The radioactivity retained by islet cells during the final incubation are given as mean values ± S.E.M. for four to twelve experiments.

TABLE 2. SUBCELLULAR DISTRIBUTION OF 5-HT AND INSULIN IN PANCREATIC β-CELLS

Fraction	Protein (μg)	Insulin (μg)	Insulin (% of protein)	5-HT (nmoles)	5-HT (m-moles/kg protein)
Total homogenate	302·0 ± 51·6	46·1 ± 5·3	15·5 ± 1·2	14·70 ± 1·78	52·0 ± 13·6
400 *g*	52·0 ± 7·1	2·2 ± 0·3	4·3 ± 0·3	0·77 ± 0·05	15·3 ± 2·4
5000 *g*	60·9 ± 10·8	8·1 ± 0·8	13·6 ± 1·3	1·66 ± 0·27	28·2 ± 5·5
28,000 *g*	58·4 ± 14·1	16·9 ± 2·8	29·6 ± 2·4	2·99 ± 0·52	55·0 ± 13·5
110,000 *g*	32·4 ± 2·6	5·3 ± 0·5	16·6 ± 2·4	0·79 ± 0·18	25·4 ± 7·9
Supernatant	85·1 ± 21·0	1·5 ± 0·4	1·8 ± 0·3	8·18 ± 0·91	102·9 ± 19·3
Gross recovery	288·9 ± 54·6 (95·2 ± 1·7%)	34·0 ± 4·2 (73·6 ± 0·7%)		14·39 ± 1·79 (98·9 ± 12·2%)	

Islets were incubated and fractionated as in Fig. 4. The figures represent mean values ± S.E.M. for three separate experiments. Gross recoveries are given in absolute values as well as in percentages as compared to the total homogenate.

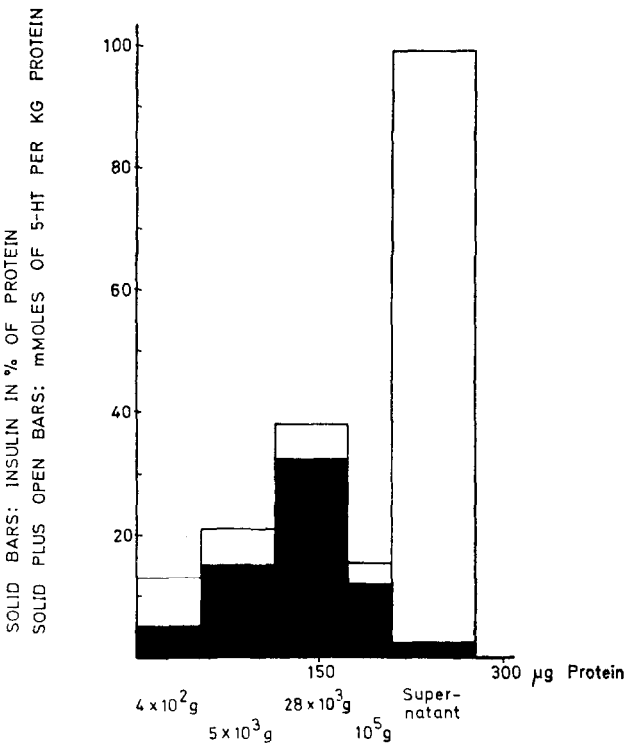

FIG. 4. Result of a representative fractionation experiment showing the subcellular distribution of insulin and 5-HT in islets preloaded by incubation for 45 min with 5 mM of the ^{14}C-labelled 5-HT. The specific activities of insulin and ^{14}C-labelled 5-HT are given for fractions sedimenting at 400 *g* for 10 min, 5000 *g* for 10 min, 28,000 *g* for 10 min, and 110,000 *g* for 30 min, as well as for the residual supernatant. The amounts of protein in the different fractions are indicated on the abscissa. In this particular experiment the total recoveries of insulin, 5-HT and protein were 74, 93 and 94 per cent as compared to the unfractionated homogenate. The latter contained 0·17 kg of insulin and 42·3 m-moles of 5-HT/kg protein.

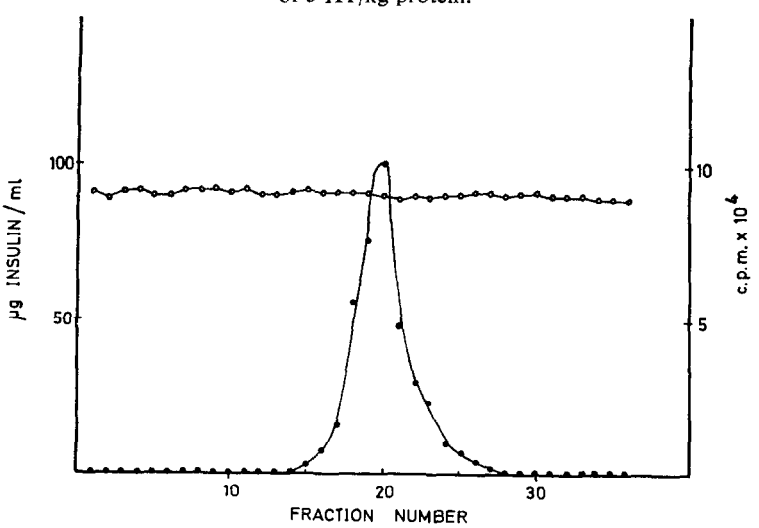

FIG. 5. Chromatogram of ox insulin (●——●) and 5-HT (○——○) in Sephadex G-50 effluents (1-ml samples) after equilibrating the column with ^{14}C-labelled 5-HT.

sucrose revealed that 2·1 ± 0·1% (mean value ± S.E.M. for 16 observations) of the radioactivity in the supernatant was trapped in each of the sedimentable fractions.

Binding of 5-HT to pure insulin. Co-sedimentation of 5-HT and insulin could theoretically be due to a great affinity of insulin for the amine. This possibility was tested by column chromatography of pure ox insulin on Sephadex G-50 equilibrated with ^{14}C-labelled 5-HT. As shown in Fig. 5, insulin was eluted as one distinct peak, whereas 5-HT was uniformly distributed over all fractions, there being no indication of 5-HT binding to insulin.

Stability of the binding of 5-HT to subcellular particles. Seven experiments were performed to test the stability of 5-HT binding to the particulate elements of the 28,000 *g* fraction. After incubating islets with ^{14}C-labelled 5-HT for 45 min, the 28,000 *g* fraction was isolated and suspended in 0·32 M sucrose. When this suspension was incubated for 15 min at 37°, subsequent re-centrifugation at 28,000 *g* revealed that almost all of its radioactivity had become soluble. A significant amount of particle-bound 5-HT was obtained only after incubation at pH 6, when 10·43 ± 2·30% ($P < 0·01$) of the radioactivity remained sedimentable. The corresponding values recorded after incubating the 28,000 *g* fraction at pH 5 and pH 7·4 were 1·89 ± 3·39 and 0·57 ± 2·87 per cent.

TABLE 3. EFFECTS OF GLUCOSE, GLIBENCLAMIDE OR DIAZOXIDE ON 5-HT EFFLUX

Incubation time (min)	No. expts.	Islet content of 5-HT (m-moles/kg dry islet)			
		Control	Glucose minus Control	HB 419 minus Control	Diazoxide minus Contro
10	7	42·6 ± 3·8	2·7 ± 1·1*	1·7 ± 1·4	2·6 ± 2·1
60	7	18·6 ± 1·7	4·6 ± 1·7*	0·6 ± 1·7	11·5 ± 5·4
60 (1 mM Pargylin present)	6	26·2 ± 3·0	5·8 ± 1·9*	4·4 ± 3·7	4·4 ± 1·5*

After preloading with ^{14}C-labelled 5-HT, islets were incubated for the given periods of time in non-radioactive media containing 20 mM glucose, 50 μg/ml glibenclamide, or 125 μg/ml diazoxide, as well as in a control medium lacking these substances. In one series of experiments all incubation media also contained 1 mM Pargylin. The radioactivities remaining after incubation were translated to the equivalent amounts of 5-HT and are given as mean values ± S.E.M. for the listed numbers of experiments. Detailed results are given for control incubation, while the effects of test substances are presented as the difference versus controls over the series of paired observations.

* $P < 0·05$.

Effects of some modifiers of insulin release on 5-HT uptake and efflux. The intimate association of 5-HT with a particle fraction rich in insulin prompted a study of the relation between 5-HT transport and insulin release. Neither glucose nor diazoxide, an insulin release inhibitor, affected the initial uptake of 5-HT. The radioactivity of islets incubated for 5 min with 20 mM glucose was equivalent to 24·8 ± 1·0 m-moles of 5-HT/kg dry islet (mean value ± S.E.M. for six experiments). The corresponding value for islets incubated with 125 μg/ml diazoxide was 21·2 ± 2·0. These values are not significantly different from 25·7 ± 4·2, which was the result obtained for parallel

control islets incubated with 5 mM 5-HT only. In the same series of experiments, islets were also incubated in sodium-deficient medium. Substituting choline for sodium resulted in a 5-min uptake of 20·6 ± 2·2 m-moles of 5-HT/kg dry islet (mean value ± S.E.M. for six experiments), which is not significantly different from the control value.

Although glucose and diazoxide could not be shown to influence the uptake of 5-HT, both substances affected the efflux of label from islets preloaded with 5-HT (Table 3). A tendency to inhibited efflux was apparent after both 10 and 60 min of incubation. This tendency was reproduced and was shown to be statistically significant when preloading was performed in the presence of 1 mM Pargylin. Glibenclamide, a potent insulin secretagogue, failed to affect the efflux of 5-HT significantly.

TABLE 4. INSULIN RELEASE IN RELATION TO THE RELEASE OF RADIOACTIVITY FROM ISLETS PRELOADED WITH ^{14}C-LABELLED 5-HT

	3 mM glucose	3 mM glucose (50 μg/ml HB 419)	20 mM glucose
Insulin release (ng/μg dry islet/hr)	0·83 ± 0·20	1·28 ± 0·11*	2·87 ± 0·28†
"5-HT" release (m-moles/kg dry islet/hr)	4·10 ± 1·05	5·88 ± 1·18	4·86 ± 1·44

After preloading with ^{14}C-labelled 5-HT, islets were incubated for 60 min in non-radioactive medium containing 3 mM glucose. In each experiment some islets were then taken for analysis of their radioactivity. The remaining islets were incubated for a further period of 60 min, using non-radioactive media containing 3 mM glucose, 3 mM glucose plus 50 μg/ml glibenclamide, or 20 mM glucose. The amounts of insulin released during this late incubation period were measured in addition to the final radioactivity of islets. By comparison with the islets incubated for only the first 60-min period, the radioactivity released during the final 60 min was calculated and translated to the equivalent amount of 5-HT ("5-HT" release). Results are given as mean values ± S.E.M. for seven experiments. From the paired observations the levels of significance vs. 3 mM glucose were calculated:

* $P < 0·02$, † $P < 0·005$.

Experiments were also carried out to test whether the radioactivity remaining in the β-cells after 60 min of washing (cf. Fig. 3) could be mobilized by subsequent stimulation of insulin release. As shown in Table 4, the release of radioactivity during further incubation for 60 min was as slow as would be expected from the efflux curve in Fig. 3. Neither glibenclamide nor a high concentration of glucose affected the release of radioactivity during this late incubation period, although both secretagogues significantly stimulated the release of insulin from the same islets.

Effect of reserpine on 5-HT uptake. In vivo treatment of lean mice with reserpine did not prevent the accumulation of 5-HT in their isolated islets. After 45 min of incubation with 5 mM ^{14}C-labelled 5-HT, the radioactivity of islets from seven reserpine-injected animals was equivalent to 91·3 ± 4·6 m-moles of 5-HT/kg dry islet (mean value ± S.E.M.), the corresponding value for vehicle-injected controls being 90·6 ± 2·7. A comparison of these results with Fig. 2 reveals that the uptake of 5-HT was the same in the islets of lean mice as in those of obese-hyperglycemic animals.

DISCUSSION

The ability to concentrate and store monoamines, either naturally or after the administration of precursors, seems to be widely distributed among endocrine cells.[19,20] The physiological meaning of this is obscure, but autoradiographic and cytochemical studies at the ultrastructural level suggest a role of monoamines in the storage of polypeptide hormones.[4,6,21,22] This idea is supported by the present *in vitro* experiments, which clearly demonstrate that the pancreatic β-cells are able to concentrate 5-HT to high levels and to incorporate the amine into a subcellular fraction characterized by an exceptionally large content of insulin per unit of protein. In conjunction with the electron microscopic data of Jaim-Etcheverry and Zieher[6] and Ericson and Ekholm,[4] the sedimentation profile after incubation with ^{14}C-labelled 5-HT suggests that the amine was incorporated into the insulin-containing secretory granules of the β-cells. Four additional observations lend further support to this interpretation. First, the subcellular distribution of label was coherent with the efflux curve showing a considerable retention of radioactivity after efflux for 60 min. Second, the incorporation of label into subcellular particles was obviously a characteristic of the living β-cell, there being no specific labelling of the insulin-rich fraction after the addition of ^{14}C-labelled 5-HT to homogenized islets. Third, pure insulin did not bind significant amounts of 5-HT during gel filtration, which also indicates that the association between ^{14}C and insulin-rich particles resulted from some biosynthetic activity in the β-cells. Fourth, the release of label from the insulin-rich subcellular fraction was pH dependent in the same way as has previously been noted for the stability of β-granules.[23] Nevertheless, it should be noted that the present evidence is circumstantial and does not provide ultimate proof for the storage of 5-HT in β-granules. The 28,000 *g* fraction was probably a mixture of different subcellular particles, whereas final evidence would seem to require the preparation of a truly pure granule fraction. The minute amount of mammalian islet tissue available makes such purification difficult,[23–25] which explains that a useful procedure has yet to be developed.

It follows from an incorporation of 5-HT into β-granules that the transport of label in intact islets must have rather complicated kinetics. It seems reasonable to assume, however, that the uptake during the first few minutes of incubation predominantly reflected properties of a transport system in the β-cell plasma membrane. In contrast, the amount of label retained by preloaded islets after 60 min of incubation in 5-HT-free medium is likely to depend essentially on the incorporation of label into β-granules. In support of this distinction, the concentration dependence of the 5-HT uptake during the first 5 min was described by a straight line upon double-reciprocal plotting of the data. The lack of significant effect of glucose or diazoxide on the initial uptake of 5-HT can therefore be interpreted as indicating that the system for amine transport across the β-cell membrane is not sensitive to these substances. The energy involved in establishing a high distribution ratio of 5-HT does not seem to derive exclusively from the sodium pump, since the 5-min uptake was not inhibited by preincubation and incubation in sodium-deficient medium. Neither does the presumably membrane-located transport system appear to be sensitive to reserpine, since treating the animals with this drug had no effect on the concentrative ability of the isolated islets.

Both glucose and diazoxide probably inhibited the efflux of label from islets preloaded with 5-HT in the presence of Pargylin. Since glucose and diazoxide have opposite effects on insulin release, it is difficult to explain these results by a common

TABLE 1 EFFECT OF CCl_4 ON HEME COMPONENTS AND METABOLIC ACTIVITY IN LIVER MICROSOMES FROM 3-MC-TREATED RATS

Time after treatment (hr)		EM-*N*-Demethyl. (nmoles HCHO formed mg protein/min)	Benzpyrene Hydroxylation (nmoles/mg protein/min)	Cyt. P-450 (Δ O.D. 450–490) (mμ/mg protein)	Cyt. P-420 (nmoles/mg protein)	Cyt. b_5 (nmoles/mg protein)	Total heme (nmoles/mg protein)	Total heme minus [Cyt. P-420 + cyt. b_5] (nmoles/mg protein)
3	Control	6·04 ± 0·92	0·393 ± 0·0424	0·065 ± 0·004	< 0·025	0·316 ± 0·032	1·35 ± 0·064	1·03 ± 0·056
	CCl_4	4·16 ± 0·16*	0·128 ± 0·0178*	0·031 ± 0·0014*	< 0·025	0·259 ± 0·0061	0·889 ± 0·027	0·553 ± 0·024*
	Per cent of control	68·8	32·6	47·6		82·0		53·5
	3-MC	7·52 ± 0·44	1·91 ± 0·10	0·162 ± 0·0036	< 0·025	0·391 ± 0·0056	2·25 ± 0·040	1·85 ± 0·13
	3-MC + CCl_4	6·86 ± 0·32	1·89 ± 0·054	0·110 ± 0·0042*	< 0·025	0·431 ± 0·010	1·86 ± 0·027	1·43 ± 0·022*
	Per cent of 3-MC	91·4	99·0	68·0		110		77·3
14	Control	6·10 ± 0·88	0·196 ± 0·036	0·073 ± 0·008	< 0·025	0·394 ± 0·037	1·39 ± 0·11	1·00 ± 0·084
	CCl_4	1·30 ± 0·28*	0·0395 ± 0·0055*	0·0167 ± 0·0024*	0·102 ± 0·0024	0·179 ± 0·013*	0·537 ± 0·033	0·255 ± 0·019*
	Per cent of control	21·3	20·1	22·8		45·5		25·5
	3-MC	7·75 ± 0·24	2·50 ± 0·058	0·170 ± 0·0067	< 0·025	0·632 ± 0·015	2·30 ± 0·074	1·67 ± 0·060
	3-MC + CCl_4	4·34 ± 0·12*	1·59 ± 0·202*	0·059 ± 0·0060*	< 0·025	0·394 ± 0·011*	1·03 ± 0·040	0·635 ± 0·029*
	Per cent of 3- MC	56·0	63·5	35·6		62·5		38·0
24	Control	7·68 ± 1·4	0·290 ± 0·037	0·084 ± 0·0051	< 0·025	0·416 ± 0·014	1·43 ± 0·05	1·01 ± 0·054
	CCl_4	0·91 ± 0·24*	0·0509 ± 0·0157*	0·0192 ± 0·0028*	0·278 ± 0·030	0·153 ± 0·016*	0·668 ± 0·088	0·237 ± 0·044*
	Per cent of control	11·8	17·6	24·0		36·8		23·5
	3-MC	8·39 ± 0·29	2·20 ± 0·078	0·173 ± 0·0058	< 0·025	0·602 ± 0·017	2·39 ± 0·065	1·78 ± 0·063
	3-MC + CCl_4	3·34 ± 0·17*	0·877 ± 0·085*	0·0439 ± 0·0052*	< 0·025	0·316 ± 0·012*	0·863 ± 0·12	0·547 ± 0·055*
	Per cent of 3-MC	40·0	40·0	25·0		52·5		30·7

* Values from the CCl_4-treated animals were significantly different from those of the corresponding controls ($P < 0·01$). The results are the mean of four animals ± S. E.

mechanism. Whereas inhibited efflux in the presence of glucose could reflect an enhanced granule neoformation and/or maturation,[26] it is perhaps possible to attribute the effect of diazoxide to its alleged stabilizing action on plasma membranes.[27]

A pronounced effect on 5-HT uptake was exhibited by Pargylin, an inhibitor of monoamine oxidase. Similar observations have been made with the histochemical fluorescence technique, and the use of tetrazolium salt has confirmed the presence of monoamine oxidase in the β-cells.[7] Since the present technique does not discriminate between 5-HT and its intracellular degradation products, it is natural to conclude either that the efflux of such products was more rapid than that of 5-HT, or that Pargylin stimulated the entry of 5-HT as well. At present, the former conclusion is favoured because a stimulating action of Pargylin on amine entry has to our knowledge not been described. In addition, it has been reported that metabolites of 5-HT are more easily washed out from different mouse tissues than is 5-HT.[28] The latter observation is consistent with our finding that islets preloaded with ^{14}C-labelled 5-HT and subsequently incubated with Pargylin for 60 min in non-radioactive medium retained significantly more label as compared to control islets incubated in the absence of monoamine oxidase inhibitor.

It has previously been reported that 5-HT is a potent inhibitor of insulin release, both when added to the medium[8,10] and when arising through intracellular decarboxylation of 5-hydroxytryptophan.[8,11] In accordance with these reports, the islets preloaded with 5-HT released only 2·87 ng of insulin/kg dry islet per hr when stimulated with 20 mM glucose. Under normal conditions this concentration of glucose stimulates the islets of obese-hyperglycemic mice to release insulin several times as rapidly.[11,29] The slow rate of insulin release after preloading with ^{14}C-labelled 5-HT may have contributed to our failure to demonstrate a correlation between this process and the mobilization of granule-bound radioactivity.

The intimate association of 5-HT with the β-granules points to this structure as a possible site for interference of the amine with insulin release. Irrespective of whether the latter process involves intracellular dissolution of the granules[26,30] or their extrusion by emiocytosis,[31] it seems plausible that insulin release should depend on the physico-chemical state of the granule sac and its contents. That this state could be profoundly altered by biogenic amines is suggested by the surprisingly large amounts of 5-HT or its metabolites that were observed in the 28,000 *g* fraction. If the molecular weight of mouse insulin is taken to be 6000, the radioactivity of this fraction was equivalent to 1·12 ± 0·27 moles of 5-HT/mole of insulin (mean value ± S.E.M. for three experiments).

Acknowledgements—This work was supported by the Swedish Medical Research Council (12x-562; 12x-2288) and M. Bergwall Foundation. The skilful technical assistance of Mrs. Marlén Carlson, Miss Karin Janze and Miss Gerd Larsson is also gratefully acknowledged.

REFERENCES

1. L. CEGRELL, B. FALCK and B. HELLMAN, in *The Structure and Metabolism of the Pancreatic Islets* (Eds. S. E. BROLIN, B. HELLMAN and H. KNUTSON) p. 429. Pergamon Press, Oxford (1964).
2. L. CEGRELL, B. FALCK and A.-M. ROSENGREN, *Life Sci.* **6,** 2483 (1967).
3. M. RITZÉN, L. HAMMARSTRÖM and S. ULLBERG, *Biochem. Pharmac.* **14,** 313 (1965).
4. L. E. ERICSON and R. EKHOLM, *J. Ultrastruct. Res.* in press.
5. B. FALCK and B. HELLMAN, *Acta endocr.* (Kbh.) **45,** 133 (1964).

6. G. JAIM-ETCHEVERRY and L. M. ZIEHER, *Endocrinology* **83,** 917 (1968).
7. H. TJÄLVE, *Acta Physiol. Scand. Suppl.* **360,** 8 (1971).
8. H. TJÄLVE, *Acta Physiol. Scand. Suppl.* **360,** 87 (1971).
9. M. TELIB, S. RAPTIS, K. E. SCHRÖDER and E. F. PFEIFFER, *Diabetologia* **4,** 253 (1968).
10. J. M. FELDMAN and H. E. LEBOVITZ, *Endocrinology* **86,** 66 (1970).
11. Å. LERNMARK, *Horm. Metab. Res.* **3,** 305 (1971).
12. W. W. UMBREIT, R. H. BURRIS and J. P. STAUFFER, *Manometric Techniques*, Burgess, Minneapolis (1964).
13. C. HELLERSTRÖM, *Acta endocr.* (Kbh) **45,** 122 (1964).
14. B. HELLMAN, J. SEHLIN and I.-B. TÄLJEDAL, *Diabetologia* **7,** 256 (1971).
15. V. NEUHOFF and M. WEISE, *Arzneimittel-Forsch.* **20,** 368 (1970).
16. J. P. HUMMEL and W. J. DREYER, *Biochim. biophys. Acta* **63,** 530 (1962).
17. C. N. HALES and P. J. RANDLE, *Biochem. J.* **88,** 137 (1963).
18. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
19. B. FALCK and C. OWMAN, *Adv. Pharmac.* **6,** 211 (1968).
20. A. G. E. PEARSE, *Proc. Roy. Soc.* **B 170,** 71 (1968).
21. L. E. ERICSON, *J. Ultrastruct. Res.* **31,** 162 (1970).
22. G. JAIM-ETCHEVERRY and L. M. ZIEHER, *Experientia* **24,** 593 (1968).
23. H. G. COORE, B. HELLMAN, E. PIHL and I.-B. TÄLJEDAL, *Biochem. J.* **111,** 107 (1969).
24. S. L. HOWELL, C. J. FINK and P. E. LACY, *J. Cell Biol.* **41,** 154 (1969).
25. W. KEMMLER and D. F. STEINER, *Biochem. biophys. Res. Commun.* **41,** 1223 (1970).
26. L. ORCI, W. STAUFFACHER, D. BEAVEN, A. E. LAMBERT, A. E. RENOLD and C. ROUILLER, *Acta diabet. lat.* **6,** (suppl. 1), 271 (1969).
27. E. ARIENS, *Acta diabet. lat* **6** (suppl. 1), 143 (1969).
28. M. D. GERSHON and L. L. ROSS, *J. Physiol.* **186,** 451 (1966).
29. Å. LERNMARK and B. HELLMAN, *Life Sci.* **8,** 53 (1969).
30. W. CREUTZFELDT, C. CREUTZFELDT and H. FRERICHS, in *The Structure and Metabolism of the Pancreatic Islets*, (Eds. S. FALKMER, B. HELLMAN and I.-B. TÄLJEDAL), p. 181. Pergamon Press, Oxford (1970).
31. P. E. LACY and S. L. HOWELL, in *The Structure and Metabolism of the Pancreatic Islets*, (Eds. S. FALKMER, B. HELLMAN and I.-B. TÄLJEDAL), p. 171. Pergamon Press, Oxford (1970).