Biochem. J. (1972) **129**, 241–254
Printed in Great Britain

Effects of Organic Mercurials on Mammalian Pancreatic β-Cells

INSULIN RELEASE, GLUCOSE TRANSPORT, GLUCOSE OXIDATION, MEMBRANE PERMEABILITY AND ULTRASTRUCTURE

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(*Received* 2 *March* 1972)

The effects of *p*-chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid on pancreatic islets were studied *in vitro*. Obese–hyperglycaemic mice were used as the source of microdissected islets containing more than 90% *β*-cells. *p*-Chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid stimulated insulin release at concentrations of 0.01 mM or above. This stimulation was significantly inhibited by the omission of Ca^{2+} or the addition of adrenaline, diazoxide or 2,4-dinitrophenol. *p*-Chloromercuribenzoic acid or chloromercuribenzene-*p*-sulphonic acid did not interfere with the insulin-releasing ability of glucose. Micro-perifusion experiments revealed that the release of insulin in response to organic mercurial occurred almost instantaneously, was reversible, and was biphasic. The two mercurials inhibited glucose transport as well as glucose oxidation, and increased the mannitol and sucrose spaces of isolated islets. Compared with the effects on insulin release, those on glucose transport and membrane permeability were characterized by a longer latency and/or required higher concentrations of organic mercurial. Apart from a seemingly higher proportion of *β*-cells exhibiting certain degenerative features, in islets exposed to 0.1 mM-chloromercuribenzene-*p*-sulphonic acid for 60 min, no significant differences with respect to *β*-cell fine structure were noted between non-incubated islets and islets incubated with chloromercuribenzene-*p*-sulphonic acid or glucose or both. It is suggested that insulin release may be regulated by relatively superficial thiol groups in the *β*-cell plasma membrane.

There is as yet no consensus about the molecular events involved in the recognition of insulin secretagogues by the pancreatic *β*-cells. Searching for the location of the glucose-sensing mechanism, we have demonstrated the presence of glucose-binding transport sites in the *β*-cell plasma membrane (Hellman *et al.*, 1971*a*). However, subsequent studies did not demonstrate any correlation between glucose-transport rates and the rates of insulin release (Hellman *et al.*, 1971*b*). It is not known if the *β*-cell plasma membrane contains other binding sites for glucose and whether they serve as a direct receptor mechanism for the triggering of insulin release (cf. Hellman *et al.*, 1972). In view of reports that the binding of glucose to cell membranes may depend on the integrity of thiol groups (Stein, 1967; Rothstein, 1970; Bode *et al.*, 1970), it is notable that *p*-chloromercuribenzoic acid has been claimed to inhibit bioassayed insulin release from the rat pancreas (Chiba, 1969). Since *p*-chloromercuribenzoic acid may affect cells in a multitude of ways, we considered it important to compare its effects with those of a thiol reagent with a poor ability to penetrate cell membranes. As will be shown in the present paper, neither *p*-chloromercuribenzoic acid nor chloromercuribenzene-*p*-sulphonic acid inhibited the stimulatory action of glucose on the release of immunoreactive insulin from microdissected mouse islets. On the contrary, both of these organic mercurials were found to stimulate insulin release strongly at both low and high glucose concentrations.

During this study it was reported that *p*-chloromercuribenzoic acid has been found to stimulate hormone release from the posterior (Douglas *et al.*, 1965) and the anterior (Schofield, 1971) pituitary gland. It thus seemed possible that membrane thiol groups may play a fundamental role in insulin release, albeit a role different from the hypothetical one that initiated the present investigation. To elucidate the nature of insulin release in response to organic mercurials we have therefore extended the study to include both membrane effects, as reflected in the transport of glucose and other non-electrolyte solutes, and metabolic effects, as reflected in glucose oxidation. The effect of chloromercuribenzene-*p*-sulphonic acid on insulin release has also been studied under conditions known to inhibit secretion in response to glucose. A micro-perifusion system made it possible to compare the dynamics of insulin release induced by organic mercurials with the time-course

of increased membrane permeability to small organic solutes. Electron microscopy was employed to assess the ultrastructure of β-cells exposed to organic mercurials.

Materials and Methods

Chemicals

p-Chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. D-[6-^{3}H]-Glucose, D-[U-^{14}C]glucose, L-[1-^{14}C]glucose, [6,6′-^{3}H]sucrose, [^{14}C]urea and insulin antibodies were from The Radiochemical Centre, Amersham, Bucks., U.K., and [1-^{3}H]mannitol was from New England Nuclear Corp., Frankfurt/Main, Germany. ^{125}I-labelled insulin was obtained from Farbwerke Hoechst A.G., Frankfurt/Main, Germany. Reagents for insulin assay were also kindly given by Novo A/S, Copenhagen, Denmark. Non-radioactive sugars and ethyleneglycolbis(aminoethylether)-tetra-acetic acid were obtained from British Drug Houses Ltd., Poole, Dorset, U.K., and urea was from Sigma Chemical Co. 2,4-Dinitrophenol was from E. Merck A.G., Darmstadt, Germany, and adrenaline from Sigma Chemical Co. Diazoxide (7-chloro-3-methyl-1,2,4-benzothiadiazine 1,1-dioxide) was given by Schering Corp., Bloomfield, N.J., U.S.A. Human serum albumin was from Kabi AB, Stockholm, Sweden, and dextran T 70 (average mol. wt. 70000) from Pharmacia Fine Chemicals, Uppsala, Sweden. Crystalline mouse insulin was prepared by Novo A/S. All reagents were of analytical grade. For electron microscopy, glutaraldehyde was obtained from Taab Laboratories, Reading, Berks., U.K., and Epikote resin 812 from George T. Gurr Ltd., London S.W.6, U.K.

Animals and microdissection of islets

Adult obese–hyperglycaemic mice (gene symbol *ob*/*ob*) of both sexes were obtained from the Umeå colony. Unless otherwise stated, the animals were starved overnight. Fresh pancreatic islets were isolated by free-hand microdissection (Hellerström, 1964) in gassed (O_2+CO_2, 95:5) Krebs–Ringer bicarbonate buffer (Umbreit *et al.*, 1964). This dissection medium contained glucose, albumin and dextran at the same concentration as used during subsequent incubations.

General aspects of incubations

All incubations were performed at 37°C, in gassed (O_2+CO_2, 95:5) Krebs–Ringer bicarbonate buffer as the basic medium. Except in micro-perifusion experiments (see 'Insulin release' below), the islets were allowed to equilibrate with the basic medium during a preincubation period of 30–45min. When the different incubation media contained glucose, albumin and dextran this is indicated in the legends to figures and tables.

Insulin release

Two different techniques were used for studying insulin release. In most experiments batches of two islets were incubated in 315μl of medium for 60min and insulin was determined in samples taken at the end of incubation. In other experiments the dynamics of insulin release were studied with the aid of a micro-perifusion system that permits continuous sampling of insulin at various intervals during incubation. Two islets were placed in a small polythene–nylon chamber (4.5μl volume) and were perifused at a rate of 33–35μl/min. The use of a screen oxygenator and the design of the medium inlet ensured optimum equilibration of the medium with O_2 and CO_2. Two different reservoirs were connected with the perifusion chamber in such a way as to allow sudden alterations of the medium fed to the perifused islets. A specially constructed valve was used to minimize the fluctuations of hydrodynamic pressure caused by changing the perifusion medium. Direct measurements of the system pressure as well as other control experiments revealed that this precaution was necessary to avoid artificial peaks of insulin release. In each experiment three different perifusion chambers were loaded with islets from a single animal and run in parallel. Results given represent mean values from these three chambers.

Insulin was assayed radioimmunologically. Separation of free and bound insulin was achieved by precipitation with ethanol (Heding, 1969). It was checked that the organic mercurials did not affect the insulin assay or the recovery of immunoreactive insulin (Fig. 1).

Glucose oxidation

Single islets were incubated for 1h in 100μl of medium containing 10mM-[U-^{14}C]glucose (1.7mCi/mmol). Incubations were performed in liquid-scintillation vials equipped with a small glass centre well (Keen *et al.*, 1963). Blank values were obtained by incubating medium without islets. Metabolism was stopped and $^{14}CO_2$ was determined as previously described (Hellman *et al.*, 1971*c*).

Uptake of glucose, mannitol, sucrose and urea

All uptake studies were based on the principle of double-labelling, by using one ^{14}C-labelled and one ^{3}H-labelled compound in each incubation medium. L-[1-^{14}C]Glucose, which does not normally penetrate the β-cell plasma membrane (Hellman *et al.*, 1971*a*),

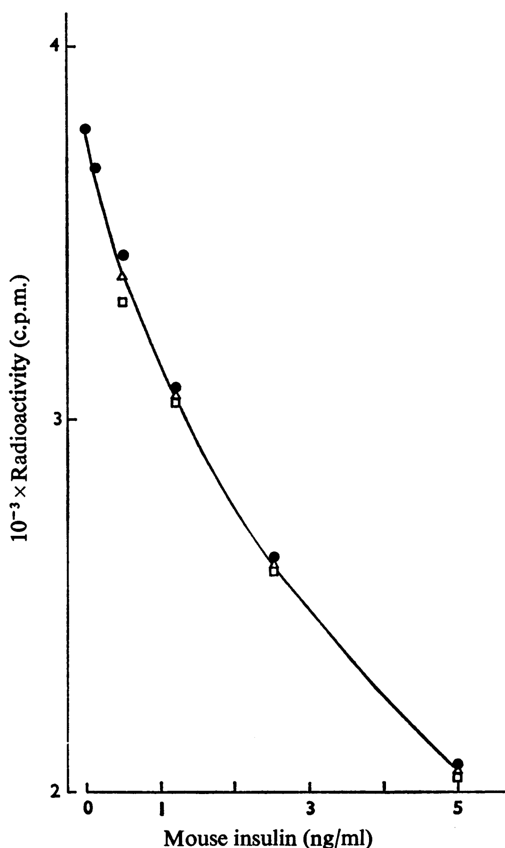

Fig. 1. *Control experiment showing lack of effect of chloromercuribenzene-p-sulphonic acid on the recovery of immunoreactive insulin*

The 0.1 M-phosphate buffer (pH 7.4) containing 5 mg of albumin/ml was supplemented with crystalline mouse insulin at concentrations ranging from 0.6 to 5 ng/ml. One set of tubes also contained 0.1 mM-chloromercuribenzene-*p*-sulphonic acid. After incubation for 15 min at 37°C, insulin was sampled and assayed radioimmunologically. Each point represents the mean of three determinations of insulin incubated without (□) or with (△) chloromercuribenzene-*p*-sulphonic acid. An ordinary standard curve (●) for non-incubated insulin is also shown.

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was used as extracellular-space marker in studies of D-[6-^{3}H]glucose uptake. Uptake of D-[6-^{3}H]glucose was also studied in relation to [^{14}C]urea, which equilibrates in the intracellular water of pancreatic islets (Hellman *et al.*, 1971*d*). Since studies of D-glucose uptake were aimed at revealing properties of the D-glucose-transport system, incubations were performed for only 45 s. Uptake of [1-^{3}H]mannitol and [6,6′-^{3}H]sucrose in relation to [^{14}C]urea was studied as an index of β-cell permeability to small organic solutes, which are normally restricted to the extracellular space of the present type of islets (Hellman *et al.*, 1971*a,d,e*). Incubations with sucrose and mannitol were therefore generally performed for 15 or 60 min. Batches of three islets were incubated in all uptake experiments. Details of the uptake experiments are given in the legends to tables. After freeze-drying and weighing, the islets were dissolved in Hyamine and analysed for ^{3}H and ^{14}C as previously described (Hellman *et al.*, 1971*a,b,d*).

Weighing of islets

Except in the perifusion experiments, incubated islets were placed on aluminium foil, and with the aid of a micropipette they were immediately freed of as much contaminating fluid as possible. The time required for this procedure was less than 5 s, after which the islets were quickly plunged into melting isopentane (−160°C). After freeze-drying [−40°C, 0.1 N/m^2 (0.001 mmHg)] overnight, the islets were weighed on a quartz-fibre balance (Lowry, 1953).

Perifused islets were frozen and freeze-dried while remaining in the perifusion chamber. When removed from the chamber, the islets were surrounded by freeze-dried medium of a fluffy consistency. This fluffy rim was gently and carefully brushed off under a stereo-microscope before the islets were weighed as described above.

Statistical evaluation of results

All quantitative results were expressed per unit dry weight of islet tissue. In studies of the effects of altered medium composition, notably the addition of organic mercurials, parallel control incubations were performed as a routine with islets isolated from the same animals as used for test incubations. This design made it possible to use each pancreas as its own control and to calculate statistical significances from the mean differences between paired test and control incubations over a series of repeated experiments. In this paper the expression 'experiment' refers to a set of incubations with islets from a single animal. Within each experiment all factors were usually tested in multiple incubations, the mean result of which was entered as one observation in the statistical treatment.

Light-microscopy and electron microscopy

Islets were taken for morphological examination both directly after microdissection in basal medium supplemented with 3 mM-glucose and 5 mg of albumin/ml and after incubation for 60 min with or without

0.1 mM-chloromercuribenzene-*p*-sulphonic acid. Islets were fixed in ice-cold 4% (w/v) glutaraldehyde in 0.1 M-sodium phosphate buffer (pH 7.3) for 2h (Sabatini *et al.*, 1963). After being rinsed in the same buffer, they were post-fixed for 1 h at 4°C in 1% (w/v) OsO_4 dissolved in 0.1 M-phosphate buffer (pH 7.3) containing 0.2 M-sucrose. The islets were then washed for 1 h in water. Stepwise dehydration in ethanol was carried out at room temperature, and embedding was performed in Epon 812 (Luft, 1961). The blocks were polymerized at 37°C overnight and for an additional period of 24 h at 60°C. Sections about 1 μm thick for light-microscopy and ultra-thin ones for electron microscopy were cut on an LKB Ultratome, by using glass knives. Sections for light-microscopy were stained with Toluidine Blue. The thin sections were post-stained with uranyl acetate and lead citrate and were examined in a Philips EM 300 electron microscope.

Results

Effects of p-chloromercuribenzoic acid and chloromercuribenzene-p-sulphonic acid on insulin release

Dose–response relationships and the effect of glucose. Fig. 2 shows the effect of different concentrations of chloromercuribenzene-*p*-sulphonic acid on the insulin release during incubation for 60 min in the presence of 3 mM-glucose and 5 mg of albumin/ml. A significant stimulation of insulin release was observed with 0.05 mM-chloromercuribenzene-*p*-sulphonic acid. Raising the chloromercuribenzene-*p*-sulphonic acid concentration above 0.05 mM resulted in an increas-

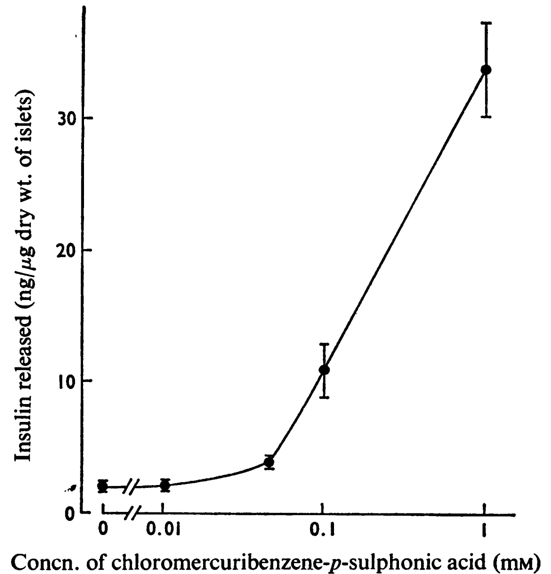


Fig. 2. *Effects of different concentrations of chloromercuribenzene-p-sulphonic acid on the amount of insulin released during incubation for* 60 *min in the presence of* 3 mM-*glucose*

Before being exposed to chloromercuribenzene-*p*-sulphonic acid, the islets were preincubated for 40 min. The preincubation medium as well as the subsequent chloromercuribenzene-*p*-sulphonic acid-containing media were supplemented with 5 mg of albumin/ml and 3 mM-glucose. Results are given as mean values ± S.E.M. for eight or nine different experiments.

Table 1. *Stimulation of insulin release by p-chloromercuribenzoic acid and chloromercuribenzene-p-sulphonic acid at different concentrations of glucose*

Islets from fed mice were preincubated for 45 min in basal medium supplemented with 0.1 mg of albumin/ml and 3 mM-glucose. The results represent amounts of insulin released during subsequent incubation for 60 min in the presence of 0.1 mg of albumin/ml and glucose, *p*-chloromercuribenzoic acid or chloromercuribenzene-*p*-sulphonic acid as indicated. Results are given as mean values ± S.E.M. for the numbers of experiments shown in parentheses. Statistical significances were judged from the mean differences between paired test and control incubations: $^{*}P<0.02$; $^{**}P<0.01$; $^{***}P<0.005$.

Test substance	Insulin released (ng/h per μg dry wt. of islets) 3 mM-Glucose	10 mM-Glucose
None (control)	0.74 ± 0.21 (7)	6.63 ± 1.57 (7)
p-Chloromercuribenzoic acid (0.01 mM)	1.64 ± 0.19** (7)	8.80 ± 3.67 (7)
p-Chloromercuribenzoic acid (0.1 mM)	11.36 ± 3.29* (6)	16.33 ± 3.73* (7)
Chloromercuribenzene-*p*-sulphonic acid (0.01 mM)	1.71 ± 0.52 (7)	7.40 ± 2.46 (7)
Chloromercuribenzene-*p*-sulphonic acid (0.1 mM)	14.57 ± 2.54*** (7)	20.94 ± 3.79*** (7)

ingly greater secretory response up to at least 1 mM, the highest concentration of chloromercuribenzene-*p*-sulphonic acid tested. At the latter concentration the amount of insulin released was more than 17 times that recorded after incubation with 3 mM-glucose alone.

The effect of chloromercuribenzene-*p*-sulphonic acid and *p*-chloromercuribenzoic acid on glucose-stimulated insulin release is shown in Table 1. The two organic mercurials appeared to be equally effective stimulants of insulin release. In this series of experiments stimulation was significant with as little as 0.01 mM-*p*-chloromercuribenzoic acid in the presence of 3 mM-glucose and only 0.1 mg of albumin/ml. The stimulatory effect of 10 mM-glucose appeared to be additive to that obtained with organic mercurials at a concentration of 0.01–0.1 mM.

Dynamics of insulin release. Micro-perifusion of isolated islets was used to study the dynamics of insulin release in response to organic mercurials. Similar results were obtained with chloromercuribenzene-*p*-sulphonic acid and *p*-chloromercuribenzoic acid, and representative records obtained with chloromercuribenzene-*p*-sulphonic acid are shown in Figs. 3 and 4. The onset of insulin release was almost instantaneous on exposure to 0.1 mM-chloromercuribenzene-*p*-sulphonic acid. Fig. 3 also shows that the maximum response to chloromercuribenzene-*p*-sulphonic acid was reached within the first few minutes, and that there was a high rate of release for as long as the perifusion with chloromercuribenzene-*p*-sulphonic acid was continued. These effects of chloromercuribenzene-*p*-sulphonic acid were obtained with both 3 mM- and 17 mM-glucose. The initial brisk response was followed by some decline of the release rate, resulting in an initial peak as shown in Fig. 3.

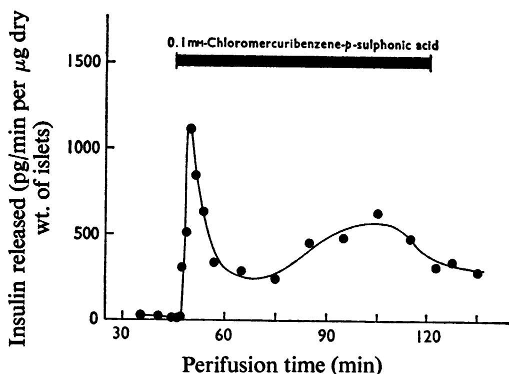


Fig. 3. *Dynamics of chloromercuribenzene-p-sulphonic acid-induced insulin release*

Islets were perifused with basal medium containing 5 mg of albumin/ml and 3 mM-glucose. After 45 min the perifusion medium was suddenly switched to the same kind of medium supplemented with 0.1 mM-chloromercuribenzene-*p*-sulphonic acid. Exposure to chloromercuribenzene-*p*-sulphonic acid (bar) was continued for 75 min, after which the experiment was completed by 15 min of perifusion with 3 mM-glucose in the absence of chloromercuribenzene-*p*-sulphonic acid. Before perifusion with chloromercuribenzene-*p*-sulphonic acid, 1 min samples of effluent were taken for insulin assay. Continuous sampling was then used, at intervals of 1 or 2 min during the first 7 min and later at intervals of 5 or 10 min. The points represent the average rate of insulin release over each sampling period. The dead space of the apparatus corresponded to a theoretical time-lag of 60 s.

Fig. 4 shows that the chloromercuribenzene-*p*-sulphonic acid induced insulin release was reversible. In islets exposed to 0.1 mM-chloromercuribenzene-*p*-sulphonic acid for only 1 min the brisk rise of insulin release was followed by a quick decline towards basal values. The true decline of insulin release may have been even more rapid than is indicated in Fig. 4, since it is not known how fast the newly released insulin was washed out from the perifused islets.

Effects of calcium deficiency, adrenaline, diazoxide and 2,4-dinitrophenol on insulin release in response to chloromercuribenzene-p-sulphonic acid. In contrast to glucose, chloromercuribenzene-*p*-sulphonic acid

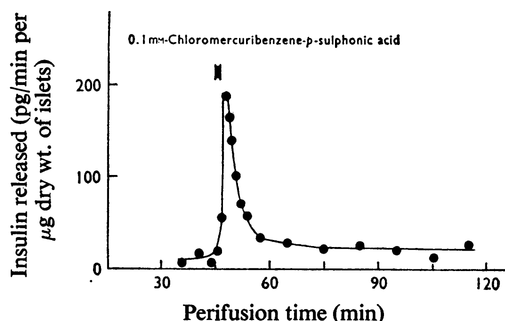


Fig. 4. *Reversibility of chloromercuribenzene-p-sulphonic acid-induced insulin release*

Islets were perifused with basal medium containing 0.1 mg of albumin/ml, 5 mg of dextran/ml and 3 mM-glucose. After 45 min the perifusion medium was suddenly switched to the same kind of medium supplemented with 0.1 mM-chloromercuribenzene-*p*-sulphonic acid. Perifusion with chloromercuribenzene-*p*-sulphonic acid (bar) lasted for only 1 min, after which the experiment was continued with the initial, chloromercuribenzene-*p*-sulphonic acid-free medium. Insulin was sampled and results were expressed as in Fig. 3.

caused a prompt release of insulin even in a Ca^{2+}-free medium supplemented with 0.5 mM-ethyleneglycolbis(aminoethylether)-tetra-acetic acid (Fig. 5). However, a comparison of Figs. 3 and 5 suggests that the response to chloromercuribenzene-*p*-sulphonic acid was depressed in the Ca^{2+}-free medium. This interpretation was confirmed by repeated measurements of insulin release over a 60 min period (Table 2). Islets incubated with 0.1 mM-chloromercuribenzene-*p*-sulphonic acid and 3 mM-glucose released only half as much insulin into a Ca^{2+}-deficient medium as into a medium containing 5 mequiv. of Ca^{2+}. Chloromercuribenzene-*p*-sulphonic acid did not reverse the inhibition of glucose-stimulated insulin release caused by calcium deficiency. As shown in Table 3, 2 μg of adrenaline/ml, 125 μg of diazoxide/ml or 0.01 mM-2,4-dinitrophenol significantly inhibited insulin release in response to chloromercuribenzene-*p*-sulphonic acid.

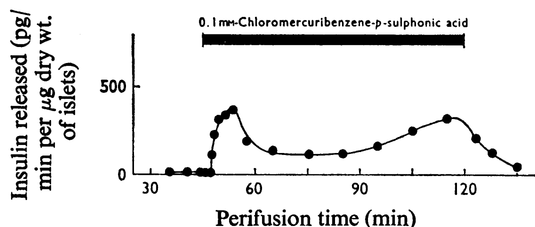


Fig. 5. *Effect of calcium deficiency on the dynamics of insulin release in response to chloromercuribenzene-p-sulphonic acid*

Islets were perifused with basal medium containing 1 mg of albumin/ml, 3 mM-glucose and 0.5 mM-ethyleneglycolbis(aminoethylether)-tetra-acetic acid. After 45 min the perifusion medium was suddenly switched to the same kind of medium supplemented with 0.1 mM-chloromercuribenzene-*p*-sulphonic acid. Exposure to chloromercuribenzene-*p*-sulphonic acid (bar) was continued for 75 min, after which the experiment was completed by 20 min of perifusion with the initial medium free from chloromercuribenzene-*p*-sulphonic acid. Insulin was sampled and results were expressed as in Fig. 3.

Effects of p-chloromercuribenzoic acid and chloromercuribenzene-p-sulphonic acid on glucose oxidation

Table 4 shows that chloromercuribenzene-*p*-sulphonic acid and *p*-chloromercuribenzoic acid markedly influenced the production of $^{14}CO_2$ from islets incubated with 10 mM-[U-^{14}C]glucose. Qualitatively different effects were obtained, depending on the concentration used. Whereas glucose oxidation was significantly stimulated by 0.05 mM-*p*-chloromercuribenzoic acid or 0.05 mM-chloromercuribenzene-*p*-sulphonic acid, oxidation was significantly inhibited when the concentration of *p*-chloromercuribenzoic acid was increased tenfold. Chloromercuribenzene-*p*-sulphonic acid seemed to be a less-potent inhibitor of glucose oxidation than was *p*-chloromercuribenzoic acid.

Effects of p-chloromercuribenzoic acid and chloromercuribenzene-p-sulphonic acid on glucose transport

Dose–response relationships. Two series of experiments were performed to study the effect of different

Table 2. *Effects of calcium deficiency on insulin release in response to chloromercuribenzene-p-sulphonic acid or glucose*

Islets from fed mice were preincubated as described in Table 1 with the modification that half the islets were preincubated in the absence of Ca^{2+}. Calcium deficiency was achieved by substituting NaCl for $CaCl_2$ in the basal medium. The results represent amounts of insulin released during subsequent incubation for 60 min in the presence of glucose, chloromercuribenzene-*p*-sulphonic acid and Ca^{2+} as indicated. Results are given as mean values ± S.E.M. for nine different experiments. Effects of calcium deficiency were judged from the mean differences between paired incubations in the presence and absence of Ca^{2+}: * $P<0.025$; ** $P<0.005$.

Organic mercurial tested	Insulin released (ng/h per μg dry wt. of islets)	
	Ca^{2+} (5 mequiv.)	No Ca^{2+}
Incubations in 3 mM-glucose		
None	0.73 ± 0.19	1.49 ± 0.38
Chloromercuribenzene-*p*-sulphonic acid (0.1 mM)	13.17 ± 2.46	6.43 ± 1.39*
Incubations in 10 mM-glucose		
None	13.15 ± 2.44	1.86 ± 0.62**
Chloromercuribenzene-*p*-sulphonic acid (0.1 mM)	19.22 ± 3.32	5.48 ± 1.05**

Table 3. *Effects of adrenaline, diazoxide and 2,4-dinitrophenol on insulin release in response to chloromercuribenzene-p-sulphonic acid*

Islets from fed mice were preincubated for 45 min in basal medium supplemented with 5 mg of albumin/ml and 3 mM-glucose. The results represent amounts of insulin released during subsequent incubations for 60 min in the same kind of medium, and in medium containing 0.1 mM-chloromercuribenzene-*p*-sulphonic acid as well. Control incubations were performed without further additives. In each experiment, parallel incubations were also performed in media supplemented with 2 μg of adrenaline/ml, 125 μg of diazoxide/ml or 0.01 mM-2,4-dinitrophenol. Results are given as mean values ± S.E.M. for the numbers of experiments stated. The statistical significances of effects were judged from the mean differences between paired test and control incubations: *$P<0.02$; **$P<0.005$.

Test substance	No. of expts.	Insulin released (ng/h per μg dry wt. of islets) Control (*a*)	Test (*b*)	(*b*)–(*a*)
Incubations without chloromercuribenzene-*p*-sulphonic acid				
Adrenaline (2 μg/ml)	6	0.76 ± 0.38	0.39 ± 0.10	−0.37 ± 0.30
Diazoxide (125 μg/ml)	10	1.14 ± 0.43	1.54 ± 0.42	0.40 ± 0.34
2,4-Dinitrophenol (0.01 mM)	4	2.87 ± 0.47	1.82 ± 0.90	−1.05 ± 0.92
Incubations with 0.1 mM-chloromercuribenzene-*p*-sulphonic acid				
Adrenaline (2 μg/ml)	6	9.89 ± 2.34	4.24 ± 0.88	−5.65 ± 1.56*
Diazoxide (125 μg/ml)	10	8.93 ± 1.39	4.47 ± 0.89	−4.46 ± 1.16**
2,4-Dinitrophenol (0.01 mM)	6	8.35 ± 1.17	4.60 ± 0.92	−3.75 ± 0.78**

Table 4. *Effects of p-chloromercuribenzoic acid and chloromercuribenzene-p-sulphonic acid on glucose oxidation*

Islets were preincubated for 45 min in basal medium containing 3 mM-glucose. Incubation was then performed for 60 min in basal medium supplemented with 10 mM-D-[U-^{14}C]glucose (1.7 mCi/mmol) and *p*-chloromercuribenzoic acid or chloromercuribenzene-*p*-sulphonic acid as indicated. Amounts of $^{14}CO_2$ produced are expressed as mmol of glucose equivalents oxidized/h per kg dry wt. of islets. Results are given as mean values ± S.E.M. for the stated numbers of experiments. In addition to test values, the differences between paired test and control incubations are presented. Statistical significances were judged from these differences: *$P<0.05$; **$P<0.02$.

Concn. of organic mercurial (mM)	No. of expts.	Rate of oxidation (mmol/h per kg dry wt. of islets): With *p*-chloromercuribenzoic acid: Test	Test minus control	Change (%)	With chloromercuribenzene-*p*-sulphonic acid: Test	Test minus control	Change (%)
0.05	7	33.5 ± 3.6	11.5 ± 3.5**	+52	30.3 ± 2.3	8.3 ± 3.2*	+38
0.10	5	25.0 ± 4.2	0.8 ± 5.0	+3	27.8 ± 1.4	3.6 ± 1.3	+15
0.50	5	9.9 ± 0.8	−20.2 ± 5.1**	−67	25.4 ± 5.7	−4.9 ± 5.8	−16
1.0	4	4.6 ± 4.2	−26.3 ± 5.0**	−85	10.4 ± 1.9	−21.2 ± 8.5	−67

concentrations of *p*-chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid on the 45 s uptake of glucose. In the first of these, the transport of D-[6-^{3}H]glucose into islet cells was studied with L-[1-^{14}C]glucose as extracellular marker (Table 5). When tested at concentrations below 0.1 mM, neither chloromercuribenzene-*p*-sulphonic acid nor *p*-chloromercuribenzoic acid significantly affected glucose transport. A significant inhibition was, however, observed when the concentration of *p*-chloromercuribenzoic acid or chloromercuribenzene-*p*-sulphonic acid was raised to 0.5 mM or 1 mM.

Since the above transport results could have been influenced by an increased β-cell permeability to L-glucose, a second series of experiments was performed. In these experiments the uptake of D-[6-^{3}H]-glucose was studied in relation to the uptake of [^{14}C]urea, which is known to enter the intracellular

Table 5. *Effects of p-chloromercuribenzoic acid and chloromercuribenzene-p-sulphonic acid on D-glucose transport*

Islets were preincubated for 45 min in basal medium supplemented with 3 mM-glucose and the listed concentrations of *p*-chloromercuribenzoic acid or chloromercuribenzene-*p*-sulphonic acid. Control islets were preincubated in the absence of organic mercurial. Incubations were then performed for 45 s in basal medium containing the same concentration of organic mercurial as used during preincubation as well as 2.0 mM-D-[6-^{3}H]glucose (10 mCi/mmol) and a ^{14}C-labelled space marker. In one series of experiments ('L-glucose') this space marker was 2.5 mM-L-[1-^{14}C]glucose (3.0 mCi/mmol). In another series ('urea') 0.5 mM-[^{14}C]urea (20 mCi/mmol) was used. To obtain a parameter reflecting mediated glucose transport, the islet uptake of D-glucose was corrected with respect to the spaces occupied by L-glucose or urea at the end of the 45 s incubation. Thus 'D-glucose transport' = [D-glucose] × (D-glucose space − L-glucose space), or [D-glucose] × (D-glucose space − urea space). D-Glucose was taken up to a greater extent than was L-glucose, resulting in positive transport values. Fast penetration of urea, on the other hand, yielded negative expressions in the second series of experiments. Calculated as above, the glucose uptake was expressed per unit dry weight of islets incubated in parallel with (test) and without (control) organic mercurial. Effects of organic mercurials are presented as mean values ± S.E.M. for the differences between paired test and control incubations, the numbers of which are given in parentheses. In addition, the percentage changes in relation to controls are presented. Since inhibition of D-glucose transport decreased the difference between D-glucose and L-glucose but increased that between D-glucose and urea, these percentages are negative in the first series of experiments but positive in the second. Statistical significances were judged from the paired test and control incubations: $^{*}P<0.05$; $^{**}P<0.005$; $^{***}P<0.001$.

	D-Glucose (mmol/kg dry wt. of islets)							
	With *p*-chloromercuribenzoic acid				With chloromercuribenzene-*p*-sulphonic acid			
	L-Glucose		Urea		L-Glucose		Urea	
Concn. of organic mercurial (mM)	Test minus control	Change (%)	Test minus control	Change (%)	Test minus control	Change (%)	Test minus control	Change (%)
0.01	−0.16 ± 0.17 (6)	−13	−0.24 ± 0.14 (5)	+23	−0.18 ± 0.39 (7)	−8	−0.20 ± 0.10 (6)	+17
0.05	−0.10 ± 0.22 (6)	−8	−0.11 ± 0.22 (6)	+9	−0.23 ± 0.30 (7)	−10	−0.10 ± 0.27 (6)	+10
0.1	−0.44 ± 0.12** (12)	−27	−0.02 ± 0.20 (6)	+1	−0.25 ± 0.40 (7)	−11	−0.57 ± 0.31 (6)	+39
0.5	−1.26 ± 0.18*** (12)	−68	−1.74 ± 0.56* (6)	+74	−1.20 ± 0.15*** (12)	−80	−1.56 ± 0.19*** (6)	+107
1.0	−1.10 ± 0.21** (6)	−56	−2.19 ± 0.18*** (6)	+92	−1.57 ± 0.16*** (12)	−100	−2.03 ± 0.29*** (6)	+140

space of the islets under investigation (Hellman *et al.*, 1971*d*). In control experiments performed in the absence of organic mercurials, the uptake of urea was faster than that of D-glucose. This difference in penetration rate was significantly increased by 0.5mM- and 1mM-*p*-chloromercuribenzoic acid or 0.5mM- and 1mM-chloromercuribenzene-*p*-sulphonic acid as would be expected if the organic mercurials inhibited glucose transport (Table 5).

Latency of the chloromercuribenzene-p-sulphonic acid-induced inhibition of glucose transport. To study whether there was any latency in the effect of organic mercurials on glucose transport, islets were preincubated with chloromercuribenzene-*p*-sulphonic acid for various periods of time before being incubated with D-[6-^{3}H]glucose and [^{14}C]urea. As shown in Table 6, exposure to 0.5mM-chloromercuribenzene-*p*-sulphonic acid for 1 or 5min had no effect on glucose transport, whereas a significant inhibition was observed after a preincubation period of 30min.

Effects of p-chloromercuribenzoic acid and chloromercuribenzene-p-sulphonic acid on the permeability of islet cells

Dose–response relationships. The effects of *p*-chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid on islet-cell permeability was studied by comparing the uptake of [^{14}C]urea with that of [1-^{3}H]mannitol or [6,6′-^{3}H]sucrose. Previous experiments have shown that urea quickly enters the β-cells, whereas mannitol and sucrose are normally excluded and therefore restricted to the extracellular space (Hellman *et al.*, 1971*d,e*). A smaller difference between the urea space on the one hand and the mannitol or sucrose spaces on the other would therefore indicate an increased permeability of the β-cell plasma membrane. Table 7 shows that 1mM-*p*-chloromercuribenzoic acid or 1mM-chloromercuribenzene-*p*-sulphonic acid markedly increased the sucrose and mannitol spaces. There was no effect on the distribution of mannitol with chloromercuribenzene-*p*-sulphonic acid at concentrations of 0.1mM and below. Further, 0.1mM-*p*-chloromercuribenzoic acid or chloromercuribenzene-*p*-sulphonic acid had no significant effect on the sucrose space, despite the fact that in this series of experiments the organic mercurials were present during a 45min preincubation period.

Latency of the chloromercuribenzene-p-sulphonic acid-induced permeability to sucrose. Islets were exposed to 1mM-chloromercuribenzene-*p*-sulphonic acid for various periods of time before being incubated for 45s with [6,6′-^{3}H]sucrose and [^{14}C]urea. Table 6 shows that preincubation for 1 or 5min had no effect on the uptake of sucrose. After exposure to chloromercuribenzene-*p*-sulphonic acid for 45min, the difference in uptake between urea and sucrose was significantly decreased, suggesting an increased β-cell permeability to sucrose. A comparison with the glucose-uptake results of Table 6 reveals that this increased membrane permeability was not enough to obscure the chloromercuribenzene-*p*-sulphonic-acid-induced inhibition of facilitated glucose transport.

Effects of chloromercuribenzene-p-sulphonic acid on the morphology of β-cells

The fine structure of pancreatic β-cells in various animal species has been reported on by several

Table 6. *Latency of chloromercuribenzene-p-sulphonic acid-induced effects on glucose transport and sucrose space*

Islets were preincubated for 45min in basal medium containing 3mM-glucose. During the final 0, 1, 5, 30 or 45min of the preincubation period, chloromercuribenzene-*p*-sulphonic acid was also present. Incubation was then performed for 45s in chloromercuribenzene-*p*-sulphonic acid-free medium containing either 2.0mM-D-[6-^{3}H]glucose (10mCi/mmol) or 0.5mM-[6,6′-^{3}H]sucrose (30mCi/mmol). In both cases 0.5mM-[^{14}C]urea (20mCi/mmol) was also present. The islet uptake of labelled D-glucose or sucrose in relation to that of urea was calculated as described in Table 5. Results are presented as mean values±S.E.M. for six different experiments. The concentration of chloromercuribenzene-*p*-sulphonic acid was 0.5mM in the experiments with D-glucose, and 1.0mM in those with sucrose. Comparisons with islets unexposed to chloromercuribenzene-*p*-sulphonic acid (0min): *$P<0.02$; **$P<0.005$.

Time of exposure to chloromercuribenzene-*p*-sulphonic acid (min)	Sugar (mmol/kg dry wt. of islets)	
	D-Glucose	Sucrose
0	−1.42±0.29	−1.31±0.07
1	−1.75±0.05	−1.34±0.09
5	−1.53±0.24	−1.21±0.16
30	−4.04±0.74*	—
45	—	−0.71±0.12**

Table 7. *Effects of p-chloromercuribenzoic acid and chloromercuribenzene-p-sulphonic acid on the mannitol and sucrose spaces*

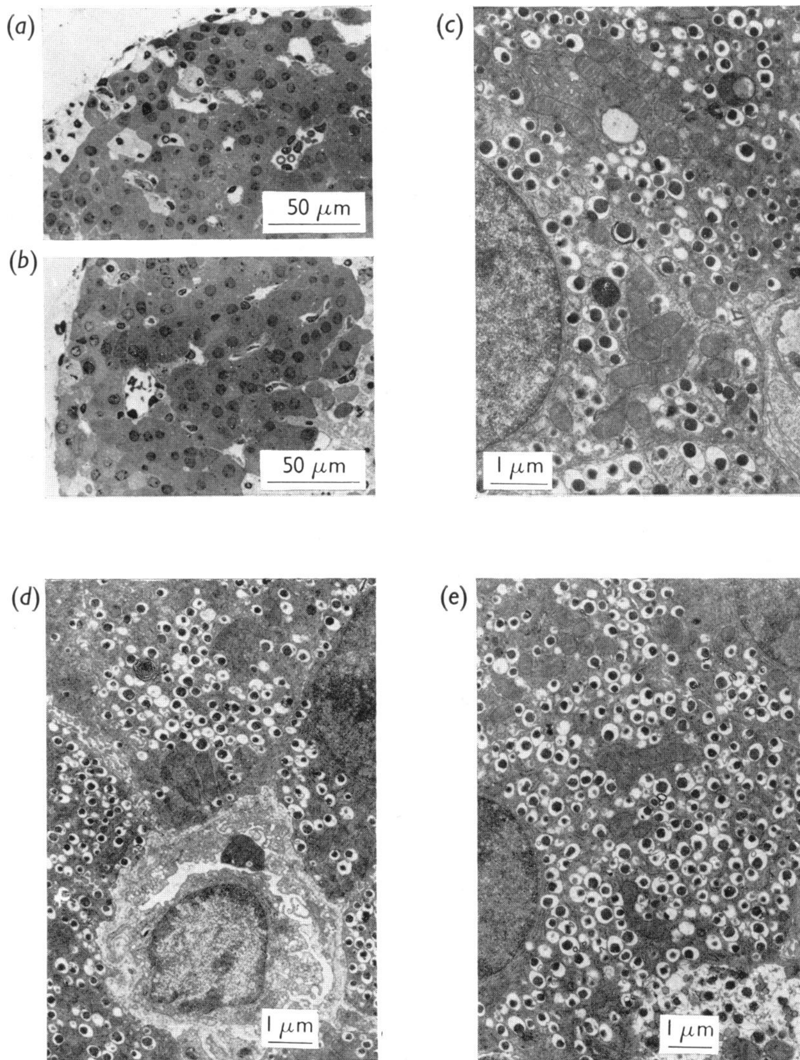
Islets were preincubated for 45min in basal medium supplemented with 3mM-glucose. In studies of sucrose uptake, the preincubation medium also contained the listed concentrations of *p*-chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid. Incubation was then performed for 15min (sucrose) or 60min (mannitol) in media containing *p*-chloromercuribenzoic acid or chloromercuribenzene-*p*-sulphonic acid as listed as well as 0.5mM-[1-^{3}H]mannitol (30mCi/mmol) or 0.5mM-[6,6'-^{3}H]sucrose (30mCi/mmol). Control incubations were performed in the absence of organic mercurial. Incubation media also contained 0.5mM-[^{14}C]urea (20mCi/mmol). The results represent islet uptake of sucrose or mannitol minus the uptake of urea. Results are given as mean values±S.E.M. for incubations in the presence of organic mercurial as well as for the differences between paired test and control incubations. The numbers of experiments are given in parentheses. Statistical significances were judged from the paired test and control data: *$P<0.01$; **$P<0.005$; ***$P<0.001$.

	Net uptake (mmol/kg dry wt. of islets)			
	With *p*-chloromercuribenzoic acid		With chloromercuribenzene-*p*-sulphonic acid	
Concn. of organic mercurial (mM)	Test	Test minus control	Test	Test minus control
Studies of mannitol uptake				
0.01	−0.79±0.05 (6)	0.02±0.08 (6)	−0.78±0.15 (5)	−0.13±0.11 (5)
0.1	−0.68±0.07 (5)	−0.04±0.09 (5)	−0.71±0.08 (5)	−0.06±0.08 (5)
1.0	−0.48±0.11 (6)	0.33±0.08* (6)	−0.31±0.05 (8)	0.30±0.06** (8)
Studies of sucrose uptake				
0.1	−1.19±0.05 (6)	0.07±0.07 (6)	−1.12±0.21 (7)	0.24±0.21 (7)
1.0	−0.58±0.06 (6)	0.67±0.07*** (6)	−0.38±0.22 (8)	1.07±0.19*** (8)

investigators. However, comparatively little information is available on the ultrastructure of the hyperplastic islets of obese–hyperglycaemic mice, and in particular on such islets which have been isolated and subjected to incubation procedures. To evaluate the possible effects of chloromercuribenzene-*p*-sulphonic acid on β-cell structure it was therefore necessary to pay considerable attention to the morphology of both non-incubated islets and islets exposed to control media.

Non-incubated islets as well as islets incubated for 60min with glucose or chloromercuribenzene-*p*-sulphonic acid or both were well preserved and were surrounded by an intact connective-tissue capsule (Plate 1*a*). All islets were devoid of exocrine cells. Although the majority of β-cells stained uniformly with Toluidine Blue, a few cells per islet, with the exception of their nuclei, stained poorly. Islets incubated with chloromercuribenzene-*p*-sulphonic acid or glucose or both had the same overall appearance as non-incubated controls. There seemed, however, to be an increased proportion of poorly stained β-cells in the islets exposed to chloromercuribenzene-*p*-sulphonic acid; the nuclei of these cells exhibited karyolytic and pycnotic features (Plate 1*b*).

The β-cells of all islets showed the classical electron-microscopic features of these cells (Plate 1*c*,*d*,*e*). The nuclei were large, round or oval, and the chromatin was evenly dispersed in the nucleoplasm with only a slight tendency towards margination. The rough endoplasmic reticulum was fairly well developed; individual profiles of its slightly widened cisternae appeared randomly distributed throughout the cytoplasm. However, in certain cytoplasmic locations, above all in the vicinity of Golgi complexes, regular arrays of ribosome-studded membrane profiles were frequently seen. Ribosomes, apparently unattached to membranes, abounded in the cytoplasm. The Golgi apparatus with its characteristic structure of stacks of parallel flattened saccules, small vesicles and large vacuoles was chiefly observed in close proximity to the nucleus. The mitochondria exhibited the classical features of this organelle and were of common occurrence.



EXPLANATION OF PLATE I

Light-micrographs and electron micrographs of isolated islets

(*a*) Incubation for 60min with 3mM-glucose. Staining with Toluidine Blue. (*b*) Incubation for 60min with 3mM-glucose and 0.1mM-chloromercuribenzene-*p*-sulphonic acid. Staining with Toluidine Blue. In both (*a*) and (*b*), note the absence of exocrine cells, the presence of the delicate connective-tissue capsule and the occasional occurrence of poorly stained β-cells with karyolytic features. (*c*) Electron micrograph of a non-incubated islet. For structural details see the text. (*d*) Electron micrograph of an islet incubated as in (*a*). (*e*) Electron micrograph of an islet incubated as in (*b*). In (*c*), (*d*) and (*e*), note the overall similarity in appearance of the β-cells irrespective of incubation. A portion of necrotic β-cell is also depicted in (*e*). Magnification is denoted by scale marks on the photographs.

Secretory granules were abundant. The majority of these were characterized by an electron-opaque matrix, which was separated from a trilaminar perigranular membrane by an electron-lucid space of varying width. Granules were also encountered, the matrix of which presented itself as rectangular crystalline bodies, frequently oriented at different angles. Microtubular structures were observed in the cytoplasmic ground substance; they were of indeterminate length in the sectioned material but measured about 20–25nm (200–250Å) in width. The β-cell cytoplasm also contained a considerable number of myelin figures as well as lysosome-like bodies of pleomorphic appearance.

The β-cells were separated from fenestrated capillaries by a sparse extracellular space. The latter was chiefly occupied by slender cytoplasmic protrusions from connective-tissue cells, by occasional non-myelinated nerve fibrils and by the attenuated basal lamellae of the capillary. β-Cell granules were frequently observed in close proximity to the plasma membrane facing the capillary. However, only rarely were configurations observed indicative of a fusion between the perigranular and plasma membranes (emiocytosis).

Those β-cells which showed certain degenerative features already in the light-microscope exhibited gross morphological changes in the electron microscope. A portion of such a cell in a chloromercuribenzene-*p*-sulphonic acid-treated islet is depicted in Plate 1(*e*). The nuclei of these cells were pycnotic and the cytoplasmic ground substance was almost completely leached out. The cells contained distorted portions of endoplasmic reticulum with adhering ribosomes, swollen mitochondria, unidentifiable membrane fragments and numerous secretory granules. The last-named were almost always still enclosed in their membrane-delimited sacs. The changes described were frequently localized to singly dispersed cells surrounded by β-cells of a completely normal appearance. Even when necrotic cells occurred in clusters, the surrounding β-cells looked perfectly normal.

Discussion

It appears to be a fairly general phenomenon that regulatory proteins such as enzymes and transport molecules depend on the integrity of thiol groups to exert full activity. This property has been frequently exploited in attempts to elucidate the topography of specific cell functions with the aid of thiol-reacting agents (Rothstein, 1970). Such agents differ characteristically in their ability to penetrate plasma membranes. A comparison of their effects on a certain thiol-dependent cell function may therefore indicate whether it is located in the plasma membrane or in the interior of the cell. It is even possible to carry the analysis so far as to correlate distinct functions with different layers of the membrane.

In the present study we took advantage of the fact that both *p*-chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid possess a high degree of specificity for thiol groups with which they form easily reversible mercaptides (Webb, 1965). Further, both these organic mercurials are known to enter cells relatively slowly. The poorest penetrating ability is displayed by chloromercuribenzene-*p*-sulphonic acid, presumably owing to its lipid solubility being decreased by the sulphonic acid group (Rothstein, 1970). This difference between *p*-chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid may explain why in the present study, although both compounds appeared equally effective as inhibitors of glucose transport, *p*-chloromercuribenzoic acid had a somewhat stronger inhibitory effect on glucose oxidation. Previous experiments with phlorrhizin have shown that there is no tight link between glucose transport and glucose oxidation in mammalian pancreatic β-cells (Hellman *et al.*, 1971*b*).

Although inhibition experiments with phlorrhizin and mannoheptulose indicated that insulin release is not governed by the total glucose transport (Hellman *et al.*, 1971*b*, 1972), the question remains whether insulin release is triggered by the binding of glucose to membrane receptors that are not transport sites or are responsible for only a fraction of the total glucose transport. Even if there is no direct evidence for such glucose receptors, their existence may explain why under appropriate conditions insulin release can be stimulated by phlorrhizin (Hellman *et al.*, 1971*b*), mannoheptulose (Kanazawa *et al.*, 1971), glucosamine (Coore & Randle, 1964; Lambert *et al.*, 1969; Landgraf *et al.*, 1971) and galactose (Lambert *et al.*, 1969; Landgraf *et al.*, 1971). The possibility that thiol groups may be essential for the function of such a hypothetical receptor initiated the present study. If glucose-stimulated insulin release could be blocked by the slowly penetrating chloromercuribenzene-*p*-sulphonic acid, this would provide suggestive evidence for the location of the receptor at the superficial layer of the β-cell plasma membrane. The results show that far from inhibiting insulin release, even temporarily, both *p*-chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid caused a rapid liberation of the hormone, resulting in a brisk initial rise followed by a sustained release at a suprabasal rate.

The value of this observation depends critically on the nature of the stimulated release. Since thiol-reacting compounds can increase the permeability of plasma membranes (Watkins *et al.*, 1970, 1971; Rothstein, 1970), the question arises whether the results were due simply to a passive diffusion of

insulin through a damaged and leaky β-cell membrane. Five observations are against such a trivial interpretation. (1) *p*-Chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid caused a pronounced insulin release at concentrations much lower than those producing a significant β-cell uptake of mannitol and sucrose. (2) Insulin release occurred within 60s after the islets were exposed to *p*-chloromercuribenzoic acid or chloromercuribenzene-*p*-sulphonic acid, whereas permeability to the much smaller sucrose molecule was not observed during the first 5min. (3) The omission of Ca^{2+}, as well as the addition of adrenaline, diazoxide or 2,4-dinitrophenol, significantly inhibited insulin release in response to chloromercuribenzene-*p*-sulphonic acid. These four modifications of the medium are all known to inhibit glucose-stimulated insulin release (Grodsky & Bennett, 1966; Coore & Randle, 1964; Malaisse & Malaisse-Lagae, 1968; Lernmark & Hellman, 1970). (4) Chloromercuribenzene-*p*-sulphonic acid appeared to affect the β-cell membrane rather mildly, since the induced insulin release was readily reversible. (5) Electron microscopy revealed that the majority of β-cells were morphologically intact after exposure to chloromercuribenzene-*p*-sulphonic acid for as long as 60min. For these reasons we are inclined to assume that at least the early effects of organic mercurials reflect some property of the physiological release mechanism. There were, however, signs of a certain increase in the proportion of necrotic β-cells after incubation with chloromercuribenzene-*p*-sulphonic acid for 60min. It is possible therefore that passive leakage from necrotic cells may have contributed to the insulin release measured over the long periods of time. This possibility could perhaps in part explain why insulin release in response to chloromercuribenzene-*p*-sulphonic acid was not completely inhibited by adrenaline, diazoxide or 2,4-dinitrophenol, or by the omission of Ca^{2+}.

The mechanism of normal insulin release may be viewed as being composed of three major components: a sensory mechanism by which the β-cells recognize and measure the stimulus; a coupling device, which provides a causal link between the sensory and discharge mechanisms; the apparatus for discharge of insulin from the cell. We wish to suggest that binding of organic mercurials to thiol groups caused the activation of one of these physiological components. The prompt action of chloromercuribenzene-*p*-sulphonic acid strongly indicates that the thiol groups involved in this activation are located near the surface of the β-cells. These thiol groups need not themselves be directly engaged in the physiological sequence of events leading to insulin release, although the simplest hypothesis at present would be that they are.

It would seem possible that the recognition of various insulin secretagogues may involve superficial thiol groups, and that the insulin-releasing effect of *p*-chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid may be due to a stimulatory interaction with these groups. However, there is as yet insufficient evidence to postulate that such thiol groups have the nature of direct-receptor sites for glucose. A qualitative difference between the action of glucose on the one hand and that of organic mercurials on the other is suggested by the observation that the initial effect of chloromercuribenzene-*p*-sulphonic acid was not blocked by the absence of Ca^{2+}. In view of the hypothesis that glucose stimulates insulin release by virtue of its metabolism (see, e.g., Ashcroft *et al.*, 1970), it is notable that low concentrations of *p*-chloromercuribenzoic acid or chloromercuribenzene-*p*-sulphonic acid stimulated glucose oxidation in our experiments. As pointed out above, the late phase of chloromercuribenzene-*p*-sulphonic acid-induced insulin release may to a certain extent represent leakage of insulin from damaged β-cells. The fact that glucose oxidation was measured over a period of 60min makes it therefore impossible to evaluate appropriately the degree of correlation between glucose oxidation and insulin release. It is quite possible that the moderate stimulation of glucose oxidation recorded over 60min represents an average of a much greater initial stimulation and a subsequent inhibition. Such an interpretation would accord with the observed latency of the effects of chloromercuribenzene-*p*-sulphonic acid on glucose transport and membrane permeability and by the significant inhibition of glucose oxidation found with higher concentrations of organic mercurials. Technical difficulties have so far precluded a study of the dynamics of glucose oxidation in isolated islets.

Several authors have suggested that calcium is essential for stimulus–secretion coupling (Douglas, 1968; Malaisse *et al.*, 1970; Milner & Hales, 1970). Observing that the omission of Ca^{2+} did not inhibit growth-hormone release in response to *p*-chloromercuribenzoic acid, Schofield (1971) suggested that *p*-chloromercuribenzoic acid could act on the coupling system by removing its calcium-sensitivity. The effects of thiol reagents on contractile proteins in muscle (Mueller, 1966) and sea-urchin eggs (Sakai, 1968) provided circumstantial support for this idea. In the present study, chloromercuribenzene-*p*-sulphonic acid could not reverse the inhibition of glucose-stimulated insulin release caused by calcium deficiency. It seems therefore necessary to assume that the organic mercurials did not act as a proxy for Ca^{2+}. In view of the postulated role of ions in stimulus–secretion coupling (Douglas, 1968; Malaisse *et al.*, 1970; Milner & Hales, 1970) it is notable that organic mercurials are known to influence the distribution of ions across cell membranes (Rothstein, 1970; Solberg & Forte, 1971). Measurements of the

membrane potential in β-cells may help to answer the question of whether the insulin-releasing action of organic mercurials was mediated by ionic fluxes.

It has been suggested that insulin can bind to membrane thiol groups in target cells, although such binding may not be a prerequisite for hormone action (Rieser, 1967; Jost *et al.*, 1968; Cuatrecasas, 1971). Provided that this idea is tenable with regard to the tertiary structure of native insulin (cf. Adams *et al.*, 1969), insulin could perhaps bind to the β-cell surface in a similar manner. Organic mercurials might then act by displacing insulin from the β-cell plasma membrane.

The present results are in conflict with a previous report that *p*-chloromercuribenzoic acid inhibited insulin release from the rat pancreas (Chiba, 1969). It is difficult to evaluate this observation, since insulin was determined by bioassays which may have been influenced by *p*-chloromercuribenzoic acid. The validity of the present results is supported by reports of a stimulatory action of *p*-chloromercuribenzoic acid on both the anterior (Schofield, 1971) and the posterior (Douglas *et al.*, 1965) pituitary gland. In Schofield's (1971) experiments the threshold for stimulation was 2μM-*p*-chloromercuribenzoic acid. It is somewhat difficult to compare this value with our own dose–response results, since our measurements of insulin release were performed in the presence of albumin. The well-known fact that albumin reacts with thiol reagents led us to attempt excluding albumin from the media. It was found that some albumin was a prerequisite for correct recoveries of insulin from the incubation vessels. When incubation was performed in the presence of only 0.1mg of albumin/ml, a significant stimulation was obtained with 10μM-*p*-chloromercuribenzoic acid. Considering that the effective concentration of *p*-chloromercuribenzoic acid may have been decreased by the presence of albumin, this value is in fair agreement with the threshold reported by Schofield (1971).

It has previously been reported that *p*-chloromercuribenzoic acid is exceptional among several thiol reagents in not affecting the permeability of fish islet cells (Watkins *et al.*, 1970). Our observations of increased mannitol and sucrose spaces in mouse islets exposed to chloromercuribenzene-*p*-sulphonic acid for 30min do not necessarily suggest that there are significant structural differences between the plasma membranes of fish and mammalian β-cells. Fish islets are known to contain relatively few β-cells (Falkmer, 1961), whereas the presently investigated islets represent a fairly pure β-cell population (Hellman, 1965).

This work was supported by the Swedish Medical Research Council (Grants 12x-562 and 12x-2288) and the City Council of Umeå. The skilful technical assistance of Miss Marianne Borg, Mrs. Lilian Karlsson and Miss Eva Lundgren is also gratefully acknowledged.

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