PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **189**, 72–78 (1988)

# $^3$H-Cyclosporine Internalization and Secretion by Human Fetal Pancreatic Islets (42782)

BENT FORMBY, LIBERTY WALKER, AND CHARLES M. PETERSON

*Sansum Medical Research Foundation, 2219 Bath Street, Santa Barbara, California 93105*

*Abstract.* Human fetal pancreatic islets were isolated from 16- to 20-week-old fetuses by a collagenase technique and cultured 48 hr in RPMI 1640 containing 10% human adult serum and unlabeled 0 to 5 μg cyclosporine A (CsA)/ml. Insulin secretory capacity of human fetal islets was expressed as a fractional stimulatory ratio FSR = $F_2/F_1$ of the fractional secretion rates during two successive 1 hr static incubations first with 2 m*M* glucose ($F_1$) to stabilize secretion followed by maximal stimulus, i.e., 25 m*M* glucose plus 10 m*M* L-leucine and 10 m*M* L-arginine ($F_2$). Unlabeled CsA at the above concentrations had no significant effects on the insulin secretory capacity expressed by FSR-values. Studies of net uptake of $^3$H-CsA by islets cultured for varying periods up to 40 hr and expressed as picomole $^3$H-CsA per picomole islet insulin content demonstrated that uptake rate was slow and did not reach isotopic equilibrium over the 40 hr of culture. When isolated fetal islets were cultured for 48 hr in the presence of $^3$H-CsA and varying concentrations of unlabeled CsA it was found during two successive 1 hr static incubations that fetal islets secrete insulin concomitantly with $^3$H-CsA following maximal stimulus for secretion. An optimal secretory molar ratio of $^3$H-CsA to insulin of 4.0 ± 1.3 ($n$ = 7) was found after islets were cultured 48 hr in the presence of a saturating 2.128 μg $^3$H-CsA per milliliter culture medium. In three successive 30-min static incubations of $^3$H-CsA loaded islets, first with low glucose, followed by high glucose plus L-arginine and L-leucine, and finally with high glucose plus L-arginine and L-leucine and 10 m*M* theophylline, the proportional fractional secretion rates of insulin and $^3$H-CsA were of the same magnitude. It is concluded that human fetal pancreatic islets during 48 hr of culture in the presence of pharmacologically relevant concentrations of CsA can internalize the drug, which is compartmentalized and concomitantly secreted with insulin following maximal stimuli. Transplanted human fetal islets utilized as delivering units for CsA could be beneficial for the induction of immunotolerance to allografted fetal islets. 

Cyclosporine A (CsA), a cyclic undecapeptide ($M_r$ 1202) of fungal origin, is a powerful immunosuppressive drug of considerable clinical importance (1, 2). Most studies of its mechanism of action have focused on T cells as primary targets, and have demonstrated that CsA inhibits proliferation of helper T cells, probably via inhibition of interleukin-2 (IL-2) production, but does not affect suppressor T cell activation (3, 4).

Patients receiving CsA by oral or intravenous administration have showed increased susceptibility to lymphomas and various opportunistic infections (5, 6). Recently, other methods for delivering CsA have been reported. Thus Hsieh *et al.* (7) demonstrated that CsA can be taken up by liposomes, which in turn may mediate immunosuppression with reduced nephrotoxicity. Sharpe *et al.* (8) were able to show that platelets that took up CsA retained their viability and released CsA into plasma. Since immune rejection is the major obstacle to successful transplantation of allogeneic fetal islets as treatment of type I diabetes mellitus (for review see (9)), a potential solution to protect transplanted islets from being destroyed by the host's immune system would be to utilize the islets themselves as a drug-delivering system without otherwise impairing their insulin secretory capacity.

In this report, we studied *in vitro* the effect of CsA on the insulin secretory capacity of human fetal pancreatic islets and their ability during 48 hr of culture to internalize $^3$H-CsA and after culture to utilize the insulin secretory machinery to secrete the drug.

**Materials and Methods.** Cyclosporine A (Sandoz, Basle, Switzerland) was dissolved in ethanol and slowly added to RPMI 1640 (GIBCO) to concentrations of 0.1 to 5.0 μg/ml. The corresponding dilutions of eth-



0037-9727/88 $1.50

anol were added to the control media. $^{3}$H-cyclosporine A (sp radioact 9 $\mu$Ci/$\mu$g) was kindly supplied by Dr. R. Voges (Sandoz, Basel, Switzerland).

The human fetal pancreata used in this study were obtained from the National Disease Research Interchange (Philadelphia, PA) following dilation and extraction. Gestational age ranged from 16 to 20 weeks. Gestational age was verified by sonography, maternal history, and fetal foot length. Pancreata were aseptically removed from surrounding tissue and immediately stored in ice-cold (0–2°C) sterile RPMI 1640 medium (GIBCO) containing 25 m*M* Hepes and 2 m*M* glutamine, pH 7.4. All islet studies were performed with fetal tissue which had been cold-stored from 18 to 24 hr as described previously (10).

*Isolation of pancreatic islets.* Prior to culture pancreata were dissected free from attachments to spleen, liver, and duodenum and chopped into fine fragments. The tissue was digested in warm (38°C) magnesium-free Hanks' buffered salt solution (HBSS) containing 7 mg/ml collagenase (Sigma grade V), 5 m*M* glucose, and 1% human albumin for 50 min. The digest was washed three times with ice-cold HBSS and samples of islets were selected under a microscope and transferred to petri dishes and cultured at 37°C in 95% air/5% $CO_2$ with RPMI 1640 supplemented with 10% human adult serum and unlabeled or radioactive CsA according to experimental protocols. A culture period of 48 hr was used and permitted further purification of islets from adherent nonislet debris. Prior to each static batch incubation cultured fetal islets were incubated in a solution of trypan blue containing 0.04% of the stain in isotonic Krebs–Ringer buffer (KRB) for 15 min and carefully washed several times in KRB. Viable unstained islets were selected as described previously (11). The large variation in the size of isolated islets (Fig. 1) precluded the expression of insulin secretory capacity as the amount of insulin secreted per islet. Therefore fractional insulin secretion rates were determined to quantify the insulin secretory capacity of a given fetal islet preparation (10).

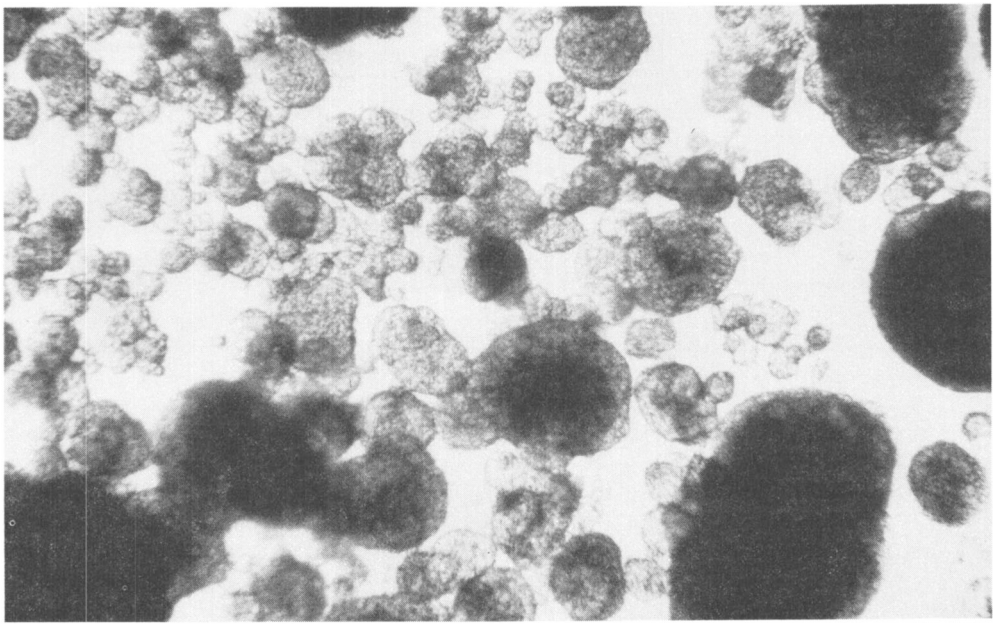

FIG. 1. Microscopic appearance of islets isolated by collagenase digestion of a single fetal pancreas and cultured for 48 hr in medium alone. Clean, intact, and viable sized islets are seen with diameters between 100 and 500 $\mu$m. Also islet-like cell clusters are seen. Unstained; magnification, ×60.

*$^3$H-Cyclosporine net uptake.* To assess total islet $^3$H-CsA net uptake as a function of time, islets were cultured for varying periods up to 40 hr. In each experiment islets were suspended in 5 ml of culture medium RPMI 1640 supplemented with 10% human adult serum plus 1.137 $\mu$g $^3$H-CsA (sp radioact 455 $\pm$ 20 cpm/ng, $n$ = 3) and aliquoted into 10 wells (24-well plates). At specific time intervals, islets were removed from the medium and washed three times in KRB. Islet content of insulin and internalized $^3$H-CsA was extracted with acidic ethanol overnight at 4°C. Insulin was assayed by radioimmunoassay and $^3$H-CsA by liquid scintillation counting (vide infra). Net uptake of $^3$H-CsA was expressed as picomole $^3$H-CsA per picomole of insulin.

*Determination of insulin secretory capacity.* Each experimental degree of freedom is represented by islets isolated from a single fetal pancreas. Insulin secretion from islets after culture in the presence of 0 to 5 $\mu$g unlabeled CsA/ml medium for 48 hr was performed as previously described by short-term static batch incubations which quantitate the capacity of the islets to secrete insulin in response to various stimuli (10). Briefly, for each experiment 200–300 trypan blue unstained islets which had been isolated and cultured from single pancreata were transferred to plastic tubes and incubated in 2.0 ml KRB, pH 7.40, containing 24 m*M* Hepes, 2 m*M* glucose, and 0.3% human serum albumin at 37°C in an atmosphere of 95% $O_2$/5% $CO_2$. After a first period of 1 hr to stabilize the basal secretion, the buffer was removed and fresh KRB buffer was added as above but with 25 m*M* glucose plus 10 m*M* L-arginine and 10 m*M* L-leucine to potentiate insulin secretion and the islets were reincubated for an additional hour. These latter conditions were necessary to measure maximal secretory capacity since we confirmed the observation of others (12) that human fetal islets do not respond optimally to high glucose alone. After each incubation, samples of buffer were removed and assayed in triplicate for insulin secreted by radioimmunoassay using human insulin as a reference standard, as were acidic ethanol extracts of the islets at the end of incubation.

*Calculation of fractional stimulatory ratio (FSR).* Insulin secretory capacity of the fetal islets was expressed as a fractional stimulatory ratio FSR = $F_2/F_1$ of the fractional secretion rates during the two successive 1 hr static batch incubations as described above, i.e., first with low glucose ($F_1$) followed by high glucose plus potentiators ($F_2$) (10). A FSR value of one theoretically represents no stimulation.

*Kinetics of insulin and $^3$H-CsA concomitantly secreted during two successive 1-hr static batch incubations.* Isolated fetal islets were cultured 48 hr in the presence of $^3$H-CsA (sp radioact 9 $\mu$Ci/$\mu$g) plus varying concentrations of unlabeled CsA. After this period culture medium and fetal islets were in isotopic equilibrium (see Results). Radioactivity in samples of the culture medium was determined by liquid scintillation counting and the various specific radioactivities of the media were expressed as counts per minute per nanogram CsA. After 48 hr of culture, trypan blue unstained islets were transferred to plastic tubes and two successive static batch incubations were performed as described above. After each incubation, samples of buffer were removed for determination of either insulin (ng/ml) or radioactivity (cpm/ml). Data of the amount of insulin (ng/ml) and $^3$H-CsA (ng/ml = cpm/ml divided by cpm/ng) released following maximal stimulus were obtained by subtracting the basal values of insulin and $^3$H-CsA released during the first hour of incubation from the values obtained during the second hour of incubation. Because of the relative large variability in islet cell mass harvested from the various fetal pancreata and likewise variability in the actual values of concomitantly secreted insulin and CsA, the molar ratio of CsA to insulin secreted following maximal stimulus was calculated ($M_r$ (CsA) = 1202, $M_r$ (insulin) = 6000). The molar ratio theoretically represents the capacity of a single $\beta$-cell to secrete internalized CsA following maximal stimulus after 48 hr of culture during which isotopic equilibrium is obtained with a given total $^3$H-CsA concentration in the medium. Only experiments to quantitate $^3$H-CsA concomitantly secreted with insulin following maximal stimulus

TABLE I. PROFILES OF CYCLOSPORINE A EFFECTS ON INSULIN FRACTIONAL STIMULATORY RATIO (FSR) OF HUMAN FETAL PANCREATIC ISLETS AFTER 48 hrs OF CULTURE

| CsA μg/ml culture medium | 0 | 0.1 | 1.0 | 5.0 |
|---|---|---|---|---|
| FSR | 3.1 ± 1.0[a] | 3.7 ± 0.8* | 2.6 ± 0.9* | 3.2 ± 0.5* |
| Gestational age | 16.3 ± 2.3 | 15.3 ± 0.6 | 17.8 ± 1.9 | 15.5 ± 0.6 |
| Percentage trypan blue exclusion | 83.2 ± 4.4 | 79.6 ± 6.9 | 85.5 ± 2.9 | 79.8 ± 5.1 |

[a] Values are means ± SD, $n = 4$.
* NS versus control.

were performed. Compartmentalizations of $^3$H-CsA in other intracellular pools than those confined to secretion of insulin were not investigated.

*Kinetics of insulin and $^3$H-CsA concomitantly secreted during three 30-min successive static incubations.* Isolated fetal islets were cultured 48 hr in the presence of 1.137 μg $^3$H-CsA (sp radioact of medium 439 ± 19 cpm/ng, $n = 3$). After culture, trypan blue unstained islets were transferred to plastic tubes and three successive 30-min static incubations were performed as described above. After a first period of 30 min in KRB with 2 m*M* glucose to stabilize the basal secretion, buffer was removed and fresh KRB was added with 25 m*M* glucose plus 10 m*M* L-arginine and 10 m*M* L-leucine during the second incubation period. The third incubation period was with 25 m*M* glucose plus 10 m*M* L-arginine and 10 m*M* L-leucine and 10 m*M* theophylline (13). After each incubation period, samples of buffer were removed for determination of either insulin or radioactivity as described above.

*Statistical analysis.* Data are expressed as means ± SD. Student's unpaired *t* test was used for comparison of data.

**Results.** No significant changes were found in insulin secretory capacity expressed by FSR-values after fetal islets were cultured for 48 hr in the presence of 0 to 5 μg unlabeled CsA/ml medium as shown in Table I. Islet variability was not affected by CsA as documented by the trypan blue exclusion data. Also shown in Table I are mean gestational age of fetuses used in the various experiments, because we previously have reported an effect of gestational age on insulin secretory capacity, which was significantly twofold higher in younger (16–18 weeks old) in comparison to older (20–24 weeks old) fetal islets (10). Table II shows the net uptake of $^3$H-CsA expressed as picomole $^3$H-CsA per picomole islet insulin content by batches of cultured islets. Uptake was slow and isotopic equilibrium between medium and islets was not convincingly achieved after a culture period of 40 hr. No corrections were made for $^3$H-CsA accummulated in the extracellular space of the islets. Shown in Table III are data of the proportional secretions of insulin and $^3$H-CsA from the same batch incubation in response to two different stimulatory mixtures, i.e., 25 m*M* glucose plus 10 m*M* L-arginine and 10 m*M* L-leucine (mix 1) followed by 25 m*M* glucose plus 10 m*M* L-arginine and 10 m*M* L-leucine with 10 m*M* theophylline (mix 2). The fractional secretion rates (ng/30 min) of insulin and $^3$H-CsA were of the same magnitude in mix 1 and mix 2, respectively. Statistical significant higher fractional secretion rates for both insulin and

TABLE II. EFFECT OF TIME ON NET UPTAKE OF $^3$H-CYCLOSPORINE BY HUMAN FETAL PANCREATIC ISLETS EXPRESSED AS PICOMOLE $^3$H-CYCLOSPORINE PER PICOMOLE ISLET INSULIN CONTENT

| Time (hr) | Uptake | *N* |
|---|---|---|
| 0.5 | 0.6 ± 0.15[a] | 3 |
| 1.0 | 0.8 ± 0.06 | 3 |
| 2.0 | 1.0 ± 0.26 | 3 |
| 6.0 | 1.1 ± 0.35[b] | 3 |
| 16.0 | 1.7 ± 0.64[c] | 3 |
| 24.0 | 2.0 ± 0.61 | 3 |
| 40.0 | 3.4 ± 1.2[d] | 3 |

*Note.* *N*, number of fetal pancreata and experiments. [a]vs[b], NS. [a]vs[c], $P < 0.05$. [c]vs[d], NS.

TABLE III. PROPORTIONAL SECRETION OF INSULIN AND $^3$H-CYCLOSPORINE FROM HUMAN FETAL PANCREATIC ISLETS

| Experiment number | Insulin secreted (ng/30 min) | | | Nonsecreted insulin (ng) | $^3$H-CyA secreted (ng/30 min) | | | Nonsecreted $^3$H-CyA (ng) | Fractional secretion of insulin (%) | | Fractional secretion of $^3$H-CyA (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | *A* | *B* | *C* | *E* | $\frac{B\text{-}A}{D} \times 100$ | $\frac{C\text{-}A}{D} \times 100$ | $\frac{B\text{-}A}{E} \times 100$ | $\frac{C\text{-}A}{E} \times 100$ |
| 1 | 9.2 | 15.2 | 49.0 | 2237 | 6.3 | 8.6 | 15.4 | 456 | 0.3 | 1.5 | 0.5 | 2.0 |
| 2 | 1.4 | 8.3 | 20.0 | 765 | 15.0 | 19.8 | 24.9 | 523 | 0.8 | 2.4 | 0.9 | 1.9 |
| 3 | 0.8 | 5.0 | 12.2 | 990 | 16.9 | 18.7 | 32.3 | 697 | 0.4 | 1.2 | 0.3 | 2.2 |
| | | | | | Mean ± SD | | | | $0.50 \pm 0.27^a$ | $1.70 \pm 0.62^b$ | $0.57 \pm 0.31^c$ | $2.03 \pm 0.15^d$ |

*Note.* Static batch incubations were performed in the following buffered solutions (see also Methods): *A*, 2 m*M* glucose. *B*, 25 m*M* glucose plus 20 m*M* L-leucine and 20 m*M* L-arginine. *C*, as *B* with 10 m*M* theophylline. Specific radioactivity in medium: 439 ± 19 cpm/ng. $^a$vs$^b$, $P < 0.04$. $^a$vs$^c$, NS. $^c$vs$^d$, $P < 0.002$. $^b$vs$^d$, NS.

$^3$H-CsA were found in response to mix 2 in comparison with mix 1 (Table III). Table IV shows results of experiments with fetal islets cultured for 48 hr in the presence of 0.128 to 5.137 μg $^3$H-CsA/ml. An optimal secretory molar ratio of $^3$H-CsA to insulin following maximal stimulus of 4.00 ± 1.3 ($n$ = 7) was found with 2.128 μg $^3$H-CsA/ml. No significant change in the molar ratio was calculated when $^3$H-CsA concentration during culture was increased to 5.137 μg/ml. Thus trypan blue viable human fetal islets can internalize and compartmentalize $^3$H-CsA and finally secrete the drug by the mechanism used for secretion of insulin. With the experimental conditions described here islets can maximally secrete four molecules of $^3$H-CsA concomitantly with one molecule of insulin following maximal stimulus.

**Discussion.** Adverse effects of high doses of CsA on rodent islets *in vivo* and *in vitro* have been reported (14, 15). On the contrary, Laube *et al.* (16) did not find toxic effects on the endocrine adult rat pancreas, when exposed to CsA concentrations up to 2000 ng/ml. Human adult islets cultured for 5 days in 100 ng CsA/ml showed a reduction in insulin release into the medium and a concomitant increase in residual insulin content. These toxic effects were partly reversed 48 hr after removal of the drug (17). Similar studies with human fetal islets have not been reported. We found that culture of human fetal islets for 48 hr in the presence of even higher concentrations of CsA resulted in no adverse effect on the insulin secretory capacity of the islets or on islet viability as assessed by trypan blue exclusion.

Studies with adult rodent islets strongly have suggested a correlation between insulin secretion in response to glucose and rates of glucose oxidation (18). A similar secretory mechanism may not be present in human fetal islets because they fail to respond to a glucose stimulus by increased insulin release although it is evident that glucose oxidation is intact (19). We have obtained an effective stimulus coupled to insulin secretion by isolated human fetal islets with a combination of L-leucine and L-arginine plus high glucose as metabolic fuel supply. With this approach, a maximal stimulatory effect of two- to four-

TABLE IV. EFFECT OF INCREASING CONCENTRATIONS OF TOTAL $^3$H-CsA DURING 48 hr OF CULTURE ON THE MOLAR RATIO OF $^3$H-CsA TO INSULIN CONCOMITANTLY SECRETED FROM HUMAN FETAL ISLETS DURING STATIC INCUBATIONS FOLLOWING MAXIMAL STIMULUS

| μg $^3$H-CsA per ml culture medium | Molar ratio $^3$H-CsA/insulin* | *n* |
|---|---|---|
| 0.125 | 1.13 ± 0.6 | 5 |
| 0.251 | $1.41 \pm 0.6^a$ | 7 |
| 1.128 | $2.53 \pm 1.0^b$ | 4 |
| 2.128 | $4.00 \pm 1.3^c$ | 7 |
| 5.137 | $4.33 \pm 1.1^d$ | 4 |

*Note.* *(see methods). *n*, number of fetal pancreata and experiments. $^a$vs$^b$, $P < 0.05$. $^b$vs$^c$, $P < 0.05$. $^c$vs$^d$, NS.

fold over basal secretion dependent on the gestational age of the fetal islets (10) is achieved. Therefore the molecular discrepancy between the insulin secretory mechanisms of adult rodent or human islets versus the human fetal islets may explain their different response to CsA whether evaluated by insulin released into culture medium or quantified by insulin secretory capacity.

The mechanisms involved in CsA internalization and compartmentalization in human fetal islets represent an important challenge. CsA is a highly lipophilic molecule and it is tempting to speculate that the drug initially is concentrated in the Golgi apparatus which itself is lipophilic (20). Our data of the net uptake of $^{3}$H-CsA by fetal islets indicate that isotopic equilibrium is not reached by 40 hr of culture. A relative long time to achieve maximal loading of the islets with CsA may point to an early structural connection in the endoplasmatic reticulum before CsA is routed into developing secretory units and finally exported throughout the cell. That during various maximal stimulatory conditions for insulin secretion, $^{3}$H-CsA and insulin are concomitantly released at fractional secretion rates of the same magnitude, assist in the interpretation of our data, i.e., CsA is compartmentalized with insulin in the secretory machinery. At conditions after 48 hr of culture the ratio of secreted CsA to insulin is four.

Since CsA has been reported to be metabolized only by the cytochrome *P*-450 oxidative system in the adult liver (21), we assume that CsA was not metabolized by fetal islets and thus, the internalized and secreted radioactivity measured is in fact CsA-related radioactivity.

The immunogenicity of allogeneic transplanted human fetal islets involves constant risks of lymphocytic infiltration of the graft and its final rejection by the recipient. Our observation that human fetal islets can internalize and secrete CsA introduces the possibility of obtaining a strictly localized immunosuppressive effect. The potential relevance of these findings to transplantation has to be demonstrated, but is heightened by our recent report that cell-associated CsA delivery by donor cells in a mixed lymphocyte culture system results in more specific and enhanced tolerance than that achieved by adding CsA directly to the media (22). Finally CsA has been reported to inhibit the induction of Class I and Class II MHC antigens in heart and kidney allografts in the rat (23). The influence of internalized CsA on the process of MHC antigen induction in fetal islets by INF-$\gamma$ is currently under our investigation.

This investigation was supported by a grant from the American Diabetes Association, Southern California Affiliate.

1. Cohen DJ, Loertscher R, Rubin MF, Tilney L, Strom TB. Cyclosporine: A new immunosuppressive agent for organ transplantation. Ann Intern Med **101**:667–692, 1984.
2. Shevach EM. The effects of cyclosporine A on the immune system. Annu Rev Immunol **3**:397–423, 1985.
3. Gauchat JF, Khandjian EW, Weil R. Cyclosproine A prevents induction of the interleukin 2 receptor gene in cultured murine thymocytes. Proc Natl Acad Sci USA **83**:6430–6434, 1986.
4. Kupiec-Weglinski JW, Filho MA, Strom TB, Tilney NL. Sparing of suppressor cells a critical action of cyclosporine. Transplantation **38**:97–101, 1984.
5. Bird AG, Maclachan SM. Cyclosporine A and Epstein–Barr virus. Lancet **2**:418, 1980.
6. Hughes WT, Smith B. Provocation of infection due to Pneumocytis Carinii by cyclosporine A. J Infect Dis **145**:767–772, 1982.
7. Hsieh H, Schreibert M, Stowe N. The use of liposome encapsulated cyclosporine in a rat model. Transplant Proc **17**:1397–1399, 1985.
8. Sharpe RJ, Schweizer TT, Moore RE, Calaluce RD. Evidence that cyclosporine-induced platelets retain function and release cyclosporine. Transplantation **40**:102–103, 1985.
9. Peterson CM, Jovanovic-Peterson L, Formby B, Eds. Fetal islet transplantation: Implications for diabetes. Springer-Verlag, 1988.
10. Formby B, Walker L, Peterson CM. Effects of duration of cold storage and gestational age on the insulin secretory capacity of human fetal pancreatic islets. Diabetes Res **4**:113–116, 1987.
11. Formby B, Walker L, Peterson CM. Rapid selection of viable transplantable human fetal pancreatic islets by trypan blue exclusion. Proc Soc Exp Biol Med **182**:245–247, 1986.
12. Hoffman L, Mandel TE, Carter WM, Koulmanda M, Martin FIR. Insulin secretion by human fetal pancreas in organ culture. Diabetologia **23**:426–430, 1982.
13. Maitland JE, Parry DG, Turtle JR. Perifusion and

culture of human fetal pancreas. Diabetes **29**(Suppl 1):57–63, 1980.
14. Robertson RP. Cyclosporine-induced inhibition of insulin secretion in isolated rat islets and HIT cells. Diabetes **35**:1016–1019, 1986.
15. Hellmchen U, Schmidt WE, Siegel EG, Creutzfeldt W. Morphological and functional changes of pancreatic B cells in Cyclosporine A-treated rats. Diabetologia **27**:416–418, 1984.
16. Laube F, Blech W, Schroder D, Besch W, Hildebrandt W, Hahn HJ. The action of cyclosporine A on the endocrine pancreas *in vitro*—Functional and morphological studies. Exp Clin Endocrinol **87**:69–78, 1986.
17. Nielsen JH, Mandrup-Poulsen T, Nerup J. Direct effects of cyclosporine A on human pancreatic B cells. Diabetes **35**:1049–1052, 1986.
18. Hedeskov CJ. Mechanisms of glucose-induced insulin secretion. Physiol Rev **60**:442–509, 1980.
19. Hoffman L. Intact glucose oxidation in human fetal pancreas. Diabetologia **28**:386–87, 1985.
20. Orci L. Macro- and micro-domains in the endocrine pancreas. Diabetes **31**:538–582, 1982.
21. Cunningham C, Burke MD, Wheatley DN, Thomson AW, Simpson JG, Whiting PH. Amelioration of cyclosporine-induced nephrotoxicity in rats by induction of hepatic drug metabolism. Biochem Pharmacol **34**:573–578, 1985.
22. Christiaansen JE, Schmid-Formby F, Peterson CM. Relative antigen-specific suppression *in vitro* of alloresponding human leukocytes by cellular delivery of cyclosporine. Transplantation **44**:119–123, 1987.
23. Milton AD, Spencer SC, Fabre JW. The effects of cyclosporine on the induction of donor class I and class II MHC antigens in heart and kidney allografts in the rat. Transplantation **42**:337–347, 1986.

---

Received October 16, 1987, P.S.E.B.M. 1988. Vol. 189.
Accepted June 10, 1988.