Growth and Hormonal Content of Human Fetal Pancreas Passaged in Athymic Mice

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SUMMARY

While the usefulness of the human fetal pancreas transplanted into diabetic humans has yet to be realized, its transplantation into nude mice has revealed some of its potential. In this animal the organ grows in size, during which time differentiation of its endocrine component and maturation of the insulinogenic response to glucose occurs. The results reported in this article expand these results by providing data on the weight and both the insulin and glucagon content of these passaged organs.

Human fetal pancreata of gestational age 14–19 wk were implanted subcutaneously (s.c.) in nude mice and maintained in vivo for 5–54 wk (absolute age 19–68 wk). The implants were then removed and their insulin and glucagon content determined. Both the weight of these implants and their insulin content were positively correlated with the absolute age of the tissue (weight: $r = 0.61$, $P < 0.001$; insulin content: $r = 0.62$, $P = 0.0003$). The glucagon content bore no relationship to the age. The maximum level of insulin extracted from 30 passaged human fetal pancreata was 0.8 U, a level far below the daily insulin requirements in adult humans. It is suggested that an explanation for this is the site of transplantation used. To compare the s.c. site with the renal subcapsular space, the explants of four fetal pancreata were evenly divided between these two sites. After 11–13 wk, the implants were removed. Those beneath the renal capsule were larger and contained a greater amount of insulin and glucagon than those transplanted s.c.

It is concluded that: (1) the human fetal pancreas implanted in the nude mouse grows and its insulin content increases with time, and (2) the renal subcapsular space is a better site for transplantation in these animals than a s.c. site. DIABETES 1986; 35:464–69.

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Human fetal pancreas has been transplanted into insulin-dependent diabetic humans since the 1970s in an attempt to ameliorate or cure the disorder of carbohydrate metabolism.[1] The success rate has been poor with only 2 of 120 patients being able to cease their exogenous insulin requirements.[2,3] There are four possible explanations for this low success rate: (1) rejection of the transplanted tissue, (2) insufficient endocrine tissue present in the implant, (3) failure of the explants to adapt to their new environment in the recipient, and (4) failure of beta cells to mature and release insulin in response to a glucose stimulus.

In an attempt to bypass the problem of rejection in diabetic humans,[4] the nude athymic mouse has been used as an animal model for xenografting human fetal pancreas of 14–20 wk gestational age. Explants of such tissue maintained in the mouse for periods of up to 37 wk have been shown to adapt to the new environment by coalescing into the one implant and increasing in size thereafter.[5] The fetal pancreas selectively differentiated into endocrine tissue, ducts, and fibrous tissue with acini being undetectable. Alpha, beta, and delta cells were observed in these implants as single cells or groups of cells budding from ducts, as well as in islets. When implants were removed and exposed in vitro to glucose, they displayed maturation of their insulinogenic response, especially when the absolute age was >55 wk (absolute age = gestational age + period in nude mouse).[6] Tissue from a single pancreas maintained in the mouse for 3–6 mo was able to normalize the hyperglycemia previously induced in the animal by administration of streptozocin.[6]

Studies with rodent fetal pancreas have shown that reversal of diabetes is possible, even with an allograft of as little as one-third of a mouse fetal pancreas.[7] Such implanted tissue also showed selective histologic differentiation into endocrine tissue ducts and fibrous tissue[8–10] and maturation of the insulinogenic response to glucose.[11–13] The insulin content of implants from the fetal mouse increased as their age ad-

vanced, eventually reaching and surpassing the insulin content of an adult mouse pancreas.[10]

It was the chief aim of this study to investigate whether the insulin content of human fetal pancreas implanted in nude mice would increase as the absolute age of the tissue advanced and whether the level would reach the 24–50 U that is secreted daily from the pancreas of an adult human.[14,15]

MATERIALS AND METHODS

Human fetal pancreas. Human fetal pancreata were obtained from the therapeutic termination of pregnancies carried out by suction curettage or prostaglandin induction between 14 and 19 wk gestation. They were diced into 1- to 2-mm^3 explants and washed three times with 0.9% saline to remove any microbiologic contamination.

Nude mice. The nude mice were crossbred from a Balb/c background and maintained in a pathogen-free environment. They were given sterile food (Barastoc irradiated feed) and water ad libitum.

Subcutaneous (s.c.) transplantation. The explants of 30 human fetal pancreata were transplanted s.c. above the iliac fossae of 25 nude mice aged 96 ± 21 days (mean ± SD), five of the animals receiving a pancreas in each iliac fossa. While the animals were still under the influence of the anesthetic (sodium pentobarbitone 65 mg/kg), their wounds were sutured.

Hormonal implants. Between 36 and 377 days after the initial surgery, the mice were killed so that their absolute age ranged from 19 to 68 wk. The tissue was weighed on its removal, freeze-dried, and reweighed before being homogenized in acid/ethanol (18 ml of 10M HCl/1 ml of 70% ethanol). Insulin and glucagon were extracted overnight at 4°C. The supernatant was stored at −20°C until it was assayed for insulin (RD12 Wellcome binding reagent) and glucagon (K5563 Novo antibody). Acid/ethanol did not interfere with either assay when diluted to the same extent as were the supernatants (1/20 to 1/1000).

Hormonal content of human fetal pancreas. For controls, 56 human fetal pancreata of gestational age 14–19 wk were obtained as described above. They were weighed and seventeen of them homogenized for extraction of insulin and glucagon.

Organ culture of passaged pancreas. Ten fetal pancreata of gestational age 14–18 wk were implanted s.c. in six nude mice (not included in the twenty-five mentioned above) and passaged for 86–415 days (absolute ages 28–73 wk). Sections of each implant were removed on 1–6 occasions (total 29 times) while the mouse was anesthetized, then diced into 1- to 2-mm^3 fragments and placed in organ culture for a maximum of 60 days. The technique used for culturing such explants has been described previously.[16] Essentially, this entailed the tissue being placed on a grid at an air-liquid interface. The culture medium used was RPMI 1640 supplemented with amino acids, penicillin, gentamicin, and stable plasma protein solution. The medium, which was changed three times a week, was stored at −20°C until it was assayed for insulin. Results are expressed both per plate of explants and per milligram of tissue (on the occasions when this was known).

Renal transplantation. The explants of four human fetal pancreata (15–17 wk gestational age) were divided into two portions and the halves of each pancreas implanted either s.c. or beneath the renal capsule of two age- and weight-matched nude mice. When the absolute age of the implants reached 26–29 wk, the animals were killed, the implants removed and weighed and then homogenized for determination of their insulin and glucagon content.

Statistics. Data obtained from the experiments was placed on file in a Digital 1170 computer and analyzed using the appropriate BMDP program (1R for regression analysis, 7D for analysis of variance, and 4F for chi-squared analysis).

RESULTS

Subcutaneous implants in nude mice. Within 2 wk of transplantation, the explants had coalesced into single mass discernable through the skin of the mouse. Thereafter, the implants were observed to increase in size. This observation was confirmed by the significant positive correlation of the net weight of the implants removed from the mice with the absolute age of the tissue: $r = 0.61$, $P < 0.001$ (Figure 1). The relationship between dry weight and absolute age was equally significant ($r = 0.57$, $P < 0.001$). These observations were obtained for pancreata of all gestational ages. They were equally valid for those with a gestational age of 14–15 wk (wet weight: $r = 0.69$, $P < 0.01$) as for those with the older gestational ages of 16–19 wk ($r = 0.56$, $P < 0.05$).

The total insulin content of the implants was significantly related to the absolute age of the tissue, so that as the tissue became older, its insulin content increased—by a factor of 51 after 1 yr in vivo (Figure 2). This was true for pancreata

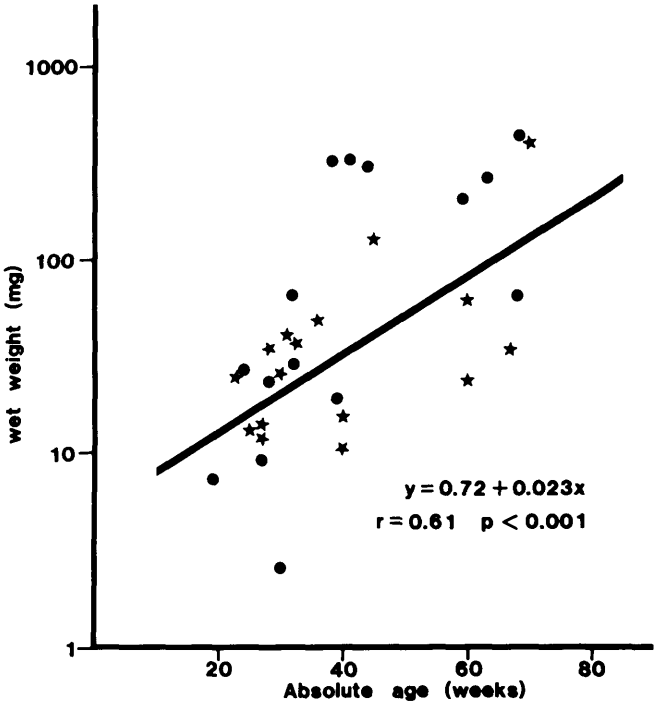

FIGURE 1. Comparison of the wet weight of s.c. implants removed from nude mice with the absolute age of the tissue. The line drawn represents the relationship between these two parameters for pancreata of all gestational ages. Significant relationships were observed for pancreata of both 14–15 wk gestational age (●, y = 0.52 + 0.03x, r = 0.69, P < 0.01) and 16–19 wk (★, y = 0.93 + 0.02x, r = 0.56, P < 0.01).

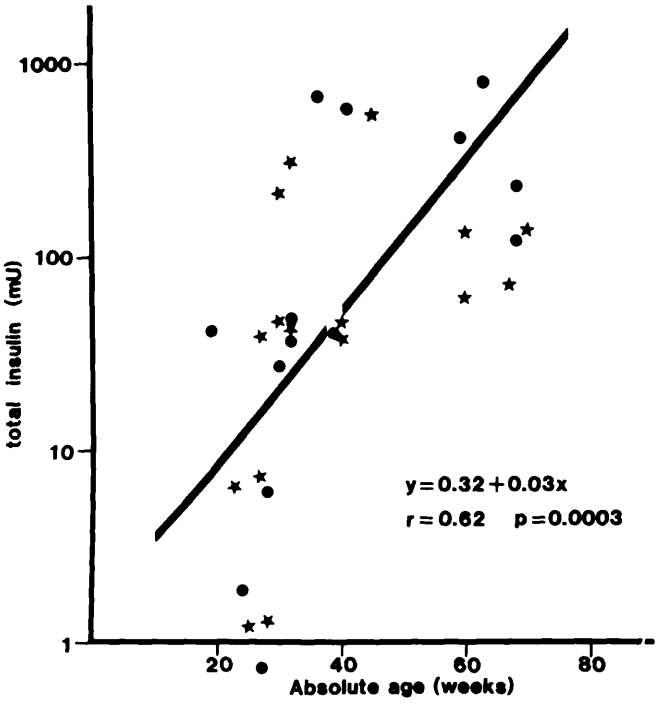

FIGURE 2. Comparison of the total insulin content of s.c. implants removed from nude mice carriers with the absolute age of the tissue. The line drawn represents the relationship between these two parameters for pancreata of all gestational ages. Significant relationships were observed for pancreata of both 14–15 wk gestational age (●, y = 0.10 + 0.04x, r = 0.70, P < 0.01) and 16–19 wk (★, y = 0.54 + 0.03x, r = 0.53, P < 0.05).

of both 14–15 wk gestational age (r = 0.70, P < 0.01) and the older ones of 16–19 wk (r = 0.53, P < 0.05). There was, however, no relationship between the insulin content per milligram of tissue (mean ± SD = 2.2 ± 2.1 U) and the absolute age (r = 0.10 for wet wt and 0.29 for dry wt). The maximum level of insulin from any one implant was 800 mU obtained from a pancreas of gestational age 14 wk that was passaged for 340 days (absolute age 67 wk).

The glucagon content of the implants bore no relation to the absolute age of the tissue, either when the levels were expressed in absolute terms (r = 0.11) or per unit weight (wet wt: r = −0.23, dry wt: r = −0.18). The insulin/glucagon ratio did not alter with age (r = 0.13).

Unpassaged human fetal pancreata. The wet weight and total insulin content of the unpassaged fetal pancreata are shown in Table 1. It can be seen by comparing this data with the corresponding values obtained for the passaged implants that there was significant overlap of the results (Figure 3). The values for passaged tissue were significantly greater for implants that had been passaged for >100 days, corresponding to an absolute age of greater than 30 wk (see Figure 3). Those implants passaged for more than this period of time were heavier and contained a greater amount of insulin than did the unpassaged tissue (upper 95% confidence limit)—for wet wt: X^2 for period in mouse = 8.28, P < 0.01; X^2 for absolute age = 5.64, P < 0.05; for total insulin content: X^2 for period in mouse = 5.63, P < 0.05; X^2 for absolute age = 8.83; P < 0.01. These results were obtained when the data from pancreata of all gestational ages were combined. When the data for pancreata of gestational age 14–15 wk was separated from that for the older pancreata and analyzed, similar findings were obtained (X^2 for period in the mouse = 4.28, P < 0.05, for wet wt; X^2 = 5.60, P < 0.05, for insulin content). No significant differences were obtained for the older pancreata.

Organ culture of passaged tissue. Insulin was secreted from the implants after sections of them were removed and placed in organ culture. Hormonal release was continuous for up to 60 days (Figure 4), showing that there were viable beta cells in the implants. The level of insulin released averaged 1.18 ± 0.21 mU/plate of explants for the entire 60 days. For the first 4 days of culture, when the explants of nine sections (mean ± SEM) of pancreatic implants were weighed (18 plates), the amount of insulin secreted was 0.25 ± 0.06 mU/mg wet wt or 1.99 ± 0.27 mU/mg dry wt.

Renal transplantation. The four implants removed from the surface of the kidney were in all cases heavier than both their matched s.c. pair and the amount of fetal pancreas transplanted (Table 2). By contrast, the s.c. implants were lighter than the tissue implanted. The insulin and glucagon content of the renal implants was also greater than that found in the matched s.c. implants. This was true both for the total hormonal levels and the levels per milligram wet or dry weight of tissue (Table 3).

DISCUSSION

Growth of the human fetal pancreas grafted into the nude mouse has been demonstrated previously. Macroscopically, this has been apparent by observing the increase in size of the s.c. transplant,[5] while microscopically there is both an

TABLE 1
Weight and insulin content of unpassaged human fetal pancreas

Gestational age (wk)	Wet wt (mg) Mean ± SD	N	Upper 95% confidence limit	Total insulin content (mU) Mean ± SD	N	Upper 95% confidence limit	Total insulin content/wet wt (mU/mg, mean ± SD)
14	17.58 ± 5.65	17	20.49	18.28 ± 9.66	6	28.42	1.01 ± 0.65
15	20.53 ± 11.31	12	27.72	22.05 ± 18.18	4	50.97	1.48 ± 1.04
16	37.54 ± 25.96	8	59.65	153.20 ± 115.73	3		3.14 ± 1.71
17	59.18 ± 46.75	9	95.12	132.79	1		3.85
18	45.04 ± 21.87	5	72.19	41.07 ± 14.20	2	193.75	0.92 ± 0.43
19	98.21 ± 33.61	5	154.69	122.24 ± 16.44	2		1.43 ± 0.72
Total (mean ± SD)							1.62 ± 1.13

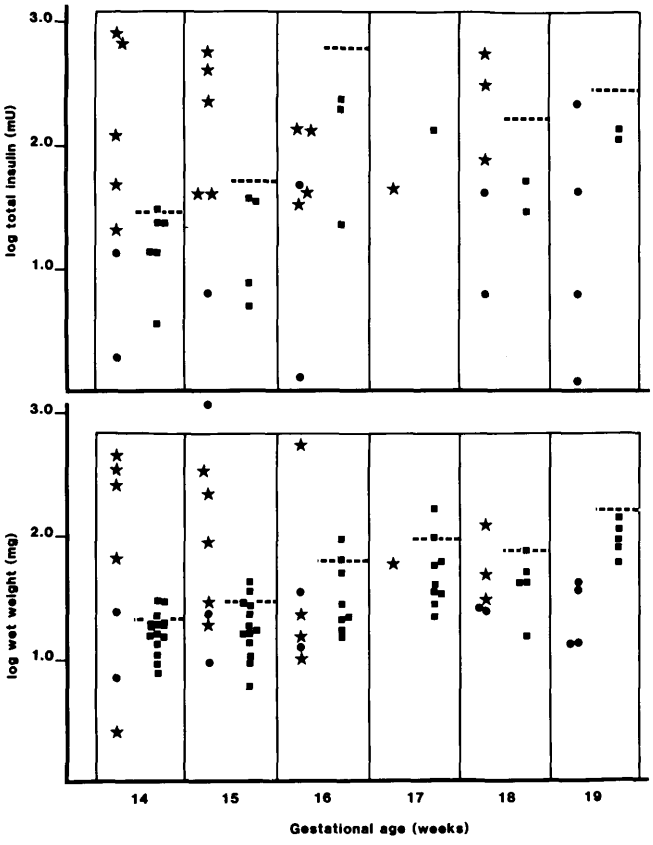

FIGURE 3. Comparison of wet weight and total insulin content of human fetal pancreatic tissue passaged in nude mouse and the corresponding values for the control unpassaged pancreata. ● For implants passaged <100 days, ★ for implants passaged >100 days, ■ for non-passaged controls, and --- upper 95% confidence limits for controls.

increase in islet size[5] and the presence of numerous mitotic figures in the islets.[17] Tissue passaged for up to 37 wk has been shown to differentiate selectively into endocrine tissue, ducts, and fibrous tissue,[5] to show maturation of the insulinogenic response to glucose, and to be capable of reversing diabetes induced in the nude mouse.[6] The data reported in this paper demonstrate an increase in weight of this tissue in vivo after transplantation, a finding consistent with the increase in size observed macroscopically. More importantly, the insulin content of the implant also increases significantly with time. This is compatible with an increase in the number of beta cells, as previously reported to occur in this type of xenograft.[18] The ability of implanted tissue to secrete insulin in vitro for up to 2 mo after its removal from the mouse also has been established (Figure 4). These new factors further illustrate the potential of this tissue after transplantation. An increase in insulin content is known to occur embryologically as the fetus becomes older[19] and is consistent with the rise in the number of beta cells that occurs during this time.[20]

The rate of increase in insulin content for the younger fetal pancreata (14–15 wk gestational age) after 1 yr was 114-fold, far greater than the 23-fold increase observed for the pancreata of older gestational age (Figure 2). This difference in the rate of increase of insulin for the two groups would explain why the insulin content of only the former group exceeded the upper 95% confidence limit for controls (Figure 3). When the data for all xenografted pancreata were combined, there was a 51-fold increase in insulin content after 1 yr in vivo (Figure 2). The increase in insulin content with time bears some similarity to what has been reported for allografted rodent fetal pancreas.[10,21] In the case of the mouse, 17-day-old fetal pancreas, which was cultured in vitro for 16 days, was passaged beneath the renal capsule of the diabetic CBA/Wehi mouse for a maximum of 12 wk.[10] Insulin content of the implant increased by a factor of some 20 times by 9 wk after surgery and plateaued thereafter. Hyperglycemia was reversed 6 wk after implantation, when the insulin content was only four times that of the implanted tissue, a level that was 20% of the pancreatic insulin content of a normal adult mouse. For allografted rat fetal pancreata, there is insufficient data reported to determine if the insulin content of the implants increased with time after transplantation.[21] The insulin content of grafted pancreata was measured only from 11 wk after surgery, a stage in the mouse when growth of the pancreatic implant had ceased. It is presumed that the content of the rat fetal implants did increase with time, since the diabetic recipients became normoglycemic. The insulin content at that stage was 23% of the total pancreatic insulin content of a normal adult rat.[21]

It was discouraging to find that the mean ± SD (N) insulin content per gram wet weight of passaged human fetal pancreas [2.2 ± 2.1 U/g (30)] was less than the insulin concentration found in the pancreas of a newborn infant[21] [12.5 ± 7.2 U/g (13), P = 0.000]. However, it was comparable to the insulin concentration of both the unpassaged fetal pancreas [see Table 1, 1.6 ± 1.3 U/g (19)] and that extracted from the adult pancreas[22,23] [1.6 ± 1.1 U/g (30) and 2.2 ± 1.0 U/g (10)]. The total amount of insulin extracted from the implants—a maximum of 0.8 U at an absolute age of 67 wk (corresponding to a postgestational age of 27 wk)—was also less than that extracted from pancreata of newborn infants, 35 U (12.5 U/g[19] × 2.8[24]), or from the homogenized adult pancreas, 147 U (1.6 U/g[24] × 91.9 g[25]) to 202 U (2.2 U/g[14] × 91.0 g[23]). Such a shortfall in the insulin content of pan-

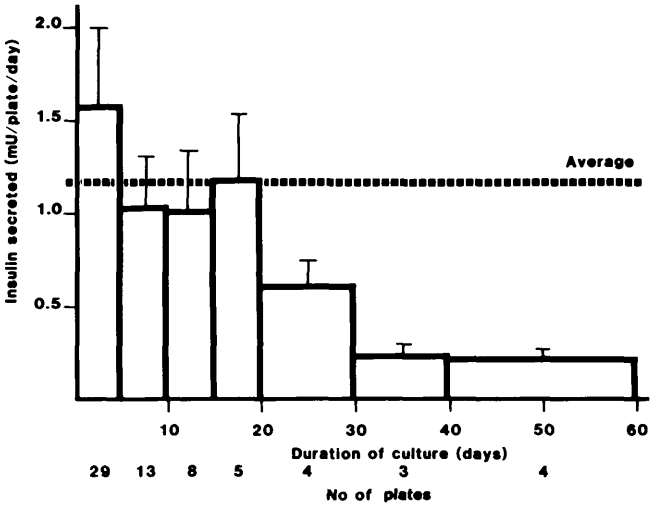

FIGURE 4. Secretion of insulin in organ culture by tissue sectioned from implants passaged in nude mice. Duration of culture was ceased on seven occasions because of infection. Results given are the mean ± SEM. There was no significant difference between the levels secreted (analysis of variance, $f_{6,65} = 0.69$).

TABLE 2
Weight of pancreatic tissue at the time of transplantation into and removal from the age- and weight-matched nude mouse carriers

Pancreas no.	Gestational age (wk)	Wt tissue implanted (mg)		Period in nude mouse (wk)	Absolute age (wk)	Wt tissue removed (mg)	
		s.c.	ren.			s.c.	ren.
1	15	9.71	8.42	11	26	7.93	33.60*
2	15	9.82	7.44	13	28	5.55	22.79
3	17	49.89	48.87	12	29	39.90	55.00
4	17	29.02	28.33	12	29	22.78	54.93

s.c., Subcutaneous site of transplantation; ren., site of transplantation was beneath renal capsule.
*The value was greater than the upper 95% confidence limit for unpassaged pancreata of a comparable gestational age.

creatic implants from the human fetus would make it unlikely that these grafts would function sufficiently in the clinical situation to reduce insulin requirements, especially since only 21% of the entire insulin content, or 24–50 U, is secreted daily from the adult organ.[14,15] If it is assumed that insulin secretion and insulin content are similarly related for the implanted human fetal pancreas, then the maximum insulin secretion per day would be 21% of 0.8 U, that is, 0.2 U. Even if the gland was able to secrete its entire insulin content daily, the amount of insulin released would be only 2% of the adult requirements. Experience gained with fetal pancreatic transplants in rodents indicates that 20–23% of the adult pancreatic insulin content is required to reverse diabetes.[10,21] Extrapolation of this data to the human situation means that the insulin content would need to be 38 U for reversal of diabetes to occur.

Affecting all of the data obtained may be the suitability of the site of transplantation to be used. The s.c. site was chosen initially because of the ease of transplantation, the copious space in which to place the pancreatic explants,[26] and most importantly the ease of observation of the implant through the hairless skin of the nude mouse. Data reported in this article show that this s.c. space was inferior to the subcapsular renal space as a site for transplantation in the nude mouse, both in terms of growth of the implant and its insulin content (Table 3). The difference between these two sites may simply be one of vasculature, with the s.c. site having a poorer blood supply.[27] Experiments with rodents, involving the transplantation of islets or cultured parathyroid fragments, have confirmed that implants at a s.c. site function poorly compared with more central sites, such as portal vein and peritoneum[28] or renal capsule.[29] The most suitable site for transplantation of human fetal pancreas in diabetic humans has yet to be determined, with sites draining into the systemic circulation (muscle and kidney capsule) and portal system (liver, spleen, and omentum) having been tried.[1,9]

The growth of fetal pancreatic implants as their absolute age increased—a maximum of 15-fold over 1 yr (Figure 1)—seems at first glance to be inconsistent with the finding of an overlap of the weight of these implants and unpassaged controls matched for gestational age. This can best be explained by the resorption of some of the original implanted tissue during the initial 2 wk after surgery, while the explants are coalescing into a single unit. This concept has been reported for other endocrine tissue previously.[26] Thereafter, growth of this lesser mass of pancreas occurred, an observation previously reported both macroscopically and in growth of islets microscopically.[5] After 100 days in vivo, corresponding to an absolute age of 30 wk the weight (and insulin content) of these implants has sufficiently increased to exceed the values found in the controls. This phenomenon was found when data from pancreata of all gestational ages were combined, as well as for the 14- to 15-wk age group. It was not found for the 16- to 19-wk age group. The discrepancy between the results obtained for the two age groups can be explained by the slower growth rate of the older pancreata: sixfold increase after 1 yr in vivo versus 39-fold increase in the younger age group (Figure 1). The greater weight of the renal subcapsular implants, compared with the s.c. sites, suggests that little loss of the transplanted tissue occurred at this site. The ability of tissue implanted at this site in streptozocin-diabetic nude mice to normalize the hy-

TABLE 3
Insulin content of pancreatic tissue removed from renal and s.c. sites in eight age- and weight-matched nude mice

Pancreas no.	Insulin content (mU)				Glucagon content (ng)			
	Total		Per mg wet wt		Total		Per mg wet wt	
	s.c.	ren.	s.c.	ren.	s.c.	ren.	s.c.	ren.
1	0.6	81.4*	0.1	2.4	4.2	855.5	0.5	25.5
2	6.7	103.1*	1.2	4.5	165.4	2827.5	29.8	124.1
3	1.1	163.6	0.03	3.0	5.5	2066.7	0.1	37.6
4	35.0	236.6*	1.5	4.3	786.2	3372.4	34.5	61.4
Mean ± SD	10.9 ± 16.3	146.2 ± 69.6	0.7 ± 0.8	3.6 ± 1.0	240.3 ± 371.7	2280.5 ± 1090.5	16.2 ± 18.5	62.1 ± 43.9

Only the hormonal content per milligram wet weight of tissue has been included. The data per milligram dry weight was measured and showed the same trend. The wet weight was 3.9 ± 1.2 (mean ± SD) times the dry weight.
*Values were greater than the upper 95% confidence limit for unpassaged pancreata of comparable gestational age.

perglycemia has been difficult to determine because of the reduction in life span of this animal.

In summary, these results demonstrate that the human fetal pancreas is capable of continuing to increase in size and insulin content up to at least 1 yr after it is removed from its natural environment and placed in a surrogate. The increase in insulin content with time offers hope that, once a suitable site for transplantation is determined and rejection has been combated, allografts of this tissue will be of functional benefit to diabetic humans.

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REFERENCES

[1] Sutherland, D. E. R.: Pancreas and islet transplantation. II. Clinical trials. Diabetologia 1981; 20:435–50.

[2] Chastan, P. H., Berjon, J. J., Gomez, H., and Meunier, J. M.: Treatment of an insulin-dependent diabetic by homograft of fetal pancreas removed before the tenth week of pregnancy: one-year follow-up. Transplant. Proc. 1980; 12 (Suppl. 2):218–22.

[3] Valente, U., Ferro, M., Barocci, S., Campisi, C., Parodi, F., Cataldi, L., Arcuri, V., and Tosatti, E.: Report of clinical cases of human fetal pancreas transplantation. Transplant. Proc. 1980; 12 (Suppl. 2):213–17.

[4] Tuch, B. E., Sheil, A. R. G., Ng, A. B. P., and Turtle, J. R.: Transplantation of cultured human fetal pancreas into insulin-dependent diabetic humans. In press. Transplant. Proc. 1986.

[5] Tuch, B. E., Ng, A. B. P., Jones, A., and Turtle, J. R.: Histologic differentiation of human fetal pancreatic explants transplanted into nude mice. Diabetes 1984; 33:1180–87.

[6] Tuch, B. E., Jones, A., and Turtle, J. R.: Maturation of the response of human fetal pancreatic explants to glucose. Diabetologia 1985; 28:28–31.

[7] Mandel, T. E., Georgiou, H., Hoffman, L., Carter, W. M., Koulmanda, M., and Dennington, P.: Proliferation of cultured and isografted fetal mouse pancreatic islets. Transplant. Proc. 1983, 15:1362–65.

[8] Hegre, O. D., Leonard, R. J., Rusin, J. D., and Lazarow, A.: Transplantation of the fetal rat pancreas: quantitative morphological analysis of islet tissue growth. Anat. Rec. 1975; 185:209–22.

[9] Brown, J., Clark, W. R., Molnar, I. G., and Mullen, Y. S.: Fetal pancreas transplantation for reversal of streptozotocin-induced diabetes in rats. Diabetes 1976; 25:56–64.

[10] Hoffman, L., Mandel, T. E., and Carter, W.: Insulin content of fetal mouse pancreas in organ culture and after transplantation. Diabetes 1982; 31:826–29.

[11] Kervran, A., and Girard, J. R.: Glucose-induced increase of plasma insulin in the rat fetus in utero. J. Endocrinol. 1974; 64:545–51.

[12] Heinze, E., Schatz, H., Nierle, C., and Pfeiffer, E. F.: Insulin biosynthesis in isolated pancreatic islets of fetal and newborn rats. Diabetes 1975; 24:373–77.

[13] Hellerstrom, C., Lewis, N. J., Borg, H., Johnson, R., and Freinkel, N.: Method for large-scale isolation of pancreatic islets by tissue culture of fetal rat pancreas. Diabetes 1979; 28:769–76.

[14] Porte, D., and Hatten, J. B.: The endocrine pancreas. *In* Textbook of Endocrinology, 6th Edit. Williams, R. H., Ed. Philadelphia, W. B. Saunders, 1981:716–843.

[15] Goldner, M. G., and Clark, D. E.: The insulin requirement of man after total pancreatectomy. J. Clin. Endocrinol. 1944; 4:194–97.

[16] Tuch, B. E., Maitland, J. E., and Turtle, J. R.: Culture and perifusion of human fetal pancreas. *In* Methods in Diabetes Research, Vol. 1. Laboratory Methods, Part B. Larner, J., and Pohl, S., Eds. New York, J. Wiley and Sons, 1984:153–63.

[17] Usadel, K. H., Maitland, J. E., Schwedes, U., Kaul, S., Bastert, G., Wacker, A., Schoffling, K., and Turtle, J. R.: Transplantation of human fetal pancreas after in vitro precultivation. Horm. Metab. Res. Suppl. 1982; 12:100–102.

[18] Mandel, T. E., and Georgiou, H. M.: Xenotransplantation of human fetal islets in nude mice. Transplant. Proc. 1984; 16:849–50.

[19] Steinke, J., and Driscoll, S. G.: The extractable insulin content of pancreas from fetuses and infants of diabetic and control mothers. Diabetes 1965; 14:573–78.

[20] Stefan, Y., Grasso, S., Perrelet, A., and Orci, L.: A quantitative immunofluorescent study of the endocrine cell populations in the developing human pancreas. Diabetes 1983; 32:293–301.

[21] Brown, J., Heininger, D., Kuret, J., and Mullen, Y.: Islet cells grow after transplantation of fetal pancreas and control of diabetes. Diabetes 1981; 30:9–13.

[22] Steinke, J., Soeldner, J. S., and Renold, A. E.: Measurement of small quantities of insulin-like activity with rat adipose tissue. IV. Serum insulin-like activity and tumor insulin content in patients with functioning islet-cell tumors. J. Clin. Invest. 1963; 42:1322–29.

[23] Rastogi, G. K., Sinha, M. K., and Dash, R. J.: Insulin and proinsulin content of pancreata from diabetic and nondiabetic subjects. Diabetes 1973; 22:804–807.

[24] Boyd, E.: Outline of Physical Growth and Development. Minneapolis, Burgess, 1941.

[25] Schaeffer, J. H.: The normal weight of the pancreas in the adult human being: a biometric study. Anat. Rec. 1926; 32:119–32.

[26] Barker, C. F.: Pancreatic islet cell transplantation. *In* Organ Transplantation. Chatterjee, S. N., Ed. Boston, J. Wright, 1982:413–15.

[27] Krohn, P. L.: Transplantation of endocrine organs: with special reference to the ovary. Br. Med. Bull. 1965; 21:157–61.

[28] Kemp, C. B., Knight, M. J., Scharp, D. W., Ballinger, W. F., and Lacy, P. E.: Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. Diabetologia 1973; 9:486–91.

[29] Naji, A., Silvers, W. K., and Barker, C. F.: Effect of culture in 95% O_2 on the survival of parathyroid allografts. Surg. Forum 1979; 30:109–11.