Transplantation of Fetal Intestine:

Survival and Function in a Subcutaneous Location in Adult Animals

STEPHEN B. LEAPMAN, M.D., ALEXANDER A. DEUTSCH, M.B., F.R.C.S., RICHARD J. GRAND, M.D.,* JUDAH FOLKMAN, M.D.

From the Departments of Surgery and Pediatrics, The Harvard Medical School, and the Departments of Surgery and Medicine (Division of Clinical Nutrition), Children's Hospital Medical Center, Boston, Massachusetts 02115

ALTHOUGH TRANSPLANTATION of fetal intestinal tissue has been carried out successfully in a variety of experimental animals, no data is available concerning the physiological function of such transplants.

In 1971, Zinzar *et al.*[14] reported that fetal intestine, when transplanted subcutaneously into an adult syngeneic host, would "grow progressively and form organ-like cavities." They indicated that the fetal transplant, although initially devoid of a blood supply in the host, would become vascularized, and remain as differentiated intestinal tissue.

Our attention was drawn to this study because of our research in the field of tumor angiogenesis. The mechanism of tumor vascularization, and the possible control of solid tumors by the blockade of angiogenesis has been the primary interest of this laboratory.[3–5] Tumor implants stimulate continuous proliferation of new capillary endothelium through the release of a diffusible material, which has been termed tumor angiogenesis factor, (TAF). Normal tissues, with but one or two possible exceptions, lack this property. Although fetal tissues can be vascularized rapidly, it is not yet clear whether they can stimulate neovascularization.

We have found that transplanted fetal gut can be made to grow as a longitudinal tube with a continuous lumen. In addition, our studies have shown that this transplant absorbs amino acids, glucose, and fat, maintains peristalsis, and exhibits normal brush border enzyme activity. These findings suggest a potential application for this system: a simple method for the transplantation and subsequent use of an accessory intestine in infants dying from a short-bowel syndrome.

Methods and Materials

A. Transplantation Technique. All transplantation experiments were done with syngeneic animals. Female Fisher strain rats* (40–50 grams) were used as recipients. Donor fetuses from the same strain were delivered by Caesarian section between the 18th to the 21st day of gestation.

The gastrointestinal tract from the stomach to the descending colon was removed *en bloc* from the fetus and stripped of its mesentery. The stomach and large bowel were discarded, and the remaining small intestine (the approximate calibre of a #0 silk suture) was flushed

Submitted for publication November 16, 1972.

Supported by U.S. Public Health Service Grants 5R01 CA08185-06 from the National Cancer Institute and AM-14523 (R.J.G.) from the National Institute of Arthritis, Metabolism, and Digestive Diseases; and by the American Cancer Society, National Chapter (IC-28), and gifts from the Merck Company and the Alza Corporation.

* Recipient of an Academic Career Development Award (5-K07-AM44590) from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

Reprint requests: Judah Folkman, M.D., Department of Surgery, Children's Hospital Medical Center, Boston, Massachusetts, 02115.

* Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

with cold Ringer's lactate solution to remove meconium and other particulate matter.

Under light ether anesthesia and with sterile technique, an air sac was formed on the dorsal surface of each recipient animal, and a longitudinal incision to the left of the midline was made in the sac. The fetal intestine from mid-jejunum to distal ileum was stretched along the midline and sutured in place at the cranial and caudal ends. The skin was closed with Michelle clips and the animals returned to their cages. No attempt was made to separate the recipients in separate cages.

A total of 160 rats received a subcutaneous fetal intestine. Of these, 120 had tubes of intestine that were acceptable for further experimentation. The remaining rats had small pieces of intestine that had either failed to grow or had formed large cysts because of improper placement in the subcutaneous space. These animals were used as controls for the studies described below.

B. Gross and Histologic Examinations. One animal was sacrificed every day for the first week and then at intervals of 2–3 days for 8 weeks. The subcutaneous space was opened, the diameter and length of the intestinal implant were measured, and frequency of peristalsis was observed. Specimens were preserved in formalin, and histologic sections were prepared from paraffin blocks and stained with hematoxylin and eosin by standard techniques.

C. Absorption Studies. Studies of absorption of glucose, amino acids, and a long-chain, fatty acid were carried out in 50 animals. Of this number, 23 rats had transplanted intestine and 27 were control animals. All perfused, subcutaneous fetal intestines had been in place for 21 days post-transplant. All control animals were 100–120 grams (4–6 weeks-old). The animals were not fasted prior to an absorption study.

Under light ether anesthesia, the subcutaneous intestine was carefully and completely cleared of a thick, pasty-yellow material that could be removed by forceps and/or saline irrigation. Glass cannulae were introduced at either end of the intestine and secured with sutures. The intestine was again irrigated with saline to identify leaks.

The intestinal transplant was perfused by a recirculation technique through its lumen at 0.2 to 0.3 ml/minute with a continuous flow pump* for 5 hours (Fig. 1). The total volume for each perfusion was 10 ml. at room temperature. An initial equilibration period of 30 minutes was allowed. Throughout the 5-hour period, the animal was kept warm with a heating lamp.

Radioactive tracers,† ^{14}C-3-O-methyl glucose (specific activity 18.5 mC/mM), ^{14}C-glycine (specific activity 91 mC/mM), and ^{14}C-oleic acid (specific activity 56.3 mC/mM) were tested individually for absorption. All tracers, except oleic acid, were added to their respective, unlabelled stock solutions which were prepared as a 5mM concentration in phosphate buffered saline. Total radioactivity added to the stock solution was 1μC/10ml. perfusate. All perfusates (except oleic acid) also contained a 5mM mannitol solution with a ^{3}H-mannitol marker (specific activity 2.65 C/mM) added as 5μC/10ml. perfusate. Since mannitol is not actively absorbed (10), it served as an internal control for water flux and loss of test substance by processes other than active absorption. Oleic acid was prepared as an 8.8mM concentration in a micellar solution of monoolein (5mM) and sodium taurocholate (40mM).* The final osmolarity of all solutions was 290–300 mOsms, and the pH range was 7.1 to 7.3.

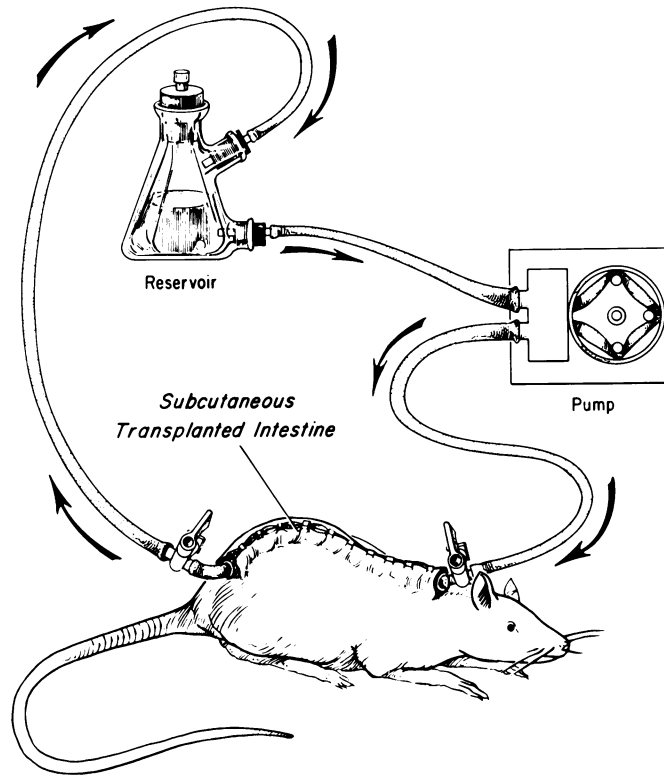

FIG. 1. Model for perfusion of subcutaneous intestine.

Duplicate samples were removed as 0.1 ml. aliquots from the reservoir at 0, ½, 1, 1½, 2, 3, 4, and 5 hours. Samples were then counted in 10 cc. of Bray's solution in a Packard liquid scintillation spectrometer. Correction of quenching was made by the external standard method; results were converted to disintegrations/minute for all

* Holter Company, Division of Estracorpored MED Spec., Inc., Mt. Laurel, New Jersey.

† New England Nuclear Company, Boston, Massachusetts.

* Prepared by Dr. Robert Glickman, Gastrointestinal Unit, Massachusetts General Hospital, Boston, Massachusetts.[6]

calculations. The amount of substance absorbed was measured by the disappearance of counts from the reservoir and is expressed by the equation:

$$\mu \text{ moles absorbed/cm.} = \frac{\{1 - (dpm_f/dpm_i)\}\ (\text{Man R})\ (\text{Ci})\ (V_c)}{\text{cm. intestine}}$$

Where: dpm_f and dpm_i are the final and initial disintegrations/minute of test substance.

Man R = dpm_f/dpm_i for mannitol. C_i = concentration of test material in μ moles/ml., and V_c = circulated volume in milliliters during each time period, corrected for sampling. Statistical analyses were performed using the standard Student's two-tailed "t" test.

Control absorption studies on rats of the same strain were conducted by perfusing a proximal jejunal loop *in situ*. Proximal jejunal loops were of the same length and were perfused in exactly the same manner as were the subcutaneous intestines. All animals were sacrificed after an absorption experiment.

D. Disaccharidase Activity. Five subcutaneous intestinal transplants and jejunal segments from three control animals of the same strain and age were removed, opened longitudinally, and the mucosa scraped on an iced glass plate with a glass slide. The tissue was then homogenized in 30 volumes of iced 5mM EDTA, pH 7.4. The disaccharidase activity was assayed by a modification[7] of the method of Messer and Dahlqvist.[9]

Results

A. Gross and Histological Examinations. In the first 24 hours, the transplant became visibly hemorrhagic and the lumen filled with extravasated blood. When the gut was observed grossly at this time, it looked dark and ischemic.

Within the next 3 days, an areolar membrane covered the intestine causing it to adhere to the underlying dorsal fascia. On day three, the normal layers of the intestine were histologically identifiable, and villi and ganglion cells were seen. Extravasated blood remained in the submucosa, and this layer was relatviely thick when compared to the others. The gut looked pink and viable by the fourth day. Large veins segmentally drained the intestine. The wall of the gut was thick and a muscular coat was identifiable. Microscopically, (Fig. 2) the villi were prominent and the epithelial cells columnar. Goblet cells were present. The intraluminal contents appeared more dense and reduced in volume. By the seventh day, small cysts developed at either end of the intestine, and the gut increased in length by approximately ½ centimeter.

During the next 2–3 weeks, the intestine continued to increase its length to an average of 4–6 cm. In the third week, one could see a straight, vascularized intestinal tube. The diameter of the tube at this time was large enough to admit readily a 3 mm. cannula. Light microscopy showed normal structures. Figure 3 demonstrates normal architecture in an intestine 21 days post-transplant. The higher power view of 25 day post-transplanted gut (Fig. 4) shows normal villi and crypts. In some histologic sections, Peyer's patches were identified. The longitudinal and circular muscle bands with accompanying intermyenteric plexi were also seen.

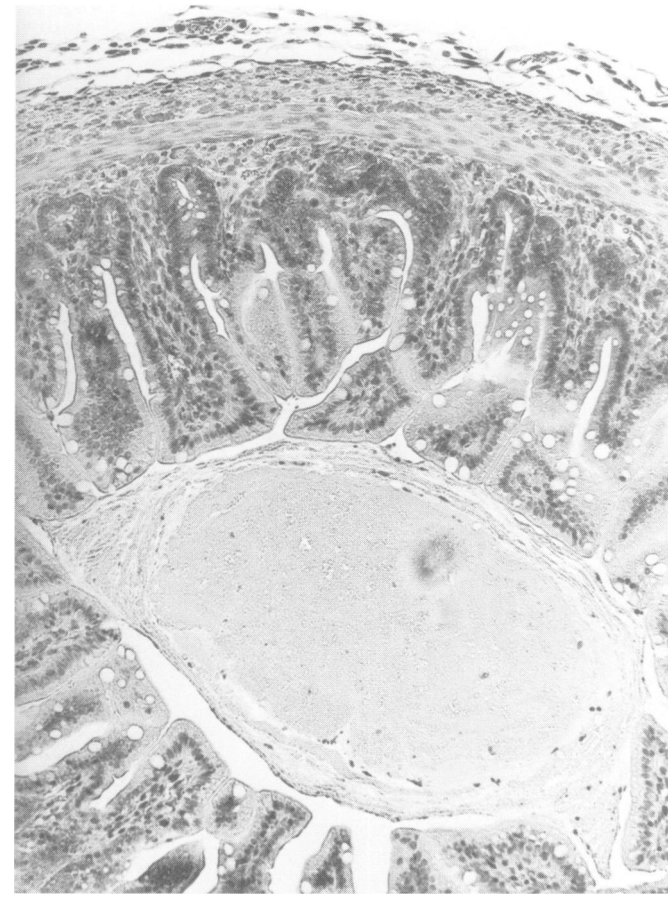

FIG. 2. Fetal small intestine 4 days after transplantation (× 187). Note normal goblet cells, intermyenteric ganglia, and muscular layers.

Transplants were followed for as long as 8 weeks. The diameter of the gut continued to increase as more material was deposited within the lumen. The length continued to increase in proportion to the growth of the animal.

The intraluminal material, made up of desquamated cells and mucous, became yellow in color after the first week. It had a very tenacious character and was exceedingly difficult to remove from the lumen after the second week. Because of its continued accumulation in the obstructed intestine, pressure increased intraluminally, and if the substance was not removed, histologic changes such as flattened, cuboidal epithelial cells and blunted villi resulted.

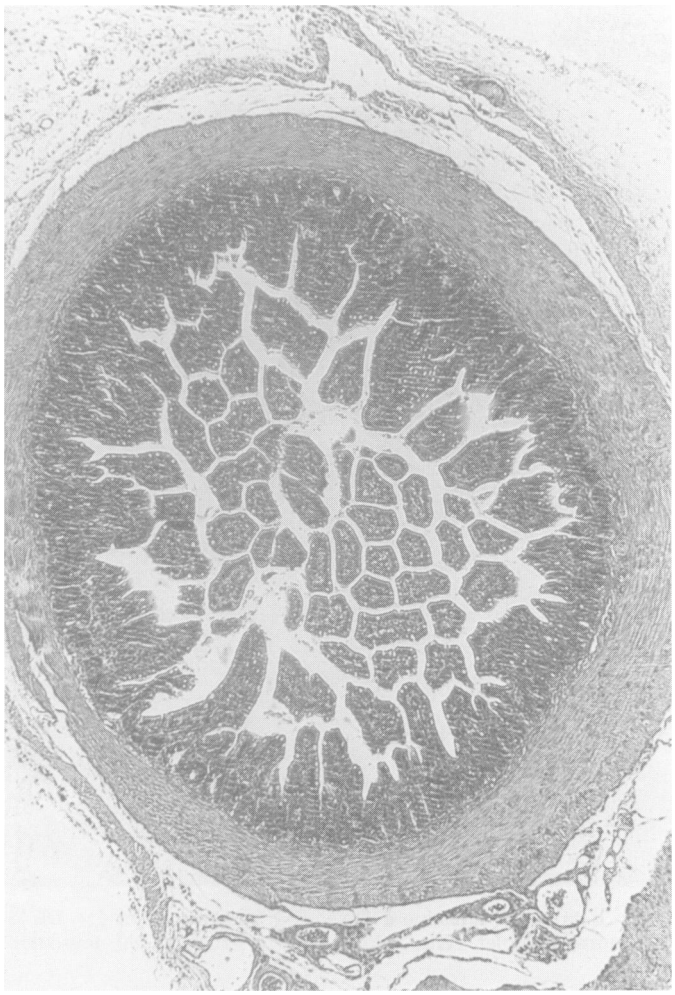

FIG. 3. Fetal small intestine 21 days after transplantation (×46.8).

B. Absorption Studies. A non-metabolizable simple sugar, ^{14}C-3-O-methyl glucose, was recirculated through the intestinal transplants and controls for 5 hours. In the first 2 hours, the control intestine absorbed significantly more glucose than did the transplant ($p < 0.02$). The difference in absorption between the transplant and the control during the third, fourth, and fifth hours was not significantly different ($p > 0.05$). The results of these studies are shown in Fig. 5.

Amino acid absorption studies were done with both glycine and α-aminoisobutyric acid (a non-metabolizable neutral amino acid). The absorption curves for glycine (Fig. 6) have similar slopes, but the amount absorbed was always, but not significantly, greater in the control intestines. The transplanted intestines did not absorb α-aminoisobutyric acid. Control intestines, however, showed absorption of this analogue in quantities comparable to results published by others.[12]

Fat absorption was examined in only one transplanted intestine and one control animal (Table 1). Oleic acid, a long-chain, unsaturated fatty acid, was perfused in a micellar preparation for 5 hours. At the end of 5 hours, oleic acid was absorbed in similar amounts by both the control and experimental intestine.

C. Disaccharidase Activity. Disaccharidase activity (Table 2) was measured in the transplanted intestine and in comparably aged controls of the same strain. Lactase activity in the 3-week-old transplanted intestine was slightly higher than that in controls. Maltase and sucrase activities were significantly higher in the control intestines (from 3-week-old and adult animals) than in the transplanted gut.

D. Peristalsis. Contractions in the smooth muscle were consistently noted in the transplants after the second week. The contractions seemed unidirectional and propulsive in nature. Stimulation by local application of neostigmine* (0.2 mg/ml) increased the activity of the muscularis, and on one occasion, a transplanted intestine was able to evacuate its entire intraluminal contents by propulsive, forceful, peristaltic contractions.

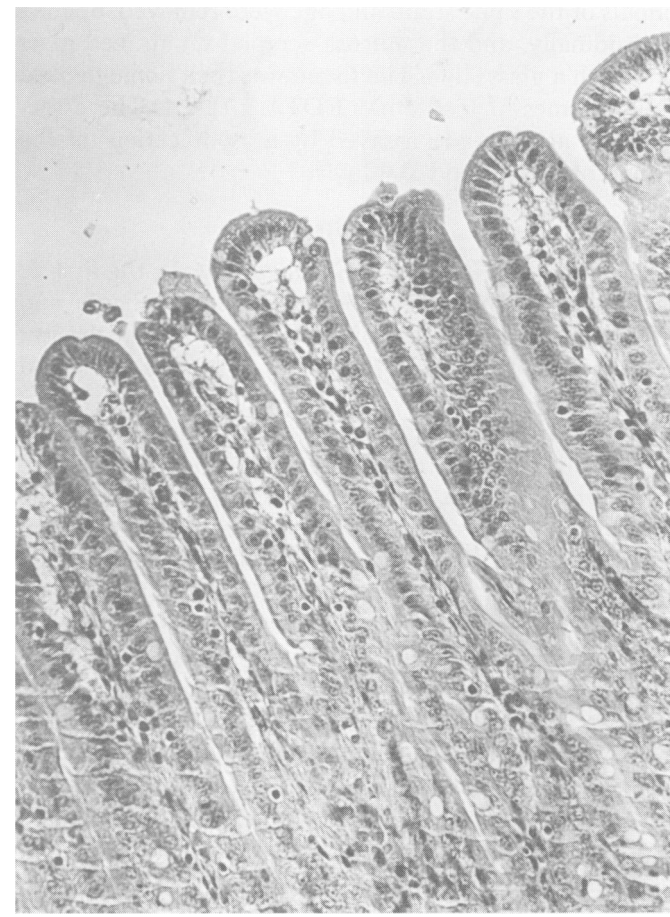

FIG. 4. Fetal small intestine 25 days after transplantation (× 468). Note normal villi and crypts.

* Elkins-Sinn, Inc., Cherry Hill, New Jersey.

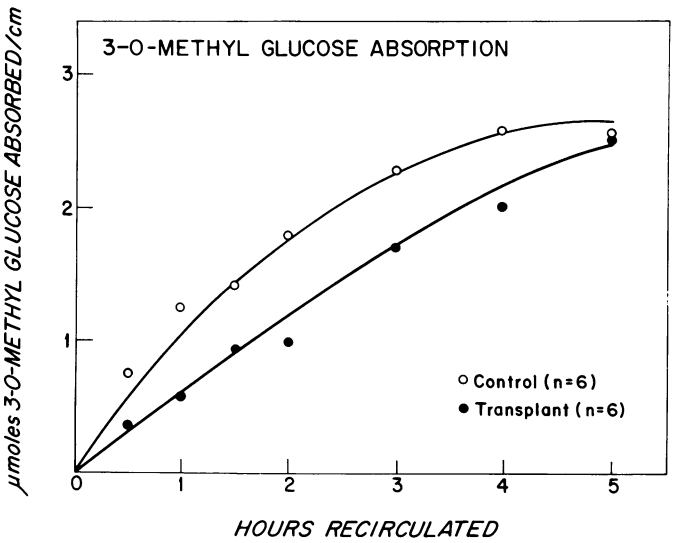

FIG. 5. ^{14}C-3-O-methyl glucose absorption curves for transplanted and control intestine.

Discussion

These studies show that free, syngeneic transplants of fetal gut into adult animals will vascularize, grow, retain peristalsis, and absorb nutrients. They maintain histological integrity, produce brush border enzymes, and exhibit ganglia and Peyer's patches.

Fetal transplants, both homologous and heterologous, survive and become vascularized in 3–4 days. Greene[8] demonstrated that stomach, small bowel, kidney, brain, lung, skin, and cartilage vascularized in the anterior chamber of the rabbit eye and remained as histologically differentiated tissues. Toolan[13] transplanted minced fetal intestine to the anterior chest wall of rabbits. These

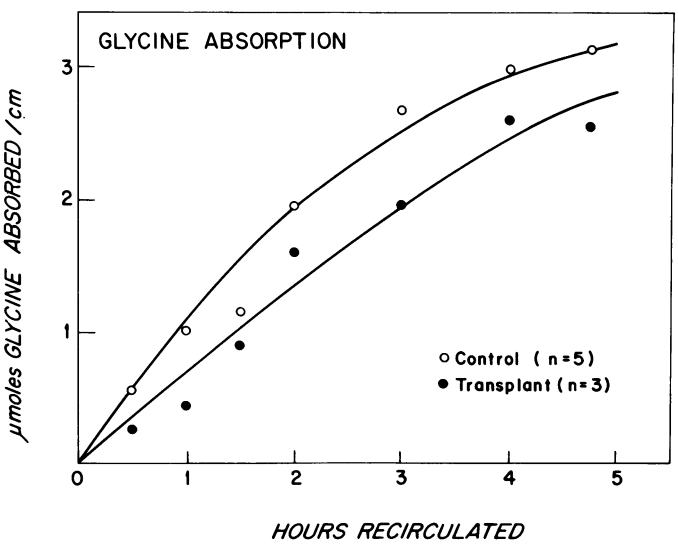

FIG. 6. ^{14}C-Glycine absorption curves for control and transplanted intestine.

TABLE 1. *Fat Absorption*
(*μ moles oleic acid absorbed per centimeter intestine*)

Time (hours)	Transplant	Control
½	1.99	0.92
1	4.30	2.28
1½	4.22	2.95
2	4.70	3.29
3	4.93	4.06
4	4.69	4.87
5	5.11	5.43

grafts remained viable for as long as 3 months if the homologous recipients were irradiated or treated with cortisone. Baxter and Goldstein[2] transplanted fetal human skin to burn patients, but noted rejection of the grafts within 46 days. Heterografts of human fetal lung and kidney have been transplanted into thymectomized, A.L.S.-treated mice. These tissues survived up to 28 days, but when intestine was transplanted into the same system, the grafts never grew.[11]

Recently, Barakat and Harrison[1] demonstrated that fetal kidney, transplanted subcutaneously into homologous rats survived and functioned. Their homografts survived up to 6 weeks, but succumbed to a pressure necrosis presumably by a mechanism like that of a closed hydronephrosis.

Our studies indicate that fetal intestine removed just before term and transplanted subcutaneously into a syngeneic host attains a normal histological appearance, grows linearly in proportion to the growth of the animal, and is capable of active peristalsis, all as a result of successful vascularization. Although the current experiments do not provide information regarding the mechanization of vascularization in transplanted fetal tissue, they do not represent a model system in which this can be evaluated.

The present experiments also demonstrate that func-

TABLE 2. *Disaccharidase Activity*
(*μ moles glucose released/min./mg. protein*)

Animal	Lactase	Maltase	Sucrase
Transplant 1	.050	.142	.048
Transplant 2	.035	.249	.058
Transplant 3	.041	.344	.043
Transplant 4	.025	.186	.021
Transplant 5	.021	.201	.038
Mean ± SE =	.034 ± .005	.224 ± .034	.042 ± .006
Control 1 (3-weeks-old)	024	.384	.087
Control 2 (3-weeks-old)	.024	.370	.082
Control 3 (3-weeks-old)	.020	.353	.082
Mean ± SE =	.024 ± .001	.369 ± .009	.084 ± .001
Control (4–6 weeks old)* Mean ± SE =	.026 ± .001	.451 ± .070	.087 ± .005

* (n = 4), values customarily obtained for rats in this laboratory.[7]

tional characteristics of the normal fetal intestine and their developmental changes survive after transplantation. At 21 days post-transplant, lactase activity is at a level comparable to that in normal animals, although sucrase and maltase levels are somewhat lower than normal. This confirms the histological evidence that the microvillus membrane is intact in the transplant. Glycine and glucose absorption are also nearly normal, although alpha-aminoisobutyric acid is not absorbed. The reason for this failure is not clear at present, but it may represent subtle biochemical defects after transplantation which might become normal later. The studies of oleic acid absorption from a micellar solution suggest that the uptake capacity of the transplanted epithelial cells is normal. We can not comment upon the utilization or disposal of the absorbed fat, since we have not yet studied the lymphatic drainage of the transplants. Presumably, uptake into the circulation of this long-chain fatty acid would require intact lymphatics.

We have recently carried out further experiments in rats which show that the transplanted intestine can be anastamosed to the recipient gut *in vivo*, and that the animal continues to thrive. These experiments will be reported separately.* It seems possible, therefore, that a similar procedure might be feasible clinically, and that a closely matched intestine from an aborted human fetus could be placed subcutaneously in a child who lacks enough bowel for survival. Since a vascular anastamosis is not required, it may greatly simplify the approach to intestinal transplantation.

Summary

Fetal rat intestine was transplanted into the subcutaneous space of a syngeneic host. The transplant was able to establish a blood supply, grow progressively, and exhibit normal histology. Studies were done to examine absorption, brush border enzymes, and peristalsis. Glucose, glycine, and oleic acid were absorbed. Peristalsis was present, and disaccharidase activity was exhibited. Clinical implications are discussed.

Acknowledgments

We are indebted to Dr. Michael Field for helpful suggestions, and to Ms. Stephanie Jaksina for her valuable help. We thank Ms. Christine Keller and Ms. Alison Mulhearn for the histologic preparations and Messrs. Paul Wesley, Frank Robinson, Scott Eder, and John Tountas for their technical assistance.

* Deutsch, A., Leapman, S., Folkman, J. Unpublished data.

Addendum

Since the submission of this manuscript, similar data regarding morphology and the development of disaccharidase activities in transplanted mouse intestine have appeared (Ferguson, A., Gerskowitch, V. P. and Russell, R. I., gastroenterology, **64**:292, 1973).

References

1. Barakat, T. I. and Harrison, R. G.: The Capacity of Fetal Neonatal Renal Tissues to Regenerate and Differentiate in a Heterotopic Allogeneic Subcutaneous Tissue Site in the Rat. J. Anat., **110**:393, 1971.
2. Baxter, H., Goldstein, M. and McMillan, G. C.: The Fate of Human Fetal Homo- and Heterografts. Plast. Reconstr. Surg., **22**:516, 1958.
3. Folkman, J., Merler, E., Abernathy, C. and Williams, G.: Isolation of a Tumor Factor Responsible for Angiogenesis. J. Exp. Med., **133**:275, 1971.
4. Folkman, J.: Anti-Angiogenesis: New Concept for Therapy of Solid Tumors. Ann. Surg., **175**:409, 1972.
5. Gimbrone, M. A., Leapman, S. B., Cotran, R. S. and Folkman, J.: Tumor Dormacy in Vivo by Prevention of Neovascularization. J. Exp. Med., **136**:261, 1972.
6. Glickman, R. M., Kirsch, K. and Isselbacher, K. J.: Fat Absorption During Inhibition of Protein Synthesis: Studies of Lymph Chylomicrons. J. Clin. Invest., **51**:356, 1972.
7. Grand, R. J., Chong, D. A. and Isselbacher, K. J.: Intracellular Processing of Disaccharidases: the Effect of Actinomycin D. Biochim. Biophys. Acta, **261**:341, 1972.
8. Greene, H. S. N.: The Heterologous Transplantation of Embryonic Mammalian Tissues. Cancer Res., **3**:809, 1943.
9. Messer, M. and Dahlqvist, A.: A One-Step Ultramicro Method for the Assay of Intestinal Disaccharidases. Anal. Biochem., **14**:376, 1966.
10. Miller, D. L. and Schedl, H. P.: Total Recovery Studies of Nonabsorbable Indicators in the Rat Small Intestine. Gastroenterology, **58**:40, 1970.
11. Phillips, B. and Gazet, J. C.: Growth of Human Foetal Tissue in Mice Treated with Antilymphocyte Serum. Nature, **222**:1292, 1969.
12. Schedl, H. P., Miller, D. L., Wilson, H. D. and Flores, P.: Aminoisobutyric Acid Transport and Tissue Concentration at Various Intestinal Sites. Am. J. Physiol., **216**:1131, 1969.
13. Toolan, H. W.: Growth of Embryonic Gut and Stomach on the Exterior Chest Wall of Adult Cortisone-treated Homologous Hosts. Cancer Res., **17**:707, 1957.
14. Zinzar, S. N., Leitina, B. I., Tumyan, B. G., Svet-Moldavsky, G. J.: Very Large Organ-like Structures Formed by Syngeneic Foetal Alimentary Tract Transplanted as a Whole or in Parts. Rev. Europ. Études Clin. et Biol., **16**:455, 1971.