with age; however, this is not the case for REM sleep percent. With respect to specific REM measures, the findings in REM latency continue to confirm and strengthen the notion that REM latency shows a striking linear relationship to age in depressed patients. In contrast to expectations (controlling for delusional states), very few differences were found between depressed inpatients and outpatients.

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Full Papers

Fetal gastric and colonic implants in syngeneic and allogeneic mice developing typical inflammatory changes

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Summary. Implants of fetal stomach and colon under the kidney capsule of syngeneic, and H-2 compatible and H-2 incompatible allogeneic mice were examined histologically at different time intervals after the procedure. According to the time of implantation typical inflammatory changes were seen in syngeneic stomach and colon implants, which resembled changes seen in chronic atrophic gastritis and chronic ulcerative colitis. Immunofluorescence studies showed that the host developed antibodies against fetal antigens, while there was no evidence for cellular immune response to fetal syngeneic antigens with the direct leukocyte migration inhibition test. Possible explanations for these results are discussed.

Introduction

There are few experimental models of non-infective inflammatory disease of the gastrointestinal tract. One of these models is the orthotopical implantation of fetal gastrointestinal tissue in adult mice. The technique of implantation of fetal mouse intestine under the kidney capsule of adult mice was first introduced by Ferguson and Parrot[3]. Fetal grafts have the great advantage that they have never been exposed to such external antigens as those of food and microorganisms. Using small intestine implants Ferguson and Parrot[3,4] and MacDonald and Ferguson[10] have shown that the morphological development parallels that of the normally sited intestine of the same age, while allografts had typical signs of rejection, such as lymphoid cell infiltration, cell damage and crypt hyperplasia.

In this paper we describe the histopathological changes in fetal colon and stomach implants in syngeneic and allogeneic mice and correlate the morphological changes with humoral and cellular immune reactions. The possible role of fetal intestinal antigens for the development of pathological changes is emphasized.

Material and methods

Mice of inbred strains BALB/c and C57bl/6J were obtained from Simonsen Laboratories, Gilroy, California. DBA/2 mice were obtained from Jackson

Laboratories, Bar Harbor, Maine. The donor material used was in all cases stomach, and colon from BALB/c fetuses 16–18 days of age. Recipient animals were female BALB/c (H-2^d) and male and female C57bl/6J (H-2^b) and DBA/2 (H-2^d) mice aged 8–16 weeks.

The preparation of donor tissue and the implantation technique were those described by Ferguson[5]. Briefly, pregnant mice were killed 1 day before parturition by means of cervical dislocation and the fetuses removed immediately by caesarean section. The stomach, small intestine and colon were removed with sterile instruments and placed in cold phosphate-buffered saline (ph 7.2) and cut into pieces of about 2 mm^2.

Recipient animals were anesthetized with an i.p. injection of Nembutal (sodium-pentobarbital, 40 mg per kg b.wt) and immobilized. The skin in the left lumbar region was shaved and cleaned with 90% alcohol. The kidney was exteriorized through a left lumbar vertical incision, the renal capsule was incised and a piece of implant material placed under the capsule by means of a teflon cannula. After replacement of the kidney, the abdominal muscle layer and skin incision were closed with silk sutures.

Groups of animals were killed at 5, 10, 15 and 20 days post-implantation. An additional group of mice receiving syngeneic implants was killed 25 days after implantation (table 1).

The mice were bled before sacrifice and the peripheral blood was used for isolation of leukocytes or preparation of serum.

In some cases host colon and stomach were removed and prepared for routine histological examination. The implanted left kidney was removed and fixed in 10% buffered formalin, the graft cut in 5-µm sections and stained with hematoxylin and eosin. In some cases the stomach graft was frozen on dry ice immediately after removal, stored at −20 °C and used for immunofluorescence studies.

Immunofluorescence. Grafted stomach tissue and fetal stomach and colon tissue of BALB/c mice were frozen on dry ice, cut in 6-µm sections on the cryostat and processed in a standard way[6].

The antisera used were purchased from Cappel Laboratories, Cochranville, PA. Fluorescein-conjugated IgG fractions of goat antisera to human immunoglobulins and fluorescein conjugated IgG fractions of rabbit anti-mouse gamma-globulin were used in the indirect Coons technique. Antisera were absorbed with rabbit liver powder and diluted 1:2 with Rhodamine-conjugated bovine serum albumin (Microbiological Associates, Bethesda, MD). For the detection of parietal-cells in stomach implants, sera of patients with pernicious anemia containing high-titer anti-parietal-cell antibodies in previous tests, were used. Undiluted mouse serum was used for tests for antibodies to fetal colon and stomach. Controls using normal human serum or normal (ungrafted) mouse serum were always included.

Table 1. Combinations of donor-host strains and numbers of implants examined[a]

	Days after implantation	BALB/c into BALB/c	BALB/c into DBA/2	BALB/c into C57bl/6J
a) Colon	5	4	4	4
	10	6	4	5
	15	5	3	3
	20	3	4	3
	25	6	-	-
b) Stomach	5	4	3	4
	10	4	4	4
	15	6	4	4
	20	4	4	3
	25	6	-	-

[a]The number of host-animals in which implants suitable for histological assessment occurred is denoted by the figures in each column. Each animal received only 1 implant.

Microscopy. A Zeiss Standard R.A. fluorescent microscope with fluorescent illuminator, Zeiss BG3 and UG5 excitor filters, and Zeiss barrier filter 50 were used, with high-speed Ektachrome film for photography.

Leukocyte migration inhibition assay. For the detection of cellular immunity of the host to fetal colon implants the agarose microdroplet leukocyte migration inhibition assay[7] with the modification of McCoy et al.[11] was used. After severing the brachial vessels peripheral blood was collected from the axillary regions into a heparinized syringe and mixed 2:1 with 6% dextran, followed by $1 \times g$ sedimentation of the red blood cells for 30 min. Usually peripheral blood from 2–3 animals of 1 group was used for 1 test. Cells were washed twice in Medium 199 supplemented with 10% fetal calf serum (FCS) (Grand Island Biological Corp., Grand Island, N.Y.) and resuspended after counting to 2×10^7/ml. Cells were spun down and mixed with 0.1 ml 0.2% agarose (Sigma Chemical Corp., St. Louis, MO, Type II) dissolved in Medium 199 supplemented with 10% FCS and penicillin (100 U/ml) streptomycin (100 mg/ml) mixture (Grand Island Biological Corps, Grand Island, N.Y.) in a 37 °C water bath. Two λ droplets were placed with a Drummond microdispenser (Drummond Scientific Corp., Broomhall, PA) into each well of a 96-well flat bottom microtest plate and covered with a lid (Falcon Plastics, Oxnard, CA). After 5 min 1 ml medium 199 supplemented with 10% FCS, penicillin and streptomycin, and 25 mM Hepes buffer (Grand Island Biological Corp., Grand Island, N.Y.) with or without antigen (protein concentration 70 µg/ml) was carefully added to each well.

After incubation at 37 °C in a 5% CO_2 humidified atmosphere for 18 h, the plates were read with a

dissecting microscope with mm scale. MI (migration index) is calculated as

$$MI = \frac{\text{migration with antigen}}{\text{migration without antigen}}$$

The antigen used in the migration inhibition assay was prepared as follows: colon, stomach, and small intestine of BALB/c fetuses aged 16–18 days were removed as described. The tissue was minced in Hank's balanced salt solution (Grand Island Biological Corp., Grand Island, N.Y.), homogenized in a sterile glass grinder and allowed to stand overnight at 4 °C. After centrifugation for 30 min at 2000 × g (4 °C) the supernatant was withdrawn, the protein content determined by the method of Lowry et al.[9] and the material stored at −20 °C. In preliminary tests we used antigen concentrations in the range of 20–120 µg protein/ml and found 70 µg/ml to allow optimal discrimination. This concentration was used in all subsequent tests.

Histopathologic grading. To quantify the changes in the graft we used a different grading system for stomach as well as for colon grafts. Grading was performed independently by the authors using coded sections. To reduce sampling errors, 2–3 different sections of one implant were graded. As the degrees of structural change and inflammatory cell infiltration were not consistently congruent, we graded structures and cellular infiltration separately, using a 1–4 grading system, although we were aware of the importance of infiltrative changes in determining the morphology. While mucosal lymphocytes can be seen normally in the colonic mucosa of the neonatal mouse, mucosal lymphocytes in the neonatal murine stomach are rare or absent. We have, therefore, used different criteria in grading the degree of cellular infiltration in colonic and gastric implants.

In stomach implants the cellular infiltration was graded according to the following criteria: grade 1, appearance of lymphocytes in the lamina propria; grade 2, lymphocyte infiltration into the mucosa; grade 3, appearance of plasma cells, neutrophil and eosinophil granulocytes; and grade 4, heavy infiltration of inflammatory cells, including many granulocytes, in the cyst lumen. Morphological changes in the stomach were compared with normal adult structures. Early changes consisted of edema, hyperemia and irregular surface epithelium (grade 1); flattening of the surface epithelium with change from tall columnar cells to more cuboidal cells (grade 2); decrease in parietal cell content, intestinal metaplasia, dedifferentiation of the glandular structure, breaks in the epithelial cell layer with extrusion of granulocytes in the cysts, cell enlargement of the squamous epithelium with higher density of nucleic material (grade 3); flat epithelium with many mucin-producing cells and absence of parietal cells (grade 4).

In the colon implants the cellular infiltrates were: grade 1, focal mucosal lymphocyte infiltration; grade 2, diffuse lymphocyte cell infiltration; grade 3, appearance of plasma cells, neutrophil and eosinophil granulocytes in addition to lymphocytes; grade 4, heavy infiltration of inflammatory cells, which appear in the cyst lumen, so-called crypt abscesses, which appeared to be less focal and contained more lymphocytes than those seen in human colonic disease.

Early changes in the morphology were: grade 1, edema, hyperemia and irregularity of surface epithelium; grade 2, increase of mucin-producing cells and goblet cells dilation of glands; grade 3, breaks in the continuity of the basal lumens and epithelial cell layer and extrusion of inflammatory cells into the graft lumen, depletion of mucin-producing cells, pseudopolyps, intramucosal hemorrhage, high content of neutrophil granulocytes in the cysts; grade 4, flat epithelium with only a small number of goblet cells.

Results

1. Developmental changes in normal BALB/c colon and stomach in situ

The stomach and colon from fetal and neonatal BALB/c mice were examined at ages corresponding with the duration of implants. These normal tissues at 5, 10, 15, 20 and 25 days were used as a comparison with the implanted tissues.

a) *Stomach.* To characterize the different stages in the fetal stomach development we used histological criteria and the expression of the canalicular parietal-cell antigen. The 16–18 days fetal stomach is characterized by high cell density, flat mucosa and the lack of clearly recognizable specialized glandular cells. One day after parturition glands are relatively flat with high columnar epithelium and specialized cells clearly visible. At 5 days of age the glands are more elongated, the abundance of surface and neck mucous cells is remarkable and parietal cells are numerous. Few mononuclear cells can be seen in the submucosa and mucosa. At 10, 15 and 20 days the glandular structure is fully developed and many parietal cells can be seen. A few lymphocytes can be seen in the submucosa, but not in the mucosa. BALB/c fetal stomachs aged 13–14, 15–16, 17–18 days, and neonatal stomachs at 5, 10, 15 and 20 days of age were examined for parietal-cells by immunofluorescence, using a specific antibody. The first clearly positive immunofluorescent staining of a parietal cell was visible in the stomachs of a 17–18-day fetus, and, subsequently, in all neonatal stomachs.

b) *Colon.* In fetal mice aged 16–18 days the glandular structure is very immature, specialized cells cannot be identified, the cell densitiy is high. Lymphocytes cannot be identified in the mucosa and surrounding connective tissue at this age. In the 5-day-old animals,

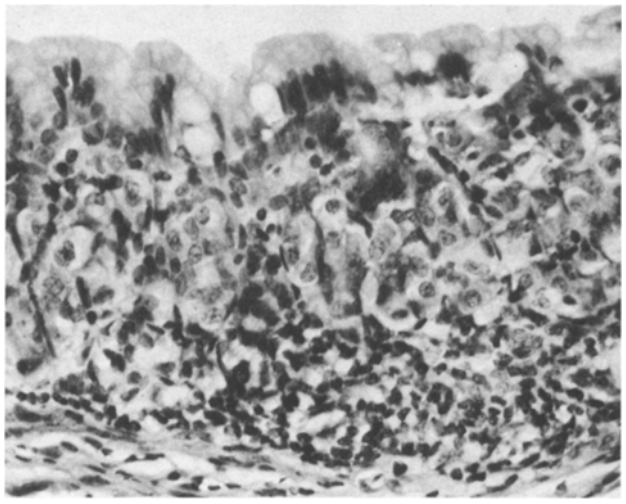

Figure 1. Syngeneic stomach implant 15 days after implantation with lymphocyte infiltration into the mucosa and irregularity of surface epithelium. H & E, × 240.

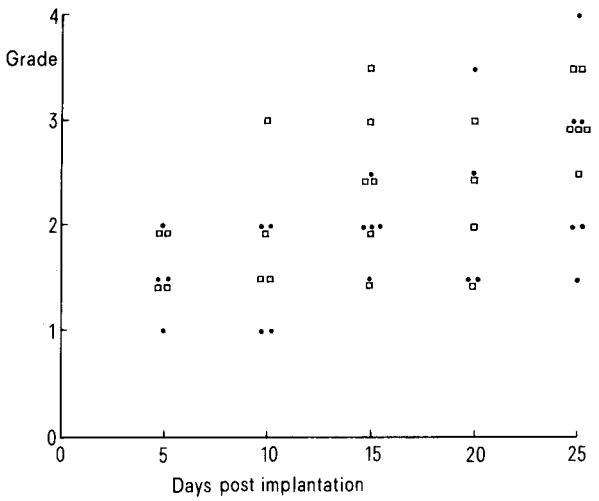

Figure 2. Fetal stomach implants - syngeneic: BALB/c into BALB/c. ●, structural change; □, grade of cellular infiltration. The grading system 1-4 is explained in the text.

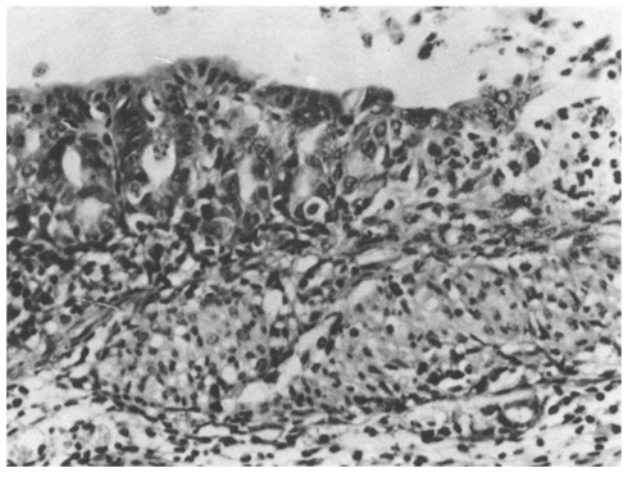

Figure 3. Syngeneic stomach implant 20 days after implantation with infiltration of lymphocytes and granulocytes and flattening of surface epithelium. H & E, × 265.

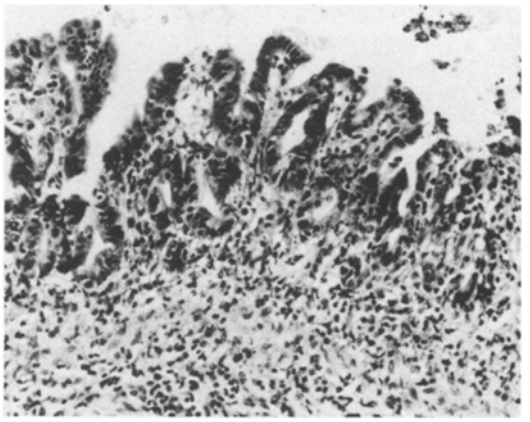

Figure 4. Allogeneic stomach implant (BALB/c into DBA/2) 15 days after implantation with mucosal destruction and loss of parietal cells. H & E, × 190.

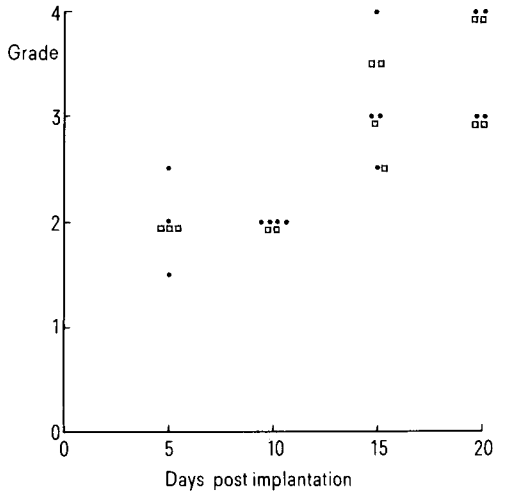

Figure 5. Fetal stomach implants - allogeneic: BALB/c into DBA/2 (H-2 compatible). Explanations see legend figure 2.

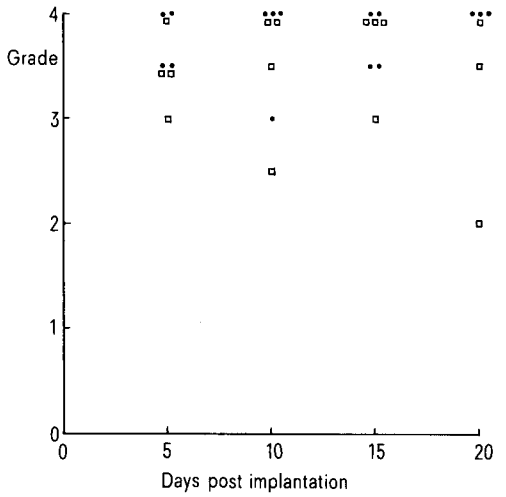

Figure 6. Fetal stomach implants - allogeneic: BALB/c into C57bl/6J (H-2-incompatible). Explanations see legend figure 2.

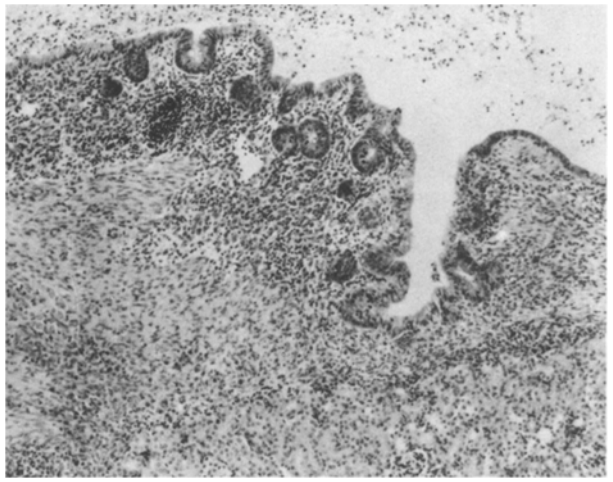

Figure 7. Syngeneic colon implant 10 days after implantation with diffuse mucosal infiltration of lymphocytes and change in surface epithelium. H&E, × 75.

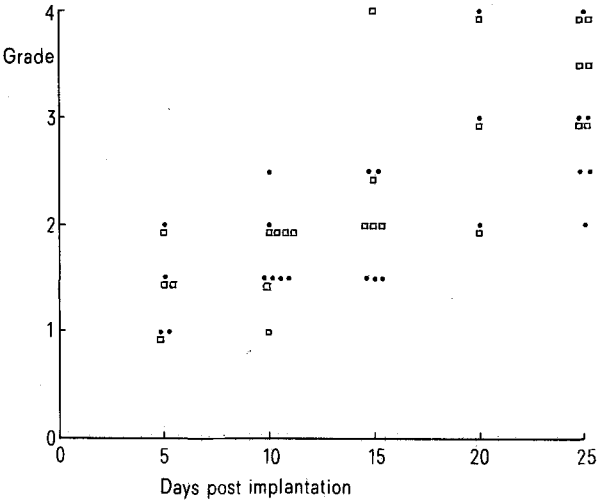

Figure 10. Fetal colon implants - syngeneic: BALB/c into BALB/c. Explanations see legend figure 2.

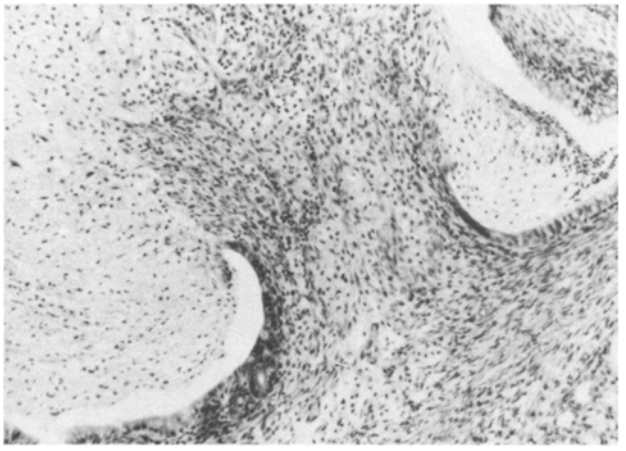

Figure 8. Syngeneic colon implant 15 days after implantation with epithelial ulceration and heavy infiltration of granulocytes. H&E, × 95.

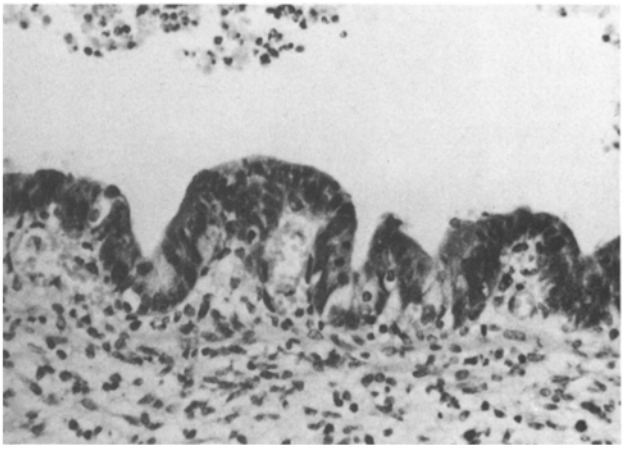

Figure 11. Allogeineic colon implant (BALB/c into DBA/2) 10 days after implantation (flat epithelium, loss of mucin producing cells, granulocytes in cyst lumen). H&E, × 215.

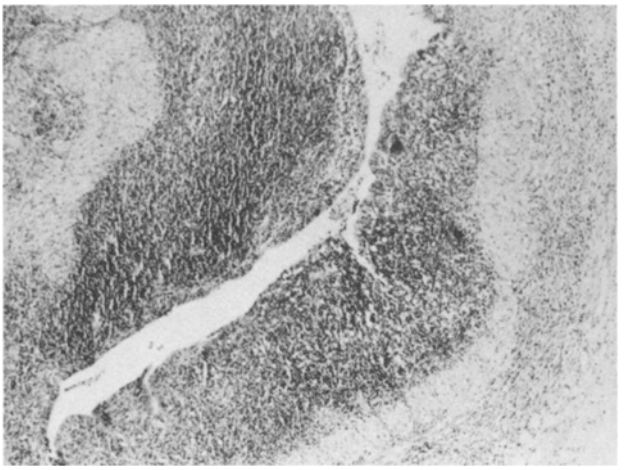

Figure 9. High cellular infiltration (lymphocytes, granulocytes) with flattened mucosa in a syngeneic colon implant 20 days after implantation. H&E, × 75.

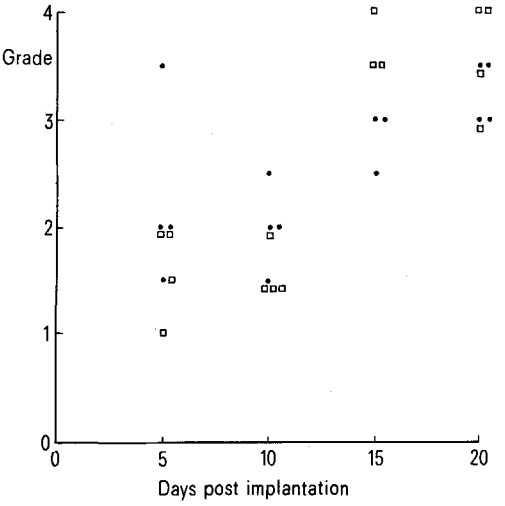

Figure 12. Fetal colon implants - allogeneic: BALB/c into DBA/2 (H-2-compatible). Explanations see legend figure 2.

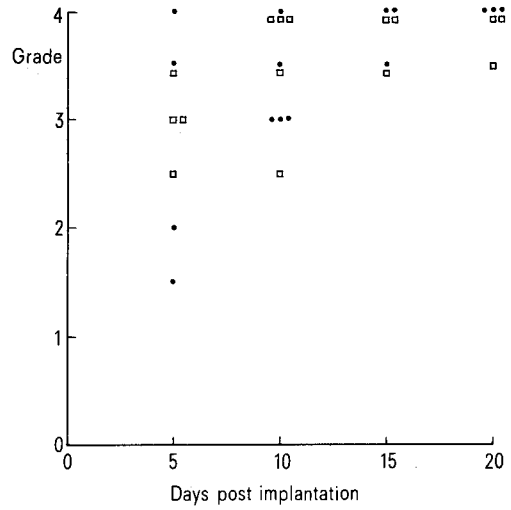

Figure 13. Fetal colon implants – allogeneic: BALB/c into C57bl/6J (H-2-incompatible). Explanations see legend figure 2.

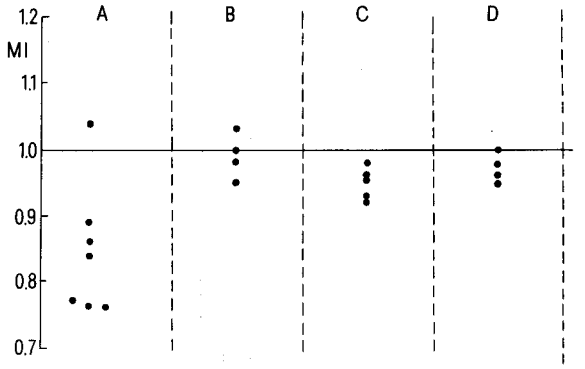

Figure 15. Leukocyte migration inhibition with fetal BALB/c colon antigen (70 µg/ml). MI, Migration index, calculated as explained in methods. *A* Leukocytes of DBA mice implanted with fetal BALB/c colon 10–20 days. *B* Leukocytes of normal, non-implanted DBA mice. *C* Leukocytes of BALB/c mice implanted with BALB/c colon 15 days. *D* Leukocytes of pregnant normal BALB/c mice.

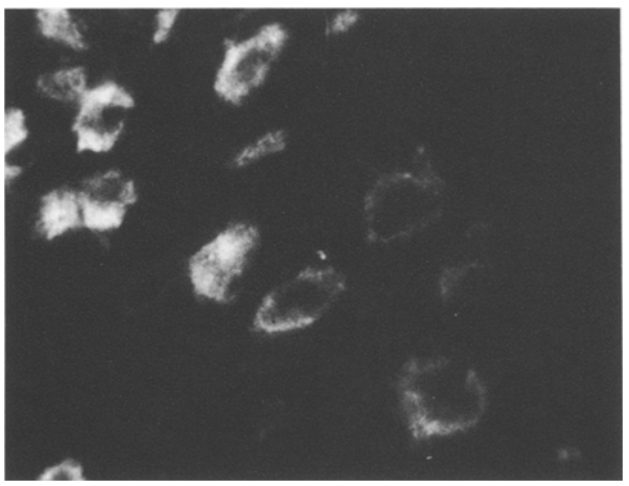

Figure 14. Immunofluorescent labeled parietal-cells in a syngeneic stomach implant 15 days after implantation, × 290.

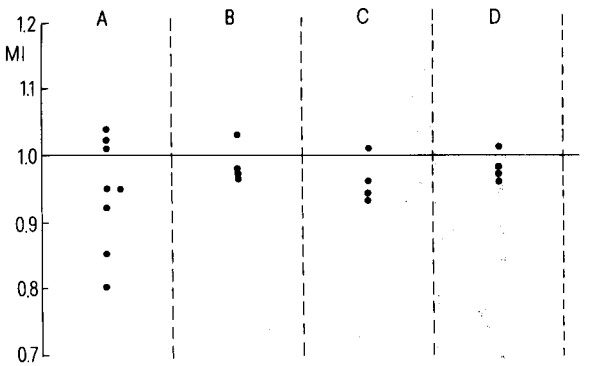

Figure 16. Leukocyte migration inhibition with fetal BALB/c stomach antigen (70 µg/ml). Explanations see figure 15.

goblet cells are clearly differentiated and a few mononuclear cells can be seen in the submucosa. At age 10 and 15 days the glandular structure is fully developed with relatively short crypts and mononuclear cells appear to infiltrate from the submucosa into the epithelium. At 20 days of age the normally developed adult structure with submucosal lymphoid follicles is seen.

Table 2. Gastric parietal cells in implants demonstrated by immunofluorescence using human parietal canalicular antibody

Days after implantation	BALB/c into BALB/c	BALB/c into DBA	BALB/c into C57bl/6J
5	+, +, Ø	+, Ø	Ø, Ø
10	+, +	Ø, +	Ø, Ø
15	+, Ø, +	+, +	Ø, Ø
20	Ø, +	+, Ø	Ø, Ø

+, positive; Ø, negative reaction in the immunofluorescence test.

2. *Histopathology of implantations*

After the implantation technique was established there was no mortality due the operative procedure, nor was there evidence of infection in any implanted grafts.

Approximately 80–90% of the implanted grafts were suitable for histological assessment. A few grafts contained too little tissue to assess and some implants did not take. Almost all grafts, both colon and stomach, formed small cysts, as early as 5 days after implantation, but in only a few cases was the cystic swelling so great that no grafted material could be found. We analyzed all stomach implantation cysts for the pH of the cyst contents immediately after removal of the kidney, using pH indicator paper (ColorpHast, E.M. Lab. Inc., Elmsford, N.Y.). In all cases we found a pH between 7 and 8.

The histology of normal adult colon and stomach of BALB/c mice was compared with the histology of the implanted grafts. Within the same group of animals there was considerable variation in the degree of structural change. Nevertheless, a clear trend emerged in each group. No single 5-day graft was graded as normal. In all implants the degree of structural change and cellular infiltration was dependent on the duration of the implantation.

Typically, changes in the syngeneic stomach grafts started with mucosal infiltration by lymphocytes and plasma cells, followed by structural changes of the gastric glands (fig. 1). The mucosa became flattened and there was an increase of mucus-producing cells with the appearance of goblet-cell types. Occasionally there was evidence for intestinal metaplasia in sections stained with alcian blue for mucin. Later there was a loss of specialized cells and the number of parietal cells decreased. Some implants showed only minor structural changes but abundant inflammatory-cell infiltration after 25 days. The inflammatory changes seen in this group were morphologically typical for chronic gastritis (figs 2 and 3). In the H-2-compatible (BALB/c into DBA/2) implants there was complete morphological change with dedifferentiation after 15–20 days (fig. 4) and in the H-2-incompatible (BALB/c into C57bl/6J) implants the tissue was completely destroyed as early as 5 days post-implantation (figs 5 and 6).

In the syngeneic colon implants the first changes were increase of diffuse mononuclear cell infiltration, dilation of the glands, increase of mucin producing cells followed by flattening of the surface epithelium from columnar to more cuboidal cell forms (fig. 7). Beginning after 15 days of implantation, neutrophil and eosinophil granulocytes appeared and accumulated in the crypts. Subsequently, there was extrusion of polymorphonuclear leukocytes into the cyst lumen with epithelial ulceration (fig. 8). Although these typical inflammatory changes occurred, there were implantations with little structural change even after 25 days. In these cases the cell infiltration was predominant (fig. 9). Taken as a group, the acute inflammatory and later ulcerative changes of the syngeneic colon implant resembled very much the changes seen in chronic ulcerative colitis (fig. 10).

In the allogeneic colon implant (H-2-compatible, BALB/c into DBA/2), infiltration and typical structural changes can be seen much earlier (complete rejection after 15–20 days) (fig. 11) and in the H-2-incompatible implants (BALB/c into C57bl/6J), complete rejection can be seen as soon as 10 days after implantation (figs 12 and 13).

3. *Morphology of stomach and colon of recipient animals*

The stomachs and colons of recipient animals were examined for evidence of pathological changes. There was no evidence of pathological change macroscopically or microscopically in the stomachs or colons of either syngeneic or allogeneic animals, when compared with the same organs of normal (no implant) animals. Two animals in each group were examined. In a few additional experiments we injected the separated lymphocytes of an animal implanted with allogeneic colon or stomach for 10 days into an animal of the same strain as the donor. No pathological change in the colon or stomach could be seen.

Two animals were inoculated by 2 i.m. injections 2 weeks apart with syngeneic and 2 with allogeneic fetal stomach homogenates emulsified in complete Freund's adjuvant. Their stomachs were examined 1 week later. There was no sign of inflammation or tissue damage in any stomach.

4. *Immunofluorescence test for parietal-cells in stomach implantations*

Stomach implants in each group were frozen immediately after removal and examined for presence of parietal-cells with the indirect immunofluorescence technique. Parietal cells could be detected in all syngeneic and H-2-compatible allogeneic implantations, but not in H-2-incompatible allogeneic implantations (table 2 and Fig. 14). The number of parietal cells seemed to decrease with the duration of implantation and was smaller in the allogeneic implants.

5. *Immunofluorescence test for fetal colon and stomach antibodies in the host animal*

Sections of fetal colon and stomach of the implanted strain were treated first with serum of implanted mice of each group and then with a fluorescein-labeled antimouse immunoglobulin. With only few exceptions, fetal colon and stomach antibodies could be detected in all animals with syngeneic and allogeneic implantations of 10 days and more in each group, whereas no antibodies could be detected in animals with 5-day grafts. Controls with adult stomach or colon and with tissue of a different organ were always included and were negative. As a specificity control we absorbed positive mouse serum with fetal colon antigen and could not demonstrate implant tissue antibodies in the immunofluorescence test thereafter.

6. *Cellular immunity against fetal colon*

We used the production of leukocyte migration inhibition factor by leukocytes of animals with colon implants in the direct agarose microdroplet method to show cellular immune reactions. Antigens were prepared from fetal BALB/c stomach or colon respectively. When leukocytes of DBA/2 mice with fetal BALB/c colon implants were incubated with fetal BALB/c colon antigen, inhibition of migration was observed in almost all cases (fig. 15). In control ex-

periments with normal (non-implanted) DBA/2 mice, no migration inhibition could be seen. No migration inhibition factor was produced either by BALB/c leukocytes of fetal syngeneic colon implanted animals or leukocytes of pregnant BALB/c mice. An additional control with adult colon BALB/c antigen and lymphocytes of DBA/2 mice with BALB/c colon implants was performed and showed negative results. Interestingly, there was migration inhibition in some cases with leukocytes of animals with allogenic colon implants incubated with fetal stomach antigen (fig. 16).

Discussion

The implantation of fetal mouse small intestine under the kidney capsule of allogeneic mice has proved to be a useful technique for demonstrating the histopathology and time course of rejection[3,4,10]. In syngeneic implants the authors showed that isografts of fetal small intestine grew well and developed morphologically similarly to normal intestine of the same age in situ. Holden and Ferguson[10], using fetal mouse colon isografts described normal development and morphology with lymphoid cell infiltrates beginning after 2 weeks. Fetal colon allografts with H-2-histocompatibility differences were rejected by 12 days. The authors report reduction in cell size, crypt shortening, and heavy lymphoid cell infiltration, but the absence of plasma cells, neutrophilic or eosinophilic leukocytes.

In our experiments we could see clearly pathological changes in all fetal colon isografts, even after 5 days of implantation. In the cellular infiltrate plasma cells and granulocytes could be differentiated in iso- and allografts. The reason for these different results is unclear, as the same technique[5] was used.

In a similar approach Ceredig et al.[1], using the same technique, showed typical morphological changes in syngeneic fetal colon implants in rats. After 6 days the isografts developed an acute inflammatory response. After 12 days there were ulceration and infiltration of plasma cells and neutrophils and after 20 days the mucosa was largely destroyed. Our own results show somewhat less acute morphological changes with early inflammation between 5 and 15 days and heavy infiltration with ulceration by 20-25 days. Some implants, however, showed relatively little structural change even after 25 days. One explanation is that the immunological inflammatory response to fetal antigens varies according to differences in fetal age, fetal antigen expression and minor antigenic differences. Such differences may have occurred in the Sprague-Dawley rat strain used by Ceredig et al.[1].

As has been reported before[1], sex differences do not appear to play a role in rejection in syngeneic colon implants. After we confirmed this with pairs of male and female recipients, host animals of either sex were used in subsequent experiments.

It appears that immature fetal stomach and colon, which lack the adult structure and cell differentiation, can develop into fully differentiated normal tissue with all specialized cell forms, when implanted heterotopically. In the particular location under the kidney capsule the potential for differentiation persists in the 2 fetal gastrointestinal organs used in the absence of humoral or cellular influences of the donor animal. It is interesting that in the colon implants lymphocytes and lymph nodes appear in spite of the lack of external antigen stimulus. These changes in differentiation are reversed to a variable degree by host reactions leading to cell infiltration and structural change. In this process specialized cells like parietal cells or goblet cells decrease according to the time of implantation.

Results of the leukocyte-migration-inhibition test show that animals with allogeneic colon implants develop cellular immunity to fetal colon of the donor strain. There was no evidence for a cellular immune reaction in the syngeneic colonimplanted animals or pregnant animals to fetal colon antigens using this test. The number of experiments was small. These results seem to us unlikely to be due to a loss of antigenicity in the preparation, since this did not occur in the tests with allogeneic implanted animals. Possibly low expression of the fetal antigen in the colon, or weak sensitization of the peripheral blood leukocytes may be responsible. On the other hand humoral immune responsiveness to fetal antigen could be demonstrated in all syngeneic implantations. More extensive studies are needed to answer this question.

In the animal system used in this study, the unique location of the implants under the kidney capsule and the 'antigen-free' environment (no food, no microorganisms) are artificial and quite different from the normal situation; the conclusions that may be drawn must, therefore, be limited to certain aspects of the system.

The stage of development at which the canalicular antigen in the parietal cell of the fetal mouse appears has been examined. It has been found that the antigen is first demonstrable at 17-18 days of fetal life. These results correspond to the ultrastructural studies of Pipan[13] who showed the typical parietal cell morphology on the 18th day of embryonic mouse life. Rugh[15] describes the first vacuoles and glandular function in stomach epithelium at 13 days. There is some controversy as to the capability of the fetal mouse parietal cell to produce acid. Pipan[13] found the gastric mucosa in 18-day-old mice fetuses to be at pH 8. Matsuyama and Suzuki[12] found the luminal content of cysts, developed after implantation of glandular stomach segments s.c. in syngeneic mice to be at pH 2.5-2.7. In

the present studies the contents of all cystic stomach implants were at pH 7-8. If acid were being produced by the implant, then buffering by proteins in the cyst contents or leakage of hydrogen ions across a faulty gastric mucosal barrier as postulated by Davenport[2] might be responsible for the neutral pH. It would be worthwhile to study acid-secretion in stomach implants using more sophisticated techniques and the use of gastrointestinal hormones and pharmacologically active substances.

In some implantation experiments we used very young fetal stomachs of age 13-14 days, when no canalicular parietal cell antigen can be demonstrated. The appearance of this antigen after 5-10 days implantation confirms the finding of other authors[12,14] that parietal cells can develop out of immature undifferentiated mucosal cells.

The decrease in expression of the canalicular parietal antigen in allogeneic implantations and the observed inflammatory and atrophic changes in the mucosa could be compared with the loss of parietal cells and humoral and cellular immune response to parietal cell antigen in chronic atrophic gastritis in humans.

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Correction

Structure of three isomeric host-specific toxins from *Helminthosporium sacchari,* Experientia *39*/4 (1983) 343-347.

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We regret a misprinting in table 3 of this article. The table should have read as follows:

Table 3. ^{1}H-NMR data[a] for aglycone moieties

Carbon No.	Isomer A		Isomer B		Isomer C	
	H_α	H_β	H_α	H_β	H_α	H_β
C-1	1.40	1.80	1.28	1.8	1.34	1.88
C-2	-	?	-	3.88	-	4.20
C-3	2.04	2.40	1.97	2.77	5.50	
C-5	-	-	1.92	-	2.10	-
C-6	2.73	2.24	1.8	1.63	1.99	1.52
C-7	-	2.61	-	2.62	-	2.64
C-8	1.64	2.00	1.8	1.8	1.83	1.93
C-9	1.37	1.37	1.43	1.32	1.45	1.29
C-12	5.11; 5.20		5.19; 5.30		5.24; 5.33	
C-13	4.03; 4.23		~4.07; 4.22		4.05; 4.23	
C-14	1.70		4.64; 4.95		1.72	
C-15	1.15		0.78		0.92	

[a] δ-Values in ppm relative to internal DSS = 0.