reabsorption of 28% of GFR and 58% of volume delivery between the sampling sites, independent of flow rate, though sensible to inhibition by volume expansion. Whether this is accomplished by gradient-driven water abstraction along the DHL or by flow-dependent reabsorption along the inaccessible portion of the proximal tubule and pars recta, cannot be established by the present data.

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Culture of presumptive epithelial cells from jejunal mucosa of axenic rats[1]

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Summary. Strains of presumptive epithelial and fibroblast cells were prepared from the jejunal mucosa of axenic rats. Cells were cultured on collagen gels, in highly enriched media supplemented with homologous sera and hormones, and were maintained for more than 7 weeks.

Very few successful attempts have been reported[2,3] for a true, dividing monolayer culture of mammalian intestinal cells, although several laboratories have been able to maintain suspensions of enterocytes[4-8] for varying lengths of time. The problem of culturing the epithelial cells of the jejunum is complicated by the presence of luminal bacteria, fungi and other microorganisms, which contaminate, overtake and overwhelm the initial culture in the absence of large doses of antibiotic and antimycotic agents. The major difficulty, however, would seem to lie in the damaged cell membranes, which cannot be readily avoided during the isolation of the cells for subsequent culture[9], and which presumably contributes to the poor attachment of these cells to the substratum. The only successful culture of intestinal epithelial cells has been obtained via explants from rat duodenum[3], which is relatively free of microorganisms[10]. Lichtenberger et al.[3] were able to promote the growth of the epithelial cells in preference to fibroblasts by treating the cultures with pentagastrin.

This report describes the use of germ-free rats for the successful culture of presumptive epithelial cells from jejunal explants, as a proliferative monolayer on collagen gel in the presence of pentagastrin. Several experimental criteria in support of the epithelial phenotype are presented and discussed.

Materials and methods. Jejuna were excised under sterile conditions from germ-free rats (male, 42-day-old, strain CD axenic, Charles River, Boston, USA), flushed and everted. Thin rings (1 mm thick) were sectioned from small segments and then further cut into 1-mm^3 pieces. These were placed in 5 ml of media in plastic tissue culture dishes (60 mm, Falcon Plastics, Culver City, USA) which pre-

Summary of growth characteristics of selected strains of intestinal jejunal cells

Strain	Original medium	Original supplements	Strain morphology	Stimulation of growth[a,b] Pentagastrin	Insulin	MEM D-valine treatment
Ia	Medium 199	Pentagastrin, cortisol	Epithelial	+	−	R[c]
Ib	Medium 199	Pentagastrin, cortisol	Epithelial	+	−	R
II	Medium 199	Pentagastrin, cortisol	Fibroblast	−	+	S[d]
III	Waymouth MD 705/1	None	Fibroblast	−		S
IV	Waymouth MD 705/1	Pentagastrin	Fibroblast	−		S
V	Waymouth MD 705/1	Pentagastrin	Fibroblast	−		S
VI	CMRL 1066	None	Fibroblast	−		S
VII	CMRL 1066	None	Fibroblast	−		S
VIII	CMRL 1066	None	Fibroblast	−		S
IXa	CMRL 1066	Pentagastrin	Epithelial	+	−	R
IXb	CMRL 1066	Pentagastrin	Fibroblast	−	+	S

[a] Tests performed between 2 to 4 weeks after strains established from original colonies. [b] Effect measured by significant increase in both diameter and number of colonies/plate compared with observations recorded on appropriate controls. [c] R = resistant (cell survived 4 weeks exposure to MEM D-valine medium + 10% dialysed fetal calf serum). Pentagastrin was provided throughout this treatment. [d] S = sensitive (cells became unattached and degenerated after exposure to MEM D-valine medium + 10% dialysed fetal calf serum). Pentagastrin was provided throughout this treatment.

viously had been coated on the bottom with a fresh layer of collagen gel[11]. Media (Waymouth MD 705/1, CMRL 1066 and Medium 199) were supplemented with 10% rat serum, 10% fetal calf serum (both heat inactivated) and 15 mM HEPES (N-2-hydroxyethylpiperazione-N'-2-ethanesulphonic acid) buffer. Media also contained penicillin (100 units/ml), streptomycin (100 µg/ml) and either fungizone (0.25 µg/ml) or mycostatin (100 units/ml). As well, the following hormones were added either singly or in combination to some of the dishes: pentagastrin (0.5 µg/ml; Ayerst Laboratories, Montreal, Canada), hydrocortisone (1 µg/ml; Sigma Chemical Co., St. Louis, USA) and insulin (8.0 µg/ml; Connaught Laboratories, Toronto, Canada). Cells were incubated at 37 °C in an atmosphere of 95% air–5% CO_2. At 4 days post inoculum, the residual tissue pieces and cellular debris were removed from the plates by rinsing with appropriate media. After 16 days when cultures were well established, selected colonies were transfered to new dishes by a modification of the single cell cloning procedure described by Ham[12]. In this way, 11 separate strains of intestinal cells were prepared for study. Several of such colonies were subcultured, and were also maintained for extended periods in MEM D-valine medium + 10% dialysed fetal calf serum, to inhibit the growth of fibroblasts[13]. All media and fetal calf sera were obtained from Grand Island Biological Co. (Grand Island, USA).

Results and discussion. In all media tested, cells rapidly migrated out from the small intestinal tissue explants onto the collagen gel. Many colonies were clearly visible by 10 days, and these were classified as distinctly epithelioid or fibroblastic in morphology. Although these cultures were derived from germ-free animals, it became necessary to add antibiotics to the medium to suppress bacterial and yeast growth. However, when a standard mixture of penicillin, streptomycin and fungizone was added, cells rapidly detached and could be seen to degenerate within a few days. Similar results were obtained with cultures of fetal intestinal cells (data not shown), where cells of an epithelioid morphology could only be induced to grow in the absence of this antibiotic/antimycotic mixture. Since fungizone has been shown to be toxic to several mammalian cell lines[14], it was thought that this agent in the antibiotic/antimycotic mixture was responsible for suppressing the growth of the epithelial cells. Therefore long-term cultures were maintained with penicillin/streptomycin as antibiotics, with the antimycotic agent, mycostatin (100 units/ml) added intermittently to treat yeast and mould growth as needed.

In these experiments large initial epithelioid cell colonies (3–4 mm in diameter) were established by day 15, but only on plates which had been supplemented with pentagastrin or pentagastrin + cortisol. The largest and most confluent colonies were seen in Medium 199 (figure 1). In dishes without pentagastrin, numerous fibroblast colonies could be detected (figure 2). The frequencies of fibroblastic colonies in media without pentagastrin were 100%, and with pentagastrin, 66%. Several of the larger colonies were selected and the cells dispersed into new dishes. For this purpose foci of proliferation containing cells of homologous phenotype were isolated by cloning rings and were transferred to new dishes where they produced exclusively one or the other phenotype. In this way, 11 strains were established, 3 epithelioid (Ia, Ib and IXa) and 8 fibroblastic. Presumptive epithelial cells were characterized by the following criteria: a) appearance of cuboidal or triangular shape, with numerous dark granules observed in the cytoplasm under phase-contrast examination; b) formation of regular monolayer sheets, both in the original explant colonies and in clones Ia, Ib and IXa; c) cells with an epithelioid morphology only became established in dishes with pentagastrin present. Pentagastrin also had a stimulatory effect on the growth of strains established from original epithelial-like colonies. In contrast, insulin had no apparent effect on these cells, and yet dramatically stimulated proliferation of several of the fibroblast strains; d) ability to survive for a minimum of 4 weeks in MEM-D-valine medium, which caused a rapid detachment of the fibroblast-like cells. The results are summarized in the table. Pentagastrin has been implicated as a trophic factor (hormone) for mucosal epithelial cell growth in the stomach and upper small intestine[15] and it was essential for establishing epithelioid cell colonies in the present experiment. In its presence, the growth of epithelial cells is apparently specifically stimulated, thereby reducing the growth of nearby fibroblasts. The criterion of D-valine resistance proved to be particularly useful, as after treatment with D-valine, only the 3 presumptive epithelial

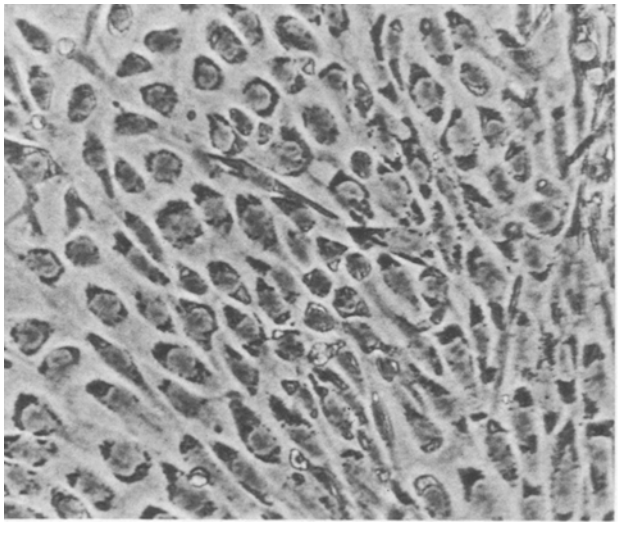

Fig. 1. Colony of rat jejunal presumptive epithelial cells at 15 days after plating in medium 199 + 10% rat serum, 10% fetal calf serum, 15 mM HEPES buffer, pentagastrin (0.5 µg/ml), cortisol (1 µg/ml) and insulin (8 µg/ml). Magnification × 250 phase-contrast.

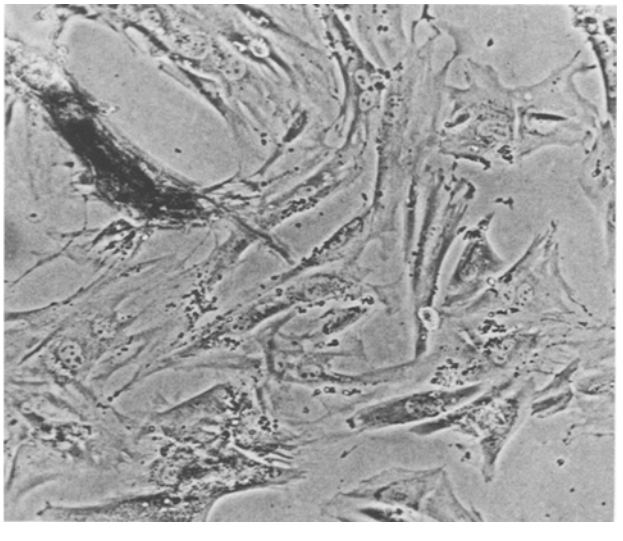

Fig. 2. Colony of rat jejunal fibroblast-like cells at 62 days after plating in CMRL 1066 medium + 10% rat serum, 10% fetal calf serum and 15 mM HEPES buffer. Magnification × 500 phase-contrast.

clones remained alive. Peters and Shio[16] have demonstrated the presence of D-valine amino acid oxidase in suspensions of pure epithelial cells from rat jejunum.

Despite the lengthy maintenance in culture, none of the original epithelioid colonies or strains were seen to form the expected characteristic columnar cells with distinct brush borders, as found in the differentiated mucosal epithelium. This absence of terminal differentiation in vitro may have been due to the presence of an inappropriate hormonal milieu[17], or, alternatively, as these cells grew in isolated colonies, to a lack of contact inhibition thought to be necessary for differentiation[18].

The present study indicates that axenic animals are suitable for the establishment of cultures of intestinal cells and that epithelioid cells can be isolated and selectively grown. The identification of these epithelioid cells as crypt cells remains to be established. However, subsequent to the original submission of this report, Quaroni et al.[19] have published a comparable method of culturing undifferentiated intestinal epithelial cells from germ-free rats. These latter workers have provided morphological and immunological evidence which suggests that these cells are originating in the crypts of the intestinal mucosa.

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The effects of parabiotic union with a normal partner on the blood tissue of the b/b rat suffering from an inheritable anemia

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Summary. The parabiotic union of a b/b rat, suffering from a red blood cell anemia, with a normal partner will restore to normal the functioning of this type of blood cell. The effect of parabiotic union is evident for several weeks following the separation of the parabiotic partners. It would be logical to conclude that the normal partner supplies a factor absent in the anemic animal needed for normal functioning of the red cell.

In 1962 after exposing a number of 8-day-old female rats to a dose of 50 R of X-rays, in the second filial of one of the irradiated animals 2 males and 1 female with an inheritable anemia were born. This anemia, recessive in nature, is characterized, among other defects, by microcytosis of red blood cells and hypochromia. Attempts to determine whether the disease appeared spontaneously or was induced by X-rays have, so far, failed. A number of results obtained from the study of this inheritable syndrome have already been published[1-4].

More recently, we have applied the standard technique of parabiosis between an adult normal rat and an anemic animal of the same age, keeping them in parabiotic union for up to 4 weeks. The technique of parabiosis applied by us involves skin union alone. From the normal rat a rectangular piece of tissue 2.5 cm in length and 2 cm in width starting from the blade-bone down is cut off. The wound left is covered by an equal piece of tissue supplied by the anemic partner. For suturing the wound we used the sterile catgut (Resorbierbarkeit normal 00, Veb Catgut, Markneukirchen), and for immobilizing the animals leucoplast No. 1517, a product of the pharmaceutic company 'Galenika', Beograd, which, allowing an intimate contact between the 2 partners, assured undisturbed healing of the wound. The operation was performed under semisterile conditions. Not 1 case of wound contamination has been recorded. 4 pairs of animals were used.

It was observed that, already after 3, 4 or 5 days of life in parabiotic union, both the peripheral blood and the bone marrow pictures of the anemic partner gave visible signs of uniform improvement. After 3-4 weeks, by applying the tests commonly used in the study of blood, no significant deviation from the standard values characteristic of the normal rat could be detected in the anemic partner (table), and the peripheral blood regained its normal appearance (figure, A and B). The capacity of even the shrunken and

Peripheral blood indices of anemic (b/b) and normal (+/+) rats before, during and after parabiotic union

	Animals	RBC ($\times 10^6$)	Hct (°/°)	Hb (g)	MCH (ng)	L ($\times 10^3$)	Range
Before	b/b	7.8	28	4.9	6	19	13-25
parabiosis	+/+	8.1	50	12.6	15	13	8-18
3 weeks in	b/b	7.1	44	10.8	15	15	8-22
parabiosis	+/+	7.1	44	10.5	14	14	12-17
3 weeks after	b/b	6.3	35	7.4	11	25	9-50
separation	+/+	8.6	50	12.4	14	11	8-16
14 weeks after	b/b	5.0	22	3.7	7	20	14-24
separation	+/+	8.5	47	12.6	16	13	8-18

Mean values for 4 pair of rats: RBC, red blood cells; Hct, hematocrit; Hb, hemoglobin per 100 ml of blood; MCH, mean corpuscular hemoglobin; L, leucocytes.