tical to that of the controls after 48 h of incubation with either isaxonine phosphate or its derivatives (data not shown). It was checked in a separate experiment that the trypsin treatment of the cells, under the conditions used, did not alter the amount of radioactivity found in the cell layer.

4) Effects of simultaneous incubation with colchicine (0.5×10^{-4} M) and increasing concentrations of isaxonine. When colchicine was added alone to the cultures, it did not significantly alter the secretion of non-collagen proteins, but it decreased the secretion of collagen by about 30% and slightly increased the intracellular collagen. The simultaneous addition of 0.5×10^{-4} M colchicine and amounts of isaxonine increasing 10^{-9} M to 10^{-5} M demonstrated a synergic effect of the two drugs (table 1).

5) Results of experiment designed for ruling out toxic effects. Table 2 shows the data obtained by incubating the fibroblasts with ^{14}C-pro firstly for 24 h in the presence of to 10^{-5} M Nerfactor and then, for an additional 24-h period, in the absence of Nerfactor. In the 2nd period, the incorporation of labeled pro into collagen and noncollagen proteins matches that of the controls.

Discussion. We used fibroblastic cells cultured from newborn child skin biopsies to study the possible effects of isaxonine phosphate (Nerfactor) and several related compounds, 2-aminopyrimidine, BN 1314, BN 1163 and BN 1041 on DNA and protein synthesis. When these compounds were incubated with the cells in the logarithmic phase of growth for 24 h, no effect was detected. On the other hand, when the total incubation period was increased to 48 h, incorporation of ^{3}H-methyl thymidine into DNA was reduced whereas the cell number was not altered. This effect was dose-dependent, increasing from concentrations of about 10^{-7} M. Addition of methotrexate (which inhibited endogenous thymidine synthesis) markedly sensitized the inhibition, demonstrating that the mechanism did not involve the stage of nucleotide synthesis. Among the compounds structurally related to isaxonine phosphate 2-aminopyrimidine, BN 1163 and BN 1314 also significantly inhibited ^{3}H-methylthymidine incorporation into DNA after 48 h incubation. These results emphasize the properties of some derivatives of isaxonine as inhibitors of DNA synthesis or repair.

Isaxonine, when incubated for 48 h with fibroblasts at concentrations over 10^{-6} M, induced a decrease in the incorporation of ^{14}C-proline into proteins and collagen secreted into the medium. The question arises whether this effect is of specific or toxic origin. We performed experiments involving double incubation of the same cultures with 10^{-5} M Nerfactor and after removal of Nerfactor. During the second period of incubation, the incorporation of ^{14}C-pro was comparable in both treated cultures and controls, ruling out a toxic effect.

The effect of colchicine in conjunction with isaxonine was studied. Colchicine was shown several years ago, by Ehrlich and Bornstein[4], to interfere with the transcellular movement of procollagen by disrupting microtubules. This effect is attested by a decrease in the secretion of collagen. On the other hand, isaxonine was shown to protect cellular microtubules from the disrupting effect of vincristine in nervous cells. Therefore the aim of this study was to examine whether isaxonine has a similar effect on non-nerve cells. In our experiments we observed that colchicine alone, at the concentration used by Ehrlich in the case of newborn rat cranial bones, has a very slight effect on fibroblasts: a significant decrease in collagen secretion was noticed, but did not amount to more than 30% in our experiments. In contrast it was about 50% in the experiment with cranial bones, with a clear increase in the amount of procollagen retained inside the cells[4].

We could not find in the literature any papers showing data for the effect of colchicine on collagen synthesis by skin fibroblasts in culture. Our results suggest that the effect is far less intense than in calvaria.

Surprisingly, very low concentrations of isaxonine enhanced the inhibiting effect of colchicine on collagen secretion. This effect may be explained by the presence of different target sites for colchicine in fibroblasts and in growing nerve fibers. The protective effect of isaxonine on nerve fibers has been shown in numerous cases, in cultures as well as with in vivo animal models and in man[1]. But if colchicine in addition to isaxonine decreases the collagen release, we observed concomitantly increases in the amount of ^{14}C-proline incorporated into the cellular proteins. It remains to be determined whether the synthesis of some structural proteins is specifically triggered.

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Morphogenesis of human colon cancer cells with fetal rat mesenchymes in organ culture[1]

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Summary. The morphogenesis and cytodifferentiation of human colon cancer cells (LS174T and HT29) were examined by combining cancer cells with fetal rat digestive-tract mesenchyme in organ culture. LS174T cells migrated into the mesenchyme to form glandular structures composed of single columnar cells with their nuclei oriented basally, while HT29 cells formed cell masses with little lumen formation. Immunohistochemical studies with antibodies against carcinoembryonic antigen and secretory components showed that the composition of cell surface glycoproteins was not necessarily reversed to the normal type, even when neoplastic cells exhibited normal glandular structures.

Key words. Human colon cancer cells; morphogenesis; carcinoembryonic antigen; secretory components; organ culture.

The importance of epithelial-mesenchymal interactions for normal morphogenesis and cytodifferentiation has been repeatedly reported[2], and alterations in the stroma have been considered as an important factor for the induction and progression of tumors[3]. Conversely, attempts have been made to induce normal differentiation of tumor cells by combining them with normal mesenchymes/stromas. Histological differentiation of tumor cells has been reported in mammary tumor cells[4], in basal cell carcinomas[5], and in transitional cell carcinomas[6] by combining tumor cells with mesenchymes/stromas. We have previously reported that epithelial morphogenesis and cytodifferentiation are affected by combination with mesenchymes both in stomach[7] and in duodenum[8]. Here we examined the effect of fetal gastrointestinal mesenchymes on the differentiation of human colon cancer cells, and found that glandular structures could be induced in a cell line by this method.

Materials and methods. Two human colon cancer cell lines were used in this experiment. LS174T (passage No. 9, modal chromosome number = 45–46) was kindly provided by Dr B. D. Kahan of the University of Texas (Houston, Texas, USA), and HT29 (passage No. 235, modal chromosome number = 69) by Dr J. Fogh, Sloan-Kettering Institute for Cancer Research (Rye, New York, USA). Cells were maintained as monolayer cultures in Dulbecco's modified Eagle's medium (GIBCO, USA) with 10% fetal bovine serum (Flow Lab., USA) and antibiotics (penicillin 100 U/ml, streptomycin 100 µg/ml, and Fungizone 0.25 µg/ml, MA Bioproducts, USA) in a humidified atmosphere of 10% CO_2 in air. The cells were cultured in a 25 cm^2 plastic flask (Lux Scientific Corp., USA) at 37 °C, and medium was changed twice weekly. They were subcultured every 2 weeks at the split ratio of 1:30 (LS174T) and 1:50 (HT29) after being suspended by treatment with 0.25% trypsin and 0.02% EDTA (GIBCO).

For combination with fetal mesenchymes, cells were seeded on membrane filters (Millipore Corp., USA; Type HAWP) at 10^4 cells/cm^2 (day 0). On day 5, mesenchymes were obtained from 16.5-day fetuses of inbred Lew/Crj rats (Charles River Japan Inc., Japan) by treating forestomach, glandular stomach, and duodenal tissue fragments with collagenase as previously described[7,8]. The fetal mesenchyme was combined with cancer cells by putting the mesenchyme on the membrane filter on which cancer cells were precultured for 5 days. Then the membrane filter with cancer cells and mesenchymes was laid on a stainless steel grid, and the combinant was organ-cultured for 4 days without medium change as described[8,9].

Combinants were fixed with Bouin's fluid on day 9, and were processed for paraffin sectioning and staining with PAS-hematoxylin. Some were also fixed with ice-cold 95% ethyl alcohol and were used for immunohistochemical study after paraffin sectioning[10]. Rabbit antisera against human carcinoembryonic antigen (CEA) (gift from Dr H. J. Hansen, Hoffmann-La Roche Inc.), and antisera against human secretory components (SC) (gift from Dr M. E. Lamm, New York University School of Medicine) were applied first, and were followed by FITC-conjugated goat antirabbit IgG antibodies (Miles Lab. Inc., USA). The localization of CEA and SC was observed using an Olympus fluorescence microscope.

Results. In cell culture in the plastic flask, LS174T cells proliferated relatively slowly with an approximate doubling time of 32 h. They adhered weakly to the substratum, and did not migrate on it, but adhered closely to each other and piled up (fig. 1). Gland formation was never observed in cell culture. In organ culture with fetal mesenchymes, LS174T cells migrated into the mesenchyme, and proliferated there to form cell masses, often (in about 20% of cases) accompanied by lumen in the center. These lumen-forming cells were simple columnar with nuclei oriented basally. Some goblet cells with PAS-positive granules were dispersed among them (fig. 2). Forestomach and glandular stomach mesenchymes were as effective as duodenal mesenchymes in inducing glandular structures, and forestomach mesenchyme was preferentially used for the combination as the forestomach epithelium could be easily distinguished from cancer cells if contaminated.

HT29 cells proliferated rapidly in cell culture, with an approximate doubling time of 18 h. They grew as tightly packed cells, and exhibited typical epithelial morphology (fig. 3). In organ culture, HT29 cells also formed cell masses in the mesenchyme,

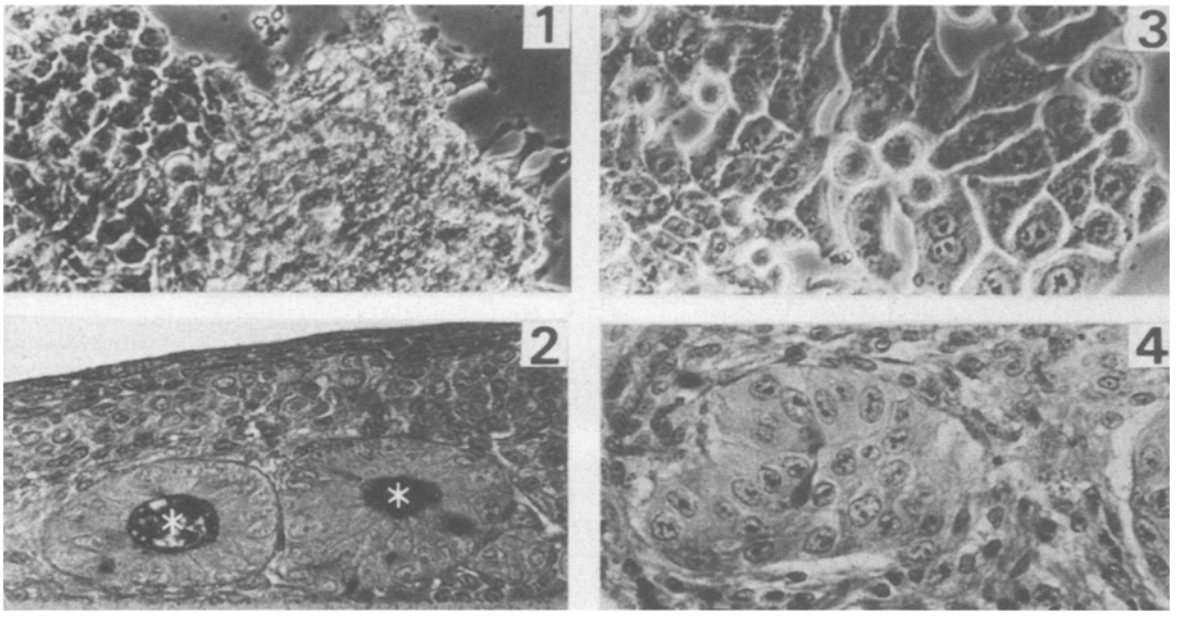
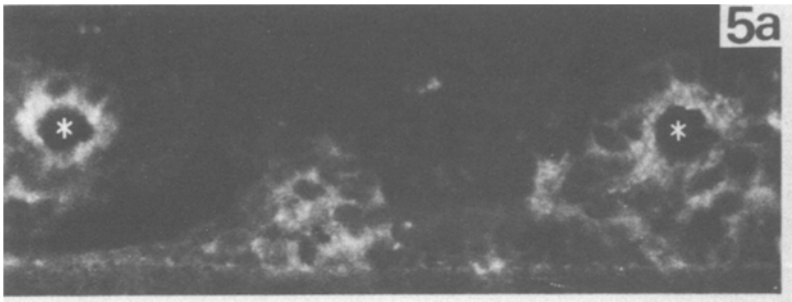


Figure 1. Phase contrast microscopy of LS174T cells in cell culture. Cells are tightly packed and some exhibit piling-up. × 300.

Figure 2. LS174T cells combined with fetal rat forestomach mesenchyme in organ culture. PAS-positive mucus is accumulated in the lumen (asterisks). × 400.

Figure 3. Phase contrast microscopy of HT29 cells in cell culture. Note typical polygonal cells. × 300.

Figure 4. HT29 cells combined with fetal rat forestomach mesenchyme in organ culture. Note undifferentiated cell masses. × 400.



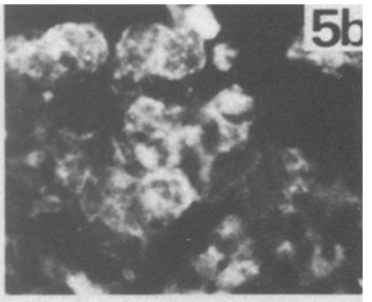
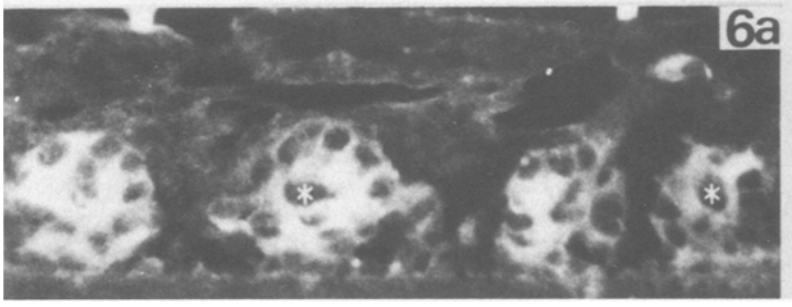


Figure 5. Immunofluorescent microscopy of LS174T cells in organ culture with anti-CEA antibodies. *a* combined with fetal rat forestomach mesenchyme. The lumen is indicated by asterisks. *b* without mesenchymes. × 400.



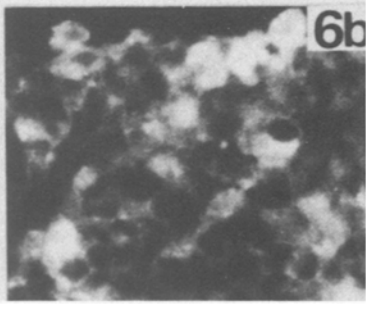


Figure 6. Immunofluorescent microscopy of LS174T cells in organ culture with anti-SC antibodies. *a* combined with fetal rat forestomach mesenchyme. The lumen is indicated by asterisks. *b* without mesenchymes. × 400.

like LS174T cells, often accompanied with intra-epithelial vacuoles, but never formed glandular structure. Cells adhered to mesenchyme so weakly that cell masses often detached from the mesenchyme during fixation and embedding. Nuclei were oriented randomly, and no polarity was observed in the cell mass (fig. 4).

Immunohistochemistry using anti-CEA and anti-SC antibodies was performed to examine whether human colon cancer cells had characteristics similar to those normal of colonic epithelial cells after combination with fetal rat mesenchymes in organ culture. When LS174T cells were grown on membrane filters without mesenchyme, CEA and SC were diffusely distributed in the cytoplasm (figs 5b, 6b). But when the cells formed glandular structures in combination with mesenchymes, CEA and SC were localized on the cell membranes. The lumen-forming apical cell membrane was stained most intensively with anti-CEA antibody, and baso-lateral membranes were only weakly stained (fig. 5a). In contrast, SC was distributed evenly all round cell membranes (fig. 6a).

HT29 cells were always negative for anti-SC antibodies in both cell and organ culture. CEA was diffusely distributed in the cytoplasm in cell culture, but was localized around the intra-epithelial vacuoles in the cell mass when HT29 cells were combined with the mesenchyme (data not shown).

Discussion. In the present investigation, we found that LS174T cells formed glandular structures comparable to normal colon glands when combined with fetal rat mesenchymes during a 4-day culture period. This indicates that some cancer cells can respond to the surrounding mesenchyme to restore and establish cellular polarity and cell-to-cell interactions. The morphogenetic potential of LS174T cells in combination with mesenchymes was first reported by Ridge and Noguchi[11] by combining cells with embryonic chick skin and culturing for 6–16 days. Our results are consistent with theirs, and support the idea that LS174T cells represent highly morphogenetic cells, and that they would be useful in studying epithelial morphogenesis in detail.

HT29 cells, in contrast, failed to form glandular structures in combination with fetal rat mesenchymes in the present study, though they can form tight junctions[12], domes[13], and intracellular lumina[14]. This shows that these mesenchymes play only a minor part in regulating epithelial morphogenesis of cancer cells, and that cells which have characteristics for epithelial morphogenesis do not always form glandular structures in combination with the mesenchyme.

Many investigators have reported the effect of normal mesenchymes/stromas on the differentiation of cancer cells[4–6], but none has succeeded in restoring the normal phenotype by combining cancer cells with normal tissues with the exception of Mintz and Illmensee[15] whose claims have not been substantiated by others. Our present results are consistent with our previous reports that mesenchymes act only permissively in regulating epithelial differentiation in normal development[7,8], and indicate that mesenchymes should be considered as providing a supportive environment for the morphogenesis of epithelial cells.

The subcellular distribution of CEA and SC has recently been described; CEA was found on the apical surface[16], and SC on the baso-lateral surface of normal colonic epithelial cells[17], while in neoplastic cells CEA and SC were found all around the cell surface[18]. In the present study, we found that LS174T cells formed glandular structures composed of simple columnar cells with nuclei oriented basally in combination with fetal mesenchymes, and we concluded that the cellular polarity was induced by combined mesenchymes. But immunohistochemical studies showed that CEA and SC were found all around the cell surface when LS174T cells formed glandular structures, suggesting that the composition of cell surface glycoproteins was not necessarily reversed to the normal epithelial type, even when neoplastic cells exhibited normal glandular structures.

The invasive behavior of cancer cells has been intensively studied[19]. Using developing chick wing bud as the assay system, Tickle et al.[20] studied the invasive behavior of cancer cells, and reported that the majority of carcinoma cells did not invade the mesenchyme but were positioned in the ectoderm. We have shown that human colon cancer cells rapidly migrate into the mesenchyme when combined with fetal rat digestive-tract mesenchymes. This indicates that digestive-tract mesenchymes may be superior to other mesenchymes in studying the invasive behavior of cancer cells in organ culture.

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Mitochondrial abnormalities in fibroblast line GM3093 defective in oxidative metabolism[1]

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Summary. Fibroblast line GM3093 deficient in the activity of the pyruvate dehydrogenase complex, was derived from a patient reported to have an inherited defect affecting the tricarboxylic acid cycle. Our results suggest a generalized defect consisting of few and abnormal mitochondria and low activities of all mitochondrial enzymes examined.
Key words. Mitochondria; mitochondrial abnormalities; mitochondrial enzymes; oxidative metabolism; fibroblast line GM3093.

Certain inherited neurological diseases have been attributed to defective oxidative metabolism. Thus, a defect in the pyruvate oxidation system has been shown in some patients with lactic acidemia and diffuse neurologic disease[5], a deficiency of mitochondrial malic enzyme in patients with Friedreich's ataxia[6], and a partial deficiency of the glutamate dehydrogenase in some patients with adult-onset olivopontocerebellar degeneration[7].

Fibroblast line GM3093 was derived from a now deceased 3-year-old girl with severe diffuse neurologic disease and persistent lactic acidosis[8]. Her fibroblasts oxidized radioactive citrate, palmitate, and pyruvate at a rate less than one third of that of the control cells. Cell homogenates had deficient activity of the pyruvate dehydrogenase complex (PDHC). It was concluded that the patient had an inherited defect affecting the tricarboxylic acid cycle. This is the only PDHC-deficient fibroblast line which is widely available and is used extensively as a reference. The objective of the present study was to further localize the defect in cell line GM3093. This led us to the measurement in cell homogenates of the activities of several mitochondrial and non-mitochondrial enzymes, and in electron microscopic examination of the mitochondria in cultured skin fibroblasts.

Materials and methods. GM3093 and control human skin fibroblast lines were obtained from the human Genetic Mutant Cell Repository, Institute for Medical Research, Camden, NJ. The cells were grown in McCoys 5A media with glutamine, supplemented with 10% fetal calf serum, 100 units/ml mycostatin, and 50 µg/ml gentamycin and were harvested at confluency. The harvested cells were subjected to three cycles of freeze-thawing and the homogenates were used immediately. Enzyme substrates, coenzymes, and all other chemicals were obtained from commercial suppliers at the highest grade available and were used without further purification.

Enzymic activities were assayed by published procedures adapted for this study. In particular, the activities of PDHC and α-ketoglutarate dehydrogenase complex (α-KGDHC) were measured radiochemically as follows: Radioactive substrates [1-C^{14}] pyruvic acid, sodium salt, 2–20 Ci/mol and [1-C^{14}] α-etoglutaric acid, sodium salt, 40–60 Ci/mol were purchased from New England Nuclear Corp. (Boston, MA) and were handled according to Blass et al.[9]. The reaction mixture (final volume 200 µl and pH 7.8) in 12 × 75 mm disposable glass test tubes, in an ice-bath, contained (final concentrations, mM), Tris buffer, 5, KCl, 25, $MgCl_2$, nicotinamide, 15, Na_2SO_3, 0.5, EDTA, 0.5, mercaptoethanol, 0.5, NAD^+, 0.3, CoA, 0.2, TPP, 0.1, pyruvate, 0.5 (5×10^5 cpm) or α-ketoglutarate, 0.1 (2×10^5 cpm), and fibroblast homogenate (50–100 µg protein). Cofactors were

Enzymic activities in GM 3093 and control fibroblast homogenates

Enzyme		GM 3093	Controls
		nmol/min/mg protein*	
Pyruvate dehydrogenase complex (non activated)		0.347 ± 151	1.45 ± 0.466
Pyruvate dehydrogenase complex (activated)		0.569 ± 0.110	2.66 ± 0.583
Dihydrolipoamide dehydrogenase	EC 1.6.4.3	17.4 ± 7	58.6 ± 12.1
α-Ketoglutarate dehydrogenase complex	EC 1.2.4.2	0.229 ± 0.043	0.912 ± 0.24
Isocitrate dehydrogenase[b] $NADP^+$	EC 1.1.1.42	17.1 ± 3.9	36.9 ± 14.3
Glutamate dehydrogenase[b] NADH	EC 1.4.1.3	887 ± 493	2554 ± 765
Cytochrome C oxidase[b]	EC 1.9.3.1	2.0 ± 0.9	10.45 ± 1.34
Malic enzyme[c] (mitochondria)	EC 1.1.1.40	0.5	1.21 ± 0.9
Lactate dehydrogenase[d, b]	EC 1.1.1.27	1137 ± 110	699 ± 91

*Values are mean ± SD; [a]Using the reverse reaction: lipoamide + NADH + H^+ → dihydrolipoamide + NAD^+, 30 °C; [b]Temperature 30 °C; [c]Data from Stumpf et al.[17]; [d]Utilizing the reaction: lactate + NAD^+ → pyruvate + NADH + H^+.