In the experiments of series IV there were no colonies and large areas of the dish were occupied by a monolayer of large cells, separated by wider intercellular spaces than in the experiments of series III (Fig. 2). The number of labeled cells in these areas varied from 12 to 22% (Fig. 2). The electron-autoradiographic investigation showed that cells which had already passed through the initial stages of differentiation could be labeled with ^{3}H-thymidine; in the perinuclear zone they formed a layer of tonofilaments, and only few keratohyalin granules were found (Fig. 3). In some smaller areas of the cultures described above the cells were smaller, did not form a monolayer, and did not take up ^{3}H-thymidine. Keratinization was expressed by the presence of a certain number of isolated horn cells.

Culture of adult human epidermocytes in medium containing 0.15 mM calcium thus inhibits differentiation of the cells and stimulates cell division. These cultures largely fill the dish 1 week after seeding. The large number of dividing cells in the cultures enables them to be used both for transplantation to a wound surface and also for subsequent culture.

LITERATURE CITED

1. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, Adaptive Reorganization of Biorhythms [in Russian], Moscow (1975).
2. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, Electron-Microscopic Autoradiography of the Cell [in Russian], Moscow (1980).
3. J. Clarke, A. Burt, A. Eldad, and B. Gusterson, Lancet, 2, No. 8510, 809 (1986).
4. M. Eisinger, Ji Soo Lee, J. Hefton, et al., Proc. Natl. Acad. Sci. USA, 76, No. 10, 5340 (1979).
5. H. Green, Cell, 15, No. 7, 801 (1978).
6. H. Hennings, D. Michael, C. Cheng, et al., Cell, 19, No. 1, 245 (1980).
7. D. Peehl and R. Ham, In Vitro, 16, No. 6, 516 (1980).
8. J. Rheinwald and H. Green, Nature, 265, 421 (1977).
9. R. Teepe, M. Ponec, R. Kreis, and R. Hermans, Lancet, 1, No. 8477, 385 (1986).

A METHOD OF OBTAINING A LONG-LIVING INTESTINAL TISSUE CULTURE

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Interest in tissue culture research has increased considerably in recent years [3, 5, 6]. A special place is occupied by the study of the properties of intestinal tissue. The small intestine (SI) has been studied by various methods: the "everted pouch," a culture of isolated intestinal cells, and the organ culture method. The "everted pouch" method is applicable under experimental conditions. Some results have been obtained with respect to isolation and culture of single intestinal cells: enterocytes in culture preserve their viability for 4 h [1, 4]. However, in such a short time interval, opportunities for solving problems of clinical importance are limited. The viability of the human intestine in organ cultures, with preservation of the synthetic and secretory functions of intestinal tissue during explant culture for 24 h have been reported [3, 7]. However, no such practical studies have been described in the Soviet literature.

The method of small intestinal tissue culture has been used to study pathological states of the intestine included under the general term of "malabsorption syndrome." Disturbance

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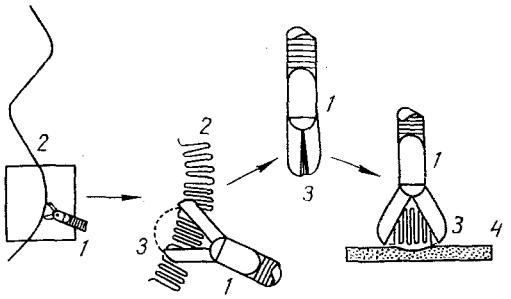

Fig. 1. Diagram showing removal of biopsy specimen from mucous membrane of SI, its transportation on a strip of filter paper, with its correct orientation. 1) Blades of biopsy forceps; 2) intestinal mucosa; 3) biopsy specimen of mucous membrane of SI: 4) strip of filter paper.

of intestinal absorption is an important problem in pediatrics today. Celiac disease has been investigated intensively as one version of the malabsorption syndrome, but results obtained with short-living tissue cultures have proved to be contradictory [2]. Lengthening the life of biopsy material naturally is not only economically important, but it also would enable more extensive investigations to be undertaken.

In the investigation described below a long-living organ culture of human SI was studied.

Biopsy specimens obtained by the method of direct vision esophagogastroduodenojejunoscopy with biopsy on children under investigation because of disturbed intestinal absorption were chosen for study. The gastroduodenojejunoscopy was carried out with A GDF-3 apparatus (Olympus, Japan), and biopsy of the jejunum was performed at the level of 30-40 cm distal to the ligament of Treitz.

The cultural investigations were carried out in another place, so that the biopsy specimens had to be transported. This was done in sterile Hanks' nutrient medium (standard commercial medium from "Flow Laboratories," England) with the addition of antibiotics, in the cold, and in the shortest possible time. In the laboratory, the biopsy specimen was laid out on metal cultural grids, lying so that the intestinal villi faced upward, in the direction of the nutrient medium, thereby simulating their natural arrangement in vivo. Explants were cultured in nutrient medium which included as its components the standard NCTC and Trowell (Flow Laboratories) commercial media, antibiotics (polymyxin, kanamycin), antifungal preparations (mycostatin), and additives (glutamine, bovine serum). Into each well of a multiwell plate (sterile plates for tissue culture) 0.5 ml of medium was added, after which the explants were aerated with a mixture of 95% O_2 and 5% CO_2 for 3-5 min, then incubated at 37°C.

A series of investigations was carried out, with incubation of the explants for different periods: 4, 16, and 24 h. After incubation the explants were fixed in formalin and histological specimens were prepared by the usual method and stained. Control examination showed that the explants cultured in this way remained viable for only 4 h. After a longer period of incubation irreversible changes took place in the explants: marked swelling and lysis of the cells and necrosis. These failures were attributed to the improper orientation of the preparations, leading to disturbance of nutrition of the tissue and to loss of its viability. To solve this problem, the biopsy specimen was oriented immediately after its removal from the organ. After removal the biopsy material was transferred from the blades of the biopsy forceps to a narrow strip of filter paper (sterile filters, prepared beforehand, treated with UV rays) measuring 5.0 × 0.5 cm, enabling the specimen to be fixed in such a way that the intestinal villi remained free (Fig. 1). As many as two to four biopsy specimens could be laid on a strip. This strip was placed in a test tube containing sterile transportation solution in a volume of 5.0 ml, and the tubes were sent to the tissue culture laboratory in a vessel with ice. Later it was sufficient to cut the strip into pieces measuring 0.5 × 0.5 cm, with a specimen on each piece, and to culture the oriented explant, well secured and correctly oriented, by the method described above without metal grids on the piece of filter

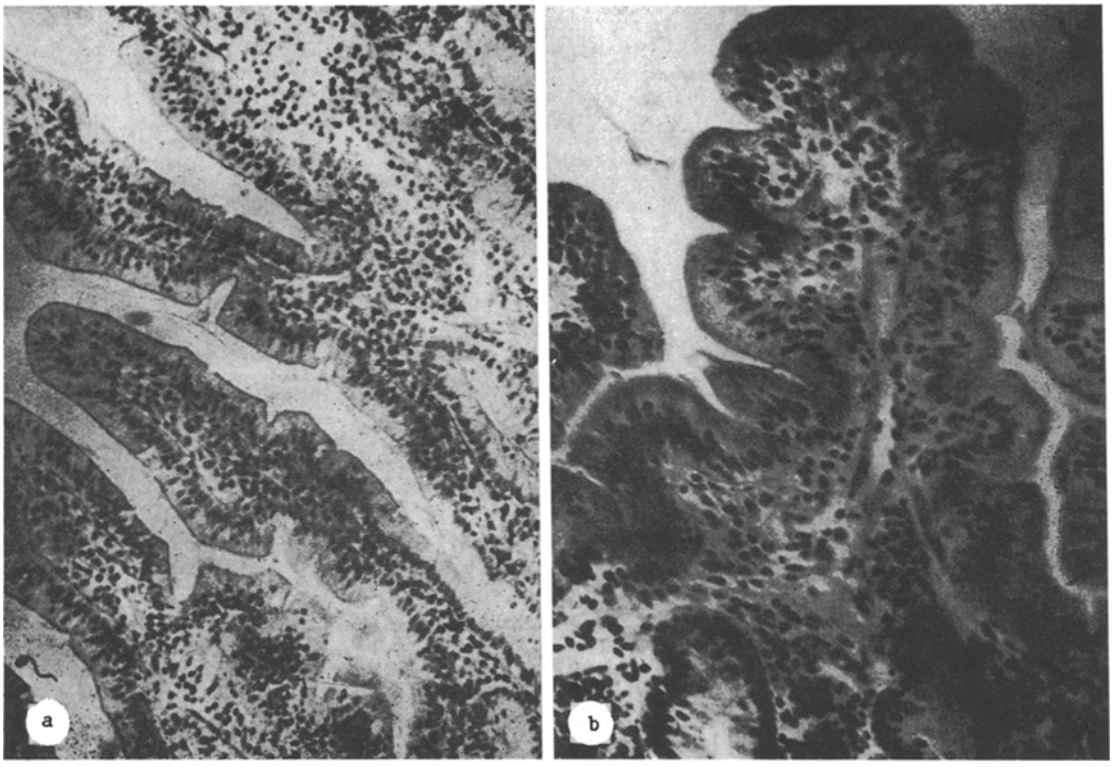

Fig. 2. Explant from human small intestine before culture (a) and after incubation for 24 h in nutrient medium (b). Hematoxylin and eosin. Magnification: a) 20, b) 40×.

paper. With this simple modification, the life of the explants could be prolonged to 24 h, disregarding transportation time, as was confirmed by the control histological investigations: the structure of the enterocytes was preserved, swelling of their nuclei was negligible, and the cell boundaries remained clear (Fig. 2). During histochemical examination, moreover, the explants demonstrated a normal level of enzyme activity in the brush border of the enterocytes: alkaline phosphatase – the principal marker of the brush border, sucrase, leucine aminopeptidase, etc. This was in agreement with the results of investigations by workers outside the USSR, who also reported viability of the intestine in organ cultures [7].

Thus biopsy specimens obtained from SI during direct vision biopsy can provide material for obtaining long-living intestinal tissue cultures. Fixation of the biopsy material on a strip of filter paper immediately after its removal enables it to be correctly oriented for subsequent successful culture for 24 h. With the aid of radionuclide precursors of different types of synthesis (^{14}C-leucine for protein synthesis, ^{3}H-thymidine for nucleic acid synthesis, ^{14}C-acetate for lipid synthesis, and so on) in the form of additives to the nutrient medium followed by isolation of the test substance from the explant, processes in the enterocyte of interest to the research worker can be studied on the resulting culture of SI. The study in vitro of explants obtained in diseases of the intestine will perhaps enable the extrinsic and intrinsic factors of pathogenesis to be distinguished and the metabolism of the different intestinal disorders to be interpreted afresh. The addition of various drugs to the medium will enable adequate treatment to be chosen.

LITERATURE CITED

1. R. Eloy, F. Raul, A. Pousse, et al., Eur. J. Surg. Res., 9, 96 (1977).
2. J. Jos and L. Rey, Arch. Franç. Mal. Dig., 64, 461 (1975).
3. L. M. Lichtenberger, J. Lechago, and T. A. Miller, Gastroenterology, 77, 1291 (1979).
4. M. P. Moyer, Proc. Soc. Exp. Biol. (New York), 174, 12 (1983).
5. A. Quaroni and R. J. May, Meth. Cell Biol., 21B, 20 (1980).
6. A. Quaroni, J. Cell Biol., 100, 1611 (1985).
7. J. S. Trier, Meth. Cell. Biol., 21, 365 (1980).